The Journal of Neuroscience, February 15, 1998, *18*(4):1187–1195

Acetylcholine Activates an α-Bungarotoxin-Sensitive Nicotinic Current in Rat Hippocampal Interneurons, But Not Pyramidal Cells

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The effects of acetylcholine on both pyramidal neurons and interneurons in the area CA1 of the rat hippocampus were examined, using intracellular recording techniques in an *in vitro* slice preparation. In current-clamp mode, fast local application of acetylcholine (ACh) to the soma of inhibitory interneurons in stratum radiatum resulted in depolarization and rapid firing of action potentials. Under voltage-clamp, ACh produced fast, rapidly desensitizing inward currents that were insensitive to atropine but that were blocked by nanomolar concentrations of the nicotinic α7 receptor-selective antagonists α-bungarotoxin (αBgTx) and methyllycaconitine. Nicotinic receptor antagonists that are not selective for α7-containing receptors had little (mecamylamine) or no effect (dihydro-β-erythroidine) on the ACh-induced currents. Glutamate receptor antagonists had no effect on the ACh-evoked response, indicating that the current was not mediated by presynaptic facilitation of glutamate release. However, the current could be desensitized almost completely by bath superfusion with 100 nM nicotine. In contrast to those actions on interneurons, application of ACh to the soma of CA1 pyramidal cells did not produce a detectable current. Radioligand-binding experiments with [^{125}I]-αBgTx demonstrated that stratum radiatum interneurons express α7-containing nAChRs, and *in situ* hybridization revealed significant amounts of α7 mRNA. CA1 pyramidal cells did not show specific binding of [^{125}I]-αBgTx and only low levels of α7 mRNA. These results suggest that, in addition to their proposed presynaptic role in modulating transmitter release, α7-containing nAChRs also may play a postsynaptic role in the excitation of hippocampal interneurons. By desensitizing these receptors, nicotine may disrupt this action and indirectly excite pyramidal neurons by reducing GABAergic inhibition.

Key words: nicotine; hippocampus; interneuron; rat; acetylcholine; electrophysiology

α-Bungarotoxin (αBgTx) is a snake toxin that binds with high affinity to nicotinic acetylcholine receptors (nAChRs) present in muscle. Although [^{125}I]-αBgTx binding sites are also abundant within the CNS, attempts to determine the functional significance of the central binding site have been unsuccessful for the most part (Clarke, 1992; McGehee and Role, 1995). The absence of αBgTx-sensitive cholinergic responses in areas with [^{125}I]-αBgTx binding (Oswald and Freeman, 1981), an inability to precipitate αBgTx binding proteins with antibodies raised against functional nAChRs (Patrick and Stallcup, 1977; Whiting et al., 1987), and numerous examples of neurons that respond to ACh with αBgTx-insensitive currents (for review, see Clarke, 1993) led many researchers to speculate that the central αBgTx binding site was unrelated to cholinergic function. That idea was not completely abandoned until the first molecular clone of an αBgTx binding protein was isolated from embryonic chicken brain (Schoepfer et al., 1990) and was shown to form a functional nAChR when expressed in *Xenopus* oocytes (Couturier et al., 1990). Since then, functional homologs of that protein, now known as the α7 nAChR subunit, have been identified in multiple species, and αBgTx-sensitive, α7-mediated nicotinic currents have been found in cultured, acutely dissociated, and immortalized cell types (Alkondon et al., 1992; Zorumski et al., 1992; Albuquerque et al., 1995; Komourian and Quik, 1996). Numerous studies on cultured hippocampal neurons have provided us with what is perhaps the best-characterized example of an αBgTx-sensitive nicotinic current in a CNS preparation (Alkondon and Albuquerque, 1991, 1993; Zorumski et al., 1992; Castro and Albuquerque, 1993; Albuquerque et al., 1995). However, it has continued to be difficult to demonstrate responses that reflect the activation of αBgTx-sensitive nAChRs in intact hippocampus or in hippocampal slices.

Several recent studies, including one in hippocampus, have demonstrated a nicotine-induced, αBgTx-sensitive presynaptic enhancement of synaptic transmission (McGehee et al., 1995; Alkondon et al., 1996b; Gray et al., 1996). Those studies, coupled with the previous difficulty involved in finding any αBgTx-sensitive cholinergic responses in the CNS, have led to the hypothesis that the predominant role of nAChRs in the brain (including the αBgTx-sensitive receptors) may be to modulate synaptic transmission via actions at presynaptic sites (McGehee and Role, 1996; Role and Berg, 1996). Indeed, outside of a few noteworthy exceptions (Curtis and Ryall, 1966), there is little evidence that postsynaptic nAChRs mediate synaptic transmission in the CNS. Nevertheless, the high level of α7-containing nAChRs in the hippocampus (Seguela et al., 1993), the robust cholinergic innervation arising from the medial septum (Woolf, 1991), and the well defined α7 responses observed in cultured hippocampal neurons (Albuquerque et al., 1995) suggest that

Received July 9, 1997; revised Nov. 20, 1997; accepted Nov. 20, 1997.

This work was supported by National Institutes of Health Grants AA11164, DA03194, MH44212, DA09457, and AG10755 and the Veterans Administration Medical Research Service.

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nAChR-mediated synaptic transmission in the hippocampus cannot be ruled out. In the present study we used intracellular recording techniques, coupled with differential interference contrast microscopy, to examine the location and pharmacology of neuronal nAChRs present in area CA1 of the rat hippocampus. Our results indicate that somatic α7-containing nAChRs mediate fast, rapidly desensitizing inward currents on stratum radiatum interneurons, but not on CA1 pyramidal cells, and present a likely postsynaptic target for ACh-containing afferents from the septal region.

