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Impaired function of α2-containing nicotinic acetylcholine receptors on oriens-lacunosum moleculare cells causes hippocampus-dependent memory impairments

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Abstract

Children of mothers who smoked during pregnancy are at significantly greater risk for cognitive impairments including memory deficits, but the mechanisms underlying this effect remain to be understood. In rodent models of smoking during pregnancy, early postnatal nicotine exposure results in impaired long-term hippocampus-dependent memory, functional loss of α2-containing nicotinic acetylcholine receptors (α2* nAChRs) in oriens-lacunosum moleculare (OLM) cells, increased CA1 network excitation, and unexpected facilitation of long-term potentiation (LTP) at Schaffer collateral-CA1 synapses. Here we demonstrate that α2 knockout mice show the same pattern of memory impairment as previously observed in wild-type mice exposed to early postnatal nicotine. However, α2 knockout mice and α2 knockout mice exposed to early postnatal nicotine did not share all of the anomalies in hippocampal function observed in wild-type mice treated with nicotine during development. Unlike nicotine-treated wild-type mice, α2 knockout mice and nicotine-exposed α2 knockout mice did not demonstrate increased CA1 network excitation following Schaffer collateral stimulation and facilitated LTP, indicating that the effects are likely adaptive changes caused by activation of α2* nAChRs during nicotine exposure and are unlikely related to the associated memory impairment. Thus, the functional loss of α2* nAChRs in OLM cells likely plays a critical role in mediating this developmental-nicotine-induced hippocampal memory deficit.

Keywords

nicotine exposure; object location memory; object recognition memory; Schaffer collateral pathway; long-term potentiation

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1. Introduction

Children of mothers who smoked during pregnancy face a significantly greater risk for numerous health and cognitive problems, including long-lasting learning and memory deficits (Fried et al., 2003; Batstra et al., 2003; Thompson et al., 2009; Bruin et al., 2010). Although cigarette smoke contains more than 8,000 chemicals, nicotine is thought to be the leading cause of these impairments (Pauly and Slotkin, 2008). Indeed, rodent models have shown that early perinatal exposure to nicotine results in persistent deficits in learning and memory, including long-term hippocampus-dependent spatial memory (Sorenson et al., 1991; Yanai et al., 1992; Ankarberg et al., 2001; Vagelnova et al., 2004; Eppolito and Smith, 2006; Nakauchi et al., 2015). However, nicotine's effects on the brain are incredibly complex, and it remains to be understood exactly how transient early life exposure to this drug causes long-lasting cognitive dysfunction.

Nicotine exerts its effects through nicotinic acetylcholine receptors (nAChRs), pentameric assemblies that are either homomers of α7,α8 or α9 subunits, or heteromers of α2-6 and β2-4 subunits. α2-, α3-, α4-, α7-containing nAChRs are expressed in the CA1 region of the hippocampus, an area critical for spatial memory that appears to be particularly sensitive to the effect of nicotine exposure (Kenney and Gould, 2008; Nakauchi et al., 2015). We have previously shown that nicotine exposure in mice during the first two postnatal weeks – a time of significant hippocampal development roughly equivalent to the third trimester of human pregnancy (Seress, 2007) – causes a persistent impairment to long-term hippocampus-dependent memory (Nakauchi et al., 2015).

Additionally, in our rodent models we observed several changes to the way in which nAChR activation modulated CA1 activity and LTP (Nakauchi et al., 2015; Chen et al., 2016). In particular, we found that early postnatal nicotine exposure resulted in the functional loss of α2-containing nAChRs (α2* nAChRs) in oriens-lacunosum moleculare (OLM) cells (Chen et al., 2016), which are located in the stratum oriens and have projections into the stratum lacunosum-moleculare (Freund and Buzsaki, 1996). These interneurons receive cholinergic inputs from the medial septum, and can facilitate LTP at SC-CA1 synapses and block LTP at temporoammonic (TA) pathway synapses (Nakauchi et al., 2007; Leao et al., 2012). They can therefore affect the relative strength of inputs to CA1 pyramidal cells from the entorhinal cortex, which conveys sensory information, and from the CA3, which conveys internal representations of the multisensory context (Kesner, 2007; Gilbert and Brushfield, 2009). Thus, OLM cells are thought to be critical mediators of the formation of spatial memories (Leao et al., 2012; Lovett-Barron et al., 2014).

These observations suggest the possibility that early life nicotine exposure leads to the functional loss of α2* nAChRs and disrupts the normal function of OLM cells to cause profound changes in CA1 function and CA1-dependent behavior. Therefore, in this study we use an α2 knockout (α2KO) mouse line to investigate whether the functional loss of α2* nAChR may underlie the memory impairments observed following early postnatal nicotine.

