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Nicotinic Receptors on Local Circuit Neurons in Dentate Gyrus: A Potential Role in Regulation of Granule Cell Excitability

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Frazier, Charles J., Ben W. Strowbridge, and Roger L. Papke. Nicotinic receptors on local circuit neurons in dentate gyrus: a potential role in regulation of granule cell excitability. J Neurophysiol 89: 3018–3028, 2003. First published February 12, 2003; 10.1152/jn.01036.2002. Although the dentate gyrus is one of the primary targets of septo-hippocampal cholinergic afferents, relatively little is known about the cholinergic physiology of neurons in the area. By combining whole cell patch-clamp recording with brief local application of exogenous agonists in horizontal slices, we found that there is robust expression of functional somatic α7-containing nicotinic acetylcholine receptors (nAChRs) on molecular layer interneurons, hilar interneurons, and the glutamatergic mossy cells of the dentate hilus. In contrast, the principal neurons of the dentate gyrus, the granule cells, are generally unresponsive to focal somatic or dentate hilus. In contrast, the principal neurons of the dentate gyrus, the granule cells, are generally unresponsive to focal somatic or dentate hilus. The principal neurons of the dentate gyrus, the granule cells, are generally unresponsive to focal somatic or dentate hilus.

INTRODUCTION

Nicotinic acetylcholine receptors (nAChRs) are expressed in abundance throughout the CNS and have been associated with positive effects on attention, cognition, and working memory. Changes in function or expression of nAChRs have been implicated in Alzheimer’s disease, schizophrenia, and some forms of epilepsy (Bertrand 1999; Elmslie et al. 1997). Although the functional neurophysiology of nAChRs has proven to be a challenging research topic, great progress has been made in recent years, particularly with respect to nAChRs that contain the α7 subunit. Several lines of evidence suggest a potential role for this receptor in volume transmission (Descaries et al. 1997; Uteshev et al. 2002), however, specific functional roles for α7 containing nAChRs have also been identified at both presynaptic and postsynaptic sites in hippocampus and elsewhere (Alkondon et al. 1998; Aramakis and Metherate 1998; Frazier et al. 1998a; Gray et al. 1996; Hatton and Yang 2002; Radcliffe et al. 1999). In CA1, these receptors are robustly expressed by local circuit neurons (Frazier et al. 1998b; Jones and Yake 1997; McQuinston and Madison 1999), where they can be activated by endogenous release of ACh (Alkondon et al. 1998; Frazier et al. 1998a) and have been implicated in both inhibition and disinhibition of pyramidal cells (Buhler and Dunwiddie 2002; Ji and Dani 2000). Similarly, functional expression of nAChRs has been reported on hilar interneurons but not on principal neurons in the dentate gyrus (Jones and Yake 1997). Recently, apparently very sparse expression of nAChRs has been detected on the apical dendrites of CA1 pyramidal cells and implicated in the induction of long-term potentiation (Ji et al. 2001). Indeed, recent immunological studies on the ultrastructural distribution and expression of α7 nAChRs in hippocampus and elsewhere have suggested that their role in modulating synaptic transmission may yet be underappreciated (Fabian-Fine et al. 2001; Levy and Aoki 2002).

This is likely to be particularly true in the dentate gyrus, an area that receives extensive cholinergic innervation and that has been a center of attention for research targeted at both age-related memory dysfunction and temporal lobe epilepsy. The principal neurons of the dentate gyrus, the granule cells, are activated by glutamatergic afferents that arrive from the entorhinal cortex via the perforant path (Johnston and Amaral 1998) and are connected by strong recurrent excitatory connections that likely occur via an unusual type of excitatory local circuit neuron in the dentate hilus, the mossy cells (Buckmaster et al. 1992, 1996; Jackson and Scharfman 1996). These cells, along with the GABAergic hilar interneurons, play a key role in regulation of granule cell excitability and are implicated in numerous theories regarding epileptogenesis. Recent dual-labeling immunoelectron microscopy studies have indicated that septohippocampal cholinergic afferents form conventional synapses on both mossy cells and hilar interneurons (Deller et al. 1999; Dougherty and Milner 1999). Based on a combination of the evidence reviewed here, we decided to examine each major cell type in the dentate gyrus for functional expression of nAChRs and to test the hypothesis that activation of such
receptors could be important in the regulation of granule cell excitability.