MATERIALS AND METHODS

Whole-cell recording. Young (18–27-d-old) male Sprague Dawley rats were used for all experiments. Housing and treatment of all animals were in accordance with institutional guidelines. Animals were decapitated, and a vibratome (Pelco) was used to prepare 300-μm-thick coronal slices of hippocampus. During incubation the slices were submerged at room temperature in artificial CSF containing (in mM): 124 NaCl, 3.3 KCl, 2.4 $MgSO_4$, 10 D-glucose, 2.5 $CaCl_2$, 1.2 KH_2PO_4, and 25.9 $NaHCO_3$ saturated with 95% O_2/5% CO_2. In experiments involving lead and cadmium, a HEPES-based (free acid) buffer, pH 7.3 (saturated with 100% O_2) was used. All experiments were performed at room temperature while the tissue was superfused with buffer at a rate of 2 ml/min. Whole-cell patch-clamp recording was accomplished by using glass pipettes pulled on a Flaming/Brown electrode puller (Sutter Instruments, Novato, CA). The resistance of the pipettes was 6–10 MΩ when filled with a potassium gluconate-based internal solution, which consisted of (in mM): 130 KOH, 130 gluconic acid, 1 EGTA, 2 $MgCl_2$, 0.5 $CaCl_2$, 2.54 ATP (di Na^+), and 10 HEPES (free acid) adjusted to pH 7.25 with additional KOH. Cells were viewed with an upright microscope equipped with Nomarski optics. Brief pulses (5–20 msec) of ACh were applied directly to the cell body via pressure microejection (5–20 psi) from pipettes identical to the recording pipettes, using a Picospritzer II (General Valve, Fairfield, NJ). Inward currents were recorded in voltage-clamped cells with an AxoClamp 2A (Axon Instruments, Foster City, CA) operating in the continuous-clamp mode. Data were recorded on a microcomputer with NeuroPro software (RC Electronics) and analyzed in Microsoft Excel with a custom-built add-in. Calibrated syringe pumps (Razel) were used to add drugs from a concentrated stock solution directly to the superfusion system. In experiments requiring double-barrel electrodes, such electrodes were prepared as previously described (Palmer et al., 1986).

[^{125}I]-αBgTx binding. Cryostat-cut frozen sections (12 μm) were collected on Probe-on slides (Fisher Scientific, Pittsburgh, PA). Slides for total [^{125}I]-αBgTx binding were incubated for 30 min at room temperature in binding buffer [0.05 M Tris-buffered saline (TBS), pH 7.4, and 0.2% bovine serum albumin]. Adjacent sections for nonspecific binding were preincubated in 5 mM nicotine bitartrate (Sigma, St. Louis, MO) in binding buffer. All slides were incubated for 3 hr at 37°C in binding buffer containing 5 nM [^{125}I]-αBgTx (specific activity, 2000 Ci/mmol; Amersham, Arlington Heights, IL) with or without the addition of 5 mM nicotine bitartrate to assess nonspecific binding. This concentration of [^{125}I]-αBgTx has been shown in rat hippocampus to bind a single site, which is presumably the α7 receptor (Clarke et al., 1985). After incubation, slides were washed at 37°C in binding buffer for 5 min, followed by TBS for 15 min, and finally in 0.05 M PBS for 5 min. Slides were dehydrated in ethanol, dried, and subjected to emulsion autoradiography (NTB-2, diluted 1:1 with water; Kodak, Rochester, NY) for 21 d.

In situ *hybridization for α7 mRNA and GABA immunoreactivity.* Sense and antisense α7-specific (GenBank number U40583; 338 base pairs) cRNA probes were transcribed (Ambion, Austin, TX) in the presence of [^{35}S]-UTP (Amersham, Arlington Heights, IL). The procedure for probe preparation, *in situ* hybridization, and GABA immunoreactivity was performed as previously described (Freedman et al., 1993; Breese et al., 1994, 1997).

RESULTS

ACh application induces an αBgTx-sensitive current in stratum radiatum interneurons

Pressure application was used to apply brief pulses of ACh (1 mM in all cases) from glass micropipettes directly onto the cell bodies of 76 identified stratum radiatum interneurons during whole-cell recording (Fig. 1*A*). Because virtually all neurons in stratum radiatum of rat hippocampus are GABAergic interneurons (Ribak et al., 1978), these cells were identified initially on the basis of their location. However, they also could be identified electrophysiologically on the basis of a distinctive resting membrane potential (−50 to −55 mV) and an ability to sustain a high firing rate (10–20 msec interspike interval) throughout a 250–300 msec depolarizing pulse (Fig. 1*B*). In contrast, CA1 pyramidal cells had more hyperpolarized (−60 to −65 mV) resting mem-