One of the significant changes that has been observed in rodent hippocampal function following early postnatal nicotine exposure is an increase in CA1 depolarization following

SC stimulation (Damborsky et al., 2012; Damborsky et al., 2015; Nakauchi et al., 2015; Chen et al., 2016). This could represent a significant restructuring of neuronal networks, caused by the persistent presence of nicotine at a time when nicotinic receptors are important modulators of the strength of newly forming excitatory synapses (Maggi et al., 2003; Maggi et al., 2004). Our previous study also showed that early nicotine exposure resulted in facilitated LTP (Nakauchi et al., 2015; Chen et al., 2016). Because LTP is a leading candidate for many forms of memory, we had expected that we would see an association of memory impairments with impaired LTP. It remains to be tested whether facilitated LTP could also contribute to memory impairments. Thus, in this study, we also address whether increased CA1 network excitation and unexpected facilitation of LTP at SC-CA1 synapses that we previously observed could be contributing to the memory deficits. The overall aim of this study is to understand which changes caused by early postnatal nicotine exposure may underlie nicotine-induced memory impairments.

2. Materials and methods

2.1. Animals and nicotine treatment

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. Efforts were made to minimize the number of animals used.

α2KO mice were generated as described (Lotfipour et al., 2013), and were compared against wild-type littermates and wild-type C57BL/6 mice. As these control mice yielded equivalent results, their data were combined for statistical analysis. In order to avoid any behavioral changes caused by differences in maternal care, for the behavior experiments the wild-type and α2KO pups were fostered with CD1 dams immediately after birth. Mouse litters were adjusted to five male or female pups.

For experiments involving early postnatal nicotine exposure, pups were exposed to nicotine through maternal milk during postnatal days 1–15 by subcutaneously implanting nursing dams with alzet osmotic minipumps (DURECT, Model 1002; approximate nicotine output: 21 mg/kg/day). Others using this model, in which lactating dams are subcutaneously implanted with alzet osmotic minipumps (DURECT) delivering 6 mg/kg/day in rats (Model 2ML2) and 21 mg/kg/day in mice (Model 1002), have previously reported plasma nicotine levels to be 102–107 ng/ml in rat dams (Chen et al., 2005) and 207 ± 40 ng/ml in mouse dams (Eugenin et al., 2008). Rat pup blood nicotine levels on P8–10 were found to be 23.9 ± 3.5 ng/ml (Chen et al., 2005), similar to blood nicotine levels achieved by pregnant women who were moderate smokers (15–45 ng/ml) (Benowitz and Jacob, 1984). Somewhat higher doses are generally required to elicit the same effects in mice, perhaps because the plasma half-life of nicotine in mice is 6–9 minutes, whereas in rats it is about 54 minutes (Matta et al., 2007). Offspring were weaned at P21 and separated by sex into cages of 2–5 mice. Here, we refer to these pups as maternal-nicotine-exposed mice. Mouse pups from dams implanted with saline minipumps were used as controls. As electophysiological recordings and behavior from male and female mice yielded equivalent results, their data were combined for statistical analysis. The same cohort of mice was used for the three different behavioral tests,

which were conducted in a consistent order (object location memory task - object recognition memory task - elevated plus-maze). Animals showing a total exploration time less than 10 s on either training or testing were removed from further analyses without knowing group identity in accord with previous studies (Okuda et al., 2004; Intlekofer et al., 2013).

2.2 Object location and object recognition memory tasks

Training and testing for object location and object recognition memory were conducted between P44 and P67 (10 male + 8 female wild-type mice and 8 male + 9 female α2KO mice), and were carried out as previously described (Vogel-Ciernia and Wood, 2014) by experimenters blind to the treatment group. Briefly, before training the mice were handled 2 minutes daily for 5 days and then habituated to the experimental arena (white rectangular open field, 30 x 23 x 21.5 cm) 5 min a day for 6 days in the absence of objects. During training, mice were placed into the experimental arena with two identical objects (100 mL beakers or metal tins) and were allowed to explore for 10 minutes. During the retention test 24 hrs later to test long-term memory, for the object location task, one of the familiar objects was placed in a novel location, and the other was placed in one of the locations used during training. For the object recognition task, a familiar and a novel object were placed in the same locations as were used during training. All combinations of locations and objects were balanced across trials to eliminate bias. Training and testing trials were videotaped and analyzed by individuals blind to the treatment condition. A mouse was scored as exploring an object when its head was oriented toward the object and within a distance of 1 cm, or when its nose was touching the object. The relative exploration time was recorded and expressed by a discrimination index ($DI=(t_{novel} - t_{familiar})/(t_{novel} + t_{familiar}) \times 100\%$).

2.3. Elevated plus-maze

The elevated plus-maze task was performed as previously described (Nakauchi et al., 2015). The maze consisted of two open arms and two enclosed arms extending from a central platform, raised to a height of 40 cm above the floor. The light level in the testing room was adjusted to 15 lux. Testing consisted of placing a mouse onto the central platform of the maze facing an open arm, and recording its locomotion within the arms of the platform for 5 min. The percentage of time spent in the closed and open arms was scored using ANY-maze version 4.99b (Stoelting). Between subjects, the maze was cleaned with 10% ethanol.

