Published in final edited form as:

Neurosci Lett. 2016 August 03; 627: 1–6. doi:10.1016/j.neulet.2016.05.043.

Genetic knockout of the $\alpha 7$ nicotinic acetylcholine receptor gene alters hippocampal long-term potentiation in a background strain-dependent manner

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Abstract

Reduced $\alpha 7$ nicotinic acetylcholine receptor (nAChR) function is linked to impaired hippocampal-dependent sensory processing and learning and memory in schizophrenia. While knockout of the *Chrna7* gene encoding the $\alpha 7$ nAChR on a C57/B16 background results in changes in cognitive measures, prior studies found little impact on hippocampal synaptic plasticity in these mice. However, schizophrenia is a multi-genic disorder where complex interactions between specific genetic mutations and overall genetic background may play a prominent role in determining phenotypic penetrance. Thus, we compared the consequences of knocking out the $\alpha 7$ nAChR on synaptic plasticity in C57/B16 and C3H mice, which differ in their basal $\alpha 7$ nAChR expression levels. Homozygous $\alpha 7$ deletion in C3H mice, which normally express higher $\alpha 7$ nAChR levels, resulted in impaired long-term potentiation (LTP) at hippocampal CA1 synapses, while C3H $\alpha 7$ heterozygous mice maintained robust LTP. In contrast, homozygous $\alpha 7$ deletion in C57 mice, which normally express lower $\alpha 7$ nAChR levels, did not alter LTP, as had been previously reported for this strain. Thus, the threshold of *Chrna7* expression required for LTP may be different in the two strains. Measurements of auditory gating, a hippocampal-dependent behavioral paradigm used to identify schizophrenia-associated sensory processing deficits, was abnormal in C3H $\alpha 7$ knockout mice confirming that auditory gating also requires $\alpha 7$ nAChR expression. Our studies highlight the importance of genetic background on the regulation of synaptic plasticity and could

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Author Contributions: Conception and design were contributed by RF, SG, SL, MD. Execution of experiments and analysis of data were performed by RF, SG, KC and KS. Writing and approval of manuscript was contributed by RF, SG, SL and MD.

The authors declare that they have no conflict of interest.

be relevant for understanding genetic and cognitive heterogeneity in human studies of $\alpha 7$ nAChR dysfunction in mental disorders.

Keywords

hippocampal slice; long-term potentiation; $\alpha 7$ nicotinic acetylcholine receptor; genetics; mouse

Introduction

Nicotinic acetylcholine receptors (nAChRs) are expressed in nearly all brain regions and are assembled from combinations of α and β subunits with the predominant subtypes being either $\alpha 7$ homomers or $\alpha 4\beta 2$ heteromers [1][2]. In the hippocampus, $\alpha 7$ nicotinic receptors ($\alpha 7$ nAChRs) are found both presynaptically, on GABAergic or glutamatergic terminals, and postsynaptically on principal neurons [3][4]. Activation of nAChRs can positively modulate NMDA receptor-(NMDAR-) dependent synaptic plasticity [5][6], and it is thought that dysfunction or loss of nAChRs in aging or neurodegenerative disorders may contribute to cognitive decline.

