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Endogenous ACh suppresses LTD induction and nicotine relieves the suppression via different nicotinic ACh receptor subtypes in the mouse hippocampus

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Abstract

Aims—Studying the normal role of nicotinic cholinergic systems in hippocampal synaptic plasticity is critical for understanding how cholinergic loss in Alzheimer's disease (AD) and tobacco use affect cognitive function. However, it is largely unknown how nicotinic cholinergic systems regulate the induction of long-term depression (LTD).

Main methods—Extracellular field potential recordings were performed in hippocampal slices prepared from wild-type, α2, α7, and β2 knockout (KO) mice. Effects of nicotine and nicotinic antagonists on LTD induction in wild-type, α2, α7, and β2 KO mice were compared.

Key findings—Activation of α7 nicotinic acetylcholine receptors (nAChRs) occurs during LTD-inducing stimulation to suppress LTD induction at CA3-CA1 synapses. Nicotine relieves this suppression, causing larger LTD. This nicotine effect was mediated by the activation of non-α7 nAChR subtypes, which were not activated by ACh released during LTD-inducing stimulation, and requires the presence of endogenous ACh-induced α7 nAChR activation. Furthermore, the effect of nicotine was prevented in the presence of mecamylamine, but not dihydro-β-erythroidine, and was still observed in both α2 KO and β2 KO mice.

Significance—This is the first report to evaluate the involvement of different nAChR subtypes in LTD induction. Findings indicate the involvement of unique non-α7 nAChR subtypes, which have not been considered in the nicotinic modulation of hippocampal long-term potentiation, in the control of LTD induction. The implication of our results is that the loss of cholinergic projections to the hippocampus, which reduces ACh release as seen in AD patients, and nicotine from tobacco smoking can differentially affect LTD induction.

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Conflict of interest statement
None

Keywords

nicotinic acetylcholine receptors; knockout mice; hippocampus; long-term depression

Introduction

Synaptic plasticity is generally thought to be the cellular substrate of learning and memory, and has been extensively investigated in the hippocampus (Bliss and Collingridge, 1993; Collingridge et al., 2010; Kemp and Bashir, 2001; Malenka and Bear, 2004; Malenka and Nicoll, 1999). Nicotinic cholinergic systems, which are implicated in cognitive function (Jones et al., 1992; Kenney and Gould, 2008; Levin and Rezvani, 2002; Newhouse et al., 2004; Picciotto and Zoli, 2002), are involved in the modulation of synaptic plasticity in the hippocampus (Fujii et al., 1999; Fujii and Sumikawa, 2001; Ge and Dani, 2005; Gu et al., 2012; Gu and Yakel, 2011; Ji et al., 2001; Mann and Greenfield, 2003; Mansvelder and McGehee, 2000; Nakauchi et al., 2007; Nakauchi and Sumikawa, 2012; Welsby et al., 2006). However, the details of the underlying mechanisms largely remain to be elucidated. Examining nicotine's effects is useful for not only for determining the normal role of nicotinic cholinergic systems in synaptic plasticity, but also for understanding how cholinergic deficiency in Alzheimer's disease (AD) and tobacco use affect synaptic plasticity. Various effects of nicotine are mediated by different nicotinic acetylcholine receptor (nAChR) subtypes. Thus, understanding distinct roles of different nAChR subtypes in synaptic plasticity is critical, if we are to understand how nicotinic cholinergic systems control synaptic plasticity.

α7 nAChR, which is the most abundant subtype in the hippocampus (Seguela et al., 1993), is expressed in CA1 pyramidal cells and many γ-aminobutyric acid (GABA)ergic interneurons in the strata oriens (SO), pyramidale, radiatum (SR) and lacunosum moleculare (SLM) of the hippocampal CA1 region (Alkondon and Albuquerque, 2001; Alkondon et al., 1998; Frazier et al., 1998a; Frazier et al., 1998b; Ji et al., 2001; McQuiston and Madison, 1999; Sudweeks and Yakel, 2000). This subtype is highly Ca^{2+}-permeable (Seguela et al., 1993) and, thus, has been proposed as contributing to synaptic plasticity (Ji et al., 2001). Indeed, this subtype is involved in the control of long-term potentiation (LTP) induction at the Schaffer collateral (SC) pathway (Gu and Yakel, 2011; Ji et al., 2001; Nakauchi and Sumikawa, 2012). In addition, it has been reported that the nicotinic cholinergic modulation of LTP induction at the SC pathway is blocked by the competitive nAChR antagonist dihydro-β-erythroidine (DHβE) and is absent in α2 knockout (KO) mice (Gu and Yakel, 2011; Ji et al., 2001; Nakauchi et al., 2007; Nakauchi and Sumikawa, 2012) and β2 KO mice (Nakauchi and Sumikawa, 2012). However, it is unknown whether nAChR subtypes involved in the nicotinic modulation of LTP induction also play roles in the control of other synaptic plasticity. Long-term depression (LTD), which is required for the consolidation of spatial memory (Ge et al., 2010), is induced at the SC pathway (Dudek and Bear, 1993; Dvorak-Carbone and Schuman, 1999; Malenka and Bear, 2004), and we have previously reported that nicotine and methyllycaconitine (MLA), an antagonist for the α7 nAChR subtype, facilitate LTD induction at the SC pathway of rats (Fujii and Sumikawa, 2001). It has been thought that effects of nicotine that are mimicked by MLA are mediated by α7

nAChR desensitization. However, the details of the underlying mechanisms were not investigated in that study. Furthermore, it remains to be examined whether similar nicotinic modulation of LTD induction occurs in mice. In the present study, we examined the nicotinic cholinergic modulation of LTD induction in the hippocampal CA1 region of wild type, α2-, α7-, and β2-KO mice in the absence and presence of nicotinic cholinergic drugs. The data obtained demonstrate that activation of α7 nAChRs during LTD-inducing stimulation suppresses LTD induction at SC pathway, and that the suppression is relieved by activation of non-α7 nAChR subtypes, which are not involved in the nicotinic control of LTP induction.

