Impaired Hippocampal Theta Oscillations in the Mice Null Alpha7 Nicotinic Acetylcholine Receptors

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Nicotinic acetylcholine receptor (nAChR) $\alpha 7$ subunits are among the most ancient of vertebrate nAChR subunits and have the capacity to assemble as homomers. Naturally expressed $\alpha 7$ -nAChRs in the central nervous system play a critical role in modulating the release of various neurotransmitters, neurite outgrowth, and even neuronal survival or death [1]. $\alpha 7$ -nAChRs also have been implicated in development, differentiation, and disease of the nervous system, as well as being targets of nicotine action.

$\alpha 7$ -nAChRs are highly expressed in the hippocampus, a brain structure associated to learning, memory, and cognitive functions. Accumulating lines of evidence demonstrate that $\alpha 7$ -nAChRs are key targets in mediating cholinergic modulations of cognition [2].

Hippocampus plays an important role in declarative memory and learning, and possesses a large amplitude rhythm at theta frequency (4–12 Hz), which is postulated to support mnemonic processes in humans and rodents [3,4]. It has been reported that nicotine modulates hippocampal theta oscillations likely through $\alpha 4\beta 2$ -nAChRs [5,6], but the role of $\alpha 7$ -nAChRs in hippocampal theta oscillations is still uncertain. Whether or not $\alpha 7$ -nAChR knockout (KO) mice exhibit hippocampal theta oscillation is unknown. Here, we present, for the first time, that $\alpha 7$ -nAChR KO mice selectively lack hippocampal theta oscillations.

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the Barrow Neurological Institute. The data were collected from 27 hippocampal slices from 8 wild-type (WT) mice (postnatal 28 ± 3 days) and 16 hippocampal slices from 6 $\alpha 7$ -nAChR KO mice (postnatal 26 ± 3 days, $P > 0.05$ between WT and $\alpha 7$ -nAChR KO mice, *t*-test). Transverse hippocampal slices (450 μ m) were prepared as previously reported [7]. External solution contained (mM) NaCl 124, NaHCO₃ 26, KCl 3, KH₂PO₄ 1.2, CaCl₂ 2.4, MgSO₄ 1.3, D-glucose 10, which was continuously bubbled with 95% O₂–5% CO₂ during the entire experimental period. Field potential recordings were performed in the CA1 cell layer using a glass electrode filled with 2 M NaCl. During recordings, the recording chamber was set to 31°C. Hippocampal theta oscillations were induced by bath application of 50 μ M carbachol (CCh) for 50–60 min, as previously described [5,8]. Under this experimental protocol, only one slice showed no response to CCh in 27 tested slices from WT mice, while six slices showed no response to CCh in 16 tested slices from $\alpha 7$ -nAChR KO mice. As shown in Figure 1, in the continuous presence of 50 μ M CCh, there were typically three types of oscillations, slow (δ : 0.5 ± 0.06 Hz, $n = 18$), theta (6.3 ± 0.46 Hz, $n = 23$), and super-fast (146.1 ± 7.0 Hz, $n = 19$) oscillations in WT hippocampal slices (Figure 1A). However, in $\alpha 7$ -nAChR KO hippocampal

Figure 1 CCh-induced field potential oscillations in hippocampal slices prepared from wild-type (WT) and $\alpha 7$ -nAChR KO mice. **(A)**: A representative trace of 50 μ M CCh-induced field oscillations in a WT slice. Fifty micromolar CCh was continuously present during recording as indicated by vertical arrow at the beginning of the trace. The theta oscillations are indicated by arrows. Insets show the expanded time scale from theta and slow oscillations. Further expanded time scale shows the super-fast oscillations. **(B)**: A representative trace of CCh-induced field oscillations in a $\alpha 7$ -nAChR KO slice. Fifty micromolar CCh was continuously present during recording as indicated by vertical arrow at the beginning of the trace. Expanded time scale shows the slow oscillations. Further expanded time scales show super-fast oscillations.