Reduced $\alpha 7$ nAChR function is also associated with schizophrenia. Schizophrenia is a complex neuropsychiatric disorder affecting 1% of the population [7], with a characteristic onset at 20–30 years of age presenting with numerous deleterious symptoms, including disorganized thought and behavior, cognitive impairments, sensory processing deficits, and hallucinations [8][9]. The *CHRNA7* gene is linked to this disorder [10], and expression of $\alpha 7$ nAChRs is decreased in hippocampus and other brain regions of schizophrenic patients [11][12]. Furthermore, schizophrenics have a high incidence of heavy smoking [13][14], suggesting that these individuals are self-medicating with nicotine to ameliorate their symptoms, which may be related to decreased $\alpha 7$ nAChR function [15][16]. Indeed, it has been demonstrated in laboratory settings that nicotine administration can normalize auditory sensory gating both in schizophrenic patients [14][17] and in rodent models of schizophrenia [18]. Furthermore, smoking is also known to regulate gene expression in human hippocampus, and differentially regulates the expression of more than 75 genes in schizophrenic postmortem brain, including *CHRNA7* [12]. Schizophrenia is strongly influenced by interactions between specific genetic mutations and environment during development. The issue of genetic background heterogeneity is particularly important in human studies, and the impact of reduced $\alpha 7$ nAChRs on brain functions in schizophrenia, such as learning and memory and sensory processing, may be modified by genetic background. Here we examined a type of hippocampal synaptic plasticity thought to underlie learning and memory—long-term potentiation (LTP)—in wild-type (WT) and $\alpha 7$ knockout (KO) mice on two genetic backgrounds, C3H and C57/Bl6, that differ in their levels of *Chrna7* gene expression; C57/Bl6 expresses approximately 15% less $\alpha 7$ nAChR than do C3H mice [19]. Previous data for $\alpha 7$ KO in C57/Bl6 mice found that LTP was not impaired [20][21], which agrees with our data presented below. However, we found that deletion of the $\alpha 7$ nAChR in C3H mice, strongly impaired LTP. Comparison of auditory gating and LTP in the C3H KO suggests that different threshold levels of $\alpha 7$ nAChRs may be required for these phenotypes. Thus, genetic background may be a significant factor in

determining the impact of reduced $\alpha 7$ nAChR function on neuronal plasticity and behavior in both mouse models and humans with schizophrenia.

Materials and methods

Animals

Mice were originally constructed as a knockout of the *Chrna7* gene on mixed background 129C57/B16 mice [22]. At the Institute for Behavioral Genetics at the University of Colorado, Boulder, CO, USA, the knockout genotype was introgressed onto C3H/1bg (C3H) by backcrossing the $\alpha 7$ KO mixed background (129C57BL/6 *Chrna7*^{-/-}) mice to C3H mice for 10 generations (maintaining six families). Mice from the last generation were used to establish a breeding colony at the University of Colorado Anschutz Medical Campus, Aurora, CO, USA. A recent genome diversity analysis (The Jackson Laboratory, Bar Harbor, ME, USA) indicates that the $\alpha 7$ KO C3H mice are at least 99.7% identical to the C3H parent strain. Similar crossings were used for $\alpha 7$ genotypes on the C57 background. Male animals of $\alpha 7$ KO, heterozygotes and WT animals on either C3H or C57 genetic backgrounds were used at 6–10 weeks of age. All animal procedures were conducted in accordance with National Institutes of Health (NIH)–United States Public Health Service guidelines and with the approval of the University of Colorado, Denver, Institutional Animal Care and Use Committee.

Electrophysiology

Mouse Hippocampal Slice Preparation—After sacrifice, the brains were rapidly removed and immersed in ice-cold, sucrose-containing cutting buffer (in mM: 87 NaCl, 2.5, KCl, 7 MgCl₂, 0.5 CaCl₂, 1.25 NaH₂PO₄, 25 D-glucose, 220 sucrose, and 25 NaHCO₃) for 40–60 sec. Transverse slices (400 μ m thickness) were made, stored and recorded as previously reported [23]. The artificial cerebrospinal fluid (aCSF) contained the following (in mM): 126 NaCl, 3.0 KCl, 1.0 MgSO₄, 2.0 CaCl₂, 1.2 NaH₂PO₄, 11 D-glucose, and 25.9 NaHCO₃.