METHODS

Horizontal slices (300 μm) were prepared from 18- to 28-day-old Sprague Dawley rats using a vibratome (Pelco, Redding, CA). Slices were incubated at 30°C for 30 min and then maintained submerged at room temperature. The artificial cerebral spinal fluid (ACSF) used for cutting and incubating slices contained (in mM) 124 NaCl, 2.5 KCl, 1.2 NaH₂PO₄, 2.5 MgSO₄, 10 d-glucose, 1 CaCl₂, and 25.9 NaHCO₃, saturated with 95% O₂-5% CO₂. After incubation, slices were transferred to a recording chamber where they were visualized with infrared differential interference contrast microscopy (IR DIC) using a Nikon E600FN microscope and superfused at a rate of 2 ml/min with ACSF that was heated to 30°C and contained (in mM) 126 NaCl, 3 KCl, 1.2 NaH₂PO₄, 15 MgSO₄, 11 d-glucose, 2.4 CaCl₂, and 25.9 NaHCO₃, saturated with 95% O₂-5% CO₂. In experiments presented in Figs. 5–7, this solution was modified slightly to contain (in mM) 124 NaCl, 5 KCl, 1.2 NaH₂PO₄, 1.2 MgSO₄, 10 d-glucose, 2.5 CaCl₂, and 25.9 NaHCO₃. Whole cell patch-clamp recordings were made with pipettes pulled on a Flaming/Brown electrode puller (Sutter Instruments, Novato, CA). Pipettes were typically 3–5 MΩ when filled with an internal solution that contained (in mM) 140 Cs-MeSO₄, 8 NaCl, 1 MgCl₂, 0.2 EGTA, 10 HEPES, 2 MgATP, 0.3 Na₂GTP, and 5 QX-314. This solution prevented action potentials from occurring and allowed for stable voltage-clamp recording at depolarized membrane potentials. Access resistance was typically between 10 and 40 MΩ. Access resistance, input resistance, whole cell capacitance, and membrane time constant were all calculated from whole cell capacitive transients generated in voltage clamp by 20 mV depolarizations lasting 10–100 ms from a holding potential of ~80 mV. For current-clamp recordings, a K-gluconate solution was used that contained (in mM) 125 K-gluconate, 1 KCl, 0.1 CaCl₂, 2 MgCl₂, 1 EGTA, 2 MgATP, 0.3 Na₂GTP, and 10 HEPES. All internal solutions were pH adjusted to 7.3 using additional CsOH or KOH, and volume was adjusted to ~285 mOsm. For experiments involving fluorescence microscopy, sulforhodamine 101 was added to the internal solution, and neurons were visualized using light from a mercury lamp filtered at 510–560 nm. For all local application experiments, a picospritzer (Gerneral Valve, Fairfield, NJ) was used to apply acetylcholine (1 mM), glutamate (1 mM), or choline (10 mM) from pipettes identical to those used for whole cell recording or from double-barreled pipettes made using theta tubing (Sutter Instruments, Novato, CA). When using single-barrel pipettes, agonists were applied for 5–15 ms using a pressure of 20–25 psi. When using double-barreled pipettes, time or pressure was occasionally increased beyond these values to obtain visual confirmation of robust application. Evoked responses were generated at 15-s intervals using a concentric bipolar stimulator (Frederick Haer, Bowdoinham, ME), connected to a constant current stimulus isolator (World Precision Instruments, Sarasota, FL). Current intensity varied between 50 and 300 μA, as necessary to generate reliable evoked responses. Stimuli lasted 0.1 ms. An Axon Multiclamp 700A amplifier (Axon Instruments, Union City, CA) was used to amplify voltage and current records. The data were sampled at 20 kHz, filtered at 2 kHz, and recorded on a computer via a Digidata 1200A A/D converter using Clampex version 8 or 9 (Axon Instruments). Data were analyzed using Clampfit version 8 or 9 (Axon Instruments), OriginPro v. 7.0 (OriginLab, Boston, MA), Graphpad Prism v. 3.0 (Graphpad Software, San Diego, CA), and Excel 2000 (Microsoft, Seattle, WA). All chemicals used in these experiments were obtained from Sigma (St. Louis, MO) except for 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzof[f]quinoxaline-7-sulfonamide disodium (NBQX), which was obtained from Tocris (Ellisville, MO).

