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Altered Hippocampal Circuit Function in C3H $\alpha 7$ Null Mutant Heterozygous Mice

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Abstract

The $\alpha 7$ subtype of nicotinic receptor is highly expressed in the hippocampus where it is purported to modulate release of the inhibitory neurotransmitter γ -aminobutyric acid (GABA). The $\alpha 7$ receptor-mediated release of GABA is thought to contribute to hippocampal inhibition (gating) of response to repetitive auditory stimulation. This hypothesis is supported by observations of hippocampal auditory gating deficits in mouse strains with low levels of hippocampal $\alpha 7$ receptors compared to strains with high levels of hippocampal $\alpha 7$ receptors. The difficulty with comparisons between mouse strains, however, is that different strains have different genetic backgrounds. Thus, the observed interstrain differences in hippocampal auditory gating might result from factors other than interstrain variations in the density of hippocampal $\alpha 7$ receptors. To address this issue, hippocampal binding of the $\alpha 7$ receptor-selective antagonist α -bungarotoxin as well as hippocampal auditory gating characteristics were compared in C3H wild type and C3H $\alpha 7$ receptor null mutant heterozygous mice. The C3H $\alpha 7$ heterozygous mice exhibited significant reductions in hippocampal $\alpha 7$ receptors levels and abnormal hippocampal auditory gating compared to the C3H wild type mice. In addition, a general increase in CA3 pyramidal neuron responsivity was observed in the heterozygous mice compared to the wild type mice. These data suggest that decreasing hippocampal $\alpha 7$ receptor density results in a profound alteration in hippocampal circuit function.

Keywords

nicotinic receptors; auditory gating; sensory inhibition; $\alpha 7$ knockout mice; autoradiography; schizophrenia

1. Introduction

The $\alpha 7$ subtype of nicotinic receptor is a rapidly desensitizing, ligand-gated ion channel (Zhang et al., 1994) that directly fluxes cations, particularly calcium (Seguela et al., 1993), resulting in increased intracellular calcium levels. The $\alpha 7$ receptor is expressed in many brain regions, but is especially dense in the hippocampus (Breese et al., 1997; Seguela et al., 1993; Whiteaker et al., 1999). This receptor has been localized to hippocampal neurons containing the inhibitory neurotransmitter γ -aminobutyric acid (GABA) (Breese et al., 1997; Fabian-Fine et al., 2001;

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Frazier et al., 1998a; Freedman et al., 1993; Khiroug et al., 2003) and activation of the receptor leads to the release of GABA in hippocampal cultures and slices (Alkondon and Albuquerque, 2001; Alkondon et al., 1999; Buhler and Dunwiddie, 2002; Radcliffe et al., 1999).

The $\alpha 7$ receptor-mediated release of GABA appears to be important for hippocampal sensory processing. Luntz-Leybman et al. (Luntz-Leybman et al., 1992) examined cholinergic regulation of sensory processing in Sprague-Dawley rat hippocampus using an auditory conditioning-testing paradigm. In this paradigm, two identical tones were presented with an intertone interval of 500 msec. Both the first (conditioning) and second (test) tones elicited an evoked potential response in hippocampal area CA3, i.e. increased the activity of CA3 pyramidal neurons. The pyramidal neuron response to the test tone was significantly less than that to the conditioning tone, suggesting that pyramidal neuron activation was inhibited or “gated” during presentation of the test tone. The decrease in pyramidal neuron response to the second tone appeared to be due to a persistent increase in CA3 hippocampal interneuron discharge that was initiated by the conditioning tone (Miller and Freedman, 1995). Intracerebroventricular infusion of the $\alpha 7$ -selective antagonist α -bungarotoxin (α -BTX) resulted in similar pyramidal neuron responses to both the conditioning and test tones, i.e. a disruption of auditory gating, while infusion of a high affinity nicotinic receptor channel blocker or a muscarinic receptor antagonist did not (Luntz-Leybman et al., 1992). These data suggest that the $\alpha 7$ receptor influences hippocampal sensory processing by modulating hippocampal inhibitory circuit function.

Individuals with schizophrenia exhibit a deficit in auditory gating relative to control subjects. Schizophrenics respond to the presentation of tone pairs with conditioning and test responses of similar amplitude, indicative of abnormal auditory gating. The schizophrenia-associated deficit in auditory gating has been genetically linked to a dinucleotide polymorphism at the chromosome 15q13-14 site of the $\alpha 7$ receptor (Freedman et al., 1997). In addition, a reduction in α -BTX binding has been observed in postmortem hippocampus of schizophrenics, including those that have never smoked or have been free of neuroleptic treatment for at least a year (Freedman et al., 1995; Pomper et al., 1999). The reduction in α -BTX binding observed in postmortem schizophrenic hippocampus may be secondary to alterations in the $\alpha 7$ receptor gene in schizophrenic brain. Point mutations in the promoter region for the $\alpha 7$ gene reduce its expression *in vitro* and are significantly associated with schizophrenia (Leonard et al., 2002), although they are also found in control hippocampus (Gault et al., 2003). Together, these studies suggest that decreased hippocampal $\alpha 7$ receptor density is associated with a disruption of normal hippocampal sensory processing.