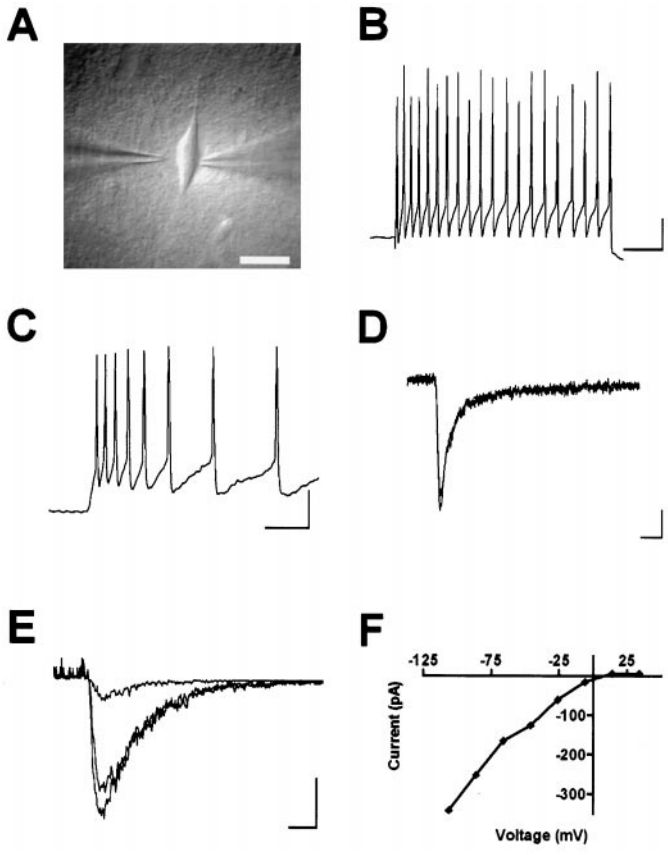

Figure 1. Electrophysiological responses from stratum radiatum interneurons in area CA1. *A*, A stratum radiatum interneuron is visualized, along with a potassium gluconate-filled patch electrode (*right*) and a drug pipette containing 1 mM ACh (*left*). The tip of the drug pipette was placed within 5 μm of the cell body. Scale bar, 20 μm. *B*, In a current-clamp mode recording from an interneuron, a 10 mV, 250 msec depolarizing pulse induced rapid firing of action potentials. Relatively little accommodation of the rate of firing was observed during the pulse. Calibration: 100 msec, 20 mV. *C*, In current-clamp mode, ACh application (5–20 msec) resulted in a depolarization sufficient to generate bursts of action potentials. Calibration: 100 msec, 100 pA. *D*, In voltage-clamp mode, pressure application of ACh to these cells produced a fast, rapidly decaying inward current (*smaller trace*), which was insensitive to treatment with 5 μM atropine (*larger trace*). The average response amplitude after treatment with atropine in five cells tested in a similar manner was 101 ± 9.4% of control. Calibration: 50 msec, 20 pA. *E*, ACh application at 5 sec intervals dramatically reduced current amplitude (*small trace*) relative to responses tested at 15 sec intervals (*large traces*). Calibration: 50 msec, 10 pA. *F*, *I–V* plot for the ACh-induced current in a stratum radiatum interneuron. The cell was voltage-clamped at −66 mV and then stepped from −106 to +36 mV in 20 mV increments. Voltage steps lasted 4 sec. ACh application occurred 2 sec after the onset of the voltage step. The resulting *I–V* curve indicates a reversal potential for the ACh-induced current of ~0 mV and strong inward rectification at depolarizing potentials. Traces in *D* and *E* are signal averages of four to nine individual responses.

brane potentials, demonstrated accommodation of firing rate during a 250–300 msec depolarizing pulse, and had comparatively slow (25–40 msec) minimum sustainable interspike intervals. In 70 of 76 interneurons, ACh application evoked depolarizations that caused strong burst firing of action potentials when recording in current-clamp mode (Fig. 1*C*) and induced large (81.0 ± 5.97 pA), rapid, inward currents under voltage-clamp conditions (Fig. 1*D*). These responses showed essentially no rundown and could be elicited repeatedly for >1 hr. Responses normally were evoked by application of ACh at 30 sec intervals, although 15 sec intervals were equally effective. If the interval was reduced to 5 sec, response amplitudes rapidly declined but recovered on returning to 15 sec intervals (Fig. 1*E*). These results suggest that the receptors can be desensitized by frequent application of ACh and that the period of desensitization is significantly longer than the duration of the current response. Current–voltage relationships for ACh-evoked responses demonstrated an extrapolated reversal potential of ~0 mV and strong inward rectification at depolarizing potentials (Fig. 1*F*).

Superfusion with 5 μM atropine had no effect on the ACh-induced current (Fig. 1*D*), indicating that the response was not mediated nor modulated by muscarinic cholinergic receptors. Superfusion with 100 nM αBgTx produced near-complete and irreversible antagonism of the ACh-evoked inward current (Fig. 2*A*). A similar extent of inhibition was observed with 10 nM αBgTx but required a longer equilibration period to become effective. Because the α7 subunit is the only known αBgTx binding protein expressed in the rat hippocampus (Seguela et al., 1993), antagonism by that toxin provided strong evidence that the current was mediated via nAChRs containing the α7 subunit. Responses also were blocked almost completely by 10 nM methyllycaconitine (MLA), a selective and potent α7 antagonist (Palma et al., 1996) (Fig. 2*B*). Although the block at 10 nM was not quite complete, at that concentration the maximal MLA effect was achieved more rapidly than with αBgTx and was reversed more readily by washout (Fig. 3). At higher concentrations (50–250 nM) MLA produced a complete antagonism with even more rapid onset but required considerably longer to wash out. In addition, the currents were reduced by 10 μM lead (65.8 ± 11.0% of control, $n = 6$), which previously has been shown to partially block currents mediated by α7-containing nAChRs (Vijayaraghavan et al., 1992; Ishihara et al., 1995).

In contrast to αBgTx and MLA, other nAChR antagonists with relatively lower affinity for the α7 receptor had little effect on interneuron responses to ACh. Dihydro-β-erythroidine was without effect (Fig. 2*C*) at a concentration (150 nM) that is 15 times higher than that previously shown to block type II, presumably α4β2-mediated, currents in cultured hippocampal neurons (Alkondon and Albuquerque, 1993; Albuquerque et al., 1995). Similarly, at a concentration that is more than sufficient to block type III (possibly α3β4-mediated) currents in cultured hippocampal neurons (2 μM) (Alkondon and Albuquerque, 1993; Albuquerque et al., 1995), mecamylamine produced only a minor reduction in the ACh response (Fig. 2*D*). Because nAChRs share a high degree of topographical and sequence homology with 5-hydroxytryptamine (5-HT_3) receptors (Eisele et al., 1993), we also examined the effects of the selective 5-HT_3 antagonist, 1αH, 3α, 5αH-tropan-3-yl-3, 5-dichlorobenzoate (MDL). A concentration of MDL that completely blocks 5-HT_3 receptor-mediated currents (500 nM; Fozard, 1984) did not affect the amplitude of the ACh-evoked current (97.9 ± 8.36% of control, $n = 4$). That result, in combination with the fact that MLA has little or no effect on 5-HT_3 receptors (Palma et al., 1996), ruled out a 5-HT_3 receptor-mediated component to the ACh response.