Electrophysiological Recordings—For each slice recording experiment, input-output (I-O) curves were generated and paired-pulse responses (PPR) were obtained, with an inter-stimulus interval of 50 ms, as previously reported [23]. Synaptic fEPSP responses were evoked with bipolar tungsten electrodes placed in the CA1 synaptic field layer. Test stimuli were delivered once every 20 sec with the stimulus intensity set to 40–50% of the maximum synaptic response. High-frequency stimulation (HFS) consisted of two trains of 100 Hz lasting 1 sec each, with an inter-train interval of 20 sec, at the control stimulus intensity. fEPSP recordings were made with a glass micropipette filled with aCSF and placed in the stratum radiatum. This stimulation produced LTP that persisted for more than 60 min in both C57 and C3H WT animals. The initial slopes of fEPSPs were calculated as the slope measured between 10–30% from the origin of the initial negative deflection. Each time point shown is an average of six 20 sec interval measurements. An average over 10 min of LTP recordings 55–65 min after HFS were used in the summary analyses in Fig. 2C. For statistical comparisons, each slice was considered a separate n.

Auditory Gating

Mice (20–27 g) were anesthetized with chloral hydrate (400 mg/kg, ip) and pyrazole (400 mg/kg, ip) to inhibit metabolism of chloral hydrate. Mice were then placed in a mouse adapter (Neuroprobe, Cabin John, MD) for the stereotaxic instrument and the scalp incised. A stainless steel, teflon-coated recording electrode (127 μ m diameter) was lowered to the CA3 region of the dorsal hippocampus, and a reference electrode was placed in the dura over the anterior cortex as previously reported [24]. Final placement was determined by the presence of complex spike activity typical of hippocampal pyramidal neurons. Miniature earphones attached to hollow ear bars, placed at the externalization of the aural canal, delivered the auditory stimuli. EEG responses to paired click stimuli were amplified and filtered at 1–500 Hz, collected and analyzed using the SciWorks acquisition and analysis program (DataWave, Loveland, CO), as previously described [18][25].

Results

Baseline electrophysiological characteristics of hippocampal CA1 synapses are not altered by $\alpha 7$ gene deletion

There were no significant differences between WT and $\alpha 7$ KO animals on either the C57 or C3H genetic background for baseline electrophysiological parameters (input-output (I-O) curves and paired-pulse ratios (PPR; Fig. 1). In particular, although differences in basal I-O curves between WT C3H and C57 mice were noted, genetic knockout of the $\alpha 7$ gene had no impact on these properties. Likewise, PPR, a measure of presynaptic function consistently produced a bigger response for the second stimulation than the first in both strains, as expected, but there were no significant PPR differences between genotypes.

LTP is impaired by $\alpha 7$ gene deletion on the C3H but not C57 background

LTP induced by HFS produced similar potentiation of fEPSPs over 60 min for both WT and $\alpha 7$ KO mice on the C57 background (Fig. 2A). In contrast, *Chrna7* gene KO on the C3H background resulted in much diminished LTP compared with its WT counterpart (Fig. 2B). However, C3H mice heterozygous for the $\alpha 7$ gene (Het) exhibited LTP at least as robust as WT. Thus, unlike for C57, in the C3H strain, LTP differences were significantly different between $\alpha 7$ KO and either WT or Het mice (for WT vs. KO, $F(1,330) = 247.23$ $p < 0.0001$; for KO vs. Het, $F(1,330) = 10.66$ $p = 0.0012$). WT C3H mice also consistently exhibited slightly greater LTP than WT C57 mice, an effect that was significant if examined over the whole time course following HFS, respectively (Fig. 2A, 2B; LTP: $F(1,29) = 46.64$, $p < 0.0001$). Average LTP data for the last 10 min are summarized in Fig. 2C.