Materials and methods

Animals

All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with protocols approved by the Institutional Animal Care and Use Committee of the University of California at Irvine. Experiments were carried out using wild-type, littermate wild-type, α2-, α7- and β2-KO mice. These mutant mice were from established colonies of heterozygous breeders in the C57BL/6J strain, originally obtained from Dr. Jim Boulter (UCLA, α2 KO mice) and Dr. Marina Picciotto (Yale University, α7- and β2-KO mice). In initial experiments, we confirmed that littermate wild-type mice behave like wild-type mice in LTD induction as in LTP study (Nakauchi and Sumikawa, 2012). Thus, data from wild-type C57BL/6J mice were used as controls for comparison between the groups.

Slice preparation

Transverse hippocampal slices (375–400 μm) were prepared from mice (4–6 weeks) anaesthetized with urethane. Slices were maintained at 30 °C for at least 1 hour before recordings in artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 124; KCl, 5; NaH_2PO_4, 1.25; $MgSO_4$, 2; $CaCl_2$, 2.5; $NaHCO_3$, 22; and glucose, 10, and oxygenated with 95% O_2 and 5% CO_2.

Extracellular field recordings

Slices were placed in a recording chamber, submerged, and continuously superfused at 2–3 ml min with oxygenated ACSF at 30 °C. A bipolar stimulating electrode (Rhodes Medical Instruments, Tujunga, CA, USA) was placed in the SR of CA1 region to stimulate the SC pathway. The SC field excitatory postsynaptic potentials (fEPSPs) were recorded from the SR of the CA1 region using glass electrodes filled with 2 M NaCl (3–8 MΩ). Stimuli were short current pulses (200 ms duration) delivered every 20 s. At the beginning of each experiment, a stimulus-response curve was established by measuring the slope of fEPSPs. The strength of the stimulus was adjusted to elicit fEPSPs that were ~ 50% of the maximum response, which fell within stimulus intensities of 30–60 μA. To avoid high variance in LTD magnitude, slices failing to meet these criteria were not used for experiments. The intensity and duration of each stimulus pulse remained invariant thereafter for each experiment. Baseline responses were recorded following delivery of test stimuli via the stimulating electrode to establish the stability of the preparation. LTD was induced by 3 × low

frequency stimulation (LFS; 200 pulses at 1 Hz) at 20 min intervals. To evaluate the magnitude of LTD, the slope of fEPSPs were measured for 35–40 min after the last LFS for 3 × LFS, and the mean value was calculated and expressed as a percentage of the mean value of the initial baseline slope of fEPSPs. Recorded signals were amplified (A-M Systems, Sequim, WA, USA), digitized, stored on a computer and analyzed using NAC 2.0 software (Theta Burst Corp., Irvine, CA, USA).

Drug application and statistical analysis

Nicotine, DHβE, 2-Amino-5-phosphonopentanoic acid (APV) and MLA were obtained from Sigma (St Louis, MO, USA). Nicotine and APV were dissolved in ACSF and bath-applied about 10 min before delivery of LFS. Other drugs were dissolved in ACSF and bath applied throughout recordings after a 10 min baseline recording. Data were normalized relative to baseline and expressed as mean ± SEM. For statistical analysis, one-way ANOVA was used, followed by *posthoc*-Tukey HSD tests to identify which groups were significantly different. Physiological data were plotted and analyzed using Origin 8.1 (OriginLab, Northampton, MA, USA).

Results

Nicotine facilitates LTD induction

Numerous examples of LFS-induced LTD have been reported at the SC pathway (Citri and Malenka, 2008; Dudek and Bear, 1993; Wagner and Alger, 1995). In many cases, small depression was observed when LFS was applied, suggesting that the SC pathway is resistant to LTD induction by LFS (Bear and Abraham, 1996). Because consecutive LFS applications can cause greater depression (Wagner and Alger, 1995), we used 3 ×LFS (200 pulses at 1 Hz) at 20 min intervals to induce LTD (Fig. 1A). However, despite this, we observed only a small depression in control wild-type mice (88.9 ± 3.8% of basal levels, n = 6; Fig. 1B). In contrast, when the same stimulation paradigm was used in the presence of nicotine (1 μM), significantly larger LTD was induced (Fig. 1B, D; 72.9 ± 5.7% of basal levels, n=7; control vs. nicotine, $F_{(1, 12)} < 5.12$, $p < 0.05$). This enhanced LTD was completely eliminated in the presence of the *N*-methyl-D-aspartate receptor (NMDAR) antagonist APV (Fig. 1C, D; 95.7 ± 4.8% of basal levels, n=4; nicotine vs. nicotine + APV, $F_{(1, 10)} < 7.36$, $p < 0.05$) to the level achieved by APV alone (Fig. 1C, D; 93.7 ± 4.1% of basal levels, n=5), indicating that the facilitation of LTD requires NMDAR activation.

Inhibition of α7 nAChRs by MLA facilitates LTD induction

The effect of nicotine on LTD could be mediated by nAChR activation, desensitization or both. If nicotine's effect is due to desensitization, it would require the activation of nAChRs. The hippocampus receives extensive cholinergic input from the medial septum/diagonal band (Aznavour et al., 2002; Frotscher and Leranth, 1985; Matthews et al., 1987). These cholinergic fibers could be stimulated to cause the release of ACh during LFS. Because α7 nAChRs appear to be activated during LTP-inducing theta burst stimulation (Nakauchi and Sumikawa, 2012), we next examined the possibility that α7 nAChRs are activated during LFS to contribute to LTD induction. When we added the selective α7 nAChR antagonist MLAs to the extracellular solution, we found significantly larger LTD (61.2 ± 10.1% of

basal levels, n=5) as compared to control LTD (Fig. 2A, D; control vs. MLA, $F_{(1, 10)} = 7.66$, $p < 0.05$). This enhanced LTD was not observed when MLA was co-applied with APV (Fig. 2B, D; 89.8 ± 5.9% of basal levels, n=6; MLA vs. MLA + APV, $F_{(1, 10)} = 6.57$, $p < 0.05$; control vs. MLA + APV, $F_{(1, 11)} = 0.016$, $p = 0.90$), indicating that the facilitation requires NMDAR activation as in the case of nicotine-induced facilitation. These findings suggest that activation of α7 nAChRs occurs during LFS and acts as a brake on the induction of LTD.