RESULTS

Identification of local circuit neurons in the dentate gyrus

Local circuit neurons in the dentate gyrus were divided into three broad categories (mossy cells, hilar interneurons, and molecular layer interneurons) on the basis of their location, morphology, and physiology. Because both hilar interneurons and the glutamatergic mossy cells are located in the hilar region, a combination of IR DIC, fluorescence microscopy, and whole cell recording in both voltage clamp and current clamp was used to make an accurate identification. Under IR DIC, mossy cells were often multipolar and were almost uniformly larger than hilar interneurons (average whole cell capacitance was 203 ± 12.2 pF for mossy cells vs. 89.3 ± 11.5 (SE) pF for hilar interneurons). On average, mossy cells also had lower input resistance and a slower membrane time constant than hilar interneurons (121 ± 10.5 MΩ, 5.1 ± 1.0 ms vs. 168 ± 9.2 MΩ, 2.7 ± 0.5 ms, respectively). Further, every cell identified as a mossy cell showed evidence of complex spines on the proximal dendrites when visualized with fluorescence microscopy (Fig. 1A), whereas hilar interneurons were usually, but not always, aspiny (Fig. 1B). In both cell types, spontaneous activity recorded under voltage clamp at ~70 mV was blocked by glutamate receptor antagonists (Fig. 1, C and D); however, every cell categorized as a mossy cell exhibited a unique population of large amplitude spontaneous events that was lacking in interneurons. A representative amplitude distribution for each cell type is shown in Fig. 1, E and F. When current-clamp recordings were made using a K-gluconate based internal solution, it became apparent that neurons identified as mossy cells always demonstrated a robust hyperpolarization activated sag and usually showed relatively long interspike intervals and modest frequency accommodation during a maintained depolarization (Fig. 1E). In contrast, most cells classified as hilar interneurons failed to show a clear hyperpolarization activated sag and demonstrated shorter interspike intervals in response to moderate depolarization (Fig. 1F). All of these characteristics are generally consistent with previously published reports (Lubke et al. 1998; Scharfman 1992; Scharfman and Schwartzkroin 1988). On average, molecular layer interneurons were smaller than hilar interneurons or mossy cells (average whole cell capacitance was 41 ± 7.4 pF). Although they also lacked the large amplitude spontaneous events and proximal dendritic spines that were characteristic of mossy cells, their identification was greatly simplified by the presence of their soma in stratum moleculare. Most stratum moleculare interneurons in our dataset were located in the inner, or middle portion of the layer, i.e., in the half closest to the granule cell layer.

Local circuit neurons in the rat dentate gyrus express somatic α7 containing nAChRs

All three types of local circuit neurons produced robust responses to local application of ACh in the presence of atropine. Inward currents measured under voltage clamp at ~70 mV had average peak amplitudes of 201 ± 24.2 pA for mossy cells (n = 11), 185 ± 41.7 pA for hilar interneurons (n = 17), and 119 ± 47.6 pA for molecular layer interneurons (n = 5, Fig. 2A). These currents showed strong inward rectification with 1–2 mM internal Mg²⁺ and a reversal potential near 0 mV.

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(Fig. 3, A and B). Average ACh-evoked current amplitudes in the presence of 20–50 nM methyllycaconitine (MLA) (an α7-selective nAChR antagonist) were ≈ 5 pA in each cell type and as such could not be distinguished from background noise (Fig. 2, A and C). In most cases, a concentration of 50 nM MLA was used due to the rapid and unambiguous nature of its block (see time course of a typical experiment in Fig. 3C). Although this concentration is largely selective for α7 nAChRs, it was possible to get a slower and yet equally thorough block, and somewhat more rapid recovery, by using lower concentrations of MLA (Fig. 3B). While this evidence clearly indicates that the ACh-induced currents that we observed in each type of local circuit neuron were mediated by α7-containing nAChRs, we did two additional experiments to further confirm this conclusion. In the first experiment, responses were elicited in both mossy cells and hilar interneurons with local application of choline (10 mM), a α7-selective agonist (Papke et al. 1996). In each case, choline produced inward currents that were comparable to those produced by ACh and that were also completely blocked by the α7-selective antagonist MLA (Fig. 2, B and C). In the second experiment, bath-applied choline was used at concentrations of 20–40 μM to desensitize α7-containing nAChRs and thus reduce or eliminate responses to local application of ACh (data not shown). Both of these control experiments further strengthen our conclusion that the vast majority of nAChRs activated in our experiments are likely to contain the α7 subunit.