The $\alpha 7$ nAChR receptor selective antagonist α -bungarotoxin (α BTX) has no effect on LTP in C3H or C57 mice

Since the lack of $\alpha 7$ nAChRs resulted in loss of LTP in C3H mice, we reasoned that this was due either to a lack of acute $\alpha 7$ nAChR activation during induction of LTP or to some pre-existing deficit in LTP induction and/or maintenance mechanisms caused by chronic lack of $\alpha 7$ nAChRs during development. We tested these possibilities by acutely blocking $\alpha 7$ nAChRs with the selective, irreversible antagonist α -bungarotoxin (α BTX) administered

30 min prior to HFS. Application of α BTX produced a blunting of the initial response to HFS in C3H slices, known as post-tetanic potentiation (PTP), but did not alter the levels of LTP over 60 min for either strain (Fig. 2). We conclude that α 7nAChRs do not have a prominent role in the acute expression of HFS-induced LTP in C3H or C57 mice. These receptors may, however, affect the organization of synaptic components during development, resulting in abnormal synaptic plasticity in adulthood.

Auditory Gating is impaired in C3H α 7 KO mice

Auditory gating is a mechanism in the brain that filters out unnecessary information and is generally linked to focus. It is measured on the skull surface in humans as a wave of latency of 50 ms occurring after an initial auditory stimulus of 70 db. A second stimulus delivered 500 ms later is filtered or gated out in normal individuals. The ratio of the test to conditioning waves is characteristically diminished in schizophrenics [15][26]. This phenomena has been studied in rodents, where it involves hippocampal circuits and depends on α 7nAChR function [27][28]. In a previous study, we found that C3H mice heterozygous for *Chrna7* had impaired sensory gating compared to their WT counterparts [25]. Here, we analyzed auditory gating for α 7 homozygous KO mice in comparison to WT on the C3H background (Fig. 3). While conditioning amplitudes were not significantly different, the average test amplitude of α 7 KO mice was significantly greater than that for WT ($***p<0.001$). Thus, WT C3H mice exhibited a test-conditioning (TC) ratio of ~ 0.5 , characteristic of normal gating, but α 7 KO mice lacked gating with TC ratio of ~ 1.0 , similar to the schizophrenic phenotype. TC ratios of WT mice were significantly less than those for the α 7 KO mice ($**p<0.01$).

Discussion

NMDAR-dependent synaptic plasticity in the hippocampus, such as LTP and LTD, is thought to represent the molecular and cellular basis for learning and memory [29][30]. LTP was expressed in WT C3H mice, but was markedly impaired in α 7 KO animals on this background. In contrast, α 7 gene deletion did not alter LTP in C57/Bl6, although WT mice in this strain exhibited slightly reduced LTP compared with WT C3H. At least two explanations are possible for the LTP impairment in C3H α 7 KO mice. α 7nAChRs may actively contribute to NMDAR-dependent LTP via actions pre- or postsynaptically (increasing glutamate release [4] or adding to postsynaptic Ca^{2+} flux [6][31] [32], respectively). Thus, the α 7AChR KO mice would accordingly manifest reduced LTP. Alternatively, the loss of *Chrna7* expression during development may result in faulty pre- and/or postsynaptic function of signaling components necessary for LTP [33][34]. Such requirements for *Chrna7* expression to form correct excitatory and inhibitory circuits during development could explain why α 7nAChRs are highly abundant early in development, but drop off markedly to adult levels by about 20 days of age [35].

To test these possibilities, we examined the effect of acute blockade of α 7nAChRs with the selective antagonist α BTX on LTP in WT C57 and C3H hippocampal slices. This α 7 irreversible antagonist blunted the initial PTP response to HFS in C3H slices, but did not alter baseline synaptic activity or LTP from the levels observed in WT mice of either strain.