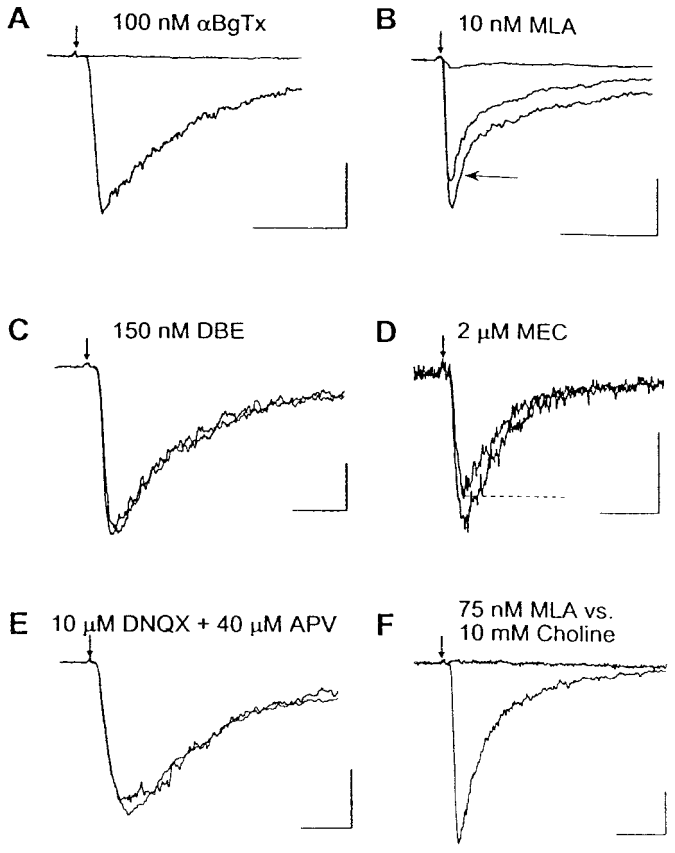

Figure 2. Pharmacological antagonism of ACh-induced currents in stratum radiatum interneurons. In each panel averaged traces (5–10 sweeps) indicate both the control response (*larger trace*) and the response during antagonist superfusion (*smaller trace*). *Vertical arrows* indicate the time of ACh application. The *horizontal arrow* in *B* indicates recovery after washout, and the *dotted line* in *D* indicates the inhibited response. Low concentrations of the highly selective α7 antagonists αBgTx and methyllycaconitine (*A*, *B*) produced near-complete blockade of the ACh-induced currents. In contrast, antagonists with relatively low affinities for the α7 receptor, such as dihydro-β-erythroidine and mecamylamine (*C*, *D*), had very little effect. *E*, DNQX and APV had no effect on ACh-induced current amplitude. *F*, Local application of choline (10 mM) produced currents that were very similar to the ACh-induced currents. Choline-induced currents were blocked almost completely by 75 nM MLA. Calibration: 100 msec, 40 pA. Figure 5 summarizes the average effects of these drugs in all cells tested.

Because several recent papers have indicated that choline may be a selective agonist of α7-containing nAChRs (Albuquerque et al., 1997; Matsubayashi et al., 1997), we also examined the ability of choline to activate currents on stratum radiatum interneurons. Although 1 mM choline applied directly to the cell body of a stratum radiatum interneuron failed to produce any detectable current, 10 mM choline evoked fast inward currents that closely resembled the ACh-induced currents in four of five cells tested. In all four of those cells the choline-induced current was antagonized completely by 75 nM MLA (Fig. 2*F*). Considered together, the results of the agonist and antagonist studies indicate that the ACh-induced current in stratum radiatum interneurons was mediated by nAChRs containing the α7 subunit.

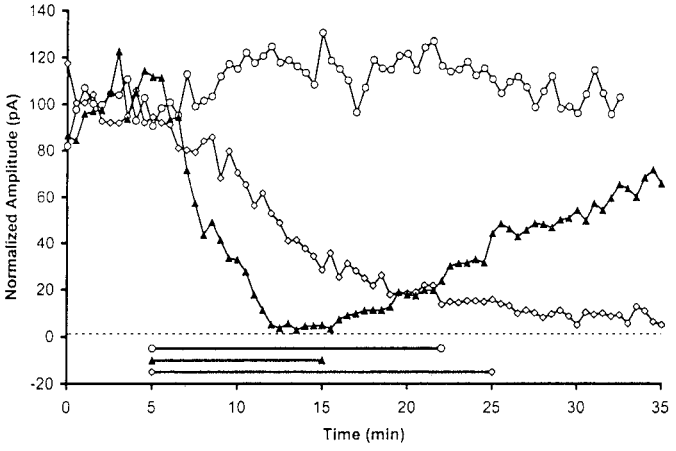

Figure 3. Comparison of αBgTx and MLA antagonism of the ACh-induced current. Superfusion with αBgTx (100 nM, *diamonds*) and MLA (10 nM, *filled triangles*) both produced near-complete antagonism of the ACh-induced current. However, the αBgTx blockade was irreversible and had a slower onset. The latter difference likely is attributable to the large size of the αBgTx [molecular weight (MW) ~8000] relative to MLA (MW = 875) and to the slow on-rate of αBgTx binding. By contrast, application of 150 nM DBE (*circles*) did not significantly affect the response. Each *line* represents the mean normalized current amplitude from three to five individual cells tested with the same drug protocol. The *horizontal bars* at the *bottom* of the figure indicate the duration of drug superfusion.

α7-Mediated current is not mediated via presynaptic receptors

Recent studies have demonstrated that some α7-containing nAChRs in brain act as presynaptic heteroreceptors on glutamatergic terminals (McGehee et al., 1995; Alkondon et al., 1996b; Gray et al., 1996). Thus, it was possible that the observed response to ACh was mediated by presynaptic facilitation of the release of glutamate, which is the primary excitatory transmitter in the hippocampus. However, inward current responses to ACh were unaffected by bath application of the ionotropic glutamate receptor antagonists 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (DNQX, 10 μM) and DL-(−)-2-amino-5-phosphonovaleric acid (APV, 40 μM; Fig. 2*E*). In addition, ACh was found to elicit inward currents of normal amplitude (128 ± 37.1 pA) in four other interneurons that were pretreated with DNQX and APV before any testing with pressure application of ACh. Finally, the ACh-evoked currents persisted even when all synaptic transmission was blocked by the combination of 500 nM TTX and 200 μM cadmium ($n = 5$). These data indicate that the ACh-induced inward currents observed in stratum radiatum interneurons were not attributable to either direct or indirect activation of glutamate receptors, nor were they dependent on any other form of synaptic transmission. Therefore, we conclude that α7-containing nAChRs are present on the soma of stratum radiatum interneurons and that those receptors are activated directly by local application of ACh.

α7-Mediated current is highly sensitive to desensitization by nicotine

Recent studies have shown that some nAChRs can be desensitized by concentrations of agonists that are too low to activate the receptor directly (Grady et al., 1994; Marks et al., 1996). In the present studies, bath superfusion of nicotine (100 nM and 1 μM) produced no significant changes in holding current but nearly abolished the response to pressure application of ACh (Figs. 4*A*, 5). Desensitization by nicotine of ACh-evoked currents persisted