In 10 of 11 mossy cells, 9 of 17 hilar interneurons, and 4 of 5 molecular layer interneurons that produced α7-like currents in the presence of atropine, additional drugs were applied to eliminate the possibility that activation of presynaptic nAChRs was facilitating release of a transmitter that in turn produced the postsynaptic response. In most cases, either 10 μM NBQX or 6,7-dinitroquinoxaline-2,3-dione (DNQX) coupled with 40 μM d-2-amino-5-phosphonovaleric acid (APV) was used for this purpose. In some cases, 1 μM TTX or a combination of NBQX, APV, and TTX was used instead. The effectiveness of these drugs was quite obvious in experiments involving mossy cells, where it was necessary to block spontaneous excitatory events to resolve the responses to local application of ACh (see Fig. 1C). Figure 3C shows a typical experiment on a hilar interneuron in which NBQX and APV failed to reduce an ACh-evoked response that was subsequently blocked by MLA.

Note that the α7-expressing local circuit neurons reported here represent a strong majority of all neurons tested. In total, 11 of 11 mossy cells, 17 of 21 hilar interneurons, and 5 of 7 molecular layer interneurons produced currents mediated by somatic α7-containing nAChRs in response to local application of ACh.
of ACh. Of the remaining cells, two hilar interneurons and two molecular layer interneurons were not responsive (mean current amplitude collectively /H11005 4.84 /H11006 1.66 pA), and two hilar interneurons produced small slow currents. One of these currents was not sensitive to antagonism by 50 nM MLA and the other was not tested. Extensive additional experiments would be necessary to determine if there are any common anatomical or histological features among these rare α7-negative hilar neurons, as no predictive features were apparent in our experiments.

Granule cells do not respond to focal somatic or dendritic application of ACh

We observed that like hilar interneurons, granule cells also failed to exhibit large spontaneous excitatory events when voltage clamped at −70 mV (Fig 4B, top). However, spontaneous inhibitory events that were mediated by GABA_A receptors and blocked by bicuculline were readily apparent when voltage clamped at 0 mV (Fig. 4B, bottom). Although these cells were routinely identified by the presence of their soma in the granule cell layer, when current-clamp recordings were performed using a K-glutamate internal solution, granule cells could also often be distinguished from mossy cells based on their lack of a clear hyperpolarization-activated sag and from interneurons (hilar or molecular layer) by their pronounced fast afterhyperpolarization (Fig. 4C) (also see Lubke et al. 1998).

We found that granule cells are unresponsive to focal somatic application of ACh, regardless of whether their soma was located in the inner or outer granule cell layer. In 13 granule cells that received somatic applications of ACh while voltage clamped at −70 mV, the mean current amplitude was 4.8 ± 1.0 pA, a value comparable to that obtained from local circuit neurons in the presence of MLA (see examples in Fig. 4D). In 5 of the 13 granule cells tested, double-barreled application pipettes were used, and one barrel was loaded with 1 mM ACh, while the other barrel contained 1 mM glutamate. Each of those five cells responded to local application of glutamate (average current amplitude was 152 ± 49 pA, see example in Fig. 4E) and yet failed to respond to ACh delivered from the same location.

To further confirm that somatic and dendritic responses to focal application of ACh were indistinguishable from the...
noise, we calculated the current integral of both types of responses over a 500-ms time period, starting with the time of ACh application and determined that neither were significantly different from a hypothesized mean value of 0 (P = 0.06, n = 13 for somatic responses, and P = 0.11, n = 8 for dendritic responses). This data are summarized in Fig. 4A. Overall, these results clearly indicated that granule cells lack robust expression of somatic or dendritic nAChRs.