2.4. Slice preparation

Transverse hippocampal slices (300–400 μm) were prepared from mice (age 4–6 weeks) anesthetized with urethane. Slices were maintained at 30°C for at least 1 h to recover in artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 124; KCL, 4; NaH_2PO_4, 1.25; $MgSO_4$, 2; $CaCl_2$, 2.5; $NaHCO_3$, 22; glucose, 10; and oxygenated with 95% O_2 and 5% CO_2.

2.5. Extracellular field recordings

Slices were submerged in a recording chamber and continually superfused at 2–3 mL/min with oxygenated ACSF at 30°C. A bipolar stimulating electrode was placed at the Schaffer

collateral (SC) pathway, and the slice stimulated with short current pulses (200 ms duration) every 20 s. Field excitatory postsynaptic potentials (fEPSPs) were recorded from the stratum radiatum of the CA1 region using glass electrodes filled with ACSF (3–8 MΩ). 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 30–50% of the maximum response (requiring stimulus intensities of 40–80 μA). The intensity and duration of each stimulus pulse remained invariant thereafter for each experiment. Baseline responses were recorded to establish the stability of the slice. Weak theta burst stimulation (weak TBS; two theta bursts of four pulses at 100 Hz) was used to monitor the induction of LTP. To evaluate LTP magnitude, the mean values of the slopes of fEPSPs from 40–50 min after weak TBS were calculated and expressed as a percentage of the mean baseline fEPSPs slopes.

2.6. Voltage-sensitive dye imaging

Voltage-sensitive dye (VSD) imaging with Di-4-ANEPPS (0.2 mM, Molecular Probes, Carlsbad, CA, USA) was performed as previously described (Tominaga et al., 2000; Nakauchi et al., 2007). Briefly, slices were submerged in a recording chamber mounted on the stage of a fluorescence microscope (BX51WI; Olympus). A 4x objective lens (0.28 NA; Olympus) focused the excitation light on the CA1 region of the hippocampus. VSD imaging was performed with a CCD camera (MiCAM02; BrainVision) which has a 6.4 x 4.4 mm^2 imaging area. To avoid bleaching of the dye, an electronically controlled shutter remained closed until 100 ms before the start of each recording. In each stimulation trial, frames were recorded at 250 Hz for 1024 ms. Eight or 16 trials were averaged to improve the signal-to-noise ratio. Extracellular potential recordings were preformed simultaneously with the optical recordings to ensure that the optical response was consistent with the electrical response. The fractional change in fluorescence intensity (ΔF/F) was used to normalize the difference in the amount of VSD in each slice, and signal gain and threshold levels were adjusted to optimize the signal-to-noise ratio of the response relative to background. Activated areas were smoothed by averaging images with spatial and cubic filters. Data was analyzed and displayed using BV-Analyser (BrainVision). To quantitatively compare optical responses across different slices, the maximum optical responses to a single stimulus were measured and compared. The magnitude of voltage changes was illustrated using pseudocolor.

2.7. Statistical analysis

Behavior datasets were expressed as mean ± SEM and analyzed with Student's *t*-tests. *p* values of less than 0.05 were considered statistically significant. Electrophysiological and optical data were normalized relative to baseline, expressed as mean ± SEM, and analyzed for significance using one-way ANOVA. Sample size *n* refers to the number of hippocampal slices analyzed in electrophysiological or optical recordings from different mice. In all graphs, *p* values are depicted as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Data were plotted and analyzed using Origin 8.1 (OriginLab).

3. Results

3.1. α2KO mice have impaired hippocampus-dependent memory

We first tested whether α2KO mice, like wild-type (WT) mice that are exposed to early postnatal nicotine, show impairments in long-term object location memory (Fig. 1A, B), a dorsal CA1-dependent task (Assini et al., 2009; Barrett et al., 2011; McQuown et al., 2011; Haettig et al., 2013; Lopez et al., 2016). We found that α2KO mice exhibited significant deficits in long-term object location memory as compared to WT mice (Fig. 1B; WT, 14.74 ± 3.82; α2KO, −2.17 ± 5.91; $t_{31} = 2.33$, $p = 0.03$). Importantly, there were no differences between the two groups with regards to total exploration time (WT, 29.48 s ± 2.39; α2KO, 29.05 s ± 3.02; $t_{31} = 0.11$ $p = 0.91$). These data suggest that α2* nAChRs are crucial for the formation of long-term, hippocampus-dependent spatial memory.

To determine whether the memory impairment in α2KO mice was global or, as in WT mice exposed to nicotine during development, was not, we tested them with the object recognition memory task (Fig. 1C, D), which is hippocampus-independent (Norman and Eacott, 2004; Moses et al., 2005). α2KO mice showed similar long-term memory for object recognition compared to WT mice (Fig. 1D; WT, 13.19 ± 3.94; α2KO, 17.28 ± 4.40; $t_{34} = -0.69$, $p = 0.49$). Both groups showed similar total exploration times (WT, 38.67 s ± 1.57; α2KO, 34.37 s ± 2.67; $t_{34} = 1.41$, $p = 0.17$). Thus, α2KO mice show normal memory for object recognition, and share the same pattern of hippocampus-specific memory impairment as do early-postnatal-nicotine-exposed WT mice.