The inhibition of PTP with α BTX in C3H slices is likely due to acute block of presynaptic α 7nAChRs because PTP results from short-term enhancement of presynaptic function; this effect was not observed in C57 slices. However, since after 60 min LTP remained unchanged, we can conclude that α 7nAChRs do not play a major acute signaling role in LTP in adult animals. Instead, the developmental hypothesis is supported, i.e., animals that develop without these receptors may have altered glutamatergic and/or GABAergic synapses, such that normal LTP cannot be induced effectively. Indeed, a recent study observed that C57 mice lacking the α 7nAChR have abnormal glutamatergic synapses accompanied by functional deficits during early postnatal development [34]. It should be noted, however, that the latest time investigated in Lozada et al. [34] was 25 days of age, and it is not known whether these synaptic functional deficits persist beyond that age or are “corrected” or compensated for by the time when we measured LTP (6–10 weeks of age). Nonetheless, any such abnormal basal synaptic functions were not severe enough to alter I-O or PPR responses measured in our study for either young or older α 7 KO mice on either the C3H or C57 backgrounds. Thus, the deficit in LTP for C3H KO mice could reflect impaired activation of signaling pathways, such as phosphorylation of AMPA receptors [36], that are acutely required for LTP but not for maintaining basal synaptic transmission. Future studies could address the role of developmental signaling alterations by using conditional α 7nAChR knockout in adult mice [37].

Taken together, these findings suggest that the absence of α 7nAChRs in the C3H mouse likely affects synaptic development to cause persistent alterations in signaling mechanisms necessary for LTP. Activation of α 7nAChRs can promote removal of NMDAR-Mg²⁺ block and promote insertion of AMPARs into NMDAR-only “silent” synapses in development [33]. In addition, other forms of developmental regulation of NMDAR-containing synapses by neuronal nAChRs have also been identified [37][38]. In particular, activation of α 7nAChRs with nicotine from postnatal day 8 – 12 resulted in increased levels of mRNA for GluN2A in auditory cortex and decreased mRNA for GluN2B in thalamus [39]. Indeed, GABAergic mechanisms may also be affected by α 7 KO [40]. Thus, it is possible that loss of α 7nAChRs during this critical developmental period might lead to alterations in both glutamatergic and GABAergic function that persist into adulthood resulting in impaired plasticity and aberrant behavior. Accordingly, investigation of auditory gating confirmed that KO of *Chrna7* on the C3H background results in a loss of gating, consistent with impaired hippocampal processing of sensory information.

Regardless of the underlying mechanisms, the absence of α 7nAChRs clearly prevented LTP in C3H animals. To the best of our knowledge the present data represents the first report of a deficit in this prototypical form of hippocampal synaptic plasticity caused by genetic knockout of the *Chrna7* gene. By contrast, in α 7 knockouts on the C57 background, LTP was unchanged from that observed in WT mice. Thus our data confirms other studies that knockout of the α 7nAChR does not impair LTP in adult C57 mice [20][21][41], although LTP impairment was noted in aged α 7 KO mice on this background [41]. As mentioned above, WT *Chrna7* gene expression is approximately 15% higher in C3H than in C57 [19]. However, other differentially-expressed genes between these two strains might also account for plasticity differences in WT animals, as well as for differential impacts and compensatory and/or adaptive mechanisms in α 7 KO animals.

In conclusion, we found that loss of $\alpha 7$ nAChR expression significantly impaired NMDAR-dependent LTP at hippocampal CA1 synapses in C3H mice, while C57 mice were functionally resistant to loss of receptor expression. Interestingly, the more profound effects on synaptic plasticity in C3H mice were also accompanied by a loss of auditory gating, a hippocampal-dependent behavior that is impaired in schizophrenics and known to be more robust in WT C3H compared to C57 mice [24]. This correspondence between LTP and gating, however, did not extend to C3H heterozygotes, which had normal LTP but abnormal gating [25]. Perhaps reduced $\alpha 7$ nAChR expression can be compensated for in the limited setting of LTP at CA1 excitatory synapses in a way not possible for gating behavior that requires overall function of hippocampal excitatory and inhibitory circuits. Expression of the human *CHRNA7* gene is reduced in multiple brain regions in most schizophrenic patients. Importantly, other genetic changes in this disorder include multiple genes in the NMDAR postsynaptic density [42][43]. However, not every schizophrenic has the same gene expression changes. This heterogeneity in gene expression suggests that the underlying genetic background in human individuals may be important for the penetration and manifestation of any one given mutation. Our current study is relevant for understanding the complex interactions between $\alpha 7$ nAChR dysfunction and genetic background.

