Behavioral/Cognitive

Cholinergic-Sensitive Theta Oscillations in Memory Encoding in Mice

Zhenglin Gu, Korey D. Stevanovic, Jesse D. Cushman, and Jerrel L. Yakel

Neurobiology Laboratory, Department of Health and Human Services, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709

Cholinergic regulation of hippocampal theta oscillations has long been proposed to be a potential mechanism underlying hippocampus-dependent memory encoding processes. However, cholinergic transmission has been traditionally associated with type II theta under urethane anesthesia. The mechanisms and behavioral significance of cholinergic regulation of type I theta in freely exploring animals is much less clear. In this study, we examined the potential behavioral significance of cholinergic regulation of theta oscillations in the object location task in male mice that involves training and testing trials and provides an ideal behavioral task to study the underlying memory encoding and retrieval processes, respectively. Cholinergic regulation of hippocampal theta oscillations and the behavioral outcomes was examined by either intrahippocampal infusion of cholinergic receptor antagonists or knocking out cholinergic receptors in excitatory neurons or interneurons. We found that both muscarinic acetylcholine receptors (mAChRs) and α7 nicotinic AChRs (α7 nAChRs) regulated memory encoding by engaging excitatory neurons and interneurons, respectively. There is a transient upregulated theta oscillation at the beginning of individual object exploration events that only occurred in the training trials, but not in the testing trials. This transient upregulated theta is also the only theta component that significantly differed between training and testing trials and was sensitive to mAChR and α7 nAChR antagonists. Thus, our study has revealed a transient cholinergic-sensitive theta component that is specifically associated with memory encoding, but not memory retrieval, in the object location task, providing direct experimental evidence supporting a role for cholinergic-regulated theta oscillations in hippocampus-dependent memory encoding processes.

Key words: **acetylcholine; hippocampus; memory encoding; muscarinic receptor; nicotinic receptor; theta oscillation**

Significance Statement

In this study, we made several important findings regarding cholinergic mechanisms regulating hippocampal memory formation in the object location task. Firstly, both hippocampal muscarinic acetylcholine receptors (mAChRs) and the α7 nicotinic AChRs (nAChRs) regulated the memory encoding process in the object location task by engaging glutamatergic neurons and interneurons, respectively. Secondly, upregulated theta oscillations were observed in the pre-exploration period in training trials, but not in the testing trials, suggesting a role in novelty encoding/learning. Thirdly, the upregulated theta was sensitive to mAChR and α7 nAChR antagonists and receptor knock-outs in neuronal subpopulations. Taken together, these observations provide direct experimental evidence supporting a role for cholinergic-regulated theta oscillations in hippocampal memory encoding process.

Introduction

The hippocampus plays a central role in higher cognitive functions, including learning and memory (Neves et al., 2008; Opitz, 2014; Moser et al., 2015; Raynal et al., 2020). The hippocampus receives major excitatory inputs from the entorhinal cortex and extensive cholinergic and GABAergic inputs from the medial septum/diagonal band of Broca (MS/DBB) (Witter et al., 2000; Amaral et al., 2007). Synchronized rhythmic activities referred to as theta oscillations emerge among these three regions during active exploration, which play a critical role in memory encoding, especially in spatial memory encoding (Buzsaki, 2002, 2005; Buzsaki and Moser, 2013; Lopez-Madrona et al., 2020; Nunez and Buno, 2021; Qasim et al., 2021; Zheng et al., 2021). Cholinergic transmission is also known for its role in memory encoding (Hasselmo, 2006; Micheau and Marighetto, 2011; Easton et al., 2012; Hersman et al., 2017; Newman et al., 2017; Zhang et al., 2021). Thus, cholinergic regulation of hippocampal theta oscillations has long been proposed as a potential

Received July 13, 2023; revised Jan. 23, 2024; accepted Jan. 24, 2024.

Author contributions: Z.G. designed research; Z.G., K.D.S., and J.D.C. performed research; Z.G. analyzed data, Z.G. and J.L.Y. wrote the manuscript.

We thank Patricia Lamb for animal genotyping and management and Arvind Prasad for technical assistance in processing the behavioral data. This research was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences/NIH/DHHS.

The authors declare no competing financial interests.

Correspondence should be addressed to Jerrel L. Yakel at yakel@niehs.nih.gov.

https://doi.org/10.1523/JNEUROSCI.1313-23.2024

mechanism for hippocampus-dependent memory encoding (Hasselmo et al., 2002; Hasselmo, 2005; Douchamps et al., 2013; Teles-Grilo Ruivo and Mellor, 2013; Gu and Yakel, 2022). However, direct experimental evidence is scarce. Traditionally cholinergic transmission has been mostly associated with the regulation of type II theta oscillations that occur under urethane anesthesia and REM sleep or under stressed situation, but not the regulation of type I theta oscillations during active exploration. Type I theta is largely preserved after atropine application and thus also has been referred to as atropine-resistant form of theta (Kramis et al., 1975; Montoya and Sainsbury, 1985; Sainsbury et al., 1987; Buzsaki, 2002). Some studies suggest that even though cholinergic antagonists cannot eliminate type I theta generation, cholinergic transmission may still regulate certain aspects of type I theta oscillations during active exploration (Yoder and Pang, 2005; Balakrishnan and Pearce, 2014). Furthermore, many studies have also observed higher septal cholinergic neuronal firing rate and hippocampal acetylcholine (ACh) release during both type II and type I theta states (Marrosu et al., 1995; Giovannini et al., 2001; Bianchi et al., 2003; Zhang et al., 2010, 2021; Ma et al., 2020). Our recent studies also suggest that cholinergic transmission can regulate type I theta power during open-field exploration through both muscarinic ACh receptors (mAChRs) and α7 nicotinic AChRs (nAChRs; Gu et al., 2017, 2020). Therefore, there may coexist cholinergic-sensitive and cholinergic-resistant theta components during active exploration (Oddie et al., 1997; Yoder and Pang, 2005).

In this study, we examined the contributions of cholinergic receptor subtypes and theta oscillations to hippocampal memory encoding in the object location task (Dere et al., 2007; Vogel-Ciernia and Wood, 2014), which is a test that engages hippocampal spatial memory and is sensitive to cholinergic regulation (Cai et al., 2012; Okada et al., 2022). Cholinergic antagonists including mAChRs, α7 nAChRs, and non-α7 nAChRs antagonists, were infused into the hippocampus before either training or testing trials to examine the roles of these receptors in memory encoding or memory retrieval, respectively. The underlying neuronal subpopulations were revealed by knocking out mAChRs or α7 nAChRs in either glutamatergic neurons or interneurons individually. Theta power during individual object exploration events was examined with subsecond precision. We found that both mAChRs and α7 nAChRs are involved in memory encoding, but not memory retrieval by engaging excitatory and inhibitory neurons, respectively. Meanwhile, we observed a transient occurrence of significantly upregulated theta oscillations at the beginning of individual object exploration events that only appeared in the training trials, but not in the testing trials. Furthermore, these upregulated theta oscillations can be prevented by mAChR and α7 nAChR antagonists. Thus, this study provides direct experimental observations that strongly support the critical role of cholinergic-sensitive theta oscillations in hippocampus-dependent memory encoding process.

Materials and Methods

Animals and materials. Wild-type C57BL/6 mice, floxed M1 mAChR knock-out mice (C57BL/6-Chrm1tm1Stl/J), floxed α7 nAChR knock-out mice [B6(Cg)-Chrna7tm1.1Ehs/YakelJ], Gad2-cre [B6N.Cg-Gad2tm2(cre)Zjh/J], and CaMK2a-cre [B6.Cg-Tg(Camk2a-cre)T29–1Stl/J] transgenic mice were originally purchased from Jackson Laboratory and then bred at NIEHS. Male mice (4–5-month-old) were used for experiments. All mice were housed under normal light/dark cycle. All procedures were approved and performed in compliance with NIEHS/NIH Humane Care and Use of Animals in Research protocols.

Selective AChR subtype antagonists were from Tocris or Sigma. Intrahippocampal infusion guide cannulas (gauge 26, cut to 2.5 mm), corresponding internal cannulas (with 0.5 mm protrusion out of guide cannula), and dummy cannulas (without protrusion) were from P1 Technologies (formerly Plastics One). Recording electrodes were made by Microprobes as microwire tetrode bundles consisting of four polyimide-coated 50 μm stainless steel wires cut to 3 mm length. The wires were connected to a five-channel Omnetics connector, with an external Ag ground wire that was also connected to the unused fifth channel. The cannula–electrode combo was formed by attaching the microwire tetrode bundle next to the guide cannula with 0.5 mm protrusion.

Object location task. Before object location tasks, mice were habituated by being exposed to an open-field arena (36 cm L × 21 cm W × 18 cm H) with normal bedding on the floor for 10 min/day for 3 consecutive days. An overhead camera and a video recording/tracking system were used to monitor and record the behaviors for later offline analyses. On the task day, two identical objects (50 ml conical glass flasks) were placed symmetrically along the long side of the arena (~1 cm away from the long side and 9 cm away from the near short side). Mice were first exposed to the open-field arena with objects for 8 min and then put back into home cage for 3 h and then exposed to the open-field arena again for 5 min but this time with one object in the original place (stationary object) and the other one moved to the other side (displaced object). Due to the innate curiosity, mice tend to spend more time on investigating (sniffing) the object with new location than the object with the familiar location. The object location discrimination index is calculated as the difference of the time spent on displaced object and stationary object over the total time spent on both objects. Discrimination index (%) = (displaced object − stationary object) / (displaced + stationary) × 100%.

Intrahippocampal cannula and electrode implantation. The procedures were similar as described before (Gu et al., 2017, 2020). To study the effects of cholinergic receptor antagonists on object location task performance and hippocampal theta oscillations in wild-type mice, adult male mice (4–5 months old) underwent intrahippocampal cannula and electrode implantation using sterile surgical techniques 1 week before theta recording and behavioral testing. Mice were anaesthetized with ketamine (100 mg/kg) and xylazine (7 mg/kg), and the surgical pain was further alleviated with one dosage of buprenorphine (0.1 mg/kg, s.c.). Stereotaxic coordinates navigation was assisted by NeuroDrive from Neurostar. A guide cannula (for later behavioral test only) or a guide cannula–electrode combo [for simultaneous local field potential (LFP) recording] was implanted into the right dorsal hippocampal area CA1 aiming at hippocampal fissure for strong theta oscillations (−2.3 mm anteroposterior, 2.0 mm mediolateral, and 1.80 mm dorsoventral from bregma). The electrodes were grounded to the skull through a miniature screw. Implanted cannula–electrode combos were secured to the skull with dental acrylic. The cannula and electrode locations were verified by postmortem histological examination of the electrode tracks. To compare theta oscillations among different mouse strains with mAChRs and α7 nAChRs knocked out in excitatory or inhibitory neurons, male mice (4–5 months old) from these strains went through the same surgical procedures as described above for wild-type mice with the same stereotaxic coordinates, but only with intrahippocampal electrode implantation (without cannula).

Intrahippocampal infusion and hippocampal local field potential recording. Experiments were done at least 1 week after surgery to allow mice to recover. Besides the 3 d habituation to the open-field arena before object location task, the mice also received extra handling that mimicked intrahippocampal drug infusion and LFP recording the day before object location task, including the insertion of internal cannula without drug infusion and the wearing of the wireless headstage for 10 min while freely moving in home cages.