Additionally, because we had previously observed that WT mice exposed to nicotine during development were more anxious than controls (Nakauchi et al., 2015), we also used the elevated plus-maze task to test for anxiety. α2KO mice spent significantly more time in the open arms of the platform than did WT mice (Fig. 1E; WT, 3.91% ± 0.5; α2KO, 9.2% ± 1.03; $t_{33} = -4.77$, $p < 0.001$) and less time in the closed arms (Fig. 1E; WT, 85.71% ± 0.92; α2KO, 71.16% ± 2.65; $t_{33} = 5.44$, $p < 0.001$), suggesting that they are actually less anxious than controls. This, in combination with the normal exploration times and normal object recognition memory performance of the α2KO mice, suggests that their poor performance in the object location memory task is indeed a reflection of impaired long-term, hippocampus-dependent memory.

3.2. α2KO mice show normal CA1 network activation in response to SC stimulation

In order to determine whether nicotine-induced activation of α2* nAChRs during hippocampal development causes increased CA1 excitatory activity, and whether this change may underlie the memory impairments that result from developmental nicotine exposure, we used VSD imaging to visualize network activation in response to SC stimulation in hippocampal slices from wild-type maternal-saline-treated (WT-SAL), wild-type maternal-nicotine-treated (WT-MATN), maternal-saline-exposed α2KO (α2KO-SAL), and maternal-nicotine-treated α2KO (α2KO-MATN) mice.

Stimulation of the SC pathway, the intensity of which was adjusted to evoke similar sizes of fEPSPs across slices, produced depolarizing optical signal in all anatomical layers of CA1, which reached its peak around 12 ms and could be presented as pseudo-colored images of

the ΔF/F signals (Fig. 2A; warmer colors indicate depolarization and red indicates the strongest depolarization). To determine the effect of maternal nicotine exposure on CA1 excitatory activity in wild-type and α2KO mice, the maximum amplitude of SC-evoked depolarizing VSD signals was measured and expressed as the ΔF/F (Fig. 2B). As we have previously shown (Nakauchi et al., 2015), depolarizing optical signal was significantly stronger in WT-MATN mice than in WT-SAL mice (WT-MATN mice; 3.93 ± 0.07, n = 13 vs. WT-SAL mice; 2.65 ± 0.07, n = 9; $F_{(1, 21)} = 155.99$, $p < 0.001$; Fig. 2A, B). These observations confirm that maternal nicotine treatment increases CA1 excitatory activity in wild-type mice.

We next measured the maximum amplitude of SC-evoked depolarizing VSD signals in hippocampal slices from α2KO-SAL and α2KO-MATN mice. The difference between the optical responses recorded from α2KO-SAL (2.68 ± 0.06, n = 6) and α2KO-MATN (2.92 ± 0.07, n = 9) mice did not reach statistical significance ($F_{(1, 14)} = 3.80$, $p = 0.052$; Fig. 2A, B), and peak signals were significantly weaker in α2KO-MATN mice as compared to WT-MATN mice ($F_{(1, 21)} = 97.12$, $p < 0.001$; Fig. 2A, B). Additionally, there was no significant difference between the optical responses recorded from WT-SAL and α2KO-SAL mice ($F_{(1, 14)} = 0.09$, $p = 0.77$; Fig. 2A, B).

These findings indicate that it is not the downregulation of α2* nAChRs that causes increased CA1 excitatory activity in mice exposed to nicotine during development. However, as seen by the near absence of effect of maternal nicotine exposure in slices from α2KO-MATN mice as compared to those from WT-MATN mice, the nicotine-induced activation of α2* nAChRs does appear to be necessary to trigger the changes that ultimately do affect CA1 excitatory activity.

3.3. Maternal-nicotine-treated a2KO mice do not have facilitated induction of LTP

Because we previously found that LTP was not diminished in hippocampal slices from early-postnatal-nicotine-exposed mice, but was unexpectedly facilitated (Nakauchi et al., 2015), we next investigated whether hippocampal slices from α2KO-MATN mice showed a similar change (Fig. 3).

As we have previously shown (Nakauchi et al., 2015), delivery of weak TBS at the SC pathway, which is subthreshold for LTP induction in WT-SAL mice (96 ± 2%, n = 9; Fig. 3A, C), induced LTP in WT-MATN mice (122 ± 3%, n = 6, WT-SAL mice vs. WT-MATN mice, $F_{(1, 14)} = 56.70$, $p < 0.001$; Fig. 3A, C). This confirms that maternal nicotine exposure, which impairs CA1-dependent memory, facilitates LTP induction.

We next considered the impact of α2* nAChR deletion on this effect of maternal nicotine. Because LTP-inducing SC stimulation does not result in the release of endogenous acetylcholine on OLM cells, α2 subunit deletion has no effect on LTP induction (Nakauchi et al., 2007). However, α2* nAChR-containing OLM cells do play a role in modulating LTP when activated by bath application of nicotine, and hippocampal slices from α2KO mice lack normal nicotine-induced LTP facilitation (Nakauchi et al., 2007). Here we found that, as in hippocampal slices from WT-SAL mice, weak TBS in slices from α2KO-SAL mice did not induce LTP (92 ± 2%, n = 6; WT-SAL vs. α2KO-SAL, $F_{(1, 14)} = 1.84$, p = 0.199; Fig.

3B, C). However, unlike in slices from WT-MATN mice, weak TBS stimulation of the SC pathway did not induce LTP in α2KO-MATN mice (91 ± 5%, n = 8; WT-MATN vs. α2KO-MATN, $F_{(1, 13)} = 23.68$, $p < 0.001$; α2KO-SAL vs. α2KO-MATN, $F(1, 13) = 0.03$, $p = 0.87$; Fig. 3B, C). These findings indicate that it is not the downregulation of α2* nAChRs that causes facilitated LTP in mice exposed to nicotine during development. Additionally, these findings indicate that maternal-nicotine-induced facilitation of LTP induction is a consequence of inappropriate α2* nAChR activation by early postnatal nicotine exposure.

4. Discussion

Recently, work has begun to emerge suggesting that OLM cells in the CA1 region of the hippocampus are important modulators of inputs from the entorhinal cortex and CA3 (Nakauchi et al., 2007; Leao et al., 2012), and play a critical role in the formation of contextual memories (Lovett-Barron et al., 2014). OLM cells are unique in the hippocampus in that they express the α2* nAChR, which appears to be continuously activated in the presence of nicotine (Jia et al., 2009). We have previously shown that early life nicotine exposure impairs dorsal CA1-dependent memory, leads to the functional loss of α2* nAChRs in OLM cells, and disrupts the normal role of OLM cells in hippocampal CA1 function and LTP (Nakauchi et al., 2015; Chen et al., 2016).

Our previous results demonstrate that the functional loss of α2* nAChRs in OLM cells persists into adolescence and adulthood (Chen et al., 2016). Such long-lasting effects are often mediated by changes in epigenetic regulation of gene expression. This functional loss of α2* nAChRs is prevented by co-administration of the nAChR antagonist mecamylamine (Chen et al., 2016), suggesting that maternal nicotine-induced nAChR activation rather than desensitization mediates the effect. Because OLM cells are continuously activated in the presence of nicotine due to the non-desensitizing nature of α2* nAChRs (Jia et al., 2009) and α2* nAChR activation triggers Ca^{2+} signaling in these cells (Jia et al., 2010), this Ca^{2+} could evoke global genomic responses by altering chromatin structures through histone deacetylation and DNA methylation for gene silencing. Therefore, here we investigated the possibility that the functional loss of α2* nAChRs underlies the memory impairment induced by early life nicotine exposure using α2KO mice. We showed that α2KO mice, like maternal-nicotine-exposed wild-type mice, have impaired hippocampus-dependent long-term memory. Our findings suggest that α2* nAChRs play a critical role in normal hippocampus-dependent memory, and that their functional loss could be the cause of memory impairments following developmental nicotine exposure. It remains possible, however, that undesired effects due to α2 gene deletion confound the normal development of hippocampal circuits, affecting CA1 function.

We also found that unlike maternal-nicotine-treated wild-type mice, both maternal-saline-treated and maternal-nicotine-treated α2KO mice did not show unusually high CA1 depolarization following SC stimulation or facilitated LTP at SC-CA1 synapses. These findings indicate that it is not the downregulation of α2* nAChRs that causes increased CA1 network activity and facilitated LTP, but the nicotine-induced activation of α2* nAChRs during development does appear to be necessary to trigger increased CA1 activity and facilitated LTP. Additionally, because both maternal-nicotine-treated wild-type mice and un-

treated α2KO mice have hippocampus-dependent memory impairments, yet α2KO mice have normal responses to SC stimulation as saline-treated wild-type mice, the abnormal CA1 activity observed following early postnatal nicotine exposure is not likely to be the cause of the nicotine-induced memory deficits.

Because LTP is thought to be one of the cellular mechanisms underlying hippocampus-dependent memory, we had expected that we would see an association of memory impairments with impaired LTP. However, early nicotine exposure resulted in facilitated LTP, suggesting that the observed memory impairments are driven by a different mechanism or form of synaptic plasticity. Furthermore, unlike maternal-nicotine-treated wild-type mice, α2KO mice and maternal-nicotine-exposed α2KO mice did not demonstrate facilitated LTP, suggesting that facilitated LTP is not likely the cause of the maternal-nicotine-induced memory impairments. However, we would not rule out some association of facilitated LTP with impaired memory, because the lower LTP threshold could contribute to behavioral impairments by strengthening synapses which might compete with those required for object location memory.

Our previous study showed that postnatal nicotine exposure induces pervasive changes to the nicotinic modulation of CA1 activity: acute nicotine no longer increases CA1 network excitation, acute nicotine inhibits rather than facilitates LTP induction at the SC pathway by recruiting an additional nicotinic receptor subtype, and acute nicotine no longer blocks LTP induction at the TA pathway (Nakauchi et al., 2015). Interestingly, responses to acute nicotine in hippocampal slices from α2KO mice are very similar to what we observed in maternal-nicotine-treated mice: acute nicotine no longer increases CA1 network excitation, acute nicotine no longer facilitates LTP induction at the SC pathway, and acute nicotine no longer suppresses LTP induction at the TA pathway (Nakauchi et al., 2007). Thus, impaired nicotinic modulation of CA1 activity by postnatal nicotine exposure could also be explained by the functional loss of α2* nAChRs in OLM cells. Together, these findings suggest a critical role of the functional loss of α2* nAChRs in mediating developmental-nicotine-induced hippocampal memory deficit and associated physiological changes.

Given the complexity of nicotine's action on the brain, α2* nAChRs are likely not the only nAChR subtype involved in mediating the memory impairment induced by early postnatal nicotine exposure. Several other nAChR subtypes are abundantly located on both interneurons and projection neurons in the hippocampus, and there is evidence that some of those subtypes are also affected by developmental nicotine exposure. Early postnatal nicotine treatment in rodents increases hippocampal binding of epibatidine (Huang and Winzer-Serhan, 2006; Huang et al., 2007), an nAChR ligand that binds strongly to α4β2* nAChRs (Sullivan and Bannon, 1996), and nicotine also may decrease binding of low-affinity nAChRs (Nordberg et al., 1991), which include α7 nAChRs, the most common nAChR subtype in the hippocampus. Furthermore, α2* nAChRs are not the only nAChR subtype mediating OLM cell activity or TA pathway inhibition – it has also been shown that the activation of OLM cells by fimbria fornix pathway stimulation is partially mediated by α7 nAChRs (Leao et al., 2012). However, both α7 knockout mice and β2 knockout mice (which would lack the second-most abundant hippocampal nAChR subtype, α4β2) show normal memory in the hippocampus-dependent Morris water maze task (Zoli et al., 1999;

Paylor et al., 1998). It must be noted, though, that as with all studies using knockout animals, including this one, the impact of confounding compensatory effects cannot be ruled out. Therefore, though this work strongly suggests that α2* nAChRs play a critical role in the hippocampal memory impairments induced by early life nicotine exposure, it remains possible that other nAChR subtypes are also involved.

One behavioral difference that we did observe between α2KO mice and our previous findings with maternal-nicotine-treated wild-type mice (Nakauchi et al., 2015) was that the knockout mice, unlike the nicotine-treated mice, did not show increased anxiety. In fact, α2KO mice seemed less anxious than wild-type mice, though it should be noted that others using this mouse line did not observe any difference in anxiety compared to controls (Lotfipour et al., 2013). Regardless, this data suggests that α2* nAChRs do not mediate the increased anxiety observed following perinatal nicotine exposure in rodents (Vaglenova et al., 2004; Nakauchi et al., 2015). Increased anxiety could be the result of many factors, including altered maternal behavior during rearing. Though for this behavior study we fostered both the wild-type and α2KO pups with CD1 dams to ensure consistent maternal care, the increased anxiety observed in maternal-nicotine-treated mice may have been due to changes in the dams' conduct while they were exposed to, and eventually withdrawn from, nicotine (Vagelnova et al., 2004). Thus, we are unable to exclude the possibility that alterations in maternal care caused by nicotine exposure and withdrawal to dams contribute to the long-term memory impairment for object location, although there were no obvious abnormal maternal behaviors (such as nest building, nursing, retrieval, and grooming) of dams.

There are still a great many questions that remain about the effects of developmental nicotine exposure and the role of α2* nAChRs. Although the functional loss of α2* nAChRs is a likely cause of the hippocampal memory impairments induced by developmental nicotine exposure, it still needs to be determined whether it is the absence of α2* nAChRs during hippocampal development, when neuronal networks are being established (Zancanaro et al., 2001; Danglot et al., 2006; Dwyer et al., 2009), that affects the later ability to form spatial memories, or if it is the lack of α2* nAChRs at the time of memory formation itself that is important. Furthermore, though we showed that the activation of α2* nAChRs is required for early-postnatal-nicotine-induced facilitation of LTP and for heighted CA1 network activity, the mechanism underlying them remains to be understood. Because enhanced CA1 network activation correlates with the facilitation of LTP induction (Nakauchi et al., 2007; Nakauchi and Sumikawa, 2012) and this association of facilitated LTP with enhanced CA1 network activity also occurs in maternal-nicotine-treated wild-type mice (Nakauchi et al., 2015) but not maternal-nicotine-treated α2KO mice, these two changes may arise from the same cellular mechanism requiring α2* nAChR activation.

This study is thus an important first step in understanding the significant role that α2* nAChRs play in hippocampal memory, and that their absence plays in the long-term impacts of early life nicotine exposure.