Different strains of inbred mice vary considerably in their hippocampal $\alpha 7$ receptor density (Marks et al., 1989; Stevens et al., 1996). Two of these strains, C3H and DBA/2, have been used in a number of previous studies to examine how differences in hippocampal $\alpha 7$ receptor levels correlate with differences in hippocampal morphology and function. C3H mice have higher (mean 69.2 fmol/mg protein) levels of hippocampal α -BTX binding while DBA/2 mice have lower (mean 45.6 fmol/mg protein) levels of hippocampal α -BTX binding, a 35% reduction in density compared to the C3H mice (Marks et al., 1989). The differences in hippocampal α -BTX binding in C3H and DBA/2 mice appear to result from an interstrain variation in the $\alpha 7$ receptor gene (*Acra7*) locus on mouse chromosome 7 (Stitzel et al., 1996). Functionally, C3H mice exhibit robust hippocampal auditory gating while DBA/2 mice are deficient in hippocampal auditory gating (Stevens et al., 1996). The disruption of auditory gating in the mouse strain with lower hippocampal $\alpha 7$ receptor levels again suggests that the $\alpha 7$ receptor influences hippocampal sensory processing via an influence on hippocampal inhibitory circuit function. Further support for this hypothesis was provided by studies demonstrating improvement in auditory gating in DBA/2 mice with administration of agonists which bind the $\alpha 7$ nicotinic receptor (Stevens et al., 1998; Stevens and Wear, 1997). Acute

nicotine administration was also found to influence auditory gating in awake C57BL/6J and DBA/2Hsd mice (Metzger et al., 2007). In this study, however, the P20 and N40 components of the auditory evoked potentials were differentially affected, as nicotine increased the amplitude and gating of the P20 component but decreased the amplitude and gating of the P40 component in each strain.

The difficulty with this two-strain animal model, however, is that the strains have different genetic backgrounds. Bowers et al. (Bowers et al., 1999) found that the impact of a null mutation of the γ -protein kinase C gene on initial sensitivity to ethanol was modulated by the background genotype of the mouse strain examined. In some strains, the “no tolerance” phenotype was expressed as expected while in other strains, the “no tolerance” phenotype was not expressed. Thus, the function of any given gene can be altered by the effect of the strain-background genetics. It is possible, therefore, that the disparity in hippocampal auditory gating observed between C3H and DBA/2 mice occurs not as a result of differences in hippocampal $\alpha 7$ receptor levels, but as a result of undefined strain genetic background differences. Resolution of this question requires a mouse model in which hippocampal $\alpha 7$ receptor levels are decreased with no change in strain background.

Mice potentially meeting this criteria, C3H $\alpha 7$ receptor null mutant heterozygous mice, have recently become available. This new mouse could provide a superior means of examining whether decreases in $\alpha 7$ receptor density alter hippocampal sensory processing. The present study examines the relationship between hippocampal ^{125}I - α -BTX binding levels and auditory gating characteristics in C3H wild type and C3H $\alpha 7$ receptor null mutant heterozygous mice.

2. Results

Binding of ^{125}I - α -BTX

A comparable pattern of ^{125}I - α -BTX binding was observed in the C3H wild type and C3H $\alpha 7$ heterozygous mice (Figure 1, Table 1). Binding of α -BTX was highest in hippocampal area CA3, lower in the dentate gyrus and least in hippocampal area CA1 in each genotype. A pronounced band of α -BTX binding at the junction of hippocampal areas CA3 and CA1 was also detected in each group of mice (arrows, Figure 1, A and B). However, the density of α -BTX binding was significantly lower in C3H $\alpha 7$ heterozygous mice compared to the wild type mice in each region examined ($t = 5.024$; $df = 12$, $p < 0.000$ for dentate gyrus; $t = 4.913$; $df = 12$, $p < 0.000$ for area CA1; $t = 3.397$; $df = 12$, $p < 0.005$ for area CA3) (Table 1). On average, binding of α -BTX was decreased by 61% in the dentate gyrus, by 64% in area CA3 and by 54% in area CA1 of the C3H $\alpha 7$ heterozygous mice compared to the C3H wild type mice.

Hippocampal auditory gating

The wild type and heterozygous mutant mice displayed distinctly different hippocampal responses to the presentation of auditory tone pairs. In the C3H wild type mice, presentation of the first (conditioning) tone resulted in an auditory evoked response with a mean ($\pm$ S.E.M.) amplitude of $19.61 \pm 3.41 \mu\text{V}$ while presentation of the second (test) tone resulted in an auditory evoked response with a mean ($\pm$ S.E.M.) amplitude of $7.78 \pm 0.92 \mu\text{V}$ (Figure 2). Calculation of the mean ($\pm$ S.E.M.) ratio of the test and conditioning amplitudes (the TC ratio) resulted in a value of 0.46 ± 0.06 for the wild type mice (Figure 2). In contrast, in the C3H $\alpha 7$ heterozygous mice, presentation of the conditioning tone resulted in an auditory evoked response with a mean ($\pm$ S.E.M.) amplitude of $51.82 \pm 12.98 \mu\text{V}$ while presentation of the test tone resulted in an auditory evoked response with a mean ($\pm$ S.E.M.) amplitude of $46.87 \pm 6.78 \mu\text{V}$ (Figure 2). Calculation of the mean ($\pm$ S.E.M.) TC ratio in the $\alpha 7$ heterozygous mice resulted in a value of 1.17 ± 0.15 (Figure 2). Statistical analysis indicated that all three auditory gating parameters differed significantly between wild type and heterozygous mice (conditioning amplitude – $t =$

5.77, $df = 18$, $p = 0.027$; test amplitude $-t = 32.73$, $df = 18$, $p < 0.001$; TC ratio $-t = 19.98$, $df = 18$, $p < 0.001$). There was also a difference in overall CA3 pyramidal neuron responsivity between the two mouse stocks, with the heterozygous mice showing significantly higher auditory evoked response amplitudes than the wild type mice (Figure 2).