Acknowledgments

This work was supported by NS040701 to M.L.D.; and by DA09457, MH81177, and VAMC to S.L.

Abbreviations

aCSF	artificial cerebrospinal fluid
$\alpha 7$ nAChR	alpha 7 nicotinic acetylcholine receptor
<i>CHRNA7</i>	alpha 7 nicotinic acetylcholine receptor gene
fEPSP	field excitatory postsynaptic potential
HFS	high-frequency stimulation
Het	heterozygous
KO	knockout
LFS	low-frequency stimulation
LTD	long-term depression
LTP	long-term potentiation
PPR	paired-pulse response
TC ratio	test-conditioning ratio
WT	wild-type

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Highlights

- Both $\alpha 7$ nAChR function and genetic background are important in schizophrenia.
- C57/B16 and C3H mice differ in background expression of $\alpha 7$ nAChRs (C3H > C57).
- Knockout of the $\alpha 7$ nAChR gene impaired LTP in C3H but not C57 mice.
- C3H knockout mice displayed abnormal auditory gating, a schizophrenic phenotype.
- Genetic background strongly influences phenotypic expression in schizophrenia.

Fig. 1.

Baseline CA1 synaptic transmission characteristics are similar for hippocampal slices from WT, $\alpha 7$ KO and Het mice on both the C57 and C3H genetic backgrounds. A. Representative fEPSP traces from an input-output (I-O) curve. Left panel: Traces are averages of 3 data sweeps for CA1 recordings in a C3H WT slice at 2, 4, 6 and 8 V stimulation. Right panel: traces of 6 data sweeps for paired-pulse recordings at 50 ms inter-stimulus intervals. Calibration bars for both I-O and PPR are 20 ms and 0.5 mV. B. I-O curves for WT and $\alpha 7$ KO from C57 and C3H animals for 6–10 week-old adult animals used for LTP studies. C. % PPR measurements for C57 ($n = 7$ and 7 for WT and $\alpha 7$ KO, respectively) and C3H ($n = 6$, 7 and 7 for WT, $\alpha 7$ KO and Het, respectively).

Fig. 2.

LTP is only impaired in $\alpha 7$ KO on the C3H genetic background. fEPSP recordings of LTP in slices from (A.) C57 and (B.) C3H WT and $\alpha 7$ KO, as well as WT C3H in the presence of 100 nM α BTX (BTX was applied to slices 30 min prior to placing in recording chamber). fEPSP recordings were made from area CA1 of acute hippocampal slices from 6–10 week-old animals as indicated in Materials and Methods. After a stable baseline period of at least 20 min., two HFS trains (100 Hz, 1 s) were delivered at the control stimulus intensity, 20 s apart (arrow at 0 min). Amplitudes and slopes of fEPSPs were recorded for at least 60 min following HFS. Data shown are % of baseline slope before HFS. Numbers in parentheses indicate (number of slices/number of animals). Scale bars: 20 ms, 0.5 mV C: Average fEPSP slope for 55–65 min. of recording after HFS as a % of baseline slope. LTP for C3H $\alpha 7$ KO mice was significantly less than that of both WT and Het (** $p < 0.01$; *** $p < 0.005$).

Fig. 3.

Summary of auditory gating data for C3H mice. WT and KO C3H mice displayed similar conditioning amplitudes, but test amplitudes of KO mice were significantly greater ($***p < 0.001$, t -test; WT ($n = 10$), KO ($n = 7$)). WT C3H mice showed 'normal' auditory gating phenotype, with a TC ratio (amplitude of the response to the second of identical paired tones/amplitude of the response to the first tone) ≈ 0.5 . C3H $\alpha 7$ KO mice did not show gated auditory responses (TC ratio ≈ 1.0), and the TC ratios were significantly different ($**p < 0.01$, t -test).