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Highlights

α2KO mice have impaired hippocampus-dependent memory

Maternal-nicotine-exposed α2KO mice do not show increased CA1 network excitation

Maternal-nicotine-treated α2KO mice do not have facilitated induction of LTP

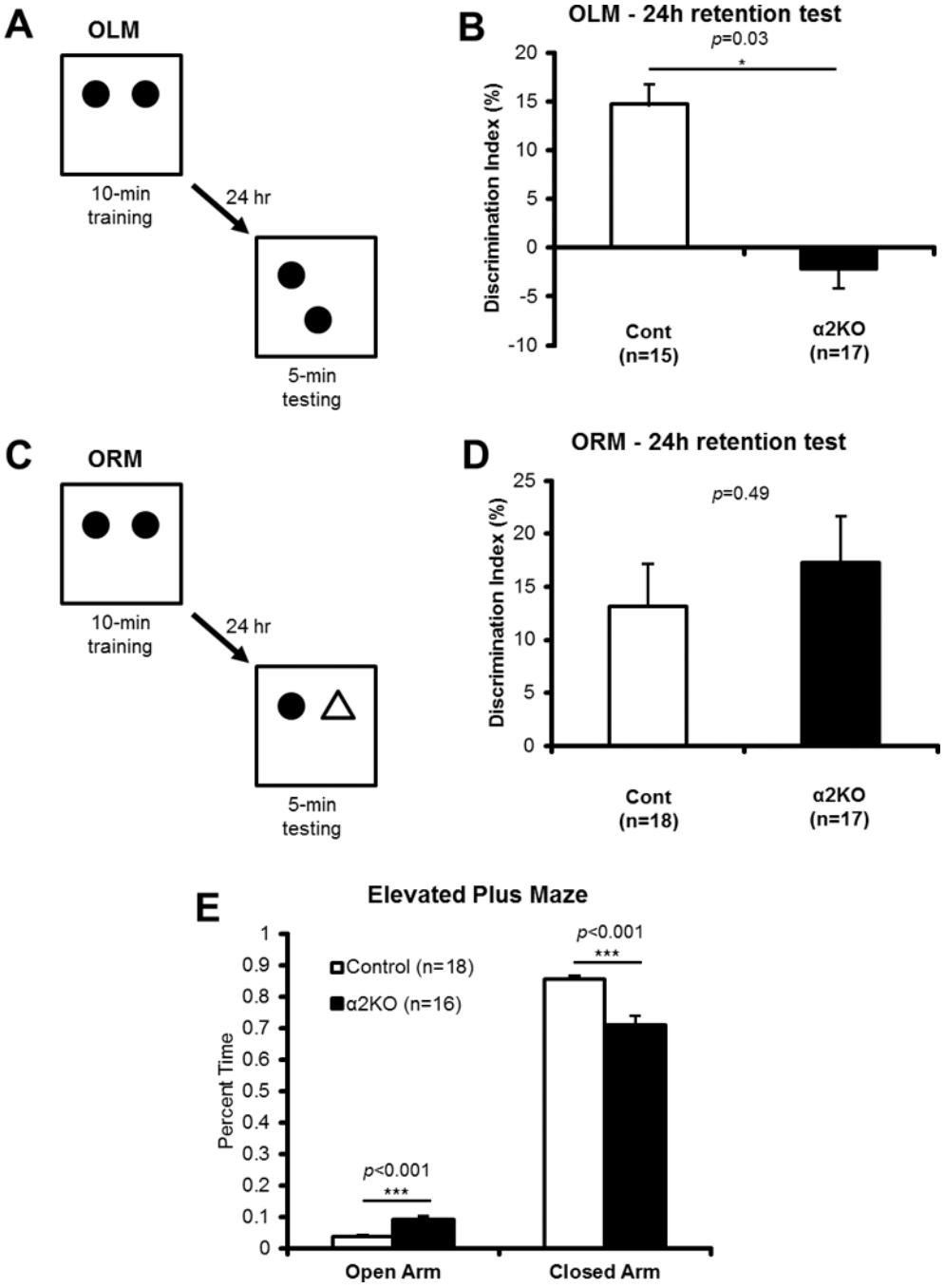

Figure 1.

α2 knockout mice have impaired long-term spatial memory and decreased anxiety. (A) For the hippocampus-dependent object location memory (OLM) task, mice were trained for 10 min with two identical objects, and tested 24 h later with one object moved to a new location. (B) α2KO mice showed significantly impaired 24-h long-term OLM compared to controls, and had a discrimination index not significantly different from zero. (C) For the hippocampus-independent object recognition memory (ORM) task, mice were trained for 10 min with two identical object, and tested 24 h later after one object was replaced by a novel

item. (D) In the ORM task, there was no difference in the 24-h long-term memory demonstrated by α2KO mice and controls. (E) α2KO mice spent significantly more time in the open arms of the elevated plus maze than did control mice, demonstrating decreased anxiety.