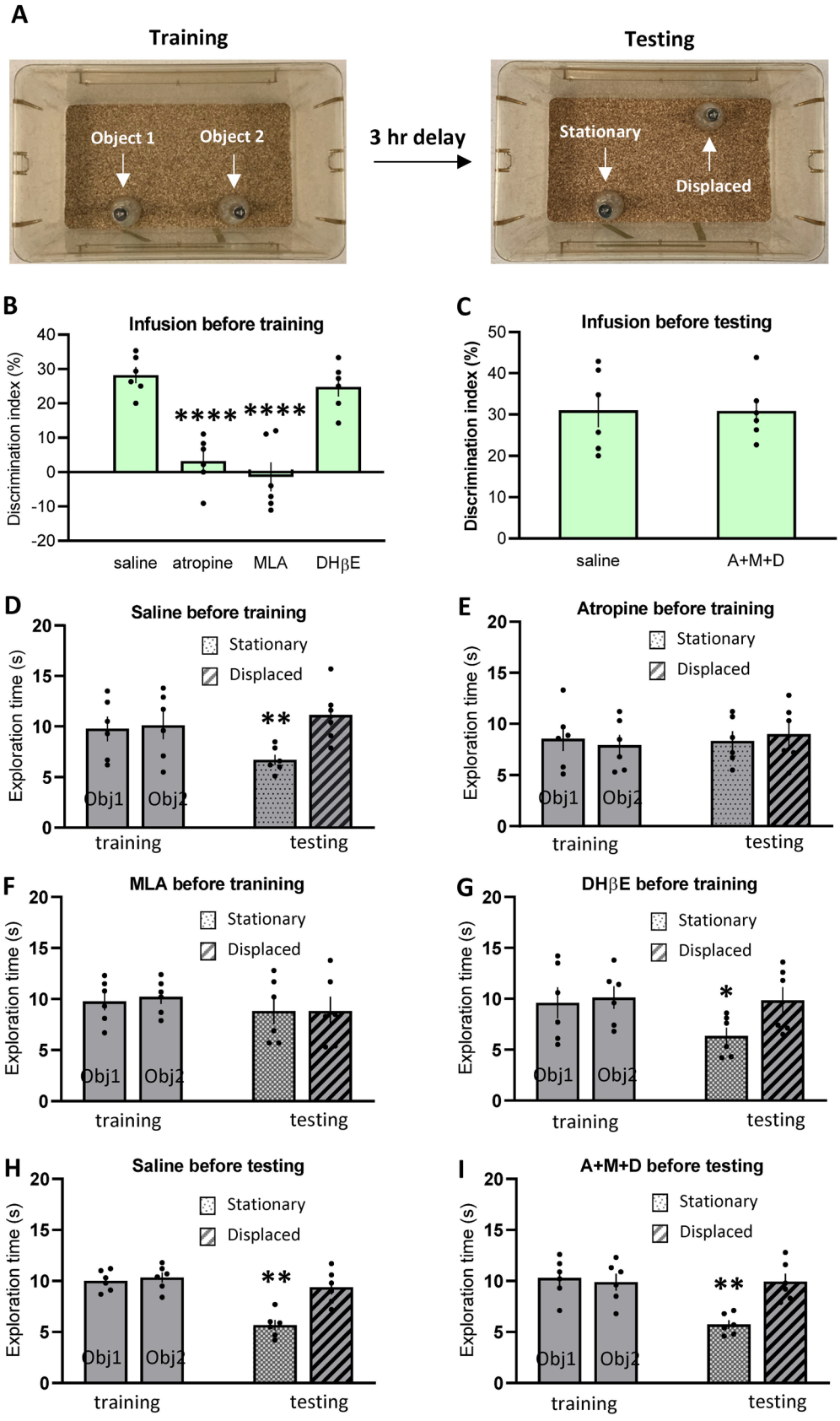

Figure 1. Continued.

On the day of object location task and electrophysiological recordings, while the mice were under isoflurane anesthesia (1.5% isoflurane in oxygen), the dummy cannula with the cap was removed and an internal cannula was inserted into the guide cannula to deliver 0.5 µl of either saline, the mAChR antagonist atropine (0.1 mM), the α7 nAChR antagonist methyllycaconitine (MLA; 0.2 µM), or the α4β2 nAChR antagonist dihydro-β-erythroidine (DHβE; 0.1 mM), at a rate of 0.1 µl/min, before the training session to study their effects on memory encoding. A cocktail including all these three antagonists was infused before the testing session to study the effects on memory retrieval. After the drug infusion, a wireless headstage was immediately connected to the probe connector that was previously implanted. Mice were then returned to their home cages and allowed to recover for 10 min from isoflurane anesthesia and then placed into the open-field arena for object location task and LFP recording. For hippocampal LFP recordings from different neuronal subpopulation mAChR and α7 nAChR knock-out mice, mice only went through a brief isoflurane anesthesia to plug the wireless headstage into the implanted connector, without drug infusion procedure. Recordings were also carried out 10 min later to allow recovery from isoflurane anesthesia. Neural activities were transmitted via the wireless headstage and were acquired with the Plexon acquisition system (Plexon). Continuous LFP data were filtered at 1,000 Hz and recorded continuously at a sampling rate of 40,000 Hz. Theta oscillations were analyzed with NeuroExplorer software (Nex Technologies).

Data analysis and statistics. Object exploration events were automatically detected with EthoVision software (Noldus), defined by the nose entering a circle that is 3 cm beyond the object. The discrimination index between two objects is calculated as the percentage of the difference of the time over the total time spent on the two objects. Values were presented as mean ± SEM. Receptor antagonist treatment groups were compared with saline control group with Student's *t* test between two groups or with ordinary one-way ANOVA in GraphPad Prism and followed by Dunnett's multiple-comparisons test when there are more than two groups.

Theta oscillations were analyzed with NeuroExplorer software as described before (Gu et al., 2017). Specifically, the spectrogram analysis was performed with 0.5 s sliding window shift, number of frequency value at 4,096, no preprocessing for each window before computing FFT, raw power spectrum density without normalization, single taper with Hann windowing function, and time corresponding to the center of sliding window. The subsecond theta analysis allows detailed examination of theta oscillations during different phases in one whole object exploration event. Individual exploration events were divided into three subsections, namely, the pre-, mid-, and postexploration periods, primarily based on the speed change. The mid-exploration period is characterized with a moving speed under 2 cm/s while the animal is sniffing an object. The pre-exploration period is defined as when the animal is approaching the object within 5 s before sniffing, usually with a moderate speed. The start of the pre-exploration period is defined by a speed increase to above 2 cm/s. The postexploration period is defined as when the animal is leaving the object in the 5 s after sniffing, usually with a high speed to retreat from the object. The end of the postexploration period is also defined by a drop of speed to under 2 cm/s. The movement speed is derived by the tracking data in EthoVision and is then used to define the boundaries of the three subsessions in object exploration events.

Hippocampal theta power varies greatly in different layers in area CA1 with the largest amplitude in hippocampal fissure. Due to the inevitable varying positions of the recording electrodes in different animals, it is therefore not ideal to directly compare theta powers across different animals. To compare theta powers across different animals in different treatments, we first normalized the peak theta power during the three periods of an object exploration event to the background activity (average of all activities between 4 and 12 Hz throughout the whole recording session). The theta powers in the three periods of the object exploration events were then compared between different groups with ordinary one-way ANOVA and followed by Dunnett's multiple-comparisons test in GraphPad Prism. Values were presented as mean ± SEM and graphs were drawn with GraphPad Prism.

Results

Hippocampal mAChRs and α7 nAChRs regulate memory encoding but not retrieval in object location task by engaging different neuronal populations

To understand the potential contribution of cholinergic regulation of theta oscillations to memory encoding, we first verified the effective impairment of the object location task performance by hippocampal infusion of cholinergic receptor antagonists, including atropine for mAChRs, MLA for α7 nAChRs, and DHβE for non-α7 (mainly α4β2 in the hippocampus) nAChRs. The mice were first exposed to two identical objects located symmetrically in an open field for 8 min (training trial) and then returned to the home cage. Three hours later, the mice were exposed to the same objects for 5 min (testing trial) but with one object moved to a new location (i.e., displaced) and the other one kept in the original position (stationary; Fig. 1*A*). The receptor antagonists were perfused to the hippocampus 15 min either before the training trial (to examine cholinergic contribution to memory encoding) or before testing trial (to examine cholinergic contribution to memory retrieval). The discrimination index was calculated by dividing the difference of the time spent on exploring the displaced object and the stationary object by the total time spent on exploring both objects. Hippocampal infusion of either the mAChR antagonist atropine or the α7 nAChR antagonist MLA before the training trial significantly impaired the location discrimination performance in the object location task, while DHβE had no significant effect on the performance with a similar discrimination index as in the saline-treated control group

←

Figure 1. Hippocampal mAChRs and α7 nAChRs regulate memory encoding but not memory retrieval in the object location task. ***A***, Object location task setting. In the training trial, mice were exposed to two identical objects that were symmetrically placed in an open arena for 8 min. In the testing trial 3 h later, one object was placed on the original place (stationary object) and the other one was placed on a new location (displaced object). ***B***, Hippocampal infusion of mAChR antagonist atropine ($p < 0.0001$) or α7 nAChR antagonist MLA ($p < 0.0001$), but not non-α7 nAChR antagonist DHβE ($p = 0.7409$) before training trial, significantly reduced discrimination index in testing trial in object location task. Statistics were performed with one-way ANOVA followed by Dunnett's multiple-comparisons test, $n = 6$ mice for each group. ***C***, Hippocampal infusion of a cocktail of cholinergic receptor antagonists before testing trial had no effect on discrimination index in object location task. $p = 0.9658$; two-tailed *t* test; $n = 6$ mice for each group. ***D***, Object exploration time showing that mice with hippocampal saline infusion before training trials spent significantly less time on stationary object than displaced object during testing trial ($p = 0.0004$) and the same object during the first 5 min of training trial ($p = 0.008$), one-way ANOVA followed by Dunnett's test, $n = 6$ mice. ***E***, ***F***, Object exploration time showing no significant difference of time spent on displaced and stationary objects during testing trial and the first 5 min of training trial in mice with hippocampal infusion of atropine (***E***; $p = 0.57$) or MLA (***F***; $p = 0.37$) before training trial, one-way ANOVA, $n = 6$ mice for each group. ***G***, Mice with DHβE infusion before training spent less time on stationary object than displaced object during testing trial ($p = 0.019$) and the same object during the first 5 min of training trial ($p = 0.028$), one-way ANOVA followed by Dunnett's test, $n = 6$ mice for each group. ***H***, Mice with saline infusion before testing spent less time on stationary object than displaced object during testing trial ($p = 0.0002$) and the same object during the first 5 min of training trial ($p = 0.002$), one-way ANOVA followed by Dunnett's test, $n = 6$ mice for each group. ***I***, Mice with a cocktail of cholinergic receptor antagonists infusion before testing spent less time on stationary object than displaced object during testing trial ($p = 0.003$) and the same object during the first 5 min of training trial ($p = 0.001$), one-way ANOVA followed by Dunnett's test, $n = 6$ mice for each group. A + M + D, atropine + MLA + DHβE. Data were presented as mean ± SEM.

(Fig. 1*B*). On the other hand, a cocktail containing all three antagonists given before the testing trial did not significantly change the discrimination performance, with a similar discrimination index as in the saline-treated control group (Fig. 1*C*). These results are consistent with previous studies showing that cholinergic receptors are involved in memory encoding, but not retrieval. Our results further suggest that in addition to mAChRs, α7 nAChRs can also play an important role in hippocampal memory encoding.

To further examine if there is any difference in the discrimination performance after atropine and MLA treatments, we then looked at the time spent on exploring the two individual objects after these receptor antagonist treatments. In pretraining saline-treated control group (Fig. 1*D*), the time spent on the stationary object (second time exposure) during the 5 min testing trial is not only significantly less than the time spent on the displaced object but also significantly less than the time spent on the same object during the first 5 min of training trial (first time exposure), indicating a correct recognition of the stationary object as a familiar object and thus with reduced interest to explore. In pretraining atropine-treated group (Fig. 1*E*) and MLA-treated group (Fig. 1*F*), there was no significant difference of the time spent on the stationary and displaced object during the testing trial and the time spent on the two objects during the first 5 min of the training trial, suggesting a lack of recognition of the objects that the mice had been previously exposed to during the training trial. In pretraining DHβE-treated group (Fig. 1*G*), the mice performed similarly to those in the saline-treated group, indicating that hippocampal DHβE treatment had no significant effects on the normal memory formation in the object location task. Mice with the pretesting infusion of a cocktail containing all three receptor antagonists (Fig. 1*I*) performed similarly to the mice treated with saline (Fig. 1*H*), with less time spent on the stationary object than the displaced object, and thus also had no significant effects on the normal memory formation. Hippocampal infusion of cholinergic antagonist before the training trial did not significantly change the general activities including the object exploration time, the total distance traveled, the percentage of active time (with a speed above 2 cm/s), and the velocity when moving around (Fig. 2). These results suggest that both mAChRs and α7 nAChRs are involved in memory encoding in the object location task.