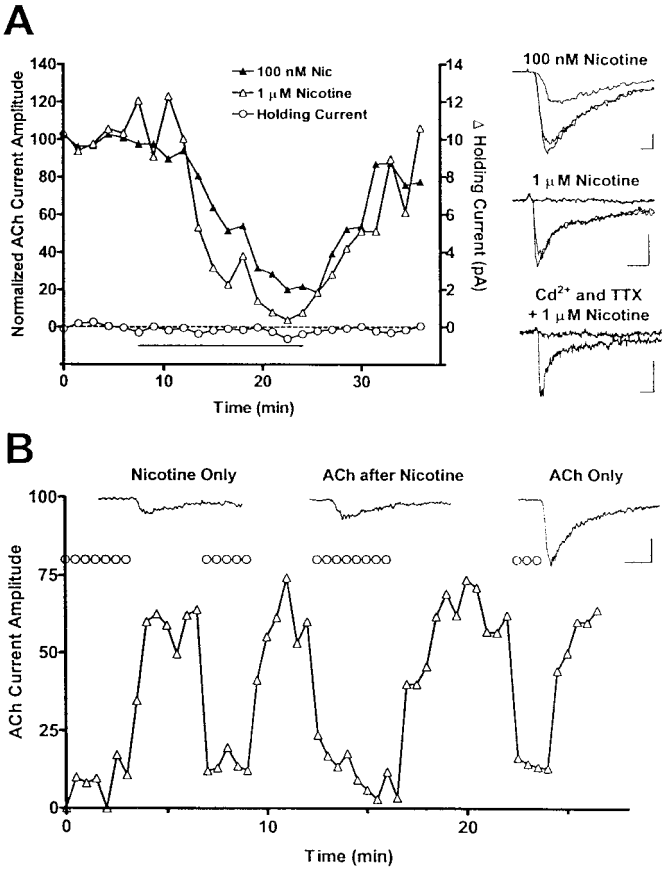

Figure 4. Nicotine-induced desensitization of ACh responses in interneurons. *A*, Bath superfusion of either 100 nM (*filled triangles*) or 1 μM (*open triangles*) nicotine reversibly desensitized the response to local application of ACh. Each *point* represents the average of three consecutive responses measured at 30 sec intervals. The *horizontal line* (*bottom*) indicates the time of nicotine superfusion. Traces on the *right* are averages of 5–10 control responses, responses during nicotine superfusion (*small traces*), and recovery. The *bottom* of the three sets of traces illustrates that the effect of 1 μM nicotine was unaltered in a slice pretreated with TTX (500 nM) and cadmium (200 μM). Calibration: 50 msec, 20 pA. Figure 5 summarizes the average effect of nicotine superfusion across all experiments. *B*, Double-barreled pipettes filled with 100 μM nicotine and 1 mM ACh also were used to demonstrate desensitization. The graph indicates the amplitude of the inward current induced by ACh application, which was tested at 30 sec intervals throughout the experiment. When nicotine was ejected 15 sec before ACh application (*open circles*), the ACh response was reduced by 80–90%. By contrast, application of ACh at 15 sec intervals ($n = 2$; data not shown) had no effect on the current amplitude. Traces at the *top* are signal averages indicating (from *left* to *right*) the response to nicotine, the response to ACh 15 sec after nicotine application, and the response to ACh without nicotine preapplication. Calibration: 100 msec, 30 pA.

in the presence of tetrodotoxin (500 nM) and cadmium (200 μM; Figs. 4*A*, 5), indicating that it was not the result of nicotine-induced synaptic release of another mediator. Desensitization also could be demonstrated with local pressure application of nicotine. When it was applied via standard drug application pipettes (tip diameter, 1–2 μm) with the same protocol as ACh, nicotine (1 mM) failed to produce any detectable inward currents. To determine whether this reflected receptor desensitization, we loaded double-barreled, high-resistance (tip diameter, <1 μm per barrel) drug application pipettes with 1 mM ACh in one barrel and 100 μM nicotine in the other. In three of three interneurons tested, ACh elicited the normal inward current response, whereas

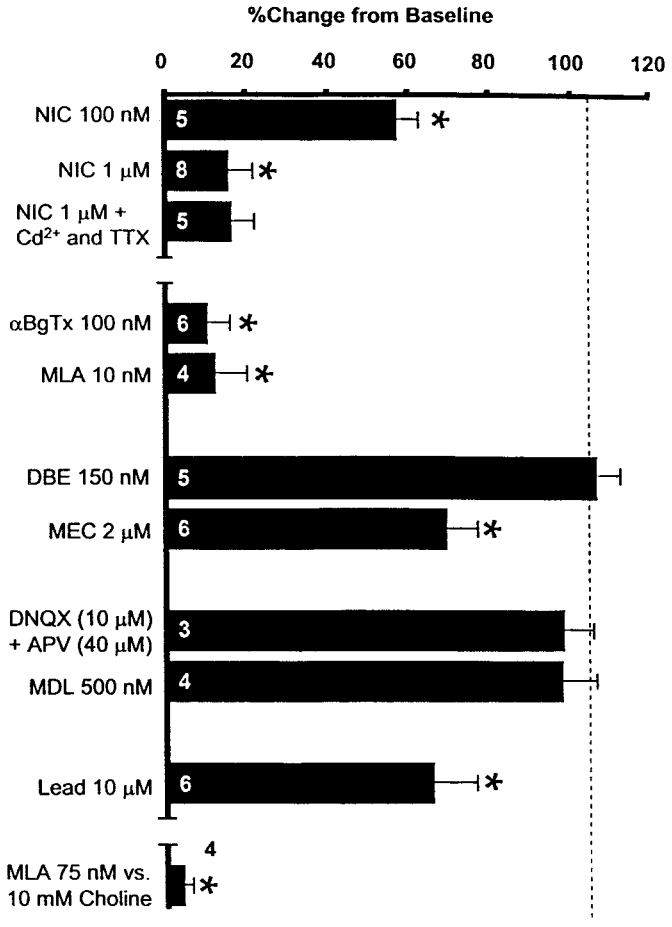

Figure 5. Summary of effects of superfusion with nicotine and nAChR antagonists on responses to locally applied ACh or choline (*bottom bar*). Each *bar* represents the average peak magnitude of the inward current response during superfusion with the indicated drug as a percentage of the control response. Error bars represent the SEM, the *number* on each bar indicates the number of cells tested, and *asterisks* indicate statistical significance versus the predrug response (paired t test, $p < 0.02$).