The magnitude of LTD induced in the presence of MLA is similar to that induced in the presence of nicotine, suggesting that the effect of nicotine is mediated by α7 nAChR desensitization. To examine this possibility, we delivered LFS in the presence of both nicotine and MLA and monitored the induction of LTD. We predicted that the magnitude of LTD would be comparable to that induced in the presence of nicotine or MLA alone, if nicotine's effect is due to desensitization of α7 nAChRs and MLA is mimicking nicotine's desensitizing effect. However, we found that the presence of both nicotine and MLA completely prevented the induction of LTD (Fig. 2C, D; 103.9 ± 11.0% of basal levels, n=5; MLA vs. MLA + nicotine, $F_{(1, 9)} = 8.25$, $p < 0.05$; nicotine vs. nicotine + MLA, $F_{(1, 11)} = 7.49$, $p < 0.05$). This suggests that the effect of nicotine is not mediated by desensitization of α7 nAChRs, but due to the action of nicotine on non-α7 nAChRs.

LTD induction is facilitated in α7 KO mice

We next used α7 KO mice to further verify the critical role for this nAChR subtype in LTD induction and the separate role for non-α7 nAChRs in LTD. When we delivered LFS in the SC pathway of α7 KO mice, we observed larger LTD (Fig. 3A, B; 67.2 ± 7.3% of basal levels, n=7) as compared to that induced in wild-type mice (wild-type control vs. α7 KO control, $F_{(1, 12)} = 6.31$, $p < 0.05$). Furthermore, the magnitude of LTD induced in α7 KO mice was comparable to that induced in the presence of MLA in wild-type mice (Figs. 2 and 3). These results not only confirm that the activation of α7 nAChRs occurs during LFS to suppress LTD induction, but also provide the reason why LFS causes only small LTD in wild-type mice.

We also delivered LFS in the presence of nicotine in α7 KO mice and found that LTD induction was completely blocked (Fig. 3A, B; 98.7 ± 5.4% of basal levels, n=6; α7 KO control vs. α7 KO nicotine, $F_{(1, 12)} = 11.46$, $p < 0.01$) as in the case of co-application of MLA with nicotine in wild-type mice (Fig. 2C, D). The presence of nicotine's effect in α7 KO mice confirms the involvement of non-α7 nAChRs in the effect. Furthermore, we noted that the effects of nicotine in wild-type and α7 KO mice on LTD were significantly different (Fig. 3B; wild-type nicotine vs. α7 KO nicotine, $F_{(1, 12)} = 10.75$, $p < 0.01$), suggesting a complex interplay between endogenous ACh- and exogenous nicotine-mediated effects in LTD induction. Nicotine's action at non-α7 nAChRs appears to relieve the α7 nAChR-mediated suppression of LTD induction. However, this effect of nicotine appeared to occur only in the presence of α7 nAChR activation, because it is absent in the presence of MLL in wild-type mice (Fig. 2) and in α7 KO mice (Fig. 3).

Nicotine facilitates LTD induction in α2 KO and β2 KO mice

α2* nAChRs (asterisk indicates the possible presence of other subunits in the nAChR complex) in O/A interneurons can continuously be activated in the presence of nicotine (Jia et al., 2009) and this subtype plays a major role in nicotinic control of LTP induction (Leao et al., 2012; Nakauchi et al., 2007). Thus, we next investigated the involvement of α2* nAChRs in the control of LTD induction. Initially, we monitored LTD induction in the absence of nicotine in α2 KO mice (Fig. 4A, C) and found that the magnitude of LTD induced was similar between wild-type (88.9 ± 3.8% of basal levels, n=6) and α2 KO mice (85.9 ± 3.9% of basal levels, n=6, p =0.58; Fig. 4A, C). This suggests that this subtype is not involved in the control of LTD induction. However, the absence of α2 KO's effect on LTD might simply reflect the lack of α2* nAChR activation by endogenous ACh, as LFS in the SC pathway might not cause ACh diffusion to the O/A region sufficient to activate α2* nAChRs on O/A interneurons (Ishii et al., 2005; Jia et al., 2009; Sudweeks and Yakel, 2000; Wada et al., 1989). We then examined the role of α2* nAChR activation in the nicotine-induced facilitation of LTD induction. In α2 KO mice, delivery of LFS in the presence of nicotine (1 μM) caused significant facilitation of LTD induction (Fig. 4A, C; α2 KO control, 85.9 ± 3.9% of basal levels, n=6; α2 KO nicotine, 64.3 ± 9.2% of basal levels, n=5; $F_{(1, 10)} = 5.29$, $p < 0.05$). The level of nicotine-induced facilitation of LTD was similar between wild-type (72.9 ± 5.7% of basal levels, n=7) and α2 KO mice (64.3 ± 9.2% of basal levels, n=5; p =0.42; Fig. 4C). Thus, nicotine is still capable of facilitating LTD induction in the absence of α2* nAChRs, demonstrating that α2* nAChRs are not involved in the nicotinic control of LTD induction.

To gain further insight into the non-α7 nAChR subtype involved in the nicotinic modulation of LTD induction, we used β2 KO mice. In the absence of nicotine, LFS did not induce LTD in β2 KO mice (97.5 ± 6.7% of basal levels, n=5; Fig. 4B, C). However, in the presence of nicotine, LTD induction was facilitated (69.2 ± 9.7% of basal levels, n=4, β2 KO control vs. β2 KO nicotine, $F_{(1, 8)} = 6.18$, $p < 0.05$) to the level observed in the presence of nicotine in wild-type mice (Fig. 4B, C). These results demonstrated that β2-containing non-α7 nAChRs are not involved in nicotine's effect on LTD induction.

Mecamylamine-sensitive non-α7 nAChRs mediate the facilitative effect of nicotine on LTD

To gain further insight into the effect of nicotine on LTD induction, we used additional nAChR antagonists. DHβE is an antagonist that blocks different non-α7 nAChRs, including the α4β2, α3β2, α2β2, α2β4, and α2β4 subtypes (Harvey et al., 1996). α4β2* nAChR subtype has the highest affinity for nicotine (Flores et al., 1992; Whiting et al., 1991) and is highly sensitive to DHβE (Khiroug et al., 2004), so that this antagonist is often used to study the role of α4β2* nAChRs in nicotine's effects. Because the effect of nicotine on LTD induction was still present in β2 KO mice, the use of DHβE would test whether non-β2-containing DHβE-sensitive nAChR subtypes are involved in the nicotinic control of LTD. In the presence of DHβE alone, the slope of fEPSPs was reduced to 87.7 ± 4.3% of basal levels (n=5; Fig. 5A, C), which is comparable to that observed in the absence of DHβE (88.9 ± 3.8%) (Fig. 5C). These results suggest either that DHβE-sensitive non-α7 nAChRs are not activated by ACh released during LFS or that their activation is not involved in LTD induction. We then tested whether nicotine is still capable of facilitating LTD induction

when DHβE-sensitive non-α7 nAChRs are blocked. As shown in Fig. 5(A, C), nicotine significantly facilitated the induction of LTD in the presence of DHβE (67.4 ± 3.4% of basal levels, n=6; DHβE vs. DHβE + nicotine, $F_{(1, 10)} = 14.30$, $p < 0.01$). The magnitudes of LTD observed in the presence of both DHβE and nicotine are very similar to those found in the presence of nicotine alone (Fig. 5C). These results indicate that the effect of nicotine is not mediated by DHβE-sensitive non-α7 nAChRs.

Because activation of DHβE-sensitive, α2-containing, and β2-containing non-α7 nAChRs, which have been demonstrated to be involved in the control of LTP induction, showed no significant effect on LTD induction, we next examined whether mecamylamine, a nonselective nAChR antagonist, blocked nicotine's effect on LTD induction. LTD induced in the presence of mecamylamine (89.5 ± 5.0% of basal levels, n=5) was similar to that induced in the absence of mecamylamine (88.9 ± 3.8%) (Fig. 5B, C), suggesting that bath application of mecamylamine (3 μM) had no effect on α7 nAChRs and, thus, α7 nAChR activation during LFS still suppresses LTD induction. In contrast, the effect of nicotine on LTD induction was significantly blocked in the presence of mecamylamine (Fig. 5B, C; 91.5 ± 4.6% of basal levels, n=7; mecamylamine vs. mecamylamine + nicotine, $F_{(1, 11)} = 0.08$, $p = 0.78$; control nicotine vs. mecamylamine + nicotine, $F_{(1, 13)} = 6.51$, $p < 0.05$). These results suggest that there are DHβE-insensitive and mecamylamine-sensitive non-α7 nAChRs that mediate nicotine's effect on LTD induction.

Discussion

Our current study suggests that cholinergic fibers in hippocampal slices are stimulated during LFS to cause the release of ACh. This results in the activation of α7 nAChRs to suppress the induction of NMDAR-dependent LTD at the SC pathway. This supports the unexpected conclusion that NMDAR-dependent LTD induced in the hippocampal CA1 region is normally affected by a nicotinic cholinergic contribution. Thus, our findings will impact the investigation of the cellular mechanism underlying LFS-induced NMDAR-dependent LTD in the hippocampal CA1 region. In addition, an important implication of the current findings is that LTD induction in the hippocampal CA1 region is most likely affected by cholinergic loss observed in AD and nicotine from tobacco use, possibly influencing cognitive function in AD patients and smokers.

Endogenous ACh-induced modulation of LTD

Previous studies show that single-stimulation-evoked α7 nAChR-mediated synaptic currents can be recorded in the hippocampal CA1 region in slices. However, the probability of recording such synaptic currents in interneurons is very low (Alkondon et al., 1998; Frazier et al., 1998a; Yamazaki et al., 2005), and recording α7-nAChR-mediated synaptic currents in pyramidal cells has never been reported. Thus, the current finding that α7 nAChRs are activated during LFS is somewhat surprising. Most cholinergic transmission in the hippocampus appears to occur through volume transmission and diffusion of ACh (Umbriaco et al., 1995). Because endogenous ACh-induced α7 nAChR responses have been more frequently recorded in both interneurons and pyramidal cells during weak tetanic stimulation (Yamazaki et al., 2005), it is possible that the concentration of ACh at α7

nAChR sites gradually increases via volume transmission and ACh diffusion, and becomes effective for receptor activation during LFS. Interestingly, LFS-induced α7 nAChR activation shifts synaptic responses towards potentiation in the same way that tetanic-stimulation-induced α7 nAChR activation does in LTP induction (Nakauchi and Sumikawa, 2012). This suggests that activation of α7 nAChRs by different stimulation paradigms still provides the same signal that crosstalks with signaling involved in LTD and LTP induction. The mechanism underlying the suppression of LTD induction through α7 nAChR activation remains to be elucidated. However, because this nAChR subtype is highly Ca^{2+}-permeable (Seguela et al., 1993), the α7-nAChR-dependent suppression of LTD induction might be due to a postsynaptic effect involving crosstalk between NMDAR- and α7-nAChR-mediated Ca^{2+} signaling pathways. It is interesting to note that α7-nAChR-activation-dependent LTP induction appears to be a postsynaptic effect involving prolongation of the NMDAR-mediated Ca^{2+} transients in pyramidal cells (Gu and Yakel, 2011), although α7-nAChR-induced Ca^{2+} transients in CA1 pyramidal cells have never been reported (see Gu and Yakel, 2011; Khiroug et al., 2003). Because both LTP-inducing stimulation and LFS appear to provide the same α7-nAChR-mediated signaling, similar postsynaptic crosstalk may mediate the suppression of LTD induction.