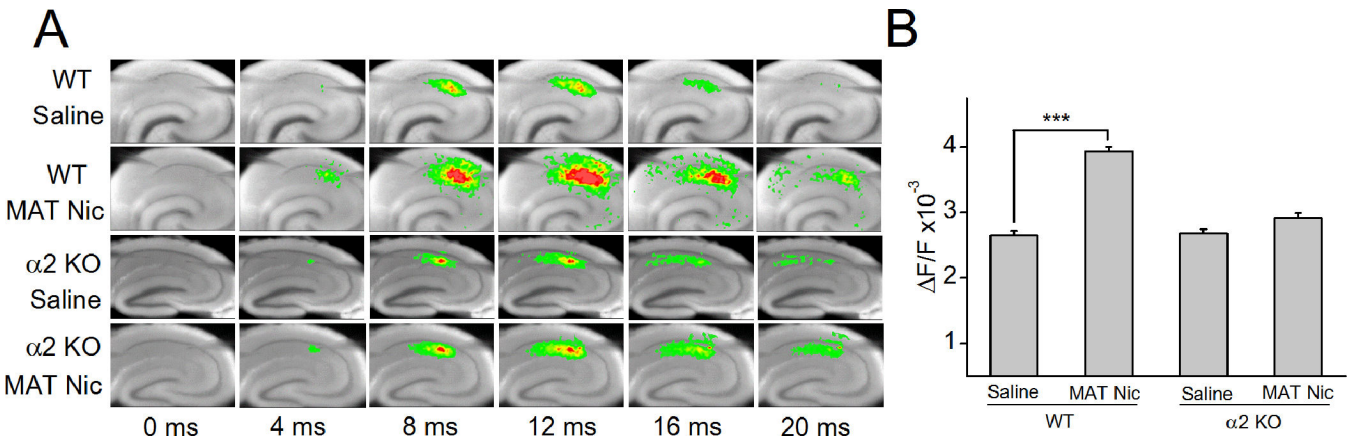

Figure 2.

Maternal nicotine exposure increases CA1 depolarization from Schaffer collateral stimulation in wild-type, but not α2 knockout mice. (A) Time courses of voltage-sensitive dye imaging detecting changes in neuronal activity following Schaffer collateral stimulation. Representative pseudocolor images of ΔF/F signals in saline-treated wild-type (WT), maternal-nicotine-treated wild-type, saline-treated α2KO and maternal-nicotine-treated α2KO mice. Maternal-nicotine-treated α2KO mice do not show the increase in depolarization detected in maternal-nicotine-treated wild-type mice. (B) Peak optical signals were significantly stronger in maternal-nicotine-treated wild-type mice than in saline-treated wild-type mice. There was no significant difference between the optical responses recorded from saline-treated α2KO and maternal-nicotine-treated α2KO mice. ***$p < 0.001$

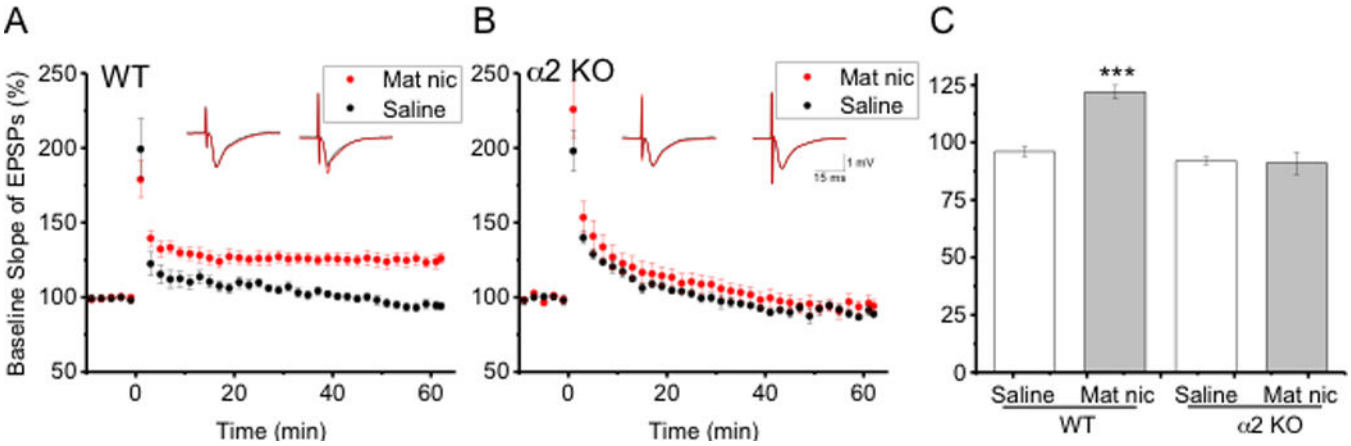

Figure 3.

Maternal-nicotine-induced facilitation of LTP occurs in wild-type, but not α2 knockout mice. (A, C) Weak TBS does not induce LTP in hippocampal slices from saline-treated wild-type (WT) mice, but does induce LTP in slices from maternal-nicotine-treated wild-type. However, (B, C) weak TBS does not induce LTP in hippocampal slices from maternal-saline-exposed and maternal-nicotine-treated α2KO mice. In A and B, changes in the slope of fEPSPs are plotted as the percentage change from initial baseline responses. Weak TBS was delivered at time 0. Traces above the graph in A and B are representative waveforms recorded before (black) and 50 min after (red) LTP-inducing stimulation. ***$p < 0.001$.