We have previously observed that mAChRs and α7 nAChRs regulate hippocampal theta power and Y-maze performance by engaging different neuronal subpopulations (Gu et al., 2017, 2020). Specifically, mAChRs expressed in glutamatergic neurons (but not interneurons) and α7 nAChRs expressed in interneurons (but not glutamatergic neurons) regulate hippocampal theta power in freely moving mice. Here we further examined the neuronal subpopulations that recruited mAChRs and α7 nAChRs during the object location task. Consistent with our previous observations, knocking out mAChRs in glutamatergic neurons (but not in interneurons) significantly impaired the object location task performance (Fig. 3*A–D*). Mice with mAChRs knocked out in interneurons (Fig. 3*C*) performed similarly to the control mice (Fig. 3*B*), with significantly less time spent on the stationary

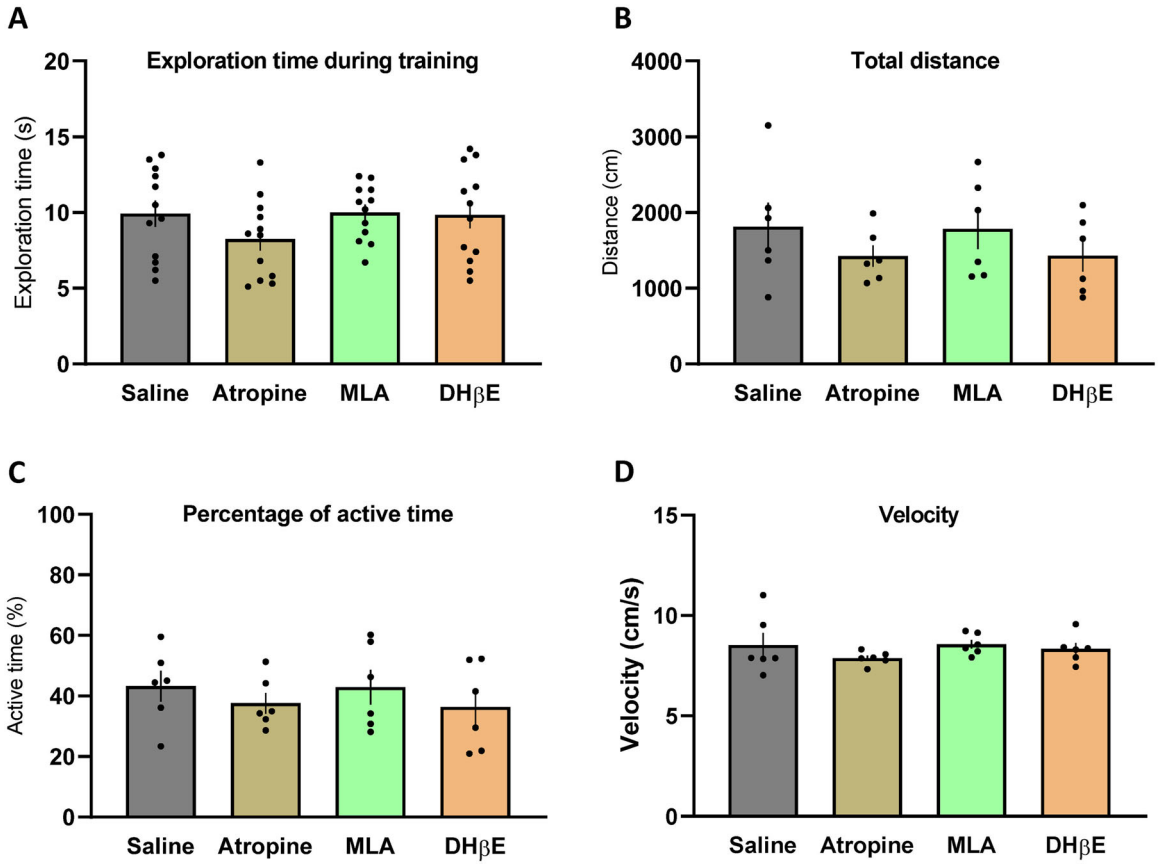

Figure 2. General activity after hippocampal infusion of cholinergic subtype antagonists. No significant difference was observed among groups in (***A***) object exploration time during training ($p = 0.33$), (***B***) total distance traveled during training ($p = 0.52$), (***C***) percentage of active time with a speed above 2 cm/s ($p = 0.69$), and (***D***) the average velocity during active time ($p = 0.50$). Statistics were done with One-way ANOVA, $n = 6$ mice for each group. Data were presented as mean ± SEM.

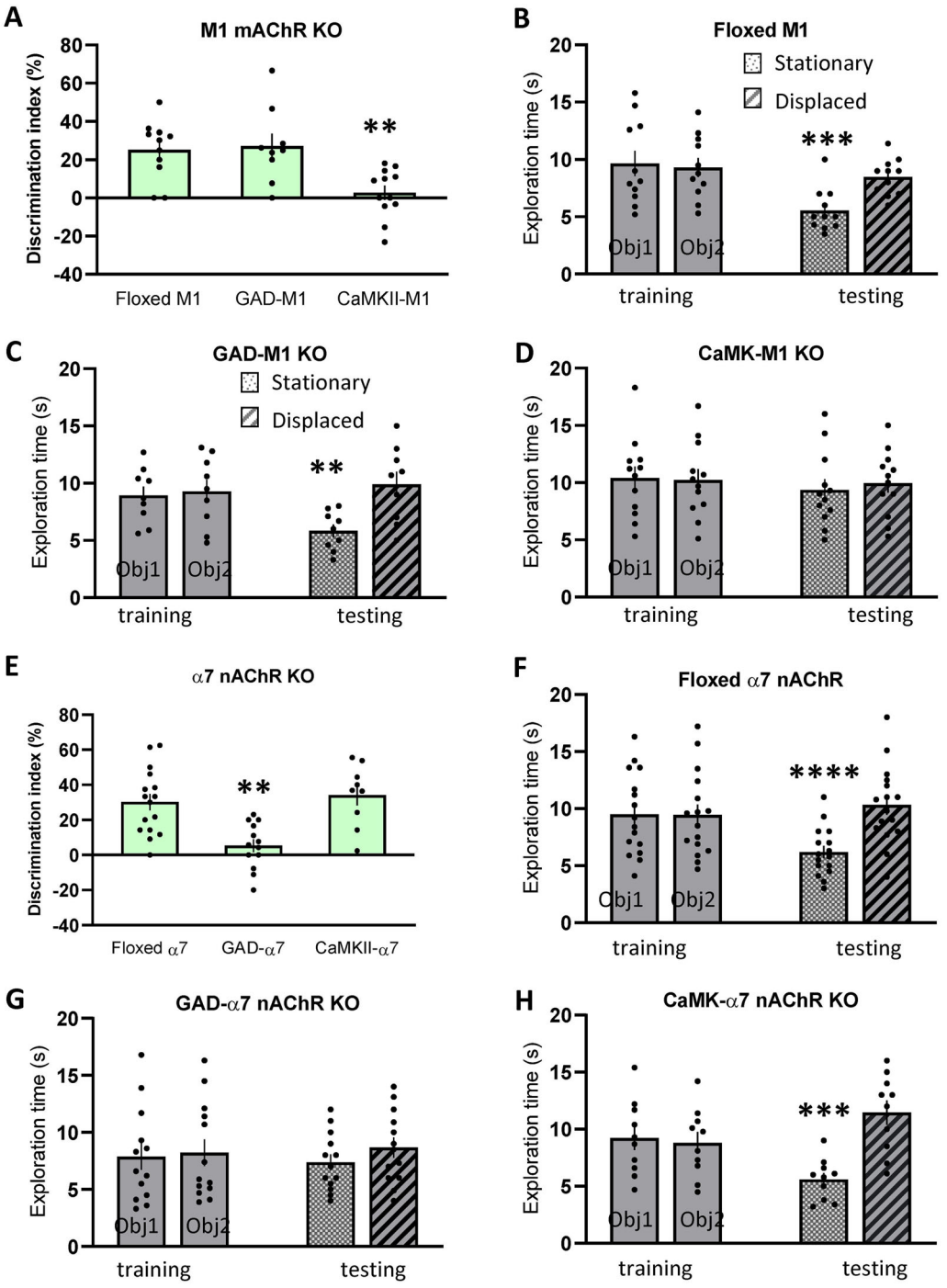

Figure 3. M1 mAChRs in glutamatergic neurons and α7 nAChRs in interneurons regulate memory encoding. ***A***, M1 mAChR knock-out in glutamatergic neurons (CaMK-M1; $p = 0.0038$; $n = 12$) but not interneurons (GAD-M1; $p = 0.9479$; $n = 9$) significantly reduced discrimination index in object location task as compared with floxed M1 control mice ($n = 11$), one-way ANOVA followed by Dunnett's multiple-comparisons test. ***B***, Object exploration time showing that floxed M1 control mice spent significantly less time on stationary object than displaced object during testing trial ($p = 0.0003$) and the same object during the first 5 min of training trial ($p = 0.002$), one-way ANOVA followed by Dunnett's test, $n = 11$ mice. ***C***, GAD-M1 knock-out mice spent significantly less time on stationary object than displaced object during testing trial ($p = 0.001$) and the same object during the first 5 min of training trial ($p = 0.015$), one-way ANOVA followed by Dunnett's test, $n = 9$ mice. ***D***, No significant difference of time spent on displaced and stationary objects during testing trial and the objects during the first 5 min of training trial in CaMK-M1 knock-out mice ($p = 0.71$), one-way ANOVA, $n = 12$ mice. ***E***, α7 nAChR knock-out in interneurons (GAD-α7; $p = 0.0018$; $n = 13$) but not glutamatergic neurons (CaMKII-α7; $p = 0.9941$; $n = 10$) significantly reduced discrimination index in object location task as compare with floxed α7 nAChR control mice ($n = 16$), one-way ANOVA followed by Dunnett's multiple-comparisons test. ***F***, floxed α7 nAChR control mice spent significantly less time on stationary object than displaced object during testing trial ($p < 0.0001$) and the same object during the first 5 min of training trial ($p = 0.015$), one-way ANOVA followed by Dunnett's test, $n = 16$ mice. ***G***, No significant difference of time spent on displaced and stationary objects during testing trial and the objects during the first 5 min of training trial in GAD-α7 nAChR knock-out mice ($p = 0.53$), one-way ANOVA, $n = 13$ mice. ***H***, CaMK-α7 nAChR knock-out mice spent significantly less time on stationary object than displaced object during testing trial ($p = 0.0004$) and the same object during the first 5 min of training trial ($p = 0.022$), one-way ANOVA followed by Dunnett's test, $n = 10$ mice. Data were presented as mean ± SEM.

versus both the displaced object in the 5 min testing trial and the same object in the first 5 min training trial, indicating a correct recognition of the stationary object and normal memory performance). Mice with mAChRs knocked out in glutamatergic neurons (Fig. 3*D*) spent similar times on stationary and displaced objects, indicating a loss of normal discrimination (Fig. 3*D*). On