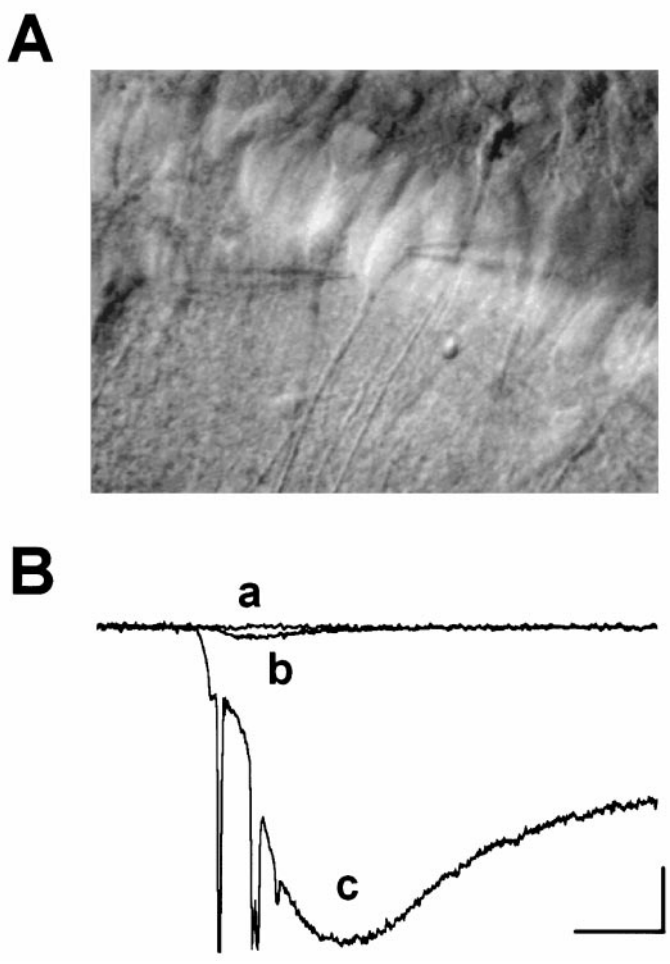

Figure 6. Whole-cell patch-clamp recording from CA1 pyramidal cells. *A*, The pipette on the *right* is the whole-cell patch-clamp recording electrode, and the pipette on the *left* is a drug application pipette. Pressure application of ACh to this neuron failed to produce fast inward currents. The total horizontal distance shown is 140 μm. *B*, Experiments using double-barreled drug application pipettes loaded with 1 mM ACh in one barrel and 1 mM glutamate in the other indicated that no ACh-evoked current could be generated (*a*), although application of an equivalent amount of glutamate elicited an extremely large response (*c*). The *downward deflections* in *c* are unclamped action potentials. *Trace b* is the response to a much lower dose of glutamate. Calibration: 50 msec, 200 pA.

pressure application of nicotine blocked subsequent responses to pressure application of ACh (Fig. 4*B*). The ACh response returned to control levels within 1–2 min of the termination of the nicotine application. In two of three cells, inward currents were observed in response to the initial nicotine application (Fig. 4*B*), but the amplitude of those responses declined rapidly with a 30 sec interapplication interval. Thus, the pharmacological studies on stratum radiatum interneurons demonstrate a directly evoked inward current subserved by somatically located α7-containing nAChRs that are subject to modulation by pharmacologically relevant concentrations of nicotine (Fig. 4).

CA1 pyramidal cells do not respond to somatic application of ACh

Because several studies have suggested that CA1 pyramidal cells are sensitive to nicotinic agonists (Rose and Engstrom, 1992; Albuquerque et al., 1995), we compared the effects of ACh on interneurons with its effects on CA1 pyramidal neurons. Somatic application of comparable amounts of ACh failed to produce comparable fast inward currents in 12 of 12 CA1 pyramidal cells (Fig. 6*A*). To confirm that the ACh pipette was located in an appropriate position to evoke a response, we used a double-barreled drug pipette containing 1 mM glutamate and 1 mM ACh to record from two of the 12 pyramidal cells. Those experiments demonstrated that CA1 pyramidal cells were unresponsive to local application of ACh, even when ejection of comparable amounts of glutamate evoked very large inward currents (>1000 pA; Fig. 6*B*). A recent study on cultured hippocampal neurons has indicated that, although nAChRs capable of generating MLA-sensitive inward currents exist on both the cell soma and the dendrites, they have a greater density at the dendrites (Alkondon et al., 1996a). For that reason, we also examined the effects of ACh application to the apical dendrites of five CA1 pyramidal cells. The majority of the cells tested was completely unresponsive to dendritic application of ACh, and none of them demonstrated reproducible inward currents that were similar to the currents generated by somatic ACh application on radiatum interneurons.