3. Discussion

This study demonstrates that significantly reducing hippocampal $\alpha 7$ receptor density within the same genetic background profoundly impacts hippocampal functioning. The observed changes in hippocampal circuit function that accompanied decreased expression of the $\alpha 7$ nicotinic cholinergic receptor included a loss of normal auditory gating as well as an apparent increase in overall CA3 pyramidal neuron responsivity. How decreasing hippocampal $\alpha 7$ receptor levels results in alterations in hippocampal functioning is currently unknown. However, we suggest the following as potential explanations:

Altered GABA release

Both the abnormal auditory gating and the increase in CA3 pyramidal neuron firing could be explained by a decrease in GABA release within hippocampal area CA3 secondary to the reduction in $\alpha 7$ receptor density. Ligand binding studies have localized α -BTX to inhibitory interneurons in all regions of the hippocampal formation (Adams, 2003; Adams et al., 2002; Frazier et al., 1998a; Freedman et al., 1995; Freedman et al., 1993). Ultrastructural evaluation of $\alpha 7$ receptor immunostaining in rat CA1 stratum radiatum found widespread labeling of both pre- and postsynaptic elements (Fabian-Fine et al., 2001). Many of these elements exhibited symmetric synaptic morphology and/or immunostaining for GABA, both of which are indicative of inhibitory synapses (Fabian-Fine et al., 2001). These findings suggest that activation of $\alpha 7$ receptors could influence GABA release in the hippocampus. Physiological studies in rat hippocampal slices have confirmed the presence of $\alpha 7$ receptors on interneurons in all layers of area CA1 (Alkondon et al., 1998; Buhler and Dunwiddie, 2001; Frazier et al., 1998b; Frazier et al., 1998a; Jones and Yakel, 1997; McQuiston and Madison, 1999) as well as GABA-mediated inhibition of CA1 pyramidal cells following activation of $\alpha 7$ receptors on CA1 interneurons (Alkondon and Albuquerque, 2001; Buhler and Dunwiddie, 2002; Ji and Dani, 2000). It seems reasonable to hypothesize that $\alpha 7$ receptors may also modulate GABA release from interneurons in hippocampal area CA3 given that α -BTX binding has been observed on CA3 interneuron subtypes (Freedman et al., 1993) that are known to make synaptic contact with CA3 pyramidal cells (Freund and Buzsaki, 1996). If $\alpha 7$ receptors do modulate GABA release in hippocampal area CA3, both the disruption in auditory gating and the increase in CA3 pyramidal neuron responsivity observed in C3H $\alpha 7$ heterozygous mice in the present study could result from a diminution in $\alpha 7$ receptor-mediated GABA release onto CA3 pyramidal cells.

Altered glutamate release

Some, but not all, data from recent studies suggest that $\alpha 7$ receptors may modulate hippocampal glutamate release. Fabian-Fine et al. (Fabian-Fine et al., 2001) observed colocalization of immunostaining for the $\alpha 7$ receptor and glutamate in presynaptic elements of stratum radiatum in hippocampal area CA1. In addition, Gray et al. (Gray et al., 1996) recorded increases in the frequency of miniature excitatory postsynaptic currents (mEPSCs) in CA3 pyramidal neurons following local application of a low concentration of nicotine. The greater mEPSC frequency was thought to occur as a result of $\alpha 7$ receptor-mediated increases in Ca^{2+} influx into mossy fiber terminals and a subsequent increase in glutamate release. This hypothesis was not supported by data from another study by Vogt and Regehr (Vogt and Regehr, 2001) as the authors did not observe an increase in mossy fiber terminal Ca^{2+} influx after nicotine administration. If $\alpha 7$ receptors do modulate glutamate release from mossy fiber terminals, a

decrease in $\alpha 7$ receptor density on these terminals would be expected to lower glutamate release and reduce CA3 pyramidal cell responsivity, not increase the responsivity as was observed in C3H $\alpha 7$ heterozygous mice in the present study. However, an observation by Acsády et al. (Acsády et al., 1998) may provide an alternate interpretation. In this study, thin, filopodial extensions of the mossy fiber terminals were found to make a greater number of synaptic contacts with GABAergic neurons than with pyramidal neurons in hippocampal area CA3. The authors suggest that the higher density of granule cell innervation of inhibitory interneurons may underlie the decreased excitability of CA3 pyramidal neurons seen following granule cell stimulation (Bragin et al., 1995a; Bragin et al., 1995b; Penttonen et al., 1997). If $\alpha 7$ receptors are present on mossy fiber filopodial presynaptic inputs to interneurons in area CA3, a decrease in $\alpha 7$ receptor levels would hypothetically result in decreased glutamate release onto the interneurons, decreased interneuron activity and a disinhibition of CA3 pyramidal cell responsivity similar to that observed in the C3H $\alpha 7$ heterozygous mice in the current study. A decrease in glutamate-mediated inhibitory neuron activity could also explain the observed deficit in auditory gating in the heterozygote mice.

Altered development

Hippocampal circuit formation is initially driven by spontaneous electrical discharges termed giant depolarizing potentials (GDPs) (Ben-Ari et al., 2004). GDPs are regulated first by GABA alone and later by both GABA and glutamate. GABA acts as an excitatory neurotransmitter early in hippocampal development because neuronal chloride gradients are immature. As expression of the mature potassium/chloride co-transporter increases, the adult neuronal chloride gradient is established and GABA switches from being an excitatory to an inhibitory neurotransmitter. GABA itself may control potassium/chloride co-transporter maturation, as the process may require binding of GABA to GABA_A receptors (Ben-Ari et al., 2004).