Nicotine-induced modulation of LTD induction

Bath application of nicotine reduces α7-nAChR-mediated responses due to desensitization, but does not inactivate the receptors completely (Frazier et al., 1998b; McQuiston and Madison, 1999; Yamazaki et al., 2006; Yamazaki et al., 2005). Thus, endogenous ACh can still activate α7 nAChRs in the presence of nicotine, and their reduced activation still contributes to LTP induction at the SC pathway (Nakauchi and Sumikawa, 2012). In the present study, we found that bath application of nicotine and MLA facilitate LTD induction at the SC pathway in mouse hippocampal slices, as found previously with rat hippocampal slices (Fujii and Sumikawa, 2001). It has been thought that the effects of nicotine that are mimicked by MLA are mediated by α7 nAChR desensitization. However, our present findings demonstrate that nicotine's effect on LTD is not due to α7 nAChR desensitization, but is mediated by activation of non-α7 nAChRs. Our present study also demonstrates that non-α7-nAChR-mediated regulation of LTD induction depends on the presence of α7 nAChR activation, and it appears that non-α7 nAChR activation counteracts α7-nAChR-mediated regulation of LTD induction. Such interplays between non-α7 and α7 nAChR activation are not found in the regulation of LTP induction at the SC pathway (Nakauchi and Sumikawa, 2012). This might be due to the involvement of different non-α7 nAChR subtypes in the regulation of LTD induction. In the hippocampus, at least four functional nAChR subtypes, α2*, α3β4* and α4β2* and α7 nAChRs, are expressed in different locations (Alkondon and Albuquerque, 2001; Alkondon et al., 1998; Frazier et al., 1998a; Frazier et al., 1998b; Ji et al., 2001; McQuiston and Madison, 1999; Sudweeks and Yakel, 2000). However, it is largely unknown whether a specific nAChR subtype is associated with a distinct neuron type or subtype. Selective optogenetic stimulation of cholinergic fibers shows DHβE-sensitive non-α7 nAChR responses in subpopulations of SO and SLM interneurons (Bell et al., 2011) and in 20% of pyramidal cells (Gu and Yakel, 2011). Based on their high sensitivity to DHβE, non-α7 nAChRs on these interneurons are thought to be α4β2* nAChRs. The identity of non-α7 nAChRs on pyramidal cells, which are also

sensitive to DHβE, remains unknown. In addition, the α2 nAChR subtype is selectively expressed in subpopulations of O/A interneurons in the CA1 region (Ishii et al., 2005; Jia et al., 2009; Sudweeks and Yakel, 2000; Wada et al., 1989). Nicotine-induced facilitation of LTP induction is absent in the presence of DHβE and in α2 and β2 KO mice (Nakauchi et al., 2007; Nakauchi and Sumikawa, 2012). In contrast, our present study demonstrates that nicotine-induced facilitation of LTD induction remains in the presence of DHβE and in α2 and β2 KO mice. These results suggest that non-α7 nAChR subtypes involved in the modulation of LTP inductions are not contributing to the modulation of LTD induction, and that the different non-α7 nAChRs have selective roles in the different forms of synaptic plasticity. Our present study also demonstrates that nicotine's effect on LTD induction is blocked by mecamylamine. Because mecamylamine appears to have higher potency for α3β4 nAChRs than for other nAChR subtypes (Papke et al., 2001), α3β4 might be the non-α7 nAChR subtype involved in the nicotine-induced facilitation of LTD induction. The lack of a mecamylamine effect on LTD induction suggests that there is not sufficient activation of mecamylamine-sensitive non-α7 nAChRs by ACh released during LFS, perhaps due to the fact that these non-α7 nAChRs are located in different hippocampal layers. However, bath application of nicotine could activate non-α7 nAChRs in different layers more effectively, which might modulate LTD induction via circuitry-dependent mechanisms, as occurs with the α2*-nAChR-mediated facilitation of LTP induction (Nakauchi et al., 2007). nAChRs are localized both pre- and postsynaptically at glutamatergic and GABAergic synapses in the hippocampal CA1 region (Alkondon et al., 1998; Frazier et al., 1998b; McQuiston and Madison, 1999; Sudweeks and Yakel, 2000; Alkondon and Albuquerque, 2001; Ji et al., 2001). In addition, projections to the hippocampal CA1 region from other brain regions contain additional presynaptic nAChRs (Role and Berg, 1996). The identity and location of the mecamylamine-sensitive non-α7 nAChR subtype involved in the facilitation of LTD induction, however, remains to be determined.

Effects of nicotine on memory and LTD

Chronic nicotine exposure designed to mimic smoking has been shown to improve hippocampal memory (Levin et al., 1992). Nicotine has also been investigated as a treatment for AD (Wilson et al., 1995; White and Levin, 1999), resulting in small improvements in cognitive function. Although much less is known about LTD as compared to LTP, increasing evidence suggests that LTD also plays an important role in hippocampus-dependent learning and memory (Collingridge et al., 2010). Thus, the observed nicotine's effects on memory might involve altered LTD. Further studies of nicotine's effects on LTD in chronic nicotine treated animals and animal models of AD could provide insights into the involvement of LTD in the nicotine-induced memory improvement.