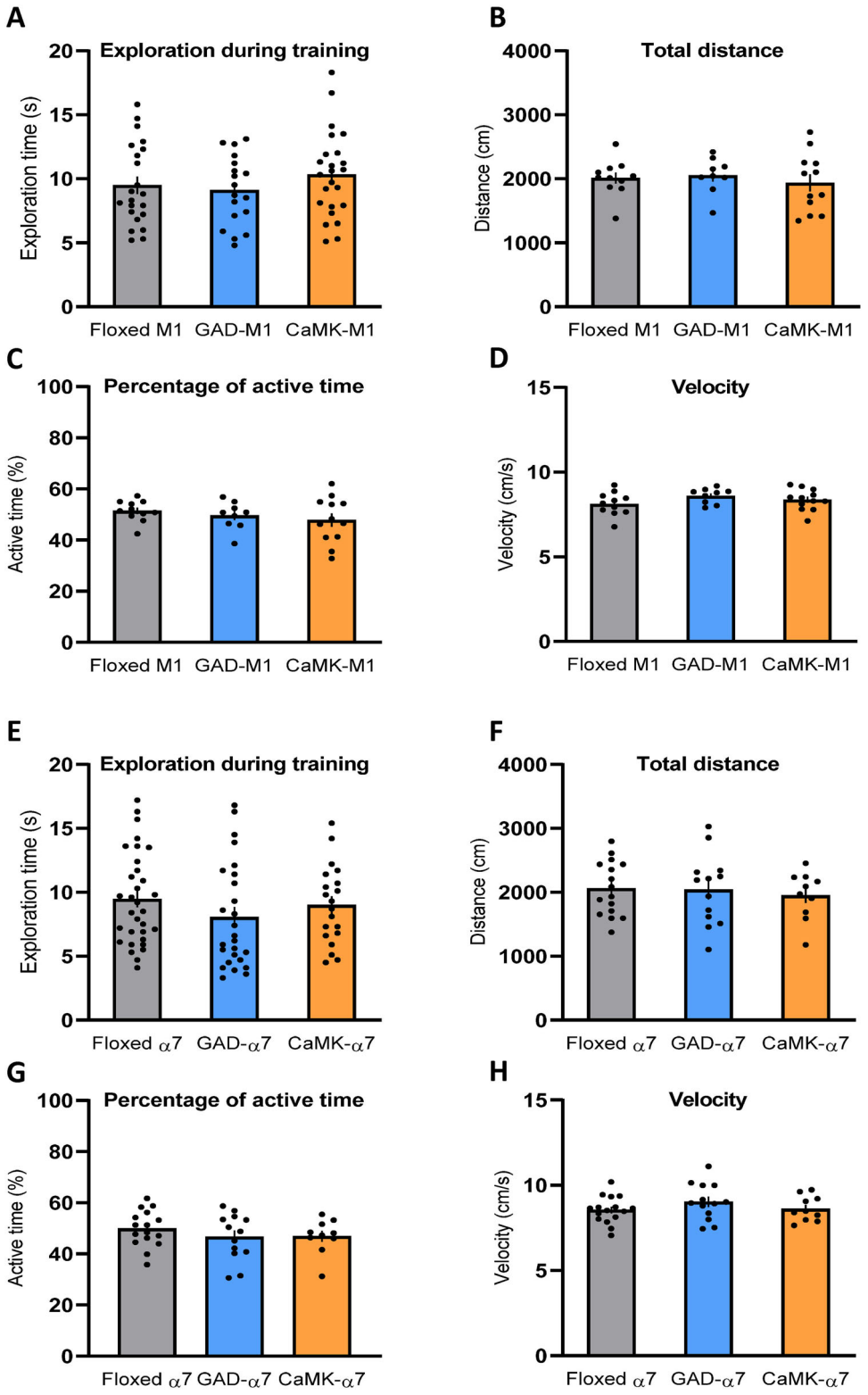

Figure 4. General activity in M1 mAChR and α7 nAChR knock-out mice. No significant difference was observed among M1 mAChR knock-out mice in (***A***) object exploration time during training ($p = 0.43$), (***B***) total distance traveled during training ($p = 0.75$), (***C***) percentage of active time with a speed above 2 cm/s ($p = 0.45$), and (***D***) the average velocity during active time ($p = 0.22$), or among α7 nAChR knock-out mice in (***E***) object exploration time during training ($p = 0.33$), (***F***) total distance traveled during training ($p = 0.83$), (***G***) percentage of active time with a speed above 2 cm/s ($p = 0.47$), and (***H***) the average velocity during active time ($p = 0.33$). Statistics were done with one-way ANOVA, $n = 11$ for floxed M1, 9 for GAD-M1, 12 for CaMK-M1, 16 for floxed α7, 13 for GAD- α7, and 10 for CaMK- α7. Data were presented as mean ± SEM.

the other hand, knocking out α7 nAChRs in interneurons but not in glutamatergic neurons also significantly impaired object location task performance (Fig. 3*E–H*). Mice with α7 nAChRs knocked out in glutamatergic neurons (Fig. 3*H*) performed similarly to the control mice (Fig. 3*E*), with more time spent on displaced versus the stationary object, indicating unimpaired memory function. On the other hand, mice with α7 nAChRs knocked out in interneurons spent similar times on stationary and displaced objects (Fig. 3*G*). Knocking out either M1 mAChRs or α7 nAChRs in neuronal subpopulations did not significantly change the general activities including the object exploration time, the total distance traveled, the percentage of active time (with a speed above 2 cm/s), and the velocity when moving around (Fig. 4). These results strongly suggest that mAChRs and α7 nAChRs may regulate memory formation in the object location task by engaging glutamatergic neurons and interneurons, respectively.

Strong correlation between theta oscillations and object exploration

Cholinergic regulation of hippocampal theta oscillations has been strongly suggested as a potential mechanism underlying hippocampus-dependent memory encoding. However, direct experimental evidence is largely lacking. To test such potential mechanisms underlying the object location task, we first examined the dynamics of theta oscillations in individual object exploration events. Individual exploration events were divided into three sections, including pre-, mid-, and postexploration periods (Fig. 5 and Extended Data Fig. 5-1). The mid-exploration period is defined as when the animal is exploring (sniffing) the object

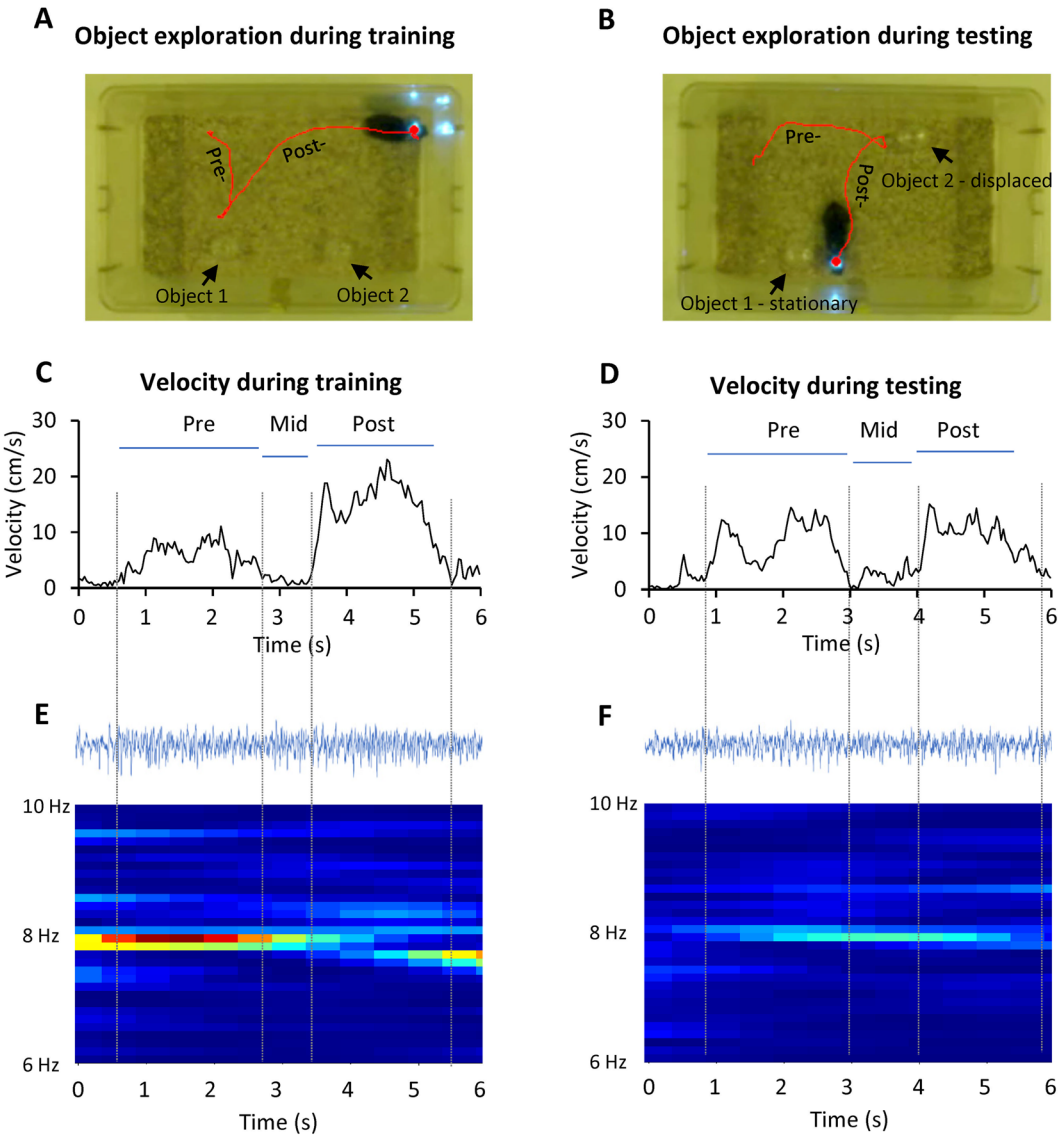

Figure 5. Strong correlation between theta oscillations and object exploration event during training trial. ***A***, ***B***, Track visualization (red lines) showing the travel path of a typical object exploration event in training trial (***A***) and testing trial (***B***), including pre-exploration and postexploration periods. ***C***, Velocity plot showing the speed distribution during the object exploration event shown in ***A***, with a moderate speed approaching the object, virtually still during exploration (sniffing), and a higher speed leaving the object. ***D***, Velocity plot showing the movement speed distribution during the object exploration event shown in ***B***. ***E***, Raw LFP recording and spectrogram analysis showing theta intensity during the object exploration event in ***A***. They are time-aligned with ***C***, showing strongest theta intensity during pre-exploration period. ***F***, Raw LFP recording and spectrum analysis showing theta intensity during the object exploration event in ***B***. They are time-aligned with ***D***, showing similar theta intensity during pre-, mid-, and postexploration periods. Data were presented as mean ± SEM. Spectrogram analysis showing longer time course can be found in Extended Data Figure 5-1.

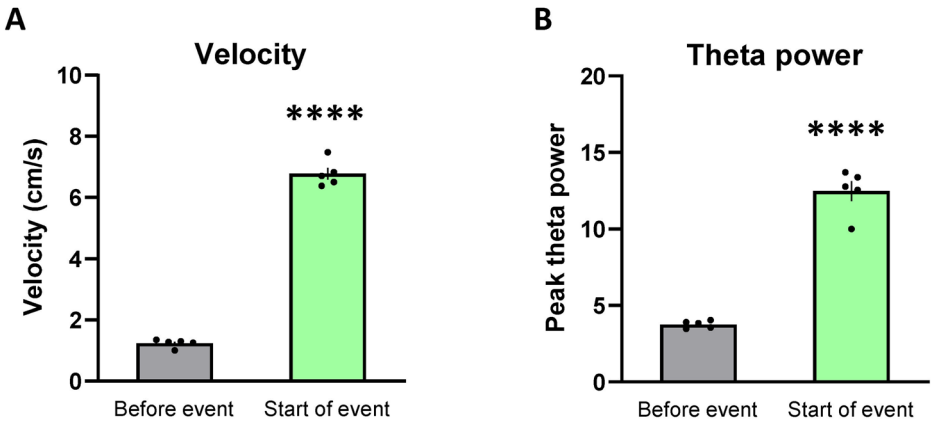

Figure 6. Velocity and theta power change at the borderline of the start of the pre-exploration period. Both velocity (***A***) and theta power (***B***) during the 1 s period immediately after the start of an event were significantly higher than those during the 1 s baseline period immediately before the start of an event. $p < 0.0001$; $n = 5$ mice; Student's t test. Data were presented as mean ± SEM.

with a moving speed under 2 cm/s. The pre-exploration period is defined as when the animal is approaching the object in the few seconds before sniffing, usually with a moderate speed. The start of the pre-exploration period is defined by a speed increase to above 2 cm/s. As shown in Figure 6, the average speed increased from 1.2 to 6.8 cm/s from the 1 s baseline immediately before the start of an event to the 1 s immediately after the start. The post-exploration period is defined as when the animal is leaving the object in the few seconds after sniffing, usually with a high speed to retreat from the object. The end of the postexploration period is defined by a drop of speed to under 2 cm/s.

Spectrogram analysis shows that theta oscillations were the strongest during the pre-exploration period (Fig. 5*E*), usually at the beginning of the period. This theta is also stronger than both the baseline theta preceding an object exploration event (Fig. 6) and the theta that occurred during other nonobject exploration activities (Fig. 7). This elevated theta intensity is not correlated with speed as the speed during the pre-exploration period is slower than that during the postexploration period. In addition, this pre-exploration high theta power only occurs during the training trial but not the testing trial (for either displaced or stationary object; Fig. 8*A*), suggesting a role in novelty detection/learning. There is also no difference in theta intensity between the two objects during the training trial, or between the stationary and the displaced objects during testing trial, suggesting that the theta oscillations may not be directly responsible for specific spatial information encoding of the individual objects. There is no significant theta frequency (Fig. 8*B*) or speed change between training and testing trials of either stationary or displaced objects during exploration (Fig. 8*C*). The fact that the elevated pre-exploration theta only occurred in the training (but not testing) trials (regardless of the spatial positioning) suggests that it may be related to novelty detection or novelty learning and involved in memory encoding in training trials, but not memory retrieval in testing trials. This also coincides well with the known cholinergic role in novelty learning and memory encoding, but not memory retrieval. To further test this idea, we compared the theta power during the early (novel) and late (less novel) object exploration events during training trial (Fig. 9). Indeed, the pre-exploration theta is significantly stronger in the first events (average of the first three events) than that in the last events (average of the last three events), supporting a role for pre-exploration theta in novelty detection or novelty learning. Theta frequency and moving velocity were not significantly changed between early and late events.

Cholinergic regulation of theta power in the object location task

We then examined the effect of the cholinergic receptor antagonists that impaired memory encoding in the object location task on the elevated theta power during training trials. Both the mAChR antagonist atropine and the α7 nAChR antagonist MLA, which impaired memory encoding in the object location task, also specifically reduced the peak theta power during the pre-exploration period; neither antagonist had any effect on the theta power during the mid- or postexploration periods in training trials as compared with the saline control treatment (Fig. 10*A*). The reduced peak theta power after both atropine and MLA treatment during the pre-exploration period was similar to the level in both the mid- and post- exploration periods, suggesting that there is a cholinergic-independent theta component throughout the whole exploration event, and the additive cholinergic-sensitive theta only occurred during the pre-exploration period. The non-α7 nAChR antagonist DHβE, which had no effect on the object location task performance, also had no effect on the occurrence of elevated theta during the pre-exploration period in the training trial (Fig. 10*A*). None of these three antagonists had any effect on theta frequency (Fig. 10*B*) or movement speed (Fig. 10*C*) during any of the three subsections of the exploration events, as compared with saline control treatment.

We also examined the behavioral performance in mice with wireless recording headstages to see how well the mice under recording can perform in the object location task. As shown in Figure 11, mice with headstages can still discriminate displaced objects against stationary objects. However, this discrimination may be less robust than that in mice without headstages. When comparing the time spent on the stationary and displaced objects in testing trials together with the time spent during the first 5 min in training trials, the difference was not significant ($p = 0.06$; one-way ANOVA; $n = 5$ mice). The exploration time and distance traveled are somewhat but not significantly reduced in mice with headstages. As expected, mice with headstages did have a significantly lower moving velocity. These results suggest that mice with recording headstages can still discriminate displaced

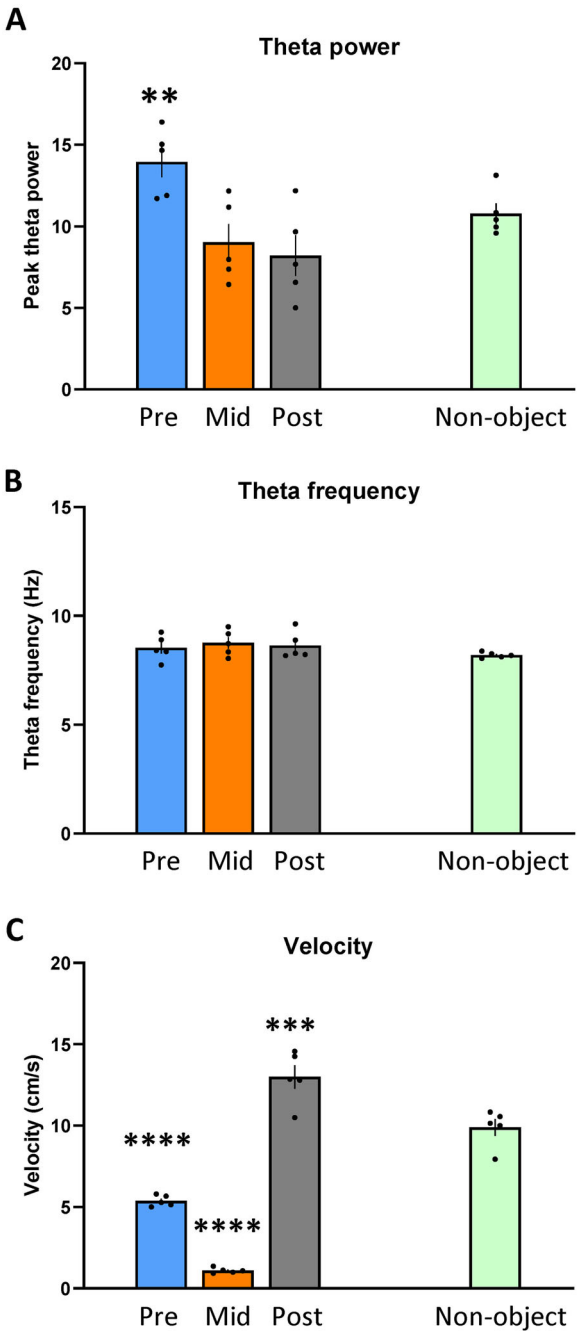

Figure 7. Theta comparison between object exploration events and general activities. ***A***, Theta power is significantly stronger during per-exploration than that during nonobject exploration activities ($p = 0.007$), while no significant difference between nonobject exploration with either mid-object ($p = 0.11$) and postobject ($p = 0.15$) exploration periods. ***B***, No significant difference of theta frequency between object and nonobject activities ($p = 0.39$). ***C***, The velocity during pre- and mid-object exploration is significantly lower than that during nonobject exploration activities ($p < 0.0001$), while higher during postexploration period than that during nonobject exploration activities ($p < 0.0005$). Statistics were done with one-way ANOVA followed by Dunnett's multiple-comparisons test. $n = 5$ mice for each group. Data were presented as mean ± SEM.

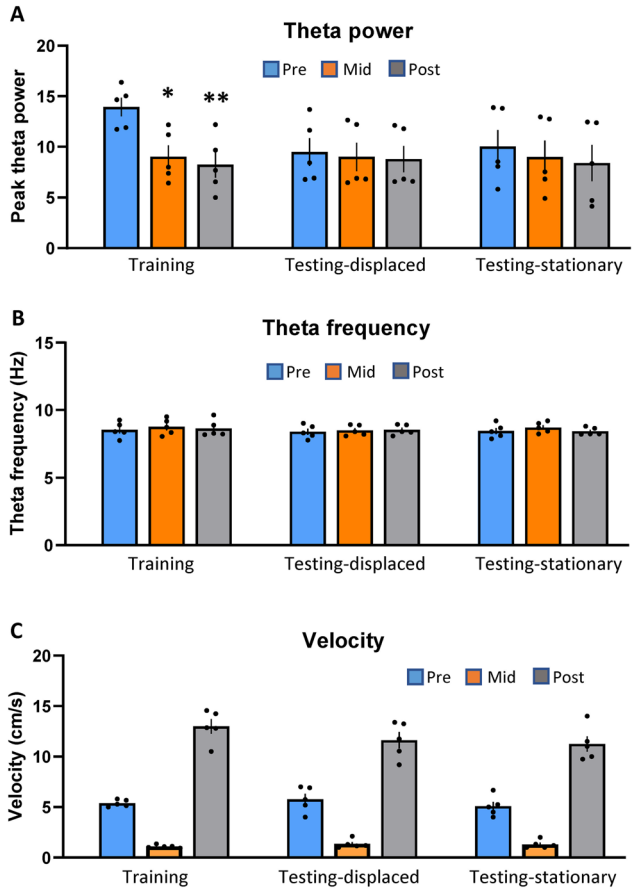

Figure 8. Elevated theta power during pre-exploration period in training trials but not testing trials. ***A***, Bar graph showing that the peak theta power is the strongest during pre-exploration period in training trials as compared with mid- ($p = 0.0156$) and postexploration ($p = 0.0061$) periods, while the peak theta power was similar among pre-, mid-, and post-exploration periods in testing trials for either displaced ($p = 0.9318$) or stationary object exploration ($p = 0.7933$), one-way ANOVA followed by Dunnett's multiple-comparisons test, $n = 5$ mice for each group. ***B***, Theta frequency is similar among pre-, mid-, and post-exploration periods in both training trials ($p = 0.8399$) and testing trials for either displaced object ($p = 0.8751$) or stationary object ($p = 0.4982$), one-way ANOVA, $n = 5$ mice for each group. ***C***, Movement speed is moderate during pre-exploration period, very low during mid-exploration period, and high during postexploration period. Speed is similar among training trials and testing trials for either displaced object or stationary object within pre- ($p = 0.5331$), mid ($p = 0.5111$), or postexploration ($p = 0.2711$) periods, one-way ANOVA, $n = 5$ mice for each group. Data were presented as mean ± SEM.

objects against stationary objects, but the discrimination may be less robust than that in the mice without headstages.

We then further evaluated the effects of mAChR and α7 nAChR knock-out in neuronal subpopulations on the elevated theta power during the pre-exploration periods in the training trials. Consistent with their effects on behavioral performance, knocking out of the mAChRs in glutamatergic neurons, which significantly impaired the object location task performance, also specifically reduced the elevated theta power during the pre-exploration periods in training trials; there was no effect on theta power during mid- and postexploration periods as compared with those in control animals (Fig. 12). Furthermore, knocking out of the mAChRs in interneurons, which had no significant effects on the object location task performance, also had no significant effects on theta power during any of the three subsections of the exploration events in the training trials (Fig. 12), as compared with control mice. On the other hand, knocking out of the α7 nAChRs in interneurons, which also significantly impaired the object location task performance, significantly reduced the elevated theta power during the pre-exploration periods in training trials and had no effect on theta power during other times in the object exploration events (Fig. 13), as compared with control mice. Knocking out of the α7 nAChRs in glutamatergic neurons,

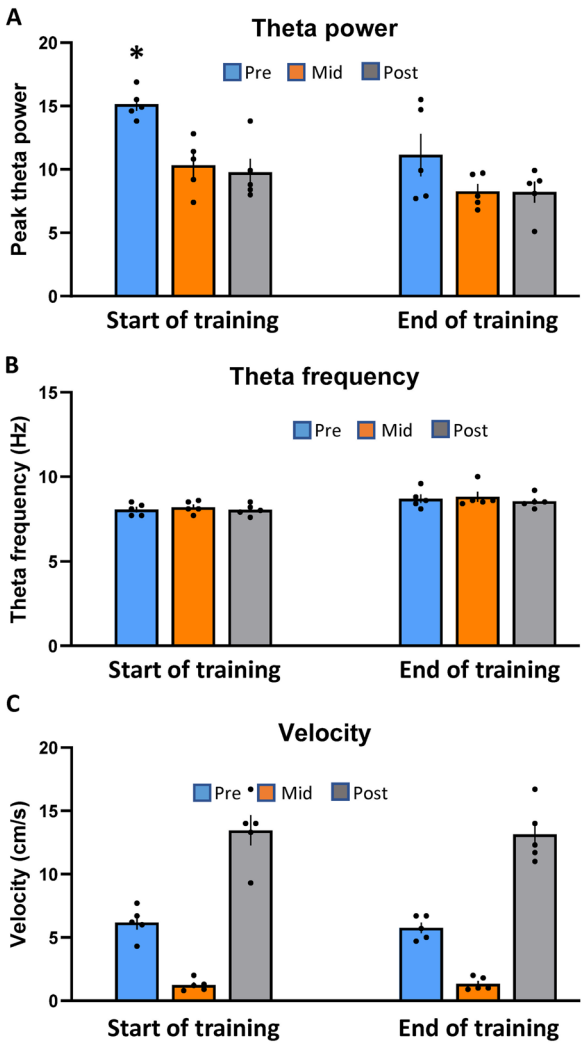

Figure 9. Stronger theta expression during pre-exploration period in early events than that in late events in training trials. ***A***, Peak theta power during pre-exploration period in early events (average of the first 3 events) is higher than that in late events (average of last 3 events; $p = 0.03$), while not significantly changed during mid- ($p = 0.10$) and postexploration ($p = 0.31$) periods between early and late events. ***B***, No significant changes of theta frequency between early and late events during pre- ($p = 0.07$), mid- ($p = 0.10$), or postexploration ($p = 0.07$) periods. ***C***, No significant changes of theta frequency between early and late events during pre- ($p = 0.56$), mid- ($p = 0.76$), or postexploration ($p = 0.84$) periods. Data were presented as mean ± SEM.

which had no significant effect on the object location task performance, also had no significant effects on theta power during the exploration periods in training trials (Fig. 13). None of these cholinergic receptor knock-outs had any significant effect on theta frequency (Figs. 12*B*, 13*B*) or movement speed (Figs. 12*C*, 13*C*) during any periods of the exploration events, as compared with control mice. Taken together, these results strongly suggest that mAChRs and α7 nAChRs both regulated the elevated theta power during the pre-exploration periods in training trials by engaging glutamatergic neurons and interneurons, respectively.