The $\alpha 7$ nicotinic receptor participates in the regulation of these developmental processes. GABA release following $\alpha 7$ receptor stimulation modulates GDP frequency which, in turn, impacts hippocampal circuit formation (Maggi et al., 2001). In addition, $\alpha 7$ receptor-mediated GABA release is required for normal expression of the mature potassium/chloride co-transporter (Liu et al., 2006) and thus, for the transformation of GABA from an excitatory to an inhibitory neurotransmitter. This transformation process impacts not only hippocampal cell firing, but may also affect hippocampal gene expression (Liu et al., 2006). Activation of the $\alpha 7$ receptor also appears to stabilize cholinergic afferent inputs to hippocampal interneurons (Zago et al., 2006). Finally, $\alpha 7$ receptor-mediated glutamate release (Maggi et al., 2003) leads to activation of “silent” AMPA glutamate receptors and enhancement of glutamatergic synaptic strength in area CA1 of the immature hippocampus. A significant decrease in $\alpha 7$ receptor density during hippocampal development might, at the very least, alter the timing of these maturational events, possibly resulting in the abnormalities in hippocampal circuit function observed in C3H $\alpha 7$ heterozygous mice in the current study.

Compensatory changes

A final possibility is that the reduction in hippocampal $\alpha 7$ receptor density in the C3H $\alpha 7$ heterozygous mice was sufficient to trigger compensatory changes in other nicotinic receptor subtypes, in other cholinergic system components and/or in components of other neurotransmitter systems. Franceschini et al. (Franceschini et al., 2002) found no difference in the relative abundance of hippocampal mRNA for the $\alpha 4$, $\alpha 5$, $\beta 2$ and $\beta 4$ subunits in adult C57BL/6 $\alpha 7$ wild type, heterozygote and null mutant mice. In contrast, Yu et al. (Yu et al., 2007) observed a significant increase in ^3H cytisine binding on postnatal day (P) 21 as well as a significant increase in $\alpha 4$ subunit protein on P10, P14 and P21 in hippocampus from C57BL/6 $\alpha 7$ heterozygote as opposed to wild type mice. No differences were observed between the two mouse groups in the levels of $\alpha 5$ and $\beta 2$ subunit proteins. These data suggest that

compensatory changes in the hippocampal expression of non- $\alpha 7$ receptor subunits in adult C3H $\alpha 7$ heterozygous mice are unlikely, although transient changes during early hippocampal development cannot be ruled out. Compensatory changes in other cholinergic system components have not been reported in mice with null mutations of nicotinic receptor subtypes. On the other hand, a gain of function mutation of the $\alpha 7$ subunit may have led to compensatory changes in developmental factors while deletion of the $\alpha 4$ subunit resulted in an apparent compensatory down regulation of glutamatergic neurotransmission (Drago et al., 2003).

Auditory gating is not restricted to the hippocampus. Filtering of repetitive auditory stimulation has also been observed in the pontine reticular formation (PRF) (Bickford et al., 1993; Moxon et al., 1999), in the medial septum (Moxon et al., 1999), in the reticular thalamic nucleus (RTN) (Krause et al., 2003) and in the amygdala (Cromwell et al., 2005). In addition, hippocampal auditory gating is influenced by stimulation of both the PRF and nucleus of the lateral lemniscus (NLL) (Bickford et al., 1993). All but one of these brain regions, the PRF, appear to express the $\alpha 7$ nicotinic receptor (Arimatsu and Seto, 1982; Azam et al., 2003; Breese et al., 1997; Happe and Morley, 2004; Hellstrom-Lindahl et al., 1998; Henderson et al., 2005; Seguela et al., 1993; Thinschmidt et al., 2005; Whiteaker et al., 1999; Zhang et al., 1998). It is currently unknown whether the $\alpha 7$ receptor modulates auditory gating in any of these extra-hippocampal areas. If so, then a decrease in $\alpha 7$ density would be expected to negatively impact the ability of these regions to effectively filter auditory input.

The loss of hippocampal auditory gating in C3H $\alpha 7$ heterozygous mice raises the possibility that these animals might also exhibit an alteration in prepulse inhibition (PPI) of startle. A study comparing PPI across eight inbred mouse strains found significant correlations between acoustic-acoustic PPI and both auditory gating and hippocampal α -BTX binding (Bullock et al., 1997). These data suggested that PPI, like auditory gating, might be regulated, at least in part, by the $\alpha 7$ subtype of nicotinic receptor. Subsequent studies, however, do not appear to provide support for this hypothesis. PPI was unaltered in DBA/2 and B6 mice following administration of GTS-21 and AR-R17779, drugs relatively selective for the $\alpha 7$ receptor (Olivier et al., 2001). Similar results were reported by Schreiber et al. (Schreiber et al., 2002) in Sprague-Dawley rats and DBA/2 mice. Finally, deletion of the $\alpha 7$ receptor had no apparent effect on levels of PPI, as $\alpha 7$ receptor-null mutant mice and their wild-type littermates exhibited similar responses (Paylor et al., 1998). Based upon these data, we would predict that the significant decrease in hippocampal α -BTX binding observed in C3H $\alpha 7$ heterozygous mice would not be associated with an alteration in PPI, although a direct examination of this hypothesis would prove interesting.

In summary, C3H $\alpha 7$ heterozygous mice exhibit a significant reduction in the density of hippocampal $\alpha 7$ receptors that is associated with a disruption of auditory gating and an increase in CA3 pyramidal neuron responsivity. The data from this study underscore the importance of the $\alpha 7$ receptor in regulating hippocampal circuit function and should provide a useful model for investigating certain hippocampal abnormalities observed in schizophrenic individuals.