Conclusions

The present study implies that the loss of cholinergic projections to the hippocampus, which is seen in AD patients, and nicotine from tobacco smoking would have a profound impact on the magnitude of LTD, reducing the flow of incoming information to the CA1 subfield. Such changes would most likely affect the formation of new memories.

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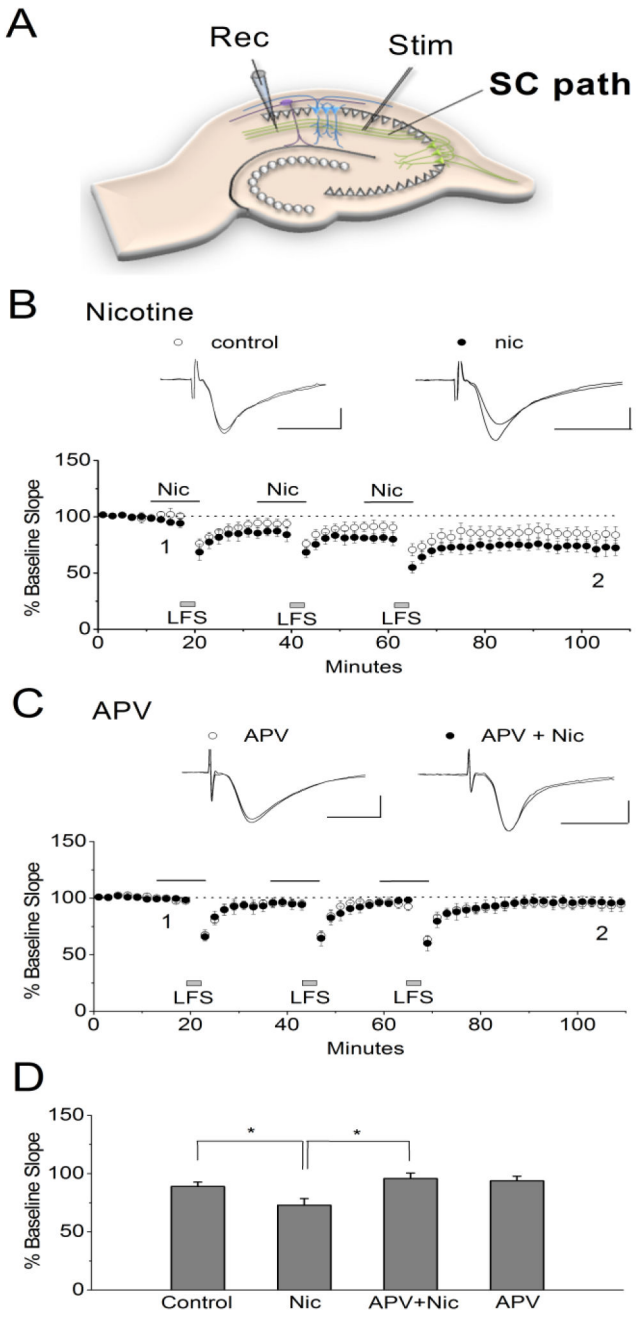

FIGURE 1. Nicotine facilitates NMDAR-dependent LTD induction

(A) Scheme of recording setup showing the position of stimulating and recording electrodes. (B) Nicotine accelerates LTD induction by 3 × LFS (200 pulses at 20 min intervals) in wild-type mice. (C) NMDAR antagonist APV (50 μM) blocked the effect of nicotine on LTD induction. In this and all subsequent graphs, changes in the slope of fEPSPs are plotted as the percentage change in initial baseline responses, and each trace above the graph was recorded at the time indicated (1 and 2). Lower bars in this and all subsequent figures indicate time at which LFS was given. In (B) and (C), administration of drugs is indicated by the upper horizontal bar, and scale bars are 10 ms and 1 mV. (D) Group data (mean ±

SEM) of the percent change in the slope of the fEPSPs. In this and all subsequent figures, data were measured 35–40 min after the third LFS. *$p < 0.05$.

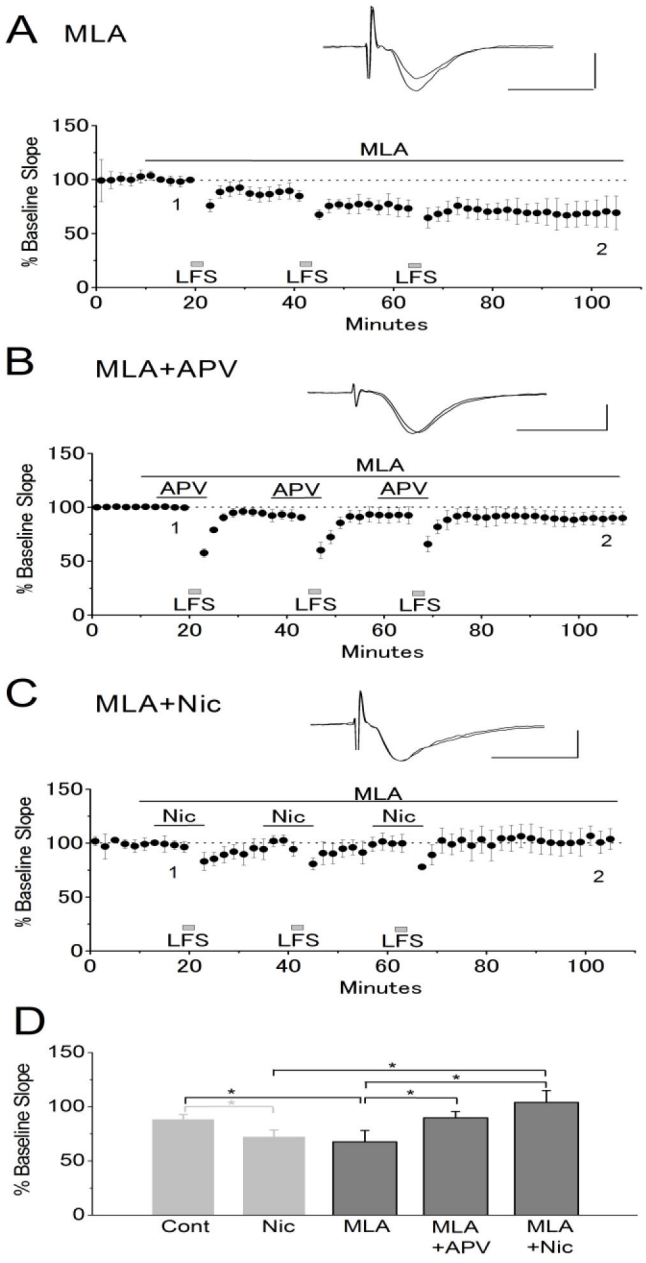

FIGURE 2. MLA facilitates NMDAR-dependent LTD induction in the absence, but not presence, of nicotine

All experiments were carried out using hippocampal slices prepared from wild-type mice. (A) LFS-induced LTD was facilitated by blocking α7-nAChR activation with MLA (20 nM). (B) APV (50 μM) prevented the effect of MLA. (C) MLA's s effect on LTD was absent in the presence of nicotine. In (A–C), scale bars are 10 ms and 1 mV, and administration of drugs is indicated by the upper horizontal bar. (D) Group data (mean ± SEM) of the percent change in the slope of the fEPSPs. *$p < 0.05$

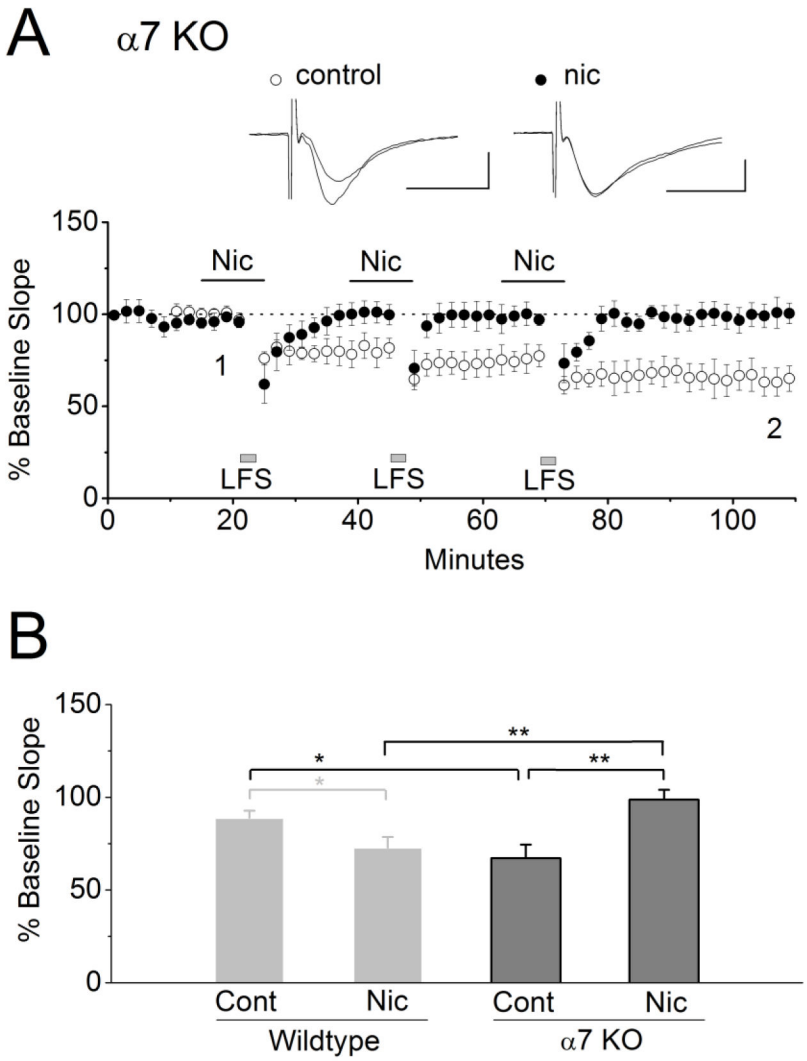

FIGURE 3. LTD induction is facilitated in the absence, but not presence, of nicotine in α7 KO mice

(A) Experiments were performed in the absence (control, open circles) and presence (filled circles) of nicotine using hippocampal slices prepared from α7 KO mice. Administration of nicotine is indicated by the upper horizontal bar, and scale bars are 10 ms and 1 mV. (B) Group data (mean ± SEM) of the percent change in the slope of the fEPSPs. Larger LTD was induced in α7 KO mice as compared to wild-type mice and nicotine' facilitative effect on LTD induction in wild-type mice was absent in α7 KO mice. *$p < 0.05$, **$p < 0.01$

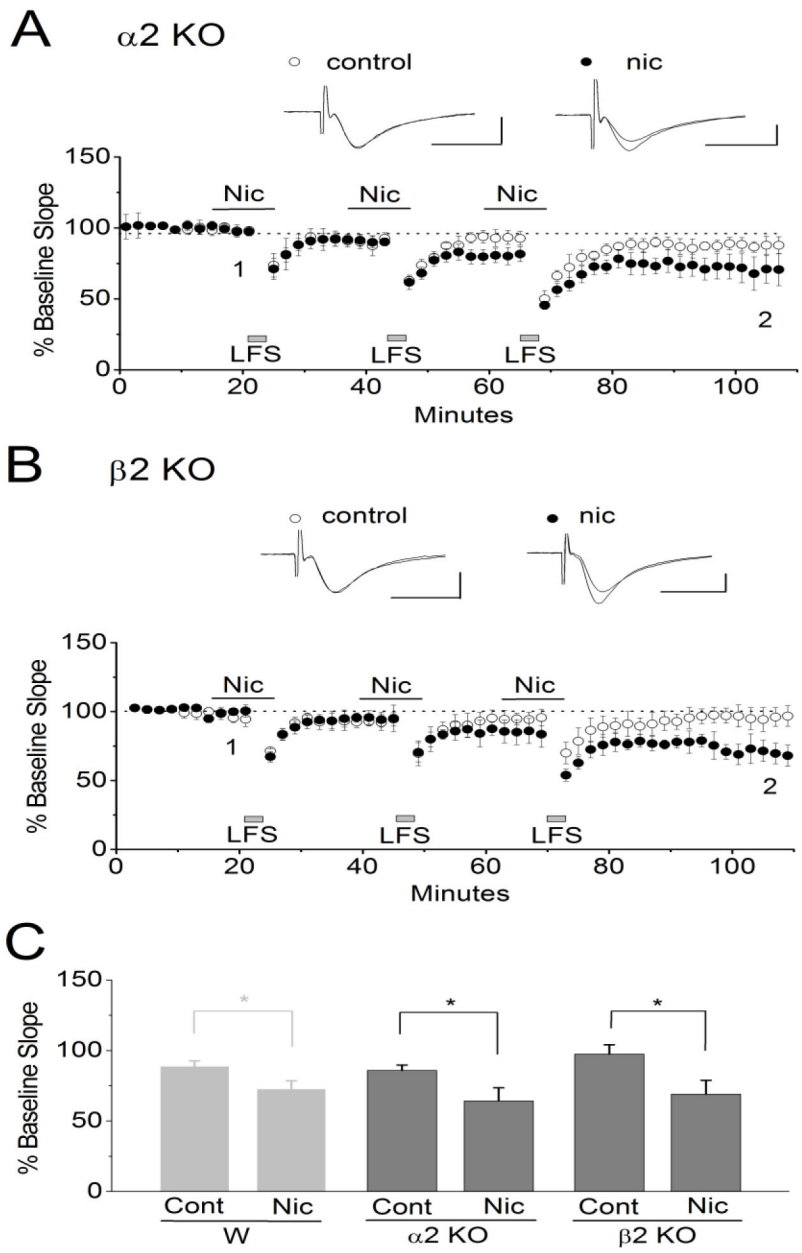

FIGURE 4. Nicotine facilitates LTD induction in 2 KO and β2 KO mice

(A) Nicotine's facilitative effect on LTD was still present in the absence of α2-containing nAChRs. (B) Nicotine still facilitates LTD induction in the absence of β2-containing nAChRs. In (A, B), scale bars are 10 ms and 1 mV. (C) Group data (mean ± SEM) of the percent change in the slope of the fEPSPs. *$p < 0.05$.

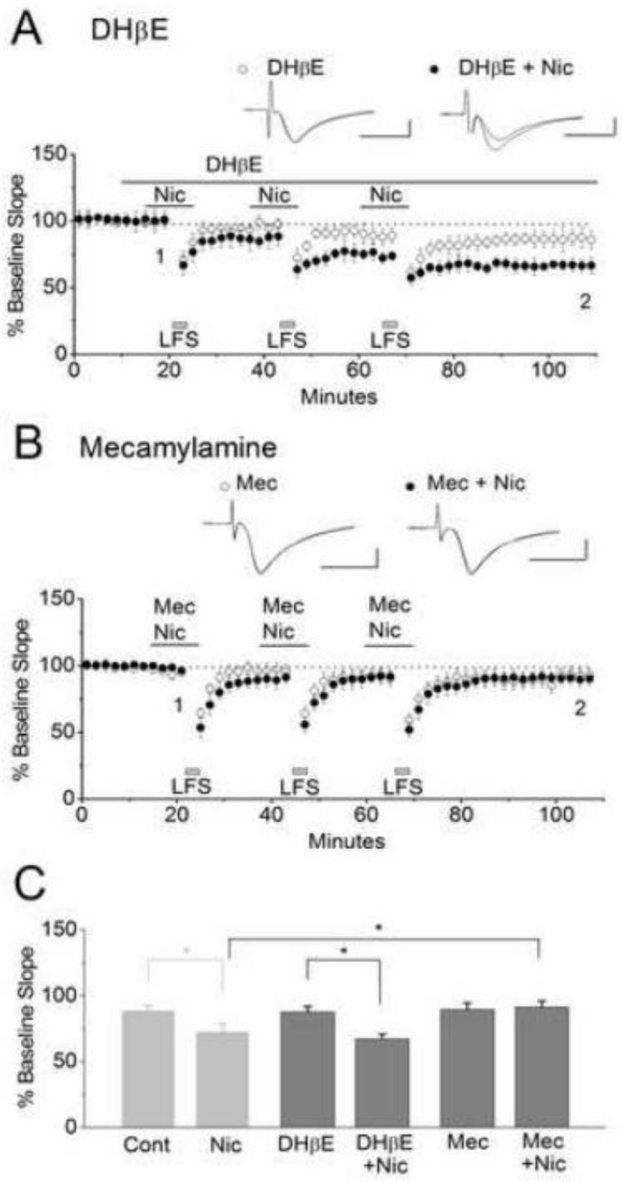

FIGURE 5. Mecamylamine, but not DHβE, prevents nicotine-induced facilitation of LTD induction

(A) DHβE (500 nM) alone had no effect on LTD induction and nicotine still facilitates LTD induction in the presence of 500 nM DHβE. (B) Mecamylamine (3 μM) alone had no effect on LTD induction, but it blocked the effect of nicotine on LTD induction. In (A, B), scale bars are 10 ms and 1 mV. (C) Group data (mean ± SEM) of the percent change in the slope of the fEPSPs. *$p < 0.05$.