Figure 2 Analysis of CCh-induced field potential oscillations in hippocampal slices. Representative figures after spectral power analysis of CCh-induced hippocampal oscillations in a WT **(A)** or a $\alpha 7$ -nAChR KO **(B)** slice. This figure shows three clear peaks of oscillatory power (indicated by vertical dashed lines) in WT slice **(A)** but only two peaks of oscillatory power in $\alpha 7$ -nAChR KO slice **(B)**. **(C)** Bar graph summarizes experimental results. Each column bar indicates the numbers of total slices tested, and the filled portion indicates the numbers that slices exhibit a corresponding oscillatory response. $\alpha 7^{-/-}$ means $\alpha 7$ -nAChR KO.

slices, the same CCh only induced two types of oscillations, slow (Δ : 0.6 ± 0.15 Hz, $n = 10$) and super-fast (153.7 ± 7.2 Hz, $n = 7$) oscillations (Figure 1B). The statistic analysis showed that the difference in frequencies of slow- or super-fast oscillations between WT and $\alpha 7$ -nAChR KO mice was not significant ($P > 0.05$, t -test). Further spectral analysis (using Clampfit 9.2, Molecular Devices, Sunnyvale, CA, USA) showed that after CCh perfusion, WT slices showed three peaks at slow-, theta- and super-fast frequency regions (Figure 2A), while $\alpha 7$ -nAChR KO slices only exhibited two peaks at slow- and super-fast frequency regions (Figure 2B), further indicating a lack of theta oscillations in $\alpha 7$ -nAChR KO slices. The $\alpha 7$ -nAChR KO mice were identified as previously described [9]. Figure 2(C) summarizes the results, in which, the ratio of each of the oscillation subtypes induced by CCh was shown. In 27 WT slice tested, 23 slices exhibited theta oscillation (85%), while in 16 $\alpha 7$ -nAChR KO slices tested, only one slice showed theta oscillations (6%). Together, our results demonstrate that $\alpha 7$ -nAChR KO mice selectively lack CCh-induced hippocampal theta oscillations in most tested slices.

It has been well known that α 7-nAChRs play an important role in the modulation of hippocampus-associated functions such as learning, memory, and cognition [10]. In this study, we compared CCh-induced hippocampal oscillations between the slices prepared from α 7-nAChR KO and WT mice. We found that under our experimental conditions, CCh induced quite similar oscillations at slow- (<1 Hz) and super-fast (>100 Hz) frequency regions in both α 7-nAChR KO and WT mice. However, most hippocampal slices prepared from α 7-nAChR KO mice (15/16) were missing theta oscillations (4–12 Hz), while most age-matched WT slices (23/27) exhibited theta oscillations. To our knowledge, this is the first report of a selective lack of hippocampal theta oscillations in α 7-nAChR KO mice. Our data suggest that α 7-nAChRs likely play an important role in the generation of hippocampal theta oscillations. Considering the importance of hippocampal theta oscillations in the processes of learning and memory, this study supports the role of α 7-nAChRs in the modulation of hippocampus-associated learning and memory.

Previous works have shown that CCh-induced hippocampal oscillations can be eliminated by α 4 β 2-nAChR antagonists [5,6], but α 7-nAChR antagonists can only partially inhibit nicotine or α 7-nAChR agonist-induced theta oscillations [6]. The present

study extends these previous works. The mechanisms of this lack of hippocampal theta oscillations in α 7-nAChR KO mice are still unclear. One possible explanation is the eliminated hippocampal GABA_A receptor function in α 7-nAChR KO mice. In α 7 KO mice, the functional switch of GABA_A receptors from depolarization to hyperpolarization is significantly delayed [10]. It has been reported that normalized GABA_A receptor function is important to generate CCh-induced theta oscillations in hippocampal slices because a GABA_A receptor antagonist (bicuculline) abolished these CCh-induced theta oscillations [5]. It will be interesting to test this possibility in future study.

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Conflict of Interest

The authors declare no conflict of interest.

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