**Alpha7-mediated activation of subgranular hilar interneurons can produce IPSCs in granule cells**

We next attempted to determine if cholinergic activation of nAChRs on local circuit neurons could lead to functional modulation of granule cell activity. Consistent with that hypothesis, we noted that long (0.5–1 s) puffs of ACh to the surface of the hilus or the molecular layer would occasionally produce long trains of IPSCs in granule cells voltage clamped at 0 mV in the presence of atropine, but in the absence of other antagonists (data not shown). There are a number of mono- and polysynaptic mechanisms that could potentially contribute to this effect. Therefore to better understand the response at a cellular level, we designed several experiments using brief focal applications of ACh.

We applied brief pulses of ACh to random subgranular interneurons (those within 50–60 μm of the granule cell layer, on the hilar side) in the hope of finding a synaptically connected pair. After numerous attempts, we found it quite difficult to drive IPSCs onto granule cells in this manner, although local application of glutamate from a double-barreled pipette was often effective. This suggested that local application of ACh was not producing an excitation of the subgranular interneurons that was sufficient to cause GABA release onto the granule cell. Therefore we recorded from nine additional subgranular interneurons using a K-gluconate internal solution and assessed the effects of local application of ACh in both voltage and current clamp directly. Consistent with previously published reports (Lubke et al. 1998), those nine interneurons had an average resting membrane potential of −61 ± 3.8 mV and an average input resistance of 205 ± 34.9 MΩ. When voltage clamped at −70 mV, they produced responses to local application of 1 mM ACh that were not significantly different from our earlier dataset, which had included interneurons from this subgranular zone as well as from deeper parts of the hilus (243 ± 58.7 vs. 191 ± 39.3 pA, respectively). In current-clamp mode, those same ACh applications produced an average depolarization of 17 ± 3.3 mV, and four of nine interneurons reached threshold for an action potential. Of those that did reach threshold, one to three action potentials were observed (data not shown). This result suggested that ACh applied to subgranular interneurons might drive IPSCs more effectively in the presence of slightly higher external KCl. Figure 5B shows three consecutive traces during which glutamate was applied to a subgranular interneuron in our standard external solution. In each case, IPSCs were detected at the granule cell. In Fig. 5B, middle, it is apparent that ACh application failed to produce a similar response under those conditions. However, when the external KCl concentration was raised to 5.5 mM, local application of ACh produced IPSCs in the granule cell similar to those previously generated with glutamate. We therefore used an extracellular solution that contained 5 mM KCl for all remaining experiments (see METHODS). Using this external solution, connected pairs could be more readily identified using ACh application alone. Figure 5C (left) shows one such experiment in which local application of ACh to a subgranular interneuron produced clear IPSCs in a granule cell. In Fig. 5C, right, it is apparent that this effect of ACh was completely blocked by MLA and thus dependent on activation of α7-containing nAChRs. The results of four such experiments are summarized in Fig. 5D.

Overall, these results clearly demonstrate that cholinergic activation of α7-containing nAChRs on subgranular interneurons can lead to synaptic release of GABA onto granule cells. However, an additional hypothesis is that cholinergic activation of these receptors can also act cooperatively with other excitatory inputs to help modulate interneuron activity and thus granule cell function. We decided to test that hypothesis by determining whether activation of α7-containing nAChRs on subgranular interneurons would be more effective at enhancing electrically evoked IPSCs than at driving IPSCs directly.

**Alpha7-mediated activation of subgranular interneurons enhances electrically evoked monosynaptic IPSCs recorded from granule cells**

IPSCs were generated using a concentric bipolar stimulator placed in one of two positions in the molecular layer. For the first set of experiments, the stimulator was placed immediately across the granule cell layer from an identified...
subgranular interneuron. This placement was chosen to maximize the probability of generating a monosynaptic IPSC by direct activation of the interneuron dendrites (Fig. 6A). Indeed, when low-intensity stimulation was delivered from this location, monosynaptic IPSCs could often be recorded from a nearby granule cell voltage clamped at 0 mV. The monosynaptic nature of these responses was indicated by the short latency between the stimulus and the onset of the response (1.2 ± 0.22 ms, n = 7) and also by insensitivity to blockade by glutamate receptor antagonists (Fig. 6B). Electrical stimuli were delivered at 15 or 20-s intervals, and ACh was co-applied in the subgranular zone near an interneuron soma with every other stimulus (see diagram in Fig. 6A). Under those conditions, we were able to observe a clear ACh-mediated enhancement of the monosynaptic IPSC (Fig. 6C). Bath application of 50 nM MLA revealed that this effect of ACh was entirely dependent on activation of α7-containing nAChRs (Fig. 6D). We also demonstrated that the NBQX- and APV-insensitive response that had been enhanced by ACh application was indeed a GABAergic IPSC by blocking it with bath application of 30 μM bicuculline (Fig. 6E). A similar series of experiments was successfully conducted in eight separate granule cells. Overall, co-application of ACh increased the amplitude of the monosynaptic IPSC to an average of 121 ± 5.99% of control. In the presence of MLA, this effect was completely eliminated. Co-application of ACh produced IPSCs with peak amplitudes of 101 ± 4.61% of control (see summary, Fig. 6A).
Alpha7-mediated activation of subgranular interneurons enhances electrically evoked disynaptic IPSCs recorded from granule cells