Discussion

In this study, we made several findings regarding cholinergic mechanism in hippocampal memory formation in the object location task. Firstly, other than the mAChRs, the α7 nAChRs also play an important role in hippocampal memory encoding and may contribute to different aspects of memory encoding from mAChRs by engaging different neuronal subpopulations. Secondly, elevated theta strength was observed in the pre-exploration period during early events in training trials. Thirdly, the elevated theta in the pre-exploration periods was reduced by mAChR and α7 nAChR antagonists. Taken together, these observations reveal a potential mechanism for cholinergic transmission and theta oscillations to contribute to the hippocampal memory encoding process.

Cholinergic transmission has long been known to play a critical role in hippocampal memory encoding (Hasselmo, 2006; Micheau and Marighetto, 2011; Easton et al., 2012; Newman et al., 2017; Zhang et al., 2021). The role of mAChRs has been well established, but the role of nAChRs is less well known (Ghoneim and Mewaldt, 1975; Broks et al., 1988; Aigner et al., 1991; Grottick and Higgins, 2000; Ge and Dani, 2005; Levin et al., 2006; Bitner et al., 2007; Dani and Bertrand, 2007; Hasselmo and Sarter, 2011). In this study, we observed that α7 nAChRs also participated in the hippocampal memory encoding process in the object location task. Like mAChRs, α7 nAChRs also had no significant effect on memory retrieval. However, α7 nAChRs primarily engaged interneurons, while mAChRs primarily engaged glutamatergic neurons. This is consistent with previous studies showing that hippocampal α7 nAChRs are highly expressed in interneurons as compared with the low expression level in pyramidal neurons (Stevens et al., 1996; Jones and Yakel, 1997; Frazier et al., 1998; McQuiston and Madison, 1999; Buhler and Dunwiddie, 2001; Adams et al., 2006; Son and Winzer-Serhan, 2008). Activation of mAChRs can regulate neuron excitability through G-protein-coupled cellular signaling, while α7 nAChRs may contribute little directly to neuronal excitability but instead have high calcium permeability and have been shown to mediate multiple forms of hippocampal synaptic plasticity (Castro and Albuquerque, 1995; Kenney and Gould, 2008; Gu and Yakel, 2011; Gu et al., 2012; Yakel, 2014; Guerreiro et al., 2022; Letsinger et al., 2022). In addition, mAChRs have high affinity to acetylcholine and are activated with relatively low levels of acetylcholine release (Kellar et al., 1985) and thus in a position to constantly regulate the excitability of the neuronal populations. On the other hand, α7 nAChRs have low affinity to acetylcholine and thus are only fully activated with higher levels of acetylcholine release (Alkondon and Albuquerque, 1993; Castro and Albuquerque, 1995) and more likely to play a role in encoding information in the presence of higher acetylcholine release. In summary, the current study reveals that cholinergic transmission can regulate hippocampal memory encoding processes through both mAChRs and α7 nAChRs that engaged glutamatergic neurons and interneurons, respectively.

Hippocampal theta oscillations have long been considered as a plausible mechanism underlying cholinergic transmission-mediated hippocampal memory encoding due to the close relationship between them (Hasselmo et al., 2002; Hasselmo, 2005; Douchamps et al., 2013; Gu and Yakel, 2022). Theta oscillations have been particularly associated with hippocampal spatial memory encoding (Buzsaki, 2010). Therefore, we choose the object location task that engages hippocampal spatial memory to investigate the contributions of cholinergic transmission and theta oscillations to the memory encoding process (Haam et al., 2018). However, cholinergic transmission has been traditionally more closely associated with type II theta, and the role of cholinergic transmission in regulating type I theta during active exploration is much less clear (Kramis et al., 1975; Montoya and

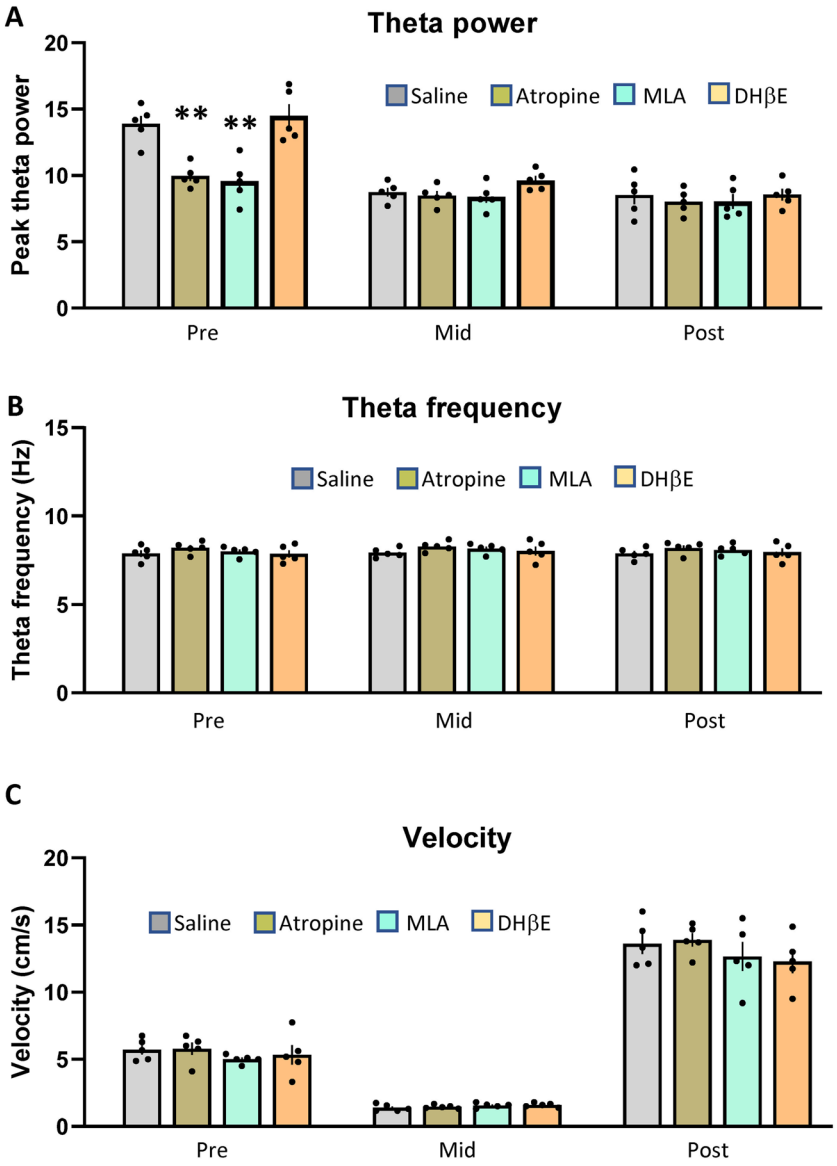

Figure 10. Elevated theta power during pre-exploration period in training trials is mediated by hippocampal mAChRs and α7 nAChRs but not by non-α7 nAChRs. ***A***, Elevated theta power during pre-exploration period in training trials is largely blocked by local hippocampal infusion of atropine ($p = 0.0025$) or MLA ($p = 0.0011$) but not by DHβE ($p = 0.8606$). Atropine or MLA did not significantly change theta power from saline infusion during mid- ($p = 0.9333$ for atropine; $p = 0.8483$ for MLA) or postexploration periods ($p = 0.8505$ for atropine; $p = 0.8698$ for MLA), one-way ANOVA followed by Dunnett's test, $n = 5$ mice for each group. ***B***, Theta frequency is not significantly changed by AChR antagonists during pre- ($p = 0.4841$), mid- ($p = 0.4678$), and postexploration ($p = 0.5839$) periods in training trials, one-way ANOVA, $n = 5$ mice for each group. ***C***, Movement speeds are also not significantly changed by AchR antagonists during pre- ($p = 0.6109$), mid- ($p = 0.2784$), and postexploration ($p = 0.4903$) periods in training trials, one-way ANOVA, $n = 5$ mice for each group. Data were presented as mean ± SEM.

Sainsbury, 1985; Sainsbury et al., 1987; Buzsaki, 2002; Gu and Yakel, 2022). Nevertheless, septal cholinergic neuronal activity and hippocampal ACh levels do closely correlate with not only type II theta, but also type I theta states (Marrosu et al., 1995; Giovannini et al., 2001; Bianchi et al., 2003; Zhang et al., 2010, 2021; Ma et al., 2020), supporting a potential role for cholinergic transmission in regulating type I theta and memory encoding. In this study, we revealed a strong association of a transient increase of theta power and novelty learning in the object location task by examining the detailed theta profile during individual object exploration events with subsecond accuracy. Theta oscillations always occurred during individual object exploration events. However, the theta strength is not evenly distributed during an object exploration event that typically can be divided into three subsections including approaching, sniffing, and quickly retreating from an object. We observed a particularly strong theta power at the beginning of an object exploration event during the training trial. Moreover, the pre-exploration theta is stronger in the early (novel) events than that in the late (less novel) events; this strongly suggests that this transient theta increase may be closely related to novelty encoding or learning. Previous study has reported an ~23–30 Hz oscillation in the hippocampus in mice exploring novel, but not familiar, environments (Berke et al., 2008). It is not clear how theta can enhance memory encoding at the cellular level, but the oscillating theta activity may provide a mechanism to selectively modulate

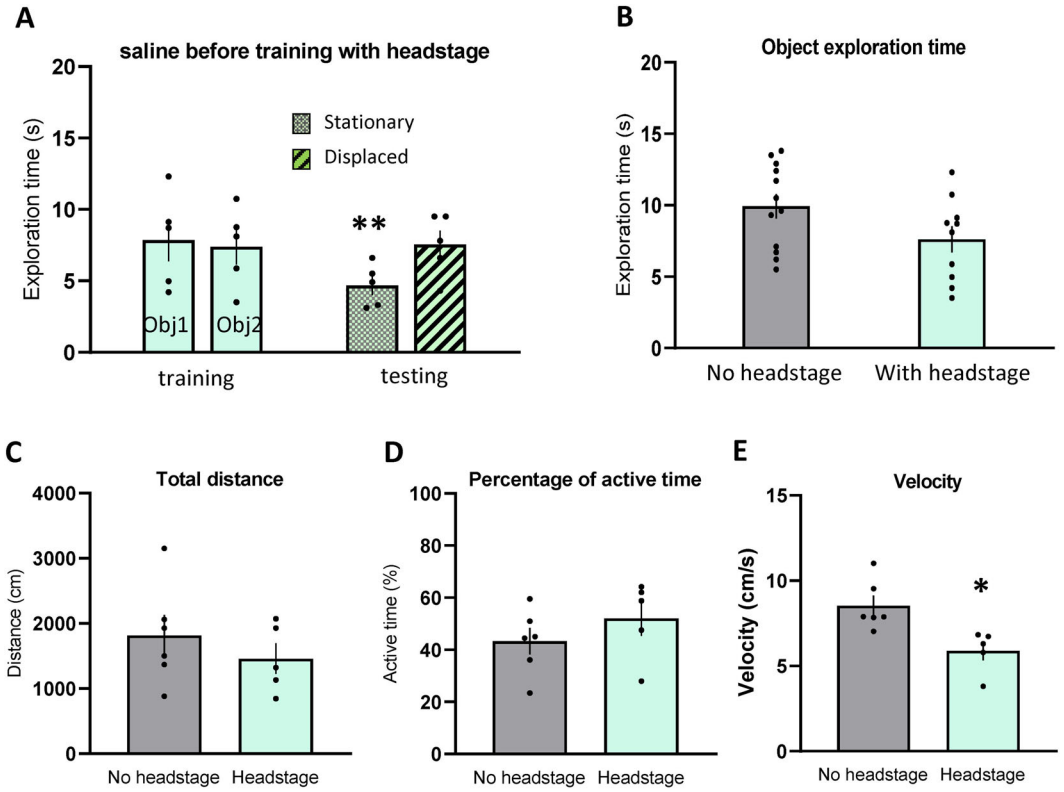

Figure 11. Mice with recording headstage were still able to discriminate between stationary and displaced objects in object location task. ***A***, Mice with recording headstages spent significantly more time on displaced object than stationary object ($p = 0.0049$) during testing trials. No significant difference between mice without headstage and mice with headstage in object exploration time during the first 5 min of training trial (***B***; $p = 0.08$), the total distance traveled during the training trial (***C***; $p = 0.41$), or the percentage of active time (with speed above 2 cm/s) during training trial (***D***; $p = 0.31$). ***E***, The mice with recording headstage had a lower velocity during active time than mice without headstage ($p = 0.01$). Statistics were done with Student's *t* test, $n = 6$ mice without headstage, 5 mice with headstage. Data were presented as mean ± SEM.