4. Experimental Procedure

Animals

All mice were obtained from the Institute for Behavioral Genetics breeding colony at the University of Colorado, Boulder, CO. The C3H $\alpha 7$ null mutant (knockout) mice were derived by mating $\alpha 7$ mixed background (129 $\times$ C57BL/6) mice with C3H mice (both sexes used). The progeny that result from this crossing are $+/+$ and $+/-$ at the $\alpha 7$ locus. The $+/-$ mice were crossed with C3H mice. The resulting $+/-$ progeny were used to create the next generation. This process was continued for 10 generations, maintaining 6 families.

Autoradiography

Tissue preparation—Adult (postnatal day 90) male and female C3H wild type ($n = 6$) and $\alpha 7$ heterozygous ($n = 8$) mice were deeply anesthetized with Isoflurane. Following decapitation, the brain was removed, frozen in dry ice snow and stored at -70°C . Sequential cryostat sections ($12\ \mu\text{m}$) through the hippocampus from animals of each genotype were cut, thaw-mounted onto gelatin-coated slides and stored at -70°C before binding with $^{125}\text{I}\alpha\text{-BTX}$.

Radioligand binding—The tissue, subdivided into total- and nonspecific-binding groups, was incubated in a solution containing 50 mM Tris-HCl, 120 mM NaCl and 2 mg/ml bovine serum albumin (TBS/BSA buffer, pH 7.4) for 30 minutes at room temperature. Nonspecific binding was determined by adding 5 mM nicotine to the TBS/BSA buffer. The two tissue sets were then incubated in the TBS/BSA buffer containing $^{125}\text{I}\alpha\text{-BTX}$ (5 nM, specific activity 2000 Ci/mmol, Amersham Pharmacia Biotech, Piscataway, NJ) at 37°C for 3 hours. Following incubation with the ligand, the tissue was rinsed in the TBS/BSA buffer for 5 min, in TBS without BSA for 15 minutes and in PBS for 5 minutes, all at 37°C . Subsequently, the slides were dipped briefly in distilled water, dried quickly under a stream of cool air and placed on radiation-sensitive Hyperfilm (Amersham, Piscataway, NJ) for 3 days with ^{14}C standards (Amersham, Piscataway, NJ) of known radioactivity in order to generate autoradiograms for quantitative analysis.

Data analysis—Autoradiograms were quantified with a computer-based image analysis system (Compix, Inc., Cranberry Township, PA) using calibrated standards of reference. A calibration curve of gray value against radioligand concentration (nCi/g tissue) was constructed using standards of known radioactivity. Specific radioligand binding was calculated by subtracting values obtained in the presence of an excess of competing ligand (nonspecific binding) from those in the absence (total binding), and was expressed as nCi/g tissue. For each brain region, measurements were taken from left and right hippocampal sections in 14 – 24 sections.

Statistical analysis—Unpaired t -tests of $\alpha\text{-BTX}$ binding levels between heterozygotes and wildtype animals were performed to determine if there were significant differences between the genotypes in regional $\alpha\text{-BTX}$ binding density.

Preparation of photomicrographs—Digital images were captured using a SpotRT digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI). Photographic plates of the digital images were subsequently made using Adobe Photoshop (Adobe Systems Inc., San Jose, CA).

Auditory gating

Acute evoked potential recordings—Adult C3H wild type ($n = 10$) and C3H $\alpha 7$ heterozygote ($n = 10$) mice were anesthetized with chloral hydrate (400 mg/kg ip) and pyrazole (400 mg/kg ip) to retard the metabolism of the chloral hydrate. The mice were placed in a stereotaxic apparatus using a mouse nose adaptor and hollow ear bars, with miniature earphones attached, were positioned adjacent to the mouse's ears. Body temperature was maintained at 37°C with a heating pad. A teflon-coated stainless-steel recording electrode was inserted into the pyramidal layer of hippocampal area CA3 at 1.8 mm posterior to bregma, 2.7 mm lateral to the midline and 1.5–1.7 mm below the surface of the dura for recording of auditory-evoked potentials. Final location was identified by the presence of complex action potentials typical of hippocampal pyramidal neurons (Miller and Freedman, 1995). A similar reference electrode was placed on the dura, contralateral to the recording electrode, just anterior to bregma. The evoked potentials were amplified 1000 times with a 1–500 Hz filter and led to an analog to digital converter for averaging and storage by a computer for later analysis. Tones, 300 Hz, 10

msec, 70 dB SPL, were presented in pairs with an intertone interval of 500 msec. Responses to 16 tone pairs were averaged at 10 minute intervals.

Auditory-evoked potential parameters measured—The N40 wave was identified as the maximum negativity in the auditory-evoked potential between 20 and 60 msec after the auditory stimulus. The P20 wave was identified as the positivity in the auditory-evoked potential immediately preceding the N40 wave. The amplitude of the N40 wave was measured relative to the peak of the P20 wave. This composite component has been shown to be less variable than either component (P20 or N40) alone (Hashimoto et al., 2005). The latency and amplitude of the response to the first tone stimulus (condition stimulus) and to the second tone stimulus (test stimulus), as well as the ratio of the amplitudes of the test and conditioning responses (TC ratio), were calculated for each set of trials by a computer algorithm (SciWorks, DataWave, Berthoud, CO). Normal auditory gating was defined as a TC ratio of 0.5 or below while a TC ratio above 0.5 was defined as deficient auditory gating.

Statistical analysis—Unpaired *t*-tests of auditory gating parameters between heterozygotes and wildtype animals were performed to determine if there were significant differences between the genotypes in conditioning and test evoked potential amplitudes and in TC ratios.

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References

- Acsady L, et al. GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. *Journal of Neuroscience* 1998;18:3386–3403. [PubMed: 9547246]
- Adams CE. Comparison of alpha 7 nicotinic acetylcholine receptor development in the hippocampal formation of C3H and DBA/2 mice. *Developmental Brain Research* 2003;143:137–149. [PubMed: 12855185]
- Adams CE, et al. Development of the alpha7 nicotinic cholinergic receptor in rat hippocampal formation. *Brain Res Dev Brain Res* 2002;139:175–187.
- Alkondon M, Albuquerque EX. Nicotinic acetylcholine receptor alpha 7 and alpha 4 beta 2 subtypes differentially control GABAergic input to CA1 neurons in rat hippocampus. *Journal of Neurophysiology* 2001;86:3043–3055. [PubMed: 11731559]
- Alkondon M, et al. alpha-Bungarotoxin- and methyllycaconitine-sensitive nicotinic receptors mediate fast synaptic transmission in interneurons of rat hippocampal slices. *Brain Research* 1998;810:257–263. [PubMed: 9813357]
- Alkondon M, et al. Choline and selective antagonists identify two subtypes of nicotinic acetylcholine receptors that modulate GABA release from CA1 interneurons in rat hippocampal slices. *Journal of Neuroscience* 1999;19:2693–2705. [PubMed: 10087082]
- Arimatsu Y, Seto A. Ontogeny of sexual difference in alpha-bungarotoxin binding capacity in the mouse amygdala. *Brain Res* 1982;234:27–39. [PubMed: 7059823]
- Azam L, et al. Co-expression of alpha7 and beta2 nicotinic acetylcholine receptor subunit mRNAs within rat brain cholinergic neurons. *Neuroscience* 2003;119:965–977. [PubMed: 12831856]
- Ben-Ari Y, et al. Interneurons set the tune of developing networks. *Trends in Neurosciences* 2004;27:422–427. [PubMed: 15219742]
- Bickford PC, et al. Auditory sensory gating in the rat hippocampus: modulation by brainstem activity. *Brain Res* 1993;607:33–38. [PubMed: 8481808]
- Bowers BJ, et al. Decreased ethanol sensitivity and tolerance development in gamma-protein kinase C null mutant mice is dependent on genetic background. *Alcoholism-Clinical and Experimental Research* 1999;23:387–397.

- Bragin A, et al. Gamma (40–100-Hz) Oscillation in the Hippocampus of the Behaving Rat. *Journal of Neuroscience* 1995a;15:47–60. [PubMed: 7823151]
- Bragin A, et al. Dentate Eeg Spikes and Associated Interneuronal Population Bursts in the Hippocampal Hilar Region of the Rat. *Journal of Neurophysiology* 1995b;73:1691–1705. [PubMed: 7643175]
- Breese CR, et al. Comparison of the regional expression of nicotinic acetylcholine receptor alpha 7 mRNA and [I-125]alpha-bungarotoxin binding in human postmortem brain. *Journal of Comparative Neurology* 1997;387:385–398. [PubMed: 9335422]
- Buhler AV, Dunwiddie TV. Regulation of the activity of hippocampal stratum oriens interneurons by alpha 7 nicotinic acetylcholine receptors. *Neuroscience* 2001;106:55–67. [PubMed: 11564416]
- Buhler AV, Dunwiddie TV. alpha 7 nicotinic acetylcholine receptors on GABAergic interneurons evoke dendritic and somatic inhibition of hippocampal neurons. *Journal of Neurophysiology* 2002;87:548–557. [PubMed: 11784770]
- Bullock AE, et al. Inbred mouse strains differ in the regulation of startle and prepulse inhibition of the startle response. *Behav Neurosci* 1997;111:1353–1360. [PubMed: 9438803]
- Cromwell HC, et al. Auditory inhibitory gating in the amygdala: single-unit analysis in the behaving rat. *Brain Res* 2005;1043:12–23. [PubMed: 15862513]
- Drago J, et al. Neuronal nicotinic receptors: insights gained from gene knockout and knockin mutant mice. *Cellular and Molecular Life Sciences* 2003;60:1267–1280. [PubMed: 12943217]
- Fabian-Fine R, et al. Ultrastructural distribution of the alpha 7 nicotinic acetylcholine receptor subunit in rat hippocampus. *Journal of Neuroscience* 2001;21:7993–8003. [PubMed: 11588172]
- Franceschini D, et al. Absence of alpha 7-containing neuronal nicotinic acetylcholine receptors does not prevent nicotine-induced seizures. *Molecular Brain Research* 2002;98:29–40. [PubMed: 11834293]
- Frazier CJ, et al. Synaptic potentials mediated via alpha-bungarotoxin-sensitive nicotinic acetylcholine receptors in rat hippocampal interneurons. *Journal of Neuroscience* 1998b;18:8228–8235. [PubMed: 9763468]
- Frazier CJ, et al. Acetylcholine activates an alpha-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. *Journal of Neuroscience* 1998a;18:1187–1195. [PubMed: 9454829]
- Freedman R, et al. Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. *Proceedings of the National Academy of Sciences of the United States of America* 1997;94:587–592. [PubMed: 9012828]
- Freedman R, et al. Evidence in Postmortem Brain-Tissue for Decreased Numbers of Hippocampal Nicotinic Receptors in Schizophrenia. *Biological Psychiatry* 1995;38:22–33. [PubMed: 7548469]
- Freedman R, et al. Alpha-Bungarotoxin Binding to Hippocampal Interneurons - Immunocytochemical Characterization and Effects on Growth-Factor Expression. *Journal of Neuroscience* 1993;13:1965–1975. [PubMed: 8478687]
- Freund TF, Buzsaki G. Interneurons of the hippocampus. *Hippocampus* 1996;6:347–470. [PubMed: 8915675]
- Gault J, et al. Comparison of polymorphisms in the alpha 7 nicotinic receptor gene and its partial duplication in schizophrenic and control subjects. *American Journal of Medical Genetics Part B- Neuropsychiatric Genetics* 2003;123B:39–49.
- Gray R, et al. Hippocampal synaptic transmission enhanced by low concentrations of nicotine. *Nature* 1996;383:713–716. [PubMed: 8878480]
- Happe HK, Morley BJ. Distribution and postnatal development of alpha 7 nicotinic acetylcholine receptors in the rodent lower auditory brainstem. *Brain Res Dev Brain Res* 2004;153:29–37.
- Hashimoto K, et al. Tropisetron improves deficient inhibitory auditory processing in DBA/2 mice: role of alpha7 nicotinic acetylcholine receptors. *Psychopharmacology* 2005;183:13–19. [PubMed: 16136299]
- Hellstrom-Lindahl E, et al. Regional distribution of nicotinic receptors during prenatal development of human brain and spinal cord. *Brain Res Dev Brain Res* 1998;108:147–160.
- Henderson Z, et al. Somato-dendritic nicotinic receptor responses recorded in vitro from the medial septal diagonal band complex of the rodent. *J Physiol* 2005;562:165–182. [PubMed: 15528250]

- Ji DY, Dani JA. Inhibition and disinhibition of pyramidal neurons by activation of nicotinic receptors on hippocampal interneurons. *Journal of Neurophysiology* 2000;83:2682–2690. [PubMed: 10805668]
- Jones S, Yakel JL. Functional nicotinic ACh receptors on interneurons in the rat hippocampus. *Journal of Physiology-London* 1997;504:603–610.
- Khiroug L, et al. Functional mapping and Ca²⁺ regulation of nicotinic acetylcholine receptor channels in rat hippocampal CA1 neurons. *Journal of Neuroscience* 2003;23:9024–9031. [PubMed: 14534236]
- Krause M, et al. Auditory sensory gating in hippocampus and reticular thalamic neurons in anesthetized rats. *Biol Psychiatry* 2003;53:244–253. [PubMed: 12559658]
- Leonard S, et al. Promoter mutations in the alpha 7 nicotinic acetylcholine receptor subunit gene are associated with an inhibitory deficit found in schizophrenia. *American Journal of Medical Genetics* 2002;114:749–749.
- Liu ZP, et al. Sequential interplay of nicotinic and GABAergic signaling guides neuronal development. *Science* 2006;314:1610–1613. [PubMed: 17158331]
- Luntz-Leybman V, et al. Cholinergic Gating of Response to Auditory-Stimuli in Rat Hippocampus. *Brain Research* 1992;587:130–136. [PubMed: 1525643]
- Maggi L, et al. Nicotine activates immature "silent" connections in the developing hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* 2003;100:2059–2064. [PubMed: 12582205]
- Maggi L, et al. Regulation of GABA release by nicotinic acetylcholine receptors in the neonatal rat hippocampus. *Journal of Physiology-London* 2001;536:89–100.
- Marks MJ, et al. Variation of Nicotinic Binding-Sites among Inbred Strains. *Pharmacology Biochemistry and Behavior* 1989;33:679–689.
- McQuiston AR, Madison DV. Nicotinic receptor activation excites distinct subtypes of interneurons in the rat hippocampus. *Journal of Neuroscience* 1999;19:2887–2896. [PubMed: 10191306]
- Metzger KL, et al. Effects of nicotine vary across two auditory evoked potentials in the mouse. *Biol Psychiatry* 2007;61:23–30. [PubMed: 16497274]
- Miller CL, Freedman R. The Activity of Hippocampal Interneurons and Pyramidal Cells during the Response of the Hippocampus to Repeated Auditory-Stimuli. *Neuroscience* 1995;69:371–381. [PubMed: 8552235]
- Moxon KA, et al. Multiple single units and population responses during inhibitory gating of hippocampal auditory response in freely-moving rats. *Brain Res* 1999;825:75–85. [PubMed: 10216175]
- Olivier B, et al. The DBA/2J strain and prepulse inhibition of startle: a model system to test antipsychotics? *Psychopharmacology (Berl)* 2001;156:284–290. [PubMed: 11549230]
- Paylor R, et al. Alpha7 nicotinic receptor subunits are not necessary for hippocampal-dependent learning or sensorimotor gating: a behavioral characterization of Acra7-deficient mice. *Learn Mem* 1998;5:302–316. [PubMed: 10454356]
- Penttonen M, et al. Feed-forward and feed-back activation of the dentate gyrus in vivo during dentate spikes and sharp wave bursts. *Hippocampus* 1997;7:437–450. [PubMed: 9287083]
- Pomper MG, et al. Hippocampal alpha 7 nicotinic cholinergic receptor, neuronal nitric oxide synthase, and glutamic acid decarboxylase mRNA co-localization in schizophrenia. *Schizophrenia Research* 1999;36:75–75.
- Radcliffe KA, et al. Nicotinic modulation of glutamate and GABA synaptic transmission in hippocampal neurons. *Molecular and Functional Diversity of Ion Channels and Receptors* 1999;868:591–610.
- Schreiber R, et al. Effects of alpha 4/beta 2- and alpha 7-nicotine acetylcholine receptor agonists on prepulse inhibition of the acoustic startle response in rats and mice. *Psychopharmacology (Berl)* 2002;159:248–257. [PubMed: 11862357]
- Seguela P, et al. Molecular-Cloning, Functional-Properties, and Distribution of Rat Brain-Alpha-7 - a Nicotinic Cation Channel Highly Permeable to Calcium. *Journal of Neuroscience* 1993;13:596–604. [PubMed: 7678857]
- Stevens KE, et al. Genetic correlation of inhibitory gating of hippocampal auditory evoked response and alpha-bungarotoxin-binding nicotinic cholinergic receptors in inbred mouse strains. *Neuropsychopharmacology* 1996;15:152–162. [PubMed: 8840351]