Another question of interest is whether ACh application to a subgranular interneuron can functionally enhance the effect of synaptically released glutamate. To address that question, it was necessary to generate disynaptic IPSCs. This was accomplished by moving the stimulator 50–200 μm away from a target interneuron, parallel to the granule cell layer (Fig. 7A).

This allowed us to activate glutamatergic inputs to subgranular interneurons that arrive from the entorhinal cortex and run through the molecular layer. However, one consequence of this arrangement was that in addition to driving glutamatergic activation of subgranular interneurons, we often also activated additional excitatory inputs to the granule cell. The effect of this monosynaptic excitatory pathway was largely eliminated by voltage clamping the granule cell at 0 mV. Figure 7B shows the response of a granule cell voltage clamped at –70 mV to

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**FIG. 5.** Alpha7 mediated activation of hilar interneurons produces inhibitory postsynaptic currents (IPSCs) in granule cells. A: schematic indicating the experimental design. All symbols are identical to those described for Fig. 4A, and a hilar neuron has been added. Granule cells were voltage clamped at 0 mV while either ACh (1 mM) or glutamate (1 mM) was applied to the soma of a nearby subgranular interneuron (oval). B, left: glutamate applied to the hilar interneuron produces IPSCs in the granule cell. ACh application failed to reproduce this effect (middle) until external KCl was raised to 5.5 mM (right). C: in 5 mM KCl, we occasionally found connected pairs using a single-barrel ACh application pipette. Left: ACh application to a subgranular interneuron produced IPSCs in the granule cell. Right: this effect was completely blocked by bath application of 50 nM MLA. D: summary plot of data from four similar experiments. The current integral was calculated over a 500-ms time period, starting with the time of ACh application. MLA completely blocked ACh-induced IPSCs. In B and C, each set of 3 traces shows individual sweeps recorded consecutively at 30-s intervals. Arrows indicate time of ACh application or glutamate application. Scale in B: 100 pA, 100 ms. Scale in C: 25 pA, 50 ms.

**FIG. 6.** Alpha7-mediated activation of subgranular interneurons enhances monosynaptic IPSCs recorded from granule cells.

**A:** schematic indicating the experimental design. Symbols are as previously described (Figs. 3A and 4A). Recordings were made from granule cells voltage clamped at 0 mV. Subgranular interneurons were activated by electrical stimulation from a bipolar stimulator placed directly across the granule cell layer from the soma, with and without concurrent application of ACh (1 mM) to the soma. B: stimulation (.1 ms, 50–300 μA) produced IPSCs that were not sensitive to 10 μM NBQX and 40 μM APV. C: co-application of ACh (10 ms) to the soma enhanced the amplitude of the monosynaptic IPSC. D: this effect of ACh was eliminated by bath application of 50 nM MLA. E: the monosynaptic IPSC that remained in the presence of NBQX, APV, 5 μM atropine, and MLA was totally blocked by 30 μM BMI, indicating that it was mediated by GABA_A receptors. Traces shown in B–E are all averages of 10 individual sweeps and were recorded from the same neuron. In C and D, stimulation was delivered every 15 s, and co-application of ACh occurred with every other stimulus. Sweeps marked with * correspond to conditions marked with * in the labels. Scale in A: 50 pA, 50 ms. Scale in C–E: 10 pA, 50 ms.
stimulation of the perforant path. Figure 7C shows the response of the same granule cell to the same stimulus when voltage clamped at 0 mV. Note that a clear outward current was produced by bath application of MLA (Fig. 7E; the effect of ACh is eliminated by bath application of 50 nM MLA). F: the disynaptic nature of the IPSC is confirmed by blocking it with bath application of 10 μM NBQX and 40 μM APV. Traces shown in B–F are all averages of 10 individual sweeps. Those in B and C were recorded from the same granule cell, while those in D–F were from another cell. In D and E, stimulation was delivered every 15 s, and co-application of ACh (15 ms) occurred with every other stimulus. Sweeps marked with * correspond to conditions marked with * in the labels. Scale in B and C: 25 pA, 25 ms. Scale in F: 20 pA, 25 ms also applies to D and E.

**DISCUSSION**

This study demonstrates that functional nAChRs containing the α7 subunit are expressed by morphologically and physiologically identified molecular layer interneurons, hilar interneurons, and mossy cells in the dentate gyrus. We also show that the principal neurons of the dentate, the granule cells, do not produce clear responses to somatic application of ACh or to focal application on their dendrites. All of these results confirm and extend the same basic dichotomy previously reported in both the dentate gyrus (Jones and Yakel 1997) and in CA1 (Frazier et al. 1998b; Jones and Yakel 1997; McQuiston and Madison 1999), whereby there is robust expression of α7-containing nAChRs on local circuit neurons but not on principal neurons. Thus these results further strengthen the hypothesis that cholinergic afferentation of nAChRs in the hippocampus and the dentate gyrus is likely to contribute to the regulation of principal cell output primarily through modula-
tion of local circuit function. Recently, the results of Ji et al. (2001) have suggested a potential exception to this generalization, i.e., that previously overlooked and sparsely expressed α7-containing nAChRs on CA1 pyramidal cell dendrites play an important role in the induction of long-term potentiation. Our results indicate that brief focal application of ACh to visually identified granule cell dendrites does not produce a response that can be reliably detected in the soma. It is possible that more widespread application of cholinergic agonists to granule cell dendrites would help to expose sparse expression of nAChRs similar to that reported in CA1 pyramidal cells by Ji et al. (2001) and that activation of such receptors could be functionally significant. However, our results also indicate that in doing such experiments it will be essential to carefully control for potential mono- and polysynaptic mechanisms that are initiated by broad application of cholinergic agonists.

Because we found that high levels of functional nAChRs in the dentate gyrus are expressed by local circuit neurons, we also tested the hypothesis that cholinergic activation of subgranular interneurons can contribute to the regulation of granule cell excitability. Specifically, we demonstrated that focal application of ACh to these neurons can drive IPSCs onto granule cells, that it can enhance an electrically evoked monosynaptic IPSC, and, perhaps most importantly, that it can enhance the ability of synaptically released glutamate to produce granule cell inhibition. Because the ACh-evoked responses that we recorded directly from mossy cells, hilar interneurons, and molecular layer interneurons were unaffected by glutamate receptor antagonists and/or TTX, we conclude that those responses were mediated by somatic nAChRs and not by nAChRs acting as presynaptic heteroreceptors to facilitate the release of other excitatory transmitters. This finding is consistent with previous reports concerning expression of α7 nAChRs by local circuit neurons in CA1 (Frazier et al. 1998b; McQuiston and Madison 1999). However, similar controls were not possible during experiments that involved recording mono- or disynaptic IPSCs directly from granule cells, and thus the question may arise as to whether activation of presynaptic α7-containing nAChRs on GABAergic terminals contributed to the ACh-mediated enhancement in mono- and disynaptic IPSCs reported here. Several lines of evidence argue against that possibility. In considering the question, it is important to note that all of the experiments summarized in Fig. 8 involved stimulation in the molecular layer. Under those conditions, we were able to observe an ACh-mediated enhancement of the mono- or disynaptic IPSC in approximately one of every five granule cells tested. That ratio is indicative of the difficulty involved in activating the same subpopulation of interneurons with two nonsaturating stimuli. Interestingly, when the stimulus was placed in the hilus, monosynaptic IPSCs were routinely generated, but we were able to observe an ACh-mediated enhancement of the IPSC in only 1 of >20 attempts (data not shown). One probable explanation for that observation is that a stimulus placed in the hilus is far more likely to activate GABAergic axons of septal afferents than a stimulus placed in the molecular layer, which will be more likely to activate the dendrites of hilar interneurons or the glutamatergic afferents of the perforant path. If facilitation of GABA release by presynaptic α7-containing nAChRs played a prominent role in our experiments, we would expect that the ACh-mediated enhancement of the monosynaptic IPSC would be as easy or easier to detect with hilar stimulation as with...
molecular layer stimulation. As that was not the case, we conclude that ACh-mediated facilitation of GABA release from presynaptic terminals is unlikely to have been a major factor in our experiments. The fact that the drug application pipette was generally between 30 and 100 μm away from the granule cell soma and on the opposite side of the granule cell layer from the dendrites further strengthens this conclusion. This interpretation is also consistent with previous reports that ACh-mediated facilitation of GABA release in area CA1 of the hippocampus is TTX-sensitive (Albuquerque et al. 1998). Nevertheless, it seems quite possible that future studies using different experimental approaches may uncover clear functional roles for presynaptic nAChRs in the dentate gyrus (Gray et al. 1996; but see Vogt and Regehr 2001).