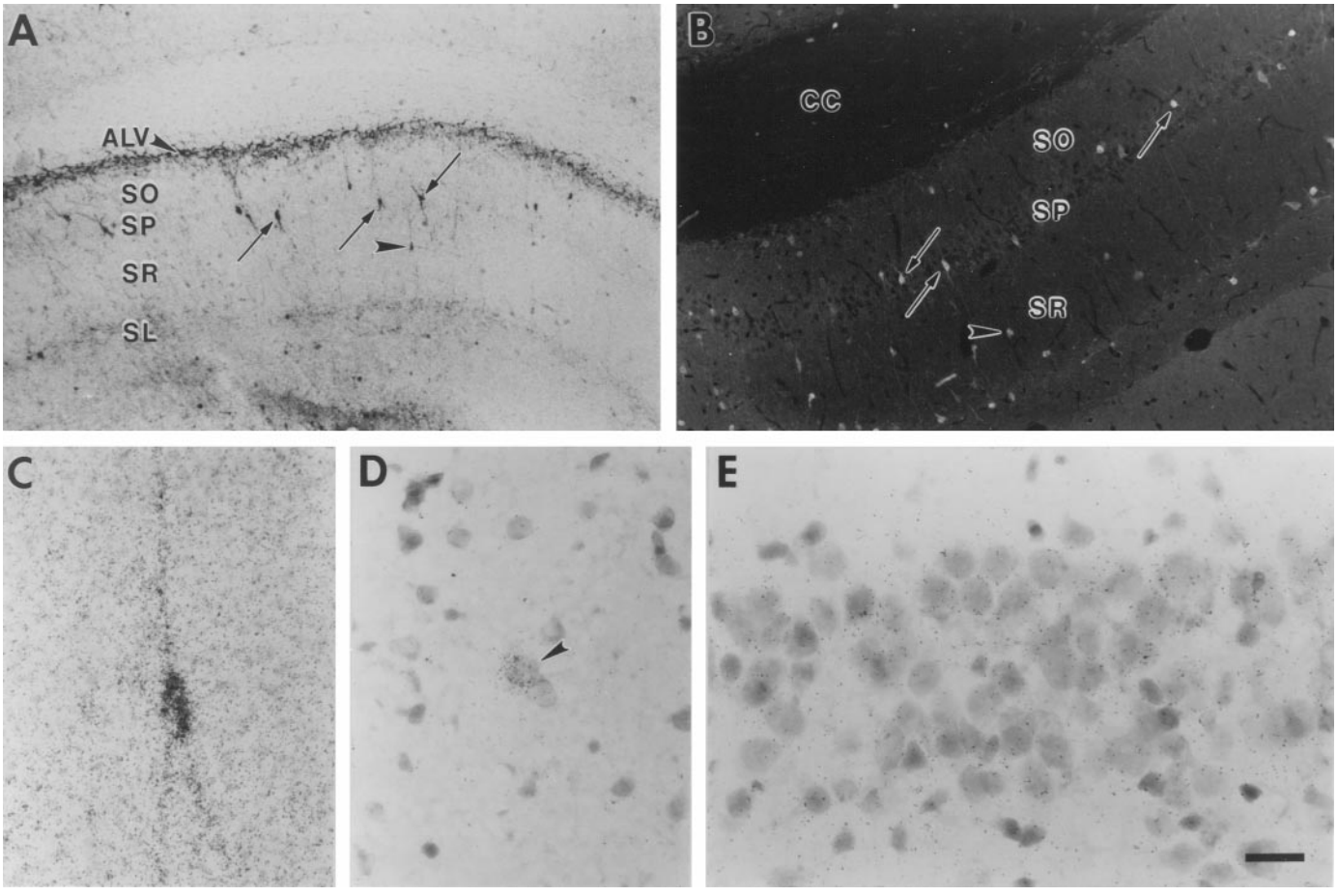

Figure 7. [^{125}I]-αBgTx binding, GABA immunoreactivity, and *in situ* hybridization in area CA1 of the hippocampus. *A*, [^{125}I]-αBgTx binding. A low-power photograph demonstrates [^{125}I]-αBgTx binding throughout various layers of the hippocampus, including the alveus (*ALV*), stratum oriens (*SO*), stratum pyramidale (*SP*), stratum radiatum (*SR*), and stratum lacunosum (*SL*). Although pyramidal cells are densely packed in stratum pyramidale, very few neurons showed specific binding (*arrows*). In contrast, nearly all of the widely scattered interneurons in stratum radiatum showed dense [^{125}I]-αBgTx binding (*arrowhead*). *B*, Immunoreactivity for GABA. GABA-positive cells occurred within stratum pyramidale (*arrows*) with approximately the same frequency as [^{125}I]-αBgTx binding neurons. The majority of neurons in stratum radiatum (*arrowhead*) also was observed to be GABA-positive. *CC*, Corpus callosum. *C*, [^{125}I]-αBgTx binding. A high-power photograph of a densely labeled interneuron in stratum radiatum indicates the presence of both somatic and dendritic [^{125}I]-αBgTx binding. *D*, *E*, *In situ* hybridization for α7 mRNA. In *D*, dense clustering of silver grains over the cell body of a stratum radiatum indicates the presence of messenger RNA for the α7 subunit. Neurons in the pyramidal cell layer (*E*) did not demonstrate the dense somatic clustering of silver grains apparent over the radiatum interneurons but did show labeling above background. The occasional cells in stratum pyramidale that did show intense hybridization for α7 mRNA (data not shown) might correspond to the GABA-positive, [^{125}I]-αBgTx binding interneurons found in this layer. Scale bar: 270 μm in *A*, *B*; 40 μm in *C*; 20 μm in *D*, *E*.

αBgTx binding, GABA immunoreactivity, and *in situ* hybridization for α7 mRNA

Although both [^{125}I]-αBgTx binding sites and α7 mRNA are present in rat hippocampus (Clarke et al., 1985; Seguela et al., 1993), few studies have examined those parameters in specific cell types. We therefore measured [^{125}I]-αBgTx binding in thin sections of rat hippocampus. Although pyramidal cells are densely packed into stratum pyramidale, only a few such cells bind [^{125}I]-αBgTx (Fig. 7*A, arrows*). Further, the number of [^{125}I]-αBgTx binding cells in stratum pyramidale corresponds closely with the number of cells that is immunoreactive for GABA (Fig. 7*B, arrows*). That result is in agreement with a previous report that nearly all [^{125}I]-αBgTx-positive cells in hippocampus are also GAD-positive (Freedman et al., 1993). Thus, it seems very likely that the few cells in the CA1 pyramidal cell layer that did bind [^{125}I]-αBgTx are stratum pyramidale interneurons (Freund and Buzsáki, 1996), and the general lack of [^{125}I]-αBgTx binding to most, if not all, pyramidal neurons is consistent with our failure to observe αBgTx-sensitive currents induced by somatic application of ACh in 12 of 12 pyramidal cells tested.

In contrast to densely packed neurons in stratum pyramidale, neuronal cell bodies are distributed only sparsely in stratum radiatum. Nevertheless, virtually all stratum radiatum neurons demonstrated immunoreactivity for GAD (Fig. 7*B, arrowhead*), as well as somatic and dendritic [^{125}I]-αBgTx binding (Fig. 7*A, arrowhead*, *C*). These data suggest that nearly all of the sparsely distributed neurons in stratum radiatum are interneurons that express nicotinic receptors containing the α7 subunit. That conclusion is quite consistent with our observation that >90% of all stratum radiatum neurons respond to ACh with MLA- and αBgTx-sensitive ACh-evoked currents.

However, in light of previous evidence that α7-containing nAChRs located at presynaptic sites function as modulators of synaptic transmission (McGehee et al., 1995; Alkondon et al.,

1996b; Gray et al., 1996), it was possible that the [^{125}I]-αBgTx binding observed on stratum radiatum interneurons could represent either receptors on the interneurons themselves or on nerve terminals forming synapses on the cell bodies of interneurons. Although the electrophysiological data already presented made this possibility appear unlikely, we localized α7 mRNA expression by *in situ* hybridization in hippocampus as well. Those studies demonstrated intense labeling of the cell bodies of stratum radiatum interneurons (Fig. 7*D*), whereas a much lower level of hybridization was observed over the pyramidal cell layer, despite the much greater density of cell bodies (Fig. 7*E*). Thus, CA1 interneurons express α7 mRNA, have high levels of [^{125}I]-αBgTx binding sites, and have αBgTx-sensitive physiological responses to ACh. The only one of those traits shared by CA1 pyramidal cells is a relatively weak expression of α7 mRNA.