the effectiveness of incoming signals depending on the timing of the inputs relative to the theta cycle, a mechanism that can effectively increase the signal/noise ratio. Oscillating theta activity may also facilitate the formation of spike-timing–dependent plasticity at certain synaptic sites depending on the timing of the inputs to the theta cycle. The theta strength during the mid- and postexploration periods was about the same in training trials and testing trials and between the stationary and displaced objects in testing trials, suggesting that theta oscillations in object location task may not be closely associated with the specific spatial information of the individual objects and the subsequent discrimination between the two object locations in the testing trials.

Our study further revealed that there is a cholinergic-sensitive theta component in this strong theta during the pre-exploration period in training trials. The cholinergic treatments that effectively impaired behavioral performance also effectively blocked the strong theta during the pre-exploration periods in the training trials. Cholinergic treatments did not eliminate the strong theta but instead reduced it to the same level as that in the testing trial, which is also similar to the levels during mid- and postexploration periods in either training trials or testing trials. These results clearly show that there is a transient cholinergic-sensitive theta component that occurred concurrently with cholinergic-insensitive theta components during the pre-exploration periods in the training trials. There is no significant difference in speed in individual exploration events between training trials and testing trials, and cholinergic treatments did not have any effect. The transient cholinergic-sensitive theta thus may not be associated with the individual object exploration events per se but more likely is associated with cholinergic-dependent novelty detection and learning that occurred primarily during the training trials when the mice were exposed to the objects for the first time. Previous study has shown that rats with impaired septal cholinergic inputs to the hippocampus had deficits in forming new spatial presentation in novel environments (Ikonen et al., 2002), also indicating a role for cholinergic transmission in novelty learning. Septal cholinergic inputs may regulate hippocampal spatial presentation through direct targeting hippocampal interneurons or indirectly through septal noncholinergic neurons (Mamad et al., 2015). However, cholinergic-sensitive theta has been closely associated with type II theta that can also occur under stress. It is also true that exposure to a novel environment may be stressful to mice. It is therefore possible that the cholinergic-sensitive theta observed here is type II theta in response to the stress of novel environment exposure. Nevertheless, type II theta usually has lower theta frequency, but the theta frequency of the cholinergic-sensitive component here is the same as that of cholinergic-resistant type I theta. Furthermore, this cholinergic-sensitive theta component is very transient and only specifically occurs at the beginning of a self-initiated voluntary exploration event. It is therefore more likely associated with initiating/planning a novel exploration event instead of being associated with the stress of novel environment exposure that should be presented constantly. The co-occurrence

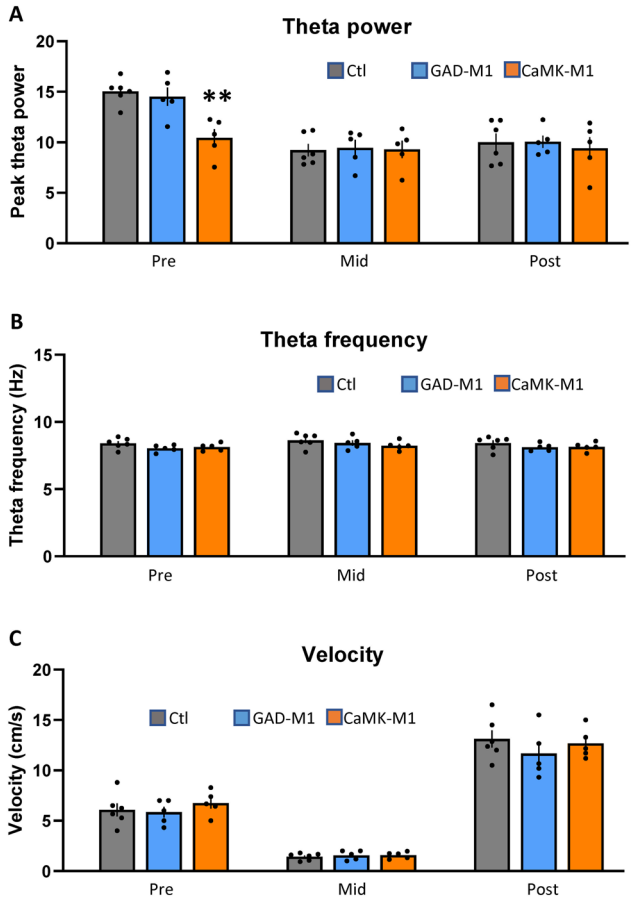

Figure 12. Elevated theta power during pre-exploration period in training trials is primarily mediated by M1 mAChRs expressed in glutamatergic neurons but not in interneurons. ***A***, Elevated theta power during pre-exploration period in training trials is significantly reduced in mice with M1 mAChR knock-out in glutamatergic neurons (CaMK-M1; $p = 0.0016$; $n = 5$ mice) but not in interneurons (GAD-M1; $p = 0.8460$; $n = 5$ mice) as compared with floxed M1 mAChR control mice ($n = 6$ mice), one-way ANOVA followed by Dunnett's multiple-comparisons test. ***B***, Peak theta frequency is not significantly changed in mice with M1 mAChR knock-out in either glutamatergic neurons ($n = 5$ mice) or interneurons ($n = 5$ mice) as compared with floxed control mice ($n = 6$ mice) during pre- ($p = 0.1671$), mid- ($p = 0.3646$), and postexploration ($p = 0.3870$) periods in training trials, one-way ANOVA. ***C***, Movement speeds are not significantly changed during pre- ($p = 0.5741$), mid- ($p = 0.7530$), and postexploration ($p = 0.7056$) periods in training trials in mice with M1 mAChR knock-out in either glutamatergic neurons ($n = 5$ mice) or interneurons ($n = 5$ mice) as compared with control mice ($n = 6$ mice), one-way ANOVA. Data were presented as mean ± SEM.

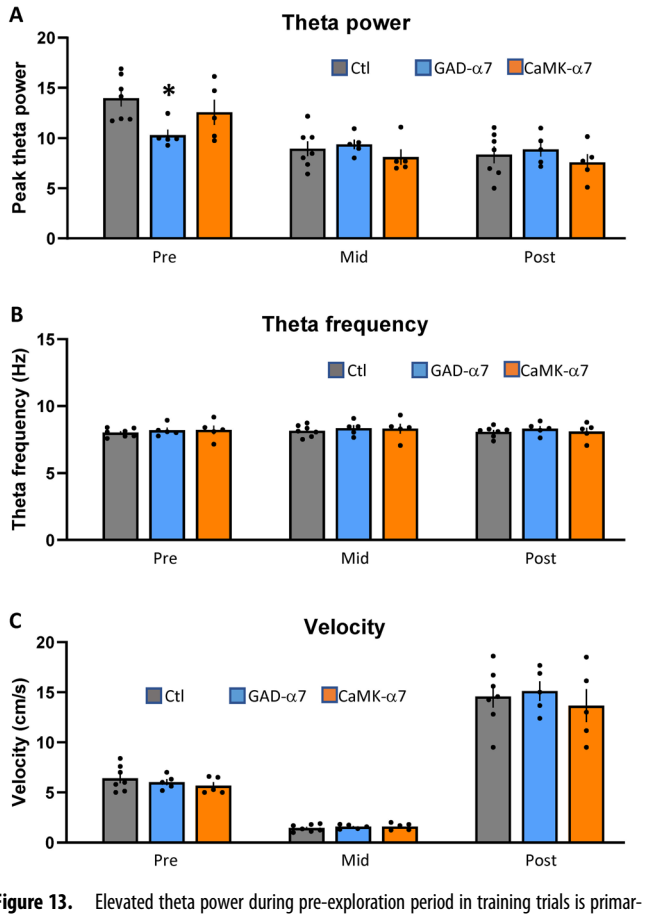

Figure 13. Elevated theta power during pre-exploration period in training trials is primarily mediated by α7 nAChRs expressed in interneurons but not glutamatergic neurons. ***A***, Elevated theta power during pre-exploration period in training trials is significantly reduced in mice with α7 nAChR knock-out in interneurons (GAD-α7; $p = 0.0224$; $n = 5$ mice) but not in mice with α7 nAChR knock-out in glutamatergic neurons (CaMK-α7; $p = 0.4602$; $n = 5$ mice) as compared with control mice ($n = 7$ mice), one-way ANOVA followed by Dunnett's multiple-comparisons test. ***B***, Peak theta frequency in mice with α7 nAChR knock-out in either interneurons ($n = 5$ mice) or glutamatergic neurons ($n = 5$ mice) are not significantly changed from control mice ($n = 7$ mice) during pre- ($p = 0.7273$), mid- ($p = 0.8259$), or postexploration ($p = 0.7117$) periods in training trials, one-way ANOVA. ***C***, Movement speeds are not significantly changed between control mice ($n = 6$ mice) and mice with α7 nAChR knock-out in either interneurons ($n = 5$ mice) or glutamatergic neurons ($n = 5$ mice) during either pre- ($p = 0.3834$), mid- ($p = 0.7439$), or postexploration ($p = 0.6954$) periods, one-way ANOVA. Data were presented as mean ± SEM.

of cholinergic-sensitive and cholinergic-resistant theta has been reported previously (Oddie et al., 1997; Oddie and Bland, 1998), where cholinergic-sensitive theta in response to sensory stimulation precedes and co-occurs with movement-related cholinergic-resistant theta and plays a role in movement planning. Together, these studies strongly suggest that even if cholinergic transmission does not directly regulate movement-related theta (cholinergic resistant) and the movement per se, it can still regulate the planning, initiation, and selection of the movements in response to sensory stimulations, including threatening inputs and novelty exposures.

Taken together, our study reveals that cholinergic transmission regulates the hippocampal memory encoding process through both mAChRs and α7 nAChRs on excitatory neurons and interneurons, respectively. Furthermore, we identified a cholinergic-sensitive theta component that clearly discriminates novel events from familiar events and therefore reveals a plausible mechanism to connect cholinergic transmission, theta oscillation, and hippocampal memory encoding.

References

Adams CE, Yonchek JC, Stitzel JA (2006) Development of hippocampal alpha7 nicotinic receptors in C3H and DBA/2 congenic mice. Brain Res 1122:27–35.

Aigner TG, Walker DL, Mishkin M (1991) Comparison of the effects of scopolamine administered before and after acquisition in a test of visual recognition memory in monkeys. Behav Neural Biol 55:61–67.

Alkondon M, Albuquerque EX (1993) Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. I. Pharmacological and functional evidence for distinct structural subtypes. J Pharmacol Exp Ther 265:1455–1473.

Amaral DG, Scharfman HE, Lavenex P (2007) The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). Prog Brain Res 163:3–22.

Balakrishnan S, Pearce RA (2014) Midazolam and atropine alter theta oscillations in the hippocampal CA1 region by modulating both the somatic and distal dendritic dipoles. Hippocampus 24:1212–1231.
Berke JD, Hetrick V, Breck J, Greene RW (2008) Transient 23-30 Hz oscillations in mouse hippocampus during exploration of novel environments. Hippocampus 18:519–529.
Bianchi L, Ballini C, Colivicchi MA, Della Corte L, Giovannini MG, Pepeu G (2003) Investigation on acetylcholine, aspartate, glutamate and GABA extracellular levels from ventral hippocampus during repeated exploratory activity in the rat. Neurochem Res 28:565–573.
Bitner RS, et al. (2007) Broad-spectrum efficacy across cognitive domains by alpha7 nicotinic acetylcholine receptor agonism correlates with activation of ERK1/2 and CREB phosphorylation pathways. J Neurosci 27:10578–10587.
Broks P, Preston GC, Traub M, Poppleton P, Ward C, Stahl SM (1988) Modelling dementia: effects of scopolamine on memory and attention. Neuropsychologia 26:685–700.
Buhler AV, Dunwiddie TV (2001) Regulation of the activity of hippocampal stratum oriens interneurons by alpha7 nicotinic acetylcholine receptors. Neuroscience 106:55–67.
Buzsaki G (2002) Theta oscillations in the hippocampus. Neuron 33:325–340.
Buzsaki G (2005) Theta rhythm of navigation: link between path integration and landmark navigation, episodic and semantic memory. Hippocampus 15:827–840.
Buzsaki G (2010) Neural syntax: cell assemblies, synapsembles, and readers. Neuron 68:362–385.
Buzsaki G, Moser EI (2013) Memory, navigation and theta rhythm in the hippocampal-entorhinal system. Nat Neurosci 16:130–138.
Cai L, Gibbs RB, Johnson DA (2012) Recognition of novel objects and their location in rats with selective cholinergic lesion of the medial septum. Neurosci Lett 506:261–265.
Castro NG, Albuquerque EX (1995) Alpha-bungarotoxin-sensitive hippocampal nicotinic receptor channel has a high calcium permeability. Biophys J 68:516–524.
Dani JA, Bertrand D (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol 47:699–729.
Dere E, Huston JP, De Souza Silva MA (2007) The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. Neurosci Biobehav Rev 31:673–704.
Douchamps V, Jeewajee A, Blundell P, Burgess N, Lever C (2013) Evidence for encoding versus retrieval scheduling in the hippocampus by theta phase and acetylcholine. J Neurosci 33:8689–8704.
Easton A, Douchamps V, Eacott M, Lever C (2012) A specific role for septohippocampal acetylcholine in memory? Neuropsychologia 50: 3156–3168.
Frazier CJ, Rollins YD, Breese CR, Leonard S, Freedman R, Dunwiddie TV (1998) Acetylcholine activates an alpha-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. J Neurosci 18:1187–1195.
Ge S, Dani JA (2005) Nicotinic acetylcholine receptors at glutamate synapses facilitate long-term depression or potentiation. J Neurosci 25: 6084–6091.
Ghoneim MM, Mewaldt SP (1975) Effects of diazepam and scopolamine on storage, retrieval and organizational processes in memory. Psychopharmacologia 44:257–262.
Giovannini MG, Rakovska A, Benton RS, Pazzagli M, Bianchi L, Pepeu G (2001) Effects of novelty and habituation on acetylcholine, GABA, and glutamate release from the frontal cortex and hippocampus of freely moving rats. Neuroscience 106:43–53.
Grottick AJ, Higgins GA (2000) Effect of subtype selective nicotinic compounds on attention as assessed by the five-choice serial reaction time task. Behav Brain Res 117:197–208.
Gu Z, Alexander GM, Dudek SM, Yakel JL (2017) Hippocampus and entorhinal cortex recruit cholinergic and NMDA receptors separately to generate hippocampal theta oscillations. Cell Rep 21:3585–3595.
Gu Z, Lamb PW, Yakel JL (2012) Cholinergic coordination of presynaptic and postsynaptic activity induces timing-dependent hippocampal synaptic plasticity. J Neurosci 32:12337–12348.
Gu Z, Smith KG, Alexander GM, Guerreiro I, Dudek SM, Gutkin B, Jensen P, Yakel JL (2020) Hippocampal interneuronal alpha7 nAChRs modulate theta oscillations in freely moving mice. Cell Rep 31:107740.
Gu Z, Yakel JL (2011) Timing-dependent septal cholinergic induction of dynamic hippocampal synaptic plasticity. Neuron 71:155–165.
Gu Z, Yakel JL (2022) Cholinergic regulation of hippocampal theta rhythm. Biomedicines 10:745.
Guerreiro I, Gu Z, Yakel JL, Gutkin BS (2022) Recurring cholinergic inputs induce local hippocampal plasticity through feedforward disinhibition. eNeuro 9:0389.
Haam J, Zhou J, Cui G, Yakel JL (2018) Septal cholinergic neurons gate hippocampal output to entorhinal cortex via oriens lacunosum moleculare interneurons. Proc Natl Acad Sci U S A 115:E1886–E1895.
Hasselmo ME (2005) What is the function of hippocampal theta rhythm?–Linking behavioral data to phasic properties of field potential and unit recording data. Hippocampus 15:936–949.
Hasselmo ME (2006) The role of acetylcholine in learning and memory. Curr Opin Neurobiol 16:710–715.
Hasselmo ME, Bodelon C, Wyble BP (2002) A proposed function for hippocampal theta rhythm: separate phases of encoding and retrieval enhance reversal of prior learning. Neural Comput 14:793–817.
Hasselmo ME, Sarter M (2011) Modes and models of forebrain cholinergic neuromodulation of cognition. Neuropsychopharmacology 36:52–73.
Hersman S, Cushman J, Lemelson N, Wassum K, Lotfipour S, Fanselow MS (2017) Optogenetic excitation of cholinergic inputs to hippocampus primes future contextual fear associations. Sci Rep 7:2333.
Ikonen S, McMahan R, Gallagher M, Eichenbaum H, Tanila H (2002) Cholinergic system regulation of spatial representation by the hippocampus. Hippocampus 12:386–397.
Jones S, Yakel JL (1997) Functional nicotinic ACh receptors on interneurones in the rat hippocampus. J Physiol 504:603–610.
Kellar KJ, Martino AM, Hall DP Jr, Schwartz RD, Taylor RL (1985) High-affinity binding of [3H]acetylcholine to muscarinic cholinergic receptors. J Neurosci 5:1577–1582.
Kenney JW, Gould TJ (2008) Modulation of hippocampus-dependent learning and synaptic plasticity by nicotine. Mol Neurobiol 38:101–121.
Kramis R, Vanderwolf CH, Bland BH (1975) Two types of hippocampal rhythmical slow activity in both the rabbit and the rat: relations to behavior and effects of atropine, diethyl ether, urethane, and pentobarbital. Exp Neurol 49:58–85.
Letsinger AC, Gu Z, Yakel JL (2022) Alpha7 nicotinic acetylcholine receptors in the hippocampal circuit: taming complexity. Trends Neurosci 45:145–157.
Levin ED, McClernon FJ, Rezvani AH (2006) Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. Psychopharmacology 184:523–539.
Lopez-Madrona VJ, Perez-Montoyo E, Alvarez-Salvado E, Moratal D, Herreras O, Pereda E, Mirasso CR, Canals S (2020) Different theta frameworks coexist in the rat hippocampus and are coordinated during memory-guided and novelty tasks. Elife 9:e57313.
Ma X, Zhang Y, Wang L, Li N, Barkai E, Zhang X, Lin L, Xu J (2020) The firing of theta state-related septal cholinergic neurons disrupt hippocampal ripple oscillations via muscarinic receptors. J Neurosci 40:3591–3603.
Mamad O, McNamara HM, Reilly RB, Tsanov M (2015) Medial septum regulates the hippocampal spatial representation. Front Behav Neurosci 9:166.
Marrosu F, Portas C, Mascia MS, Casu MA, Fa M, Giagheddu M, Imperato A, Gessa GL (1995) Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep–wake cycle in freely moving cats. Brain Res 671:329–332.
McQuiston AR, Madison DV (1999) Nicotinic receptor activation excites distinct subtypes of interneurons in the rat hippocampus. J Neurosci 19:2887–2896.
Micheau J, Marighetto A (2011) Acetylcholine and memory: a long, complex and chaotic but still living relationship. Behav Brain Res 221:424–429.
Montoya CP, Sainsbury RS (1985) The effects of entorhinal cortex lesions on type 1 and type 2 theta. Physiol Behav 35:121–126.
Moser MB, Rowland DC, Moser EI (2015) Place cells, grid cells, and memory. Cold Spring Harb Perspect Biol 7:a021808.
Neves G, Cooke SF, Bliss TV (2008) Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. Nat Rev Neurosci 9: 65–75.
Newman EL, Venditto SJC, Climer JR, Petter EA, Gillet SN, Levy S (2017) Precise spike timing dynamics of hippocampal place cell activity sensitive to cholinergic disruption. Hippocampus 27:1069–1082.
Nunez A, Buno W (2021) The theta rhythm of the hippocampus: from neuronal and circuit mechanisms to behavior. Front Cell Neurosci 15:649262.

Oddie SD, Bland BH (1998) Hippocampal formation theta activity and movement selection. Neurosci Biobehav Rev 22:221–231.
Oddie SD, Kirk IJ, Whishaw IQ, Bland BH (1997) Hippocampal formation is involved in movement selection: evidence from medial septal cholinergic modulation and concurrent slow-wave (theta rhythm) recording. Behav Brain Res 88:169–180.
Okada K, Hashimoto K, Kobayashi K (2022) Cholinergic regulation of object recognition memory. Front Behav Neurosci 16:996089.
Opitz B (2014) Memory function and the hippocampus. Front Neurol Neurosci 34:51–59.
Qasim SE, Fried I, Jacobs J (2021) Phase precession in the human hippocampus and entorhinal cortex. Cell 184:3242–3255.e10.
Raynal E, Schnider A, Manuel AL (2020) Early signal from the hippocampus for memory encoding. Hippocampus 30:114–120.
Sainsbury RS, Heynen A, Montoya CP (1987) Behavioral correlates of hippocampal type 2 theta in the rat. Physiol Behav 39:513–519.
Son JH, Winzer-Serhan UH (2008) Expression of neuronal nicotinic acetylcholine receptor subunit mRNAs in rat hippocampal GABAergic interneurons. J Comp Neurol 511:286–299.
Stevens KE, Freedman R, Collins AC, Hall M, Leonard S, Marks MJ, Rose GM (1996) Genetic correlation of inhibitory gating of hippocampal auditory evoked response and alpha-bungarotoxin-binding nicotinic cholinergic receptors in inbred mouse strains. Neuropsychopharmacology 15:152–162.
Teles-Grilo Ruivo LM, Mellor JR (2013) Cholinergic modulation of hippocampal network function. Front Synaptic Neurosci 5:2.
Vogel-Ciernia A, Wood MA (2014) Examining object location and object recognition memory in mice. Curr Protoc Neurosci 69:8–31.
Witter MP, Naber PA, van Haeften T, Machielsen WC, Rombouts SA, Barkhof F, Scheltens P, Lopes da Silva FH (2000) Cortico-hippocampal communication by way of parallel parahippocampal-subicular pathways. Hippocampus 10:398–410.
Yakel JL (2014) Nicotinic ACh receptors in the hippocampal circuit; functional expression and role in synaptic plasticity. J Physiol 592: 4147–4153.
Yoder RM, Pang KC (2005) Involvement of GABAergic and cholinergic medial septal neurons in hippocampal theta rhythm. Hippocampus 15: 381–392.
Zhang Y, Cao L, Varga V, Jing M, Karadas M, Li Y, Buzsaki G (2021) Cholinergic suppression of hippocampal sharp-wave ripples impairs working memory. Proc Natl Acad Sci U S A 118:e2016432118.
Zhang H, Lin SC, Nicolelis MA (2010) Spatiotemporal coupling between hippocampal acetylcholine release and theta oscillations in vivo. J Neurosci 30:13431–13440.
Zheng C, Hwaun E, Loza CA, Colgin LL (2021) Hippocampal place cell sequences differ during correct and error trials in a spatial memory task. Nat Commun 12:3373.