Overall, our results suggest the possibility of a complex interplay between glutamatergic inputs from the entorhinal cortex and cholinergic inputs from the medial septum in the normal regulation of granule cell function. It is tempting to speculate that endogenous activation of α7-containing nAChRs by synaptically released ACh might similarly provide direct and/or cooperative modulation of inhibitory circuits within the dentate gyrus in vivo. This hypothesis is fueled not only by the results presented in this report but also by the fact that similar receptors on interneurons in area CA1 have been shown to be activated by synaptic release of endogenous ACh (Alkondon et al. 1998; Frazier et al. 1998a) and by the recent observation that septohippocampal cholinergic afferents form conventional synapses on hilar neurons (Deller et al. 1999; Dougherty and Milner 1999). However, it is important to note that we also show in this manuscript that the α7-containing nAChRs expressed by hilar neurons can be activated by somatic application of choline (10 mM) and desensitized by physiologically relevant concentrations of bath-applied choline (40 μM). These observations are consistent with previous reports regarding the effects of choline on α7-containing nAChRs expressed in other systems (Alkondon et al. 1997; Frazier et al. 1998b; Papke et al. 1996; Uteshev et al. 2002) and suggest the possibility that choline may modulate nAChR function through volume transmission in vivo. Thus it will be extremely important for future studies to examine the extent to which fast synaptic and volume transmission contribute to the normal activation and/or desensitization of α7-containing nAChRs in the dentate gyrus. A better understanding of each of these functional modalities will be essential in developing more effective therapeutic strategies for coping with age-related degeneration of the septohippocampal cholinergic system.

While the downstream consequences of cholinergic activation of mossy cells remain, for the moment, untested, several interesting possibilities exist. The local application studies presented here provide the first demonstration of robust somatic expression of functional nAChRs by any glutamatergic cell type in the hippocampus or dentate gyrus. In addition to being glutamatergic (Soriano and Frotscher 1994), mossy cells are also unusual among local circuit neurons in the dentate in that their axons project along the septotemporal axis of the hippocampus and form a large portion of the commissural/associational projection to the inner molecular layer (Scharfman et al. 1990). This significantly complicates the study of synaptic circuits involving mossy cells as complete circuits may not exist within the hippocampal slice (Scharfman et al. 1990). However, ultrastructural studies have demonstrated that the axons of mossy cells likely form dense synaptic contacts with granule cell dendrites in the inner molecular layer (Buckmaster et al. 1996) and may also have synapses on inhibitory interneurons (Scharfman et al. 1990). These features of mossy cells, coupled with the recent observation that they are directly innervated by septohippocampal cholinergic afferents (Deller et al. 1999), and our demonstration that they express functional nAChRs, lead us to hypothesize that cholinergic input to mossy cells could be an important factor contributing to a role in the regulation and possibly synchronization of granule cell activity. Considered in combination with our results concerning cholinergic modulation of inhibitory circuits in the dentate, we believe that the septohippocampal cholinergic projection and the functional nAChRs described here could ultimately have a prominent role not only in the generation of hippocampal theta rhythms and memory formation but also in other areas that depend on tight control over granule cell activity, such as the modulation of seizure susceptibility and the etiology of temporal lobe epilepsy.

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