DISCUSSION

The present study demonstrates that stratum radiatum interneurons in area CA1 of the hippocampus respond to ACh application with a fast, rapidly desensitizing inward current. The fast kinetics and atropine insensitivity suggest that the current is mediated by nicotinic, rather than muscarinic, ACh receptors. Further, the involvement of nAChRs that contain the α7 subunit is indicated by the sensitivity of the current to blockade by αBgTx and MLA, which are both α7-selective antagonists, and by the relative insensitivity to DBE and MEC, which are more effective antagonists at other, non-α7-containing nAChRs. The partial blockade of the current by lead, the strong inward rectification at positive potentials, the ability to evoke similar currents with choline, and the reversal potential of ~0 mV are all consistent with the properties of α7-containing nAChRs (Couturier et al., 1990; Clarke, 1992; Vijayaraghavan et al., 1992; Zorumski et al., 1992; Sargent, 1993; Seguela et al., 1993; Alkondon et al., 1994; Ishihara et al., 1995; Papke et al., 1996; Zhang et al., 1996; Albuquerque et al., 1997).

In addition to demonstrating that the ACh-induced inward current on stratum radiatum interneurons is mediated by α7-containing nAChRs, the present study also establishes that these receptors are somatic (i.e., on the cell bodies of the interneurons themselves), and not on nerve terminals. This result contrasts with other studies demonstrating a presynaptic role for α7-containing nAChRs in the CNS. Studies performed in chick brain (McGehee et al., 1995), in rat hippocampus (Gray et al., 1996), and in rat olfactory bulb neurons (Alkondon et al., 1996b) have shown that activation of presynaptic nAChRs located on glutamate-containing nerve terminals results in an αBgTx-sensitive or MLA-sensitive facilitation of glutamatergic transmission. In each of those preparations the effects of presynaptic nAChR activation could be blocked by glutamate receptor antagonists. In the present study the insensitivity of the ACh-evoked current to blockade by the glutamate receptor antagonists DNQX and APV, coupled with the persistence of the current in the presence of TTX and cadmium, clearly indicated that a similar presynaptic mechanism is not responsible for generating the ACh-induced currents observed in these experiments.

In addition, the present study establishes that the somatic α7-containing nAChRs on stratum radiatum interneurons are highly susceptible to desensitization. Although application of 1 mM ACh produced robust currents that were reproducible at 15–30 sec intervals, more frequent application resulted in a decrement in response. Comparable responses to nicotine were difficult to achieve, required much longer recovery periods, and usually resulted in a loss of both ACh and nicotine sensitivity. Those experiments indicated that nicotine and ACh were acting on the same receptor population and suggested that the difficulty in generating nicotine-induced currents with our normal drug application pipettes was attributable to a leak-induced desensitization. Those results suggest that, as with other types of nAChRs (Grady et al., 1994), α7-containing receptors can be desensitized by concentrations of agonist that produce no detectable agonist response (Marks et al., 1996). It is worth noting that the ACh-evoked current was abolished almost completely by bath superfusion of 100 nM nicotine, a concentration well within the range commonly achieved when smoking (Benowitz et al., 1990). The slow application time of the superfusion system in those experiments is at least approximately comparable to the time course of the increase in nicotine concentrations likely to be experienced by tobacco users (Benowitz et al., 1990). The desensitization occurred without a change in holding current or any other detectable evidence of receptor activation. Considered together, the desensitization experiments indicate that reproducible activation of α7-containing nAChRs on radiatum interneurons requires very fast application of agonist. Although such conditions would be achieved by synaptic release of ACh and can be mimicked by rapid application of exogenous agonists, it seems unlikely that the use of tobacco products in humans would provide sufficiently rapid delivery to evoke the rapid inward currents observed in these experiments. In contrast, presynaptic α7 receptors do not appear to share this property (McGehee et al., 1995); for example (Gray et al., 1996), nicotine has been shown to modify release of glutamate for >5 min after a single local application in the CA3 region of rat hippocampus (Gray et al., 1996). These results suggest that an important determinant of the effects of nicotine in the brain may be the rate of receptor desensitization and that this can differ even for receptors that are thought to belong to the same receptor subclass.

In sharp contrast to the stratum radiatum interneurons, the CA1 pyramidal cells that were tested did not respond to somatic application of ACh. This observation was consistent with the relative lack of [^{125}I]-αBgTx binding associated with pyramidal neurons and with the relative levels of *in situ* hybridization for α7 mRNA in individual interneurons and pyramidal cells. Although our results are generally consistent with a previous report indicating that [^{125}I]-αBgTx binding in the rat hippocampus was found primarily, if not exclusively, on GABAergic neurons (Freedman et al., 1993) and with several other studies that have failed to observe [^{125}I]-αBgTx binding on hippocampal pyramidal cells (Polz-Tejera et al., 1975; Hunt and Schmidt, 1978; Breese et al., 1997), they stand in contrast to an extensive body of work in cultured hippocampal neurons, which has suggested that postsynaptic nicotinic receptors of the α7 subtype exist on pyramidal cells (for review, see Albuquerque et al., 1995). Although the reasons for this discrepancy are not clear, it seems possible that culture techniques select preferentially for interneurons, that pyramidal cells express receptors in culture that they do not express *in vivo*, or that pyramidal cells in other hippocampal subregions do express somatic nAChRs.

In summary, the present work establishes that there are functional somatic α7-containing nAChRs on hippocampal interneurons. However, there are a number of issues concerning the role of α7-containing nAChRs in the brain that remain unresolved. Still at issue is whether or not such receptors play a role in fast synaptic transmission, although several factors suggest that possibility in the current system. First, cholinergic afferents from the

medial septum have been shown to innervate the hippocampus and to form synapses with radiatum interneurons (Miettinen and Freund, 1992; Freund and Buzsáki, 1996). Second, ACh diffusion to extrasynaptic receptors unrelated to synaptic transmission seems unlikely in the current system because of the rapid action of esterases present in brain. Activation of extrasynaptic receptors by choline also seems unlikely because in the current system, as in *Xenopus* oocytes (Papke et al., 1996), receptor activation requires considerably higher concentrations of choline than ACh. Finally, αBgTx blocked virtually all of the ACh-induced current observed in stratum radiatum interneurons, indicating that unlike other systems (Zhang et al., 1996) there are no other non-α7-containing nAChRs on these neurons that could mediate synaptic responses to ACh. Nevertheless, conclusive demonstration of synaptic transmission mediated by α7-containing nAChRs in the rat hippocampus awaits the demonstration of a synaptically evoked potential that is functionally blocked by αBgTx.

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