- Stevens KE, et al. Selective alpha(7)-nicotinic agonists normalize inhibition of auditory response in DBA mice. *Psychopharmacology* 1998;136:320–327. [PubMed: 9600576]
- Stevens KE, Wear KD. Normalizing effects of nicotine and a novel nicotinic agonist on hippocampal auditory gating in two animal models. *Pharmacology Biochemistry and Behavior* 1997;57:869–874.
- Stitzel JA, et al. Linkage of strain-specific nicotinic receptor alpha 7 subunit restriction fragment length polymorphisms with levels of alpha-bungarotoxin binding in brain. *Brain Res Mol Brain Res* 1996;43:30–40. [PubMed: 9037516]
- Thinschmidt JS, et al. Medial septal/diagonal band cells express multiple functional nicotinic receptor subtypes that are correlated with firing frequency. *Neurosci Lett* 2005;389:163–168. [PubMed: 16112453]
- Vogt KE, Regehr WG. Cholinergic modulation of excitatory synaptic transmission in the CA3 area of the hippocampus. *Journal of Neuroscience* 2001;21:75–83. [PubMed: 11150322]
- Whiteaker P, et al. An autoradiographic study of the distribution of binding sites for the novel alpha 7-selective nicotinic radioligand [H-3]methyllycaconitine in the mouse brain. *European Journal of Neuroscience* 1999;11:2689–2696. [PubMed: 10457165]
- Yu WF, et al. Postnatal upregulation of [alpha]4 and [alpha]3 nicotinic receptor subunits in the brain of [alpha]7 nicotinic receptor-deficient mice. *Neuroscience* 2007;146:1618–1628. [PubMed: 17434683]
- Zago WM, et al. Nicotinic activity stabilizes convergence of nicotinic and GABAergic synapses on rilopodia of hippocampal interneurons. *Molecular and Cellular Neuroscience* 2006;31:549–559. [PubMed: 16403644]
- Zhang X, et al. Postnatal changes of nicotinic acetylcholine receptor alpha 2, alpha 3, alpha 4, alpha 7 and beta 2 subunits genes expression in rat brain. *Int J Dev Neurosci* 1998;16:507–518. [PubMed: 9881299]
- Zhang ZW, et al. Neuronal Acetylcholine-Receptors That Bind Alpha-Bungarotoxin with High-Affinity Function as Ligand-Gated Ion Channels. *Neuron* 1994;12:167–177. [PubMed: 7507338]

Figure 1.

Film images of transverse sections through the hippocampus of C3H wild type (A) and C3H $\alpha 7$ receptor heterozygous (B) mice processed for ^{125}I - α -BTX autoradiography. Note the significantly decreased level of ^{125}I - α -BTX binding in the section from the C3H $\alpha 7$ receptor heterozygous mouse compared to the section from the wild type mouse. In contrast, the pattern of ^{125}I - α -BTX binding is comparable between the two groups of mice. The dense band of ^{125}I - α -BTX binding across all layers at the CA3/CA1 border zone is still clearly observable (arrow heads), as is the greater density of labeling within CA1 stratum lacunosum/moleculare and within the hilus of the dentate gyrus. Abbreviations: D – dentate gyrus, CA3 – hippocampal area CA3, CA1 – hippocampal area CA1, O – stratum oriens, P – stratum pyramidale, R –

stratum radiatum, LM – stratum lacunosum/moleculare, Luc – stratum lucidum, M – molecular layer of the dentate gyrus, G – granule cell layer of the dentate gyrus, H – hilus of the dentate gyrus.

Figure 2.

Hippocampal auditory sensory inhibition in adult C3H wild type and C3H $\alpha 7$ heterozygous mice. TC ratios are shown in the right panel. C3H wild type mice inhibit the response to the second (test) tone, resulting in a low TC ratio. The C3H $\alpha 7$ heterozygous mice fail to inhibit the response to the test tone, resulting in a high TC ratio. These data suggest that C3H $\alpha 7$ heterozygous mice have a deficit in hippocampal inhibitory circuit function. Data are mean $\pm$ S.E.M., $n = 10$ per group.

Figure 3.

Representative auditory evoked potentials from a wildtype C3H and an $\alpha 7$ heterozygous C3H mouse. The wildtype shows a pattern similar to that seen with the parental C3H mice, that is a large response to the conditioning stimulus and a suppressed response to the test stimulus. In contrast, the heterozygous mice show an increased response to the conditioning stimulus coupled with an increased response to the test stimulus, demonstrating reduced auditory gating. The arrows note the onset of stimulus (and stimulus artifact), the ticks mark the wave of interest.

Table 1

Density of ^{125}I -BTX binding to sections of hippocampal formation from C3H wild type and $\alpha 7$ heterozygous mice

Strain	Dentate Gyrus (mean $\pm$ S.E.M. nCi/g)	Hippocampal Area CA3 (mean $\pm$ S.E.M. nCi/g)	Hippocampal Area CA1 (mean $\pm$ S.E.M. nCi/g)
C3H Wild Type Mice	1310.42 $\pm$ 143.79	1531.41 $\pm$ 182.88	899.42 $\pm$ 141.51
C3H $\alpha 7$ Heterozygous Mice	508.57 $\pm$ 77.82 *	555.76 $\pm$ 53.86 *	413.14 $\pm$ 60.57 *

* $p < 0.005$ $\alpha 7$ heterozygote *versus* wild type mice