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Early postnatal nicotine exposure disrupts the α2* nicotinic acetylcholine receptor-mediated control of oriens-lacunosum moleculare cells during adolescence in rats

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Abstract

Maternal cigarette smoking during pregnancy and maternal nicotine exposure in animal models are associated with cognitive impairments in offspring. However, the underlying mechanism remains unknown. Oriens-lacunosum moleculare (OLM) cells expressing α2* nicotinic acetylcholine receptors (nAChRs) are an important component of hippocampal circuitry, gating information flow and long-term potentiation (LTP) in the CA1 region. Here we investigated whether early postnatal nicotine exposure alters the normal role of α2*-nAChR-expressing OLM cells during adolescence in rats. We found that early postnatal nicotine exposure significantly decreased not only the number of α2-mRNA-expressing interneurons in the stratum oriens/alveus, but also α2*-nAChR-mediated responses in OLM cells. These effects of nicotine were prevented by co-administration with the nonselective nAChR antagonist mecamylamine, suggesting that nicotine-induced activation, but not desensitization, of nAChRs mediates the effects. α2*-nAChR-mediated depolarization of OLM cells normally triggers action potentials, causing an increase in spontaneous inhibitory postsynaptic currents in synaptically connected pyramidal cells. However, these α2*-nAChR-mediated effects were profoundly reduced after early postnatal nicotine exposure, suggesting altered control of CA1 circuits by α2*-nAChR-expressing OLM cells. Furthermore, these effects were associated with altered excitatory neural activity and LTP as well as the loss of normal α2*-nAChR-mediated control of excitatory neural activity and LTP. These findings suggest the altered function of α2*-nAChR-expressing OLM cells as an important target of further study for identifying the mechanisms underlying the cognitive impairment induced by maternal smoking during pregnancy.

Keywords

α2 nicotinic acetylcholine receptor; OLM cells; LTP; nicotine; development; hippocampus

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1. Introduction

Maternal smoking during pregnancy elevates the risk of attentional and cognitive deficits in children (Fried et al., 2003; Thompson et al., 2009; Heath and Picciotto, 2009), but little is known about the mechanisms. Animals exposed to prenatal or postnatal nicotine have lasting deficits in hippocampus-dependent behaviors (Ankarberg et al., 2001; Eppolito and Smith, 2006; Sorenson et al., 1991; Vaglenova et al., 2004; Yanai et al., 1992; Portugal et al., 2012; Nakauchi et al., 2015), supporting the view that nicotine, the principle neuroactive component of tobacco, plays a major role in the detrimental consequences of maternal smoking.

α2* nicotinic acetylcholine receptors (nAChRs) show distinct localization in a subset of GABAergic interneurons in the stratum oriens/alveus (O/A) (Ishii et al., 2005; Wada et al., 1989; Son and Winzer-Serhan, 2006; Jia et al., 2010). The majority of these interneurons are somatostatin-positive oriens-lacunosum moleculare (OLM) cells (Jia et. al., 2009, 2010; Leao et al., 2012) that receive cholinergic inputs from the medial septum and are involved in the modulation of brain-state-specific network oscillations (Leao et al., 2012; Pangalos et al., 2013; Lovett-Barron et al., 2014). In the CA1, OLM cell dendrites extend horizontally within the stratum oriens, and their axons ramify extensively in the stratum lacunosum moleculare, where they innervate the distal dendritic tufts of pyramidal cells. These distal dendrites are the terminus of the temporoammonic (TA) pathway from the entorhinal cortex, one of the two major excitatory inputs to CA1 pyramidal cells. Activation of α2*-nAChR-expressing O/A interneurons inhibits the TA pathway while disinhibiting the Schaffer collateral (SC) pathway, another excitatory input to CA1 pyramidal cells (Nakauchi et al., 2007; Leao et al., 2012). Moreover, their activation suppresses LTP at the TA pathway while facilitating LTP at the SC pathway (Nakauchi et al., 2007; Leao et al., 2012). Thus, activation of these α2*-nAChR-containing O/A interneurons can shift the balance of information flow into the CA1 region, decreasing direct entorhinal cortex inputs (from the TA pathway - entorhinal cortex layer III → CA1) in favor of indirect inputs (from the SC pathway - entorhinal cortex layer II → dentate gyrus → CA3 → CA1). This change could enhance rapid contextual learning (Nakashiba et al., 2008). The significant role of somatostatin-positive OLM cells in hippocampal function is highlighted by the recent finding that inactivating these cells prevents contextual fear learning in mice (Lovett-Barron et al., 2014), perhaps due to unregulated sensory information flow into CA1 pyramidal cells from the entorhinal cortex. These previous findings suggest that early postnatal nicotine exposure-induced changes in the normal function of α2*-nAChR-containing OLM cells could affect hippocampal-dependent behavior.

Importantly, the expression of α2 mRNAs in O/A interneurons reaches peak levels during the early postnatal period (~P2–15), which is a critical stage for hippocampal development (Son and Winzer-Serhan, 2006). Furthermore, unlike other nAChR subtypes, α2* nAChRs remain activated in the presence of nicotine (Jia et al., 2009). Thus, early postnatal nicotine exposure could inappropriately activate α2* nAChRs during critical postnatal development to disturb normal functioning of α2*-nAChR-containing OLM cells in later life. In the current study, we exposed early postnatal rats to nicotine through maternal milk, the treatment that impairs hippocampal CA1-dependent memory in adolescent mice (Nakauchi

et al., 2015), and examined the changes in the function of α2*-nAChR-containing OLM cells during adolescence in rats. In addition, we used the nonselective nAChR antagonist mecamylamine to investigate whether the effect of early postnatal nicotine exposure is due to nicotine-induced activation of nAChRs or blocking endogenous ACh signals by inducing nAChR desensitization.

2. Materials and methods

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 numbers of animals used.

2.1 Drug treatment

Rat litters (Sprague-Dawley, Charles River) were adjusted to eight male or female pups, and were exposed to nicotine through maternal milk during postnatal days 1–15 by subcutaneously implanting lactating dams with osmotic minipumps (approximate nicotine output: 6 mg/kg/day; Alzet model 2ML2, Durect Corporation, Cupertino, CA). Here, we refer to these pups as maternal-nicotine-exposed rats. Others using this model, have previously reported that 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). Naïve rats and rat pups from dams implanted with saline-containing minipumps were used as controls. Both controls yielded equivalent results, thus data obtained from those groups were combined for statistical analysis. Also, because initial data obtained from electrophysiological recordings were indistinguishable between male and female rats, all data were combined for statistical analysis. In some experiments, pups were exposed to nicotine (6 mg/kg/day) plus mecamylamine (2 mg/kg/day) or mecamylamine (2 mg/kg/day) alone as described above for nicotine exposure.

2.2 Slice preparation

Transverse hippocampal slices (300–400 μm) were prepared from control and maternal-nicotine-exposed adolescent and rats anesthetized with isoflurane or urethane. Slices were maintained at 30°C for at least 1 h before recordings to recover in artificial cerebrospinal fluid (ACSF) as described previously (Yamazaki et al., 2005; Nakauchi et al., 2007, 2015; Jia et al., 2009).

2.3 Electrophysiology

Current- and voltage-clamp recordings were made from the somatic region of pyramidal cells and interneurons as described previously (Yamazaki et al., 2005, 2006; Jia et al., 2009). Slices were placed in a recording chamber, submerged and continuously perfused at ~2 ml/min with oxygenated ACSF at 30°C. Neurons were visualized using a 40x water-immersion objective and infrared differential interference contrast (IR-DIC) system (Axioskop, Zeiss, Germany). Voltage-clamp recordings were obtained using an Axopatch-1D amplifier (Molecular Devices, Union City, CA) and current-clamp recordings

were obtained using an Axoclamp 2B amplifier (Molecular Devices). The patch pipettes had a resistance of 3–5 MΩ after being filled with pipette solution. For spontaneous inhibitory postsynaptic current (sIPSC) recordings, the pipette solution consisted of (in mM): 140 CsCl, 2 $MgCl_2$, 10 HEPES, 3 QX314, 1 MgATP, and 0.2 Na_2GTP. To record nicotine-induced responses, whole-cell voltage-clamp recordings (holding potential = −60 mV) were carried out in the presence of the glutamatergic antagonists 6,7-dinitroquinoxaline-2,3-dione (DNQX; 20 μM) and 2-amino-5-phosphonovaleric acid (APV; 20 μM), and the GABAergic antagonist bicuculline (10 μM). For these recordings, pipette solution contained (in mM): 117 Cs-methanesulfonate, 10 HEPES, 0.5 EGTA, 2.8 NaCl, 5 TEA-Cl, 5 QX314, 2.5 MgATP and 0.3 NaGTP. For current-clamp recordings, the pipette solution contained (in mM): 120 K-gluconate, 120, 10 KCl, 2 ATP, 0.2 Na-GTP, 2 MgCl2, 10 HEPES, 10 EGTA. All pipette solutions were adjusted to pH 7.2–7.25 and 265–275 mOsm. For extracellular field recordings, a bipolar stimulating electrode was placed at the SC pathway. Field excitatory postsynaptic potentials (fEPSPs) were recorded from the stratum radiatum of the CA1 region using glass electrodes filled with ACSF (3–8 MΩ) as previously described (Nakauchi et al., 2007, 2015). LTP was induced by weak theta burst stimulation (TBS; two theta bursts of four pulses at 100 Hz). To evaluate the magnitude of LTP, the mean value for the slope of fEPSPs recorded at 50–55 min after stimulation 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).

2.4 Voltage-sensitive dye imaging

Voltage-sensitive dye (VSD) imaging was performed as previously described (Nakauchi et al., 2007, 2015; Tominaga et al., 2000). Briefly, slices loaded with the voltage-sensitive dye Di-4-ANEPPS (Molecular Probes, Carlsbad, CA, USA) 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). 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. Images were displayed and analyzed using an acquisition and analysis software (BV-Analyser; BrainVision), and peak depolarization amplitudes were measured and compared. The magnitude of voltage changes was also illustrated using pseudocolor.

2.5 In situ hybridization

The localization of α2-mRNA-positive cells by non-radioactive in situ hybridization was carried out as previously described (Ishii et al., 2005). To enhance the sensitivity, α2 mRNA was detected using a digoxigenin-labeled riboprobe, anti-digoxigenin-horseradish peroxidase (Roche) and Tyramine signal amplification Plus Cyanine 3 reagent (Perkin Elmer). For quantification, representative coronal sections containing either dorsal or ventral hippocampi were selected at two positions (around plate 61 and plate 79) along the septotemporal axis according to Paxinos and Watson rat brain atlas. All fluorescent images

were captured using the fluorescence microscope (BIOREVO, Keyence). Identical exposure times were used for sections that were compared. The number of fluorescently labeled cells in the stratum oriens-alveus of the CA1 region was counted using 20x magnification for analysis.

2.6 Drugs

APV, DNQX, bicuculline and mecamylamine were obtained from Abcam Biochemicals (Cambridge, MA). Nicotine and other chemicals were obtained from Sigma (St. Louis, MO).

2.7 Data Analysis

Whole-cell recordings were filtered at 2 kHz and digitized at 10 kHz with a Digidata 1200 analog-digital interface (Molecular Devices) for analysis. Data were analyzed off-line using Clampfit 10.2 (Molecular Devices). Recorded sIPSCs were also analyzed using Mini Analysis (Synaptosoft Inc., Fort Lee, NJ). Events detected when recording sIPSCs were analyzed, and any events that spuriously met trigger specifications were rejected as noise. Cumulative probability distributions of spontaneous IPSCs were constructed by pooling 100 events from each cell, and statistically analyzed using Kolmogorov-Smirnov test. Data containing two groups were analyzed using Student's t-test. Data containing more than two groups were statistically analyzed using a one-way ANOVA and a post-hoc Tukey test. Statistical analyses were performed using OriginPro 8.1 (OriginLab, Northampton, MA, USA), SigmaPlot or SPSS for Windows (SPSS, Chicago, IL), with a level of significance of $p < 0.05$. Data were presented as mean ± SEM, and "n" is the number of recorded cells or brain slices.

3. Results

Experiments described below used adolescent (30–45 days old) and adult (84–98 days old) rats that had been exposed to nicotine through lactating dams during postnatal days 1–15.

3.1 Maternal nicotine exposure decreases the number of α2-mRNA-expressing O/A interneurons in adolescence

To gain insight into the impact of maternal nicotine exposure on α2*-nAChR-expressing O/A interneurons, we initially examined whether maternal nicotine exposure altered the number of α2-mRNA-expressing O/A interneurons in adolescent rats, as detected by in situ hybridization (Ishii et al., 2005; Jia et al., 2010). As previously demonstrated, α2-mRNA-positive cells were uniquely localized in the O/A region of the saline-exposed control hippocampi with variable signal intensity (Fig. 1). Maternal nicotine exposure significantly decreased the number of α2-mRNA-positive cells in both the dorsal (Fig. 1A–D; saline, 77±4, n=8, vs. maternal nicotine, 66±3, n=8, $p<0.01$) and ventral (Fig. 1F–I; saline, 137±4, n=8, vs. maternal nicotine, 87±6, n=8, $p<0.001$) hippocampus. This effect of maternal nicotine exposure was prevented by maternal co-administration of the nAChR antagonist mecamylamine with nicotine in both the dorsal (Fig. 1A–E; maternal nicotine, 66±3, n=8, vs. maternal nicotine + mecamylamine, 84±5, n=8, $p<0.001$) and ventral (Fig. 1F–J; maternal nicotine, 87±6, n=8, vs. maternal nicotine + mecamylamine, 122±6, n=8, $p<0.001$) hippocampus. These findings imply that the activation of α2* nAChRs by maternal nicotine

causes lasting decreases in α2 mRNA expression in O/A interneurons. The reduced number of α2-mRNA-positive cells could reflect the down-regulation of α2 gene expression, decreased numbers of α2-mRNA-expressing O/A interneurons, or both. We have previously reported that α2 mRNA-expressing cells also contain somatostatin mRNA (jia et al., 2010) and our recent study showed that maternal nicotine exposure does not result in a decrease in the number of somatostatin mRNA-containing cells (unpublished results). This might suggest that a decrease in the number of α2 mRNA-expressing cells observed in the current study is due to the down-regulation of α2 gene expression.

3.2 Maternal nicotine exposure decreases α2* nAChR function in O/A interneurons in adolescence

Subsequently, we examined whether maternal nicotine exposure caused the decrease in α2*-nAChR-mediated responses in O/A interneurons of adolescent rats. We have previously used IR-DIC visualization to target horizontally oriented interneurons at the O/A border for whole-cell recoding and single-cell RT-PCR, and found that these cells show non-desensitizing responses to nicotine, and that they contain α2 but not a4 mRNA (Jia et al, 2009). In separate experiments, we stained these interneurons with biocytin and showed that they displayed OLM cell morphology (Jia et al, 2010). Thus, we targeted these cells for whole-cell recording after early-life nicotine exposure. Recorded cells showed large input resistance, marked afterhyperpolarization and obvious voltage sag, which are also features of OLM cells. In the cells from saline-exposed adolescent rats, bath application of nicotine (10 μM) produced long-lasting inward currents (Fig. 2A, B; mean amplitude, 86.9±32 pA, n=8), a characteristic response associated with α2-mRNA-expressing O/A interneurons in naïve rats (Jia et al., 2009). However, in maternal-nicotine-exposed cells, the mean amplitude of elicited currents was significantly smaller (Fig. 2A, B; 6.8±15.2 pA, n= 8, $p<0.05$). This effect of maternal nicotine exposure was prevented by maternal co-administration of mecamylamine with nicotine (Fig. 2A, B; 84.6±11.5 pA, n=12, $p<0.05$), and was not mimicked by maternal mecamylamine exposure alone (Fig. 2A, B; mean amplitude, 80.7±9.7 pA, n=7, $p<0.05$). These findings demonstrate that the decreased number of α2-mRNA-expressing O/A interneurons is associated with the functional loss of α2 nAChRs. Furthermore, these results indicate that these observed effects of maternal nicotine exposure arise as a consequence of inappropriate nAChR activation during maternal nicotine exposure, and not nicotine-induced nAChR desensitization preventing the activation of nAChRs by endogenous ACh. We have previously reported a nicotine concentration-response analysis of α2 nAChRs on O/A interneurons, demonstrating that nicotine at 10 μM elicits maximum responses and 30 μM or 100 μM nicotine causes no further increases in responses (Jia et al., 2009). Based on these findings, we selected a nicotine concentration of 10 μM in the current study. Because nicotine at 10 μM elicited almost no α2 nAChR-mediated responses after maternal nicotine exposure, we did not use a higher nicotine concentration. Thus, it remains possible that the almost complete loss of α2 nAChR-mediated responses involves an unusually large decrease in agonist sensitivity and accelerated desensitization.

We also performed similar experiments with saline- and maternal-nicotine-exposed rats at a later age (P84–98), and found that nicotine-induced inward currents were also significantly

smaller in O/A interneurons from maternal-nicotine-exposed rats (Fig. 2C, D; mean amplitude, 8.8±3.4 pA, n= 8) than in those from saline-exposed control rats (Fig. 2C, D; mean amplitude, 63.1±11.3 pA, n= 8, $p<0.05$). These data demonstrate that the effect of maternal nicotine exposure persists at least into adulthood.

The observed difference in nicotinic responses between saline- and maternal-nicotine-exposed O/A interneurons could reflect on firing action potentials by α2-nAChR-mediated depolarization in these interneurons. Indeed, bath application of 10 μM nicotine caused significant increases in firing action potentials in saline-exposed controls, but not in maternal-nicotine-exposed O/A interneurons (Fig. 2E, F; maximum fold change, control, 6.3±2.4, n=13, vs. maternal-nicotine-exposed, 1.27±0.2, n=13, $p<0.05$), suggesting that maternal nicotine exposure alters nicotinic cholinergic control of GABA release from α2*-nAChR-expressing O/A interneurons onto postsynaptic membrane domains. Under the conditions, O/A interneurons recorded in control and maternal-nicotine-exposed rats exhibited similar resting membrane potentials (control, −47.5±1.6 mV, n=13; maternal-nicotine-exposed, −46.6±1.2 mV, n=13, no significant difference), spontaneously fired action potentials (control, 5.2±1.3 Hz; maternal-nicotine-exposed, 6.1±1.8 Hz, no significant difference), and similar action potential firing thresholds (control, −49.2±0.8 mV; maternal-nicotine-exposed, −47.8±0.9 mV, no significant difference).

3.3 Maternal nicotine exposure disrupts the inhibition of CA1 pyramidal cells by α2*-nAChR-expressing O/A interneurons in adolescence

The above findings indicate that maternal nicotine exposure alters the normal functioning of α2 nAChRs in O/A interneurons. However, because our recordings were targeted to horizontally oriented interneurons at the O/A border and there remain many α2-mRNA-positive O/A interneurons in maternal-nicotine-exposed adolescent rats, it is possible that the recorded interneurons were a subpopulation of α2-nAChR-expressing O/A interneurons selectively affected by maternal nicotine exposure. To gain further insight into maternal-nicotine-induced changes in the normal function of α2 nAChRs in O/A interneurons, we subsequently recorded spontaneous IPSCs (sIPSCs), which are a postsynaptic measure of the firing of GABAergic interneurons converging on the recorded cell. Because α2-nAChR-expressing O/A interneurons are synaptically connected to pyramidal cells, and bath application of nicotine increases sIPSCs in CA1 pyramidal cells via the activation of O/A interneurons (Jia et al., 2009), this postsynaptic measure could be used to determine the overall impact of maternal nicotine exposure on the function of α2 nAChRs in O/A interneurons connected to pyramidal cells. There were small, but statistically significant, differences in both baseline sIPSC frequency and amplitude between control and maternal-nicotine-exposed CA1 pyramidal cells from adolescent (P30–45) rats (Fig. 3A–C). The mean inter-event interval of sIPSCs in maternal-nicotine-exposed rats (Fig. 3B; 35.1±1.4 msec, n=12) was shorter than in saline-treated controls (Fig. 3B; 46.1±6.3 msec, n=12, $p<0.05$), but average sIPSC amplitude was smaller in maternal-nicotine-exposed rats (Fig. 3C; 39.0±1.7 pA, n=12) than that of controls (Fig. 3C; 44.8±2.5 pA, n=12, $p<0.05$). More dramatic differences in sIPSCs between saline- and maternal-nicotine-exposed hippocampi were observed during bath application of 10 μM nicotine, which is known to increase sIPSCs in CA1 pyramidal cells in naïve rats via the activation of nAChRs in the O/A region

(Jia et al., 2009). In saline-treated hippocampi, bath application of nicotine significantly shortened the mean inter-event interval of sIPSCs (Fig. 3B; baseline, 46.1±6.3 msec vs. bath nicotine, 33.1±3.9 msec, n=12, $p<0.05$) and increased the mean amplitude of sIPSCs (Fig. 3C; baseline, 44.8±2.5 pA vs. bath nicotine, 72.7±7.2 pA, n=12, $p<0.05$). In contrast, in maternal-nicotine-exposed hippocampi, bath application of nicotine had no significant effects on either mean inter-event interval of sIPSCs (Fig. 3B; baseline, 35.1±1.4 msec vs. bath nicotine, 34.1±1.7 msec, n=12), or the amplitude of sIPSCs (Fig. 3C; baseline, 39.0±1.7 pA vs. bath nicotine, 41.2±2.2 pA, n=12). Cumulative probability distributions of sIPSC inter-event interval and amplitude confirmed these differences (Fig. 3D, E). These findings indicate that maternal nicotine exposure uniformly affects the normal function of all α2-nAChR-expressing O/A interneurons connected to pyramidal cells.

We then examined whether maternal co-application of mecamylamine with nicotine, which blocked the maternal-nicotine-induced decreases in nicotinic responses in O/A interneurons (Fig. 2B), prevented the loss of nicotinic control of sIPSCs. We found that bath application of nicotine caused significant increases in sIPSCs in maternal mecamylamine co-exposed pyramidal cells (Fig. 3F; % change of maximum charge transfer, 150.2±10.7%, n=9) as found for saline-exposed pyramidal cells (Fig. 3F; % change, 159.6±14.6%, n=12). However, this increase was significantly larger than in maternal-nicotine-exposed pyramidal cells (Fig. 3F; % change, 106.9 ±6 .9%, n=16, $p<0.05$), indicating that mecamylamine blocked the effect of maternal nicotine exposure. Furthermore, we found that maternal mecamylamine exposure alone had no effect on the nicotinic control of sIPSCs. Thus, bath nicotine caused significant increases in sIPSCs that is comparable to those observed in controls and mecamylamine and nicotine co-administered pyramidal cells, but the increase was significantly larger than in maternal-nicotine-exposed pyramidal cells (Fig. 3F; % change, maternal mecamylamine, 146±9.5%, n=7, vs. maternal nicotine, 106.9 ±6 .9%, n= 16, $p<0.05$). Taken together, our results strongly suggest that the maternal nicotine's effect on the nicotinic control of sIPSCs stems from the altered function of α2-nAChRs on O/A interneurons.

We also investigated whether the altered nicotinic control of sIPSCs observed in maternal-nicotine-exposed rats at P30–45 was still maintained in adulthood, at P84–98. We found that bath application of nicotine caused a significant increase in sIPSCs in CA1 pyramidal cells from control rats, but, as in P30–45 days old rats, bath nicotine had no significant effect in O/A interneurons from older maternal-nicotine-exposed rats (Fig. 3G, H; % change, control, 162±15%, n=8, vs. maternal nicotine, 115±8%, n= 11, $p<0.05$). These data demonstrate that altered nicotinic control of sIPSCs in pyramidal cells persists well beyond adolescence.

3.4 Maternal nicotine exposure affects LTP induction and α2*-nAChR-expressing O/A-interneuron-mediated control of LTP

α2*-nAChR-expressing O/A interneurons are important for gating LTP in the CA1 region (Nakauchi et al., 2007; Leao et al., 2012). This control mechanism is disrupted in maternal-nicotine-exposed adolescent mice, which show CA1-dependent memory impairment (Nakauchi et al., 2015). Because our data demonstrated that maternal nicotine exposure disrupted the normal function of α2*-nAChR-expressing O/A interneurons, we subsequently

assessed maternal nicotine's effects on LTP at the SC pathway in adolescent rats (P30–45). There were no significant differences in the stimulus intensity-response relationships of field excitatory postsynaptic potentials (fEPSPs) between saline- and maternal-nicotine-exposed rats (Fig. 4A), indicating that basal glutamatergic synaptic transmission was not significantly affected by maternal nicotine treatment. However, we found that delivery of weak TBS, which is subthreshold for LTP induction in saline-treated control hippocampi (Fig. 4B, C; 97.2±2.4%, n=4), induced small, but statistically significant, LTP in maternal-nicotine-exposed hippocampi (Fig. 4B, C; 108.6±3.3 %, n=10, $p<0.05$). Thus, maternal nicotine exposure caused the facilitation of LTP induction as in maternal-nicotine-exposed adolescent mice (Nakauchi et al, 2015). Endogenous ACh-mediated activation of α2*-nAChR-containing O/A interneurons does not occur during LTP-inducing stimulation in the SC pathway, but the role of α2*-nAChR-containing O/A interneurons in modulating LTP becomes visible when α2*nAChRs are activated by bath application of nicotine (Nakauchi et al, 2007). Thus, in saline-treated hippocampi, weak TBS induced LTP in the presence, but not in the absence, of nicotine (Fig. 4B, C; control, 97.2±2.4%, n=4, vs. bath nicotine, 121.9±3.1 %, n=5, $p<0.05$), confirming the facilitative role of α2*-nAChR-containing O/A interneuron activation in LTP induction in naïve mice (Nakauchi et al., 2007; Leao et al., 2012). However, in hippocampal slices from maternal-nicotine-exposed rats, when the same weak TBS, which alone induces LTP in these slices, was delivered in the presence of nicotine (1 μM), LTP induction was blocked (Fig. 4B, C; control, 108.6±3.3 %, n=10, vs. bath nicotine, 96.2±1.7 %, n=8, $p<0.05$), as is observed with maternal-nicotine-exposed adolescent mice (Nakauchi et al, 2015). Thus, bath nicotine elicits opposing effects on LTP induction in saline- and maternal-nicotine-exposed hippocampi. These findings indicate that maternal nicotine exposure significantly disrupts the normal α2*-nAChR-containing O/A interneuron-mediated control of LTP induction, which is associated with impaired CA1-dependent memory in adolescent mice (Nakauchi et al, 2015).

3.5 Maternal nicotine exposure alters CA1 circuit activity and its nicotinic modulation

By monitoring CA1 network activity triggered by SC stimulation with VSD imaging, we have previously demonstrated that the enhanced intensity of excitatory neural activity correlates with facilitation of LTP induction at SC synapses of mice (Nakauchi et al, 2007; Nakauchi and Sumikawa, 2012). Indeed, facilitated LTP in maternal-nicotine-exposed adolescent mice was associated with enhanced excitatory neural activity (Nakauchi et al, 2015). Thus, to determine whether enhanced LTP induction in maternal-nicotine-exposed rat hippocampi is also associated with increased excitatory neural activity, we next used VSD imaging. We evoked excitatory activity by single electrical stimulation in the SC pathway, intensity of which was adjusted to elicit similar amplitudes of fEPSPs in different slices from saline- and maternal-nicotine-exposed rats. Such stimulation triggered the spread of excitatory neural activity in all anatomical layers of the CA1, which can be presented as traces or as pseudocolor images of the ΔF/F signals (Fig. 5A–C). We found that depolarizing responses originating from the site of stimulation peaked at 12 ms in slices from both control and maternal-nicotine-exposed rats, but the peak optical signals were significantly stronger in the slices from maternal-nicotine-exposed rats (Fig. 5D; control, 2.30±0.12, n=7, vs. maternal-nicotine-exposed, 2.85±0.05, n=7, $p<0.05$). Thus, facilitation of LTP induction at SC synapses was associated with the increased excitatory neural activity in maternal-

nicotine-exposed rats as in mice, suggesting that facilitation of LTP and enhanced neural activity observed in maternal-nicotine-exposed hippocampi might arise by the same cellular mechanism.

When CA1 network activity is evoked in the presence of nicotine (1 μM), intensity of optical signal becomes stronger in the naïve mouse due to activation of α2-nAChR-expressing O/A interneurons (Nakauchi et al, 2007), and this effect of nicotine is correlated with nicotine-induced facilitation of LTP induction at SC synapses of mice (Nakauchi et al, 2007; Nakauchi and Sumikawa, 2012). Here we observed that bath application of nicotine (1 μM) significantly increased excitatory neural activity in saline-treated rat hippocampi as compared to naïve mouse hippocampi (Fig. 5A–D; control, 2.30±0.12, n=7, vs. bath nicotine, 2.79±0.15, n=7, $p<0.05$). In contrast, bath nicotine had no significant effect on evoked excitatory neural activity in maternal-nicotine-exposed rat hippocampi (Fig. 5A–D; control, 2.85±0.05, n=7, vs. bath nicotine, 2.87±0.21, n=7, $p=0.94$) as in mice. Together, our results provide further evidence that, in adolescent rats as in mice, maternal nicotine exposure alters the operation of the hippocampal CA1 circuit involving α2-nAChR-expressing O/A interneurons.

4. Discussion

The first two weeks of postnatal development of rodents, which is roughly equivalent to the third trimester of human development (Bayer et al, 1993; Quinn, 2005), is a critical time for neurogenesis and synaptogenesis in the hippocampus (Danglot et al, 2006). This period is also a time of rapid development of the cholinergic axon network (Aznavour et al, 2005), of a transient increase in the expression of nAChRs with particular increases in α2, α5 and α7 subunits (Adams et al, 2002; Son and Wizner-Serhan, 2006; Winzer-Serhan and Leslie, 2005), and of the nAChR-mediated switch in the role of γ-aminobutyric acid (GABA) from being an excitatory to an inhibitory neurotransmitter (Liu et al, 2006; Liu et al., 2007). Maternal nicotine exposure could inappropriately mimic endogenous ACh action or disrupt ACh function by causing nAChR desensitization. Thus, it is not surprising that previous studies have found that maternal nicotine exposure upregulates α4β2* nAChRs (Huang and Winzer-Serhan, 2006; Miao et al., 1998; Narayanan et al., 2002), downregulatealters α7 nAChRs (Tizabi et al, 2000), and affects the level of other cholinergic proteins (Zahalka et al, 1992; Yanai et al., 1992) in the hippocampus. The observed increase in α4β2* nAChRs is not accompanied by altered mRNA expression of α4, β2 and α7 subunits and is not prevented by co-administration of the antagonist dihydro-β-erythroidine (DHbE) (Huang and Winzer-Serhan, 2006). Because administration of DHbE alone upregulates α4β2* nAChRs (Huang and Winzer-Serhan, 2006), the effect of nicotine appears to be mediated by receptor desensitization. In the current study we found that maternal nicotine exposure decreases α2 mRNA expression and the normal functioning of α2* nAChRs in O/A interneurons. Furthermore, this effect of maternal nicotine is prevented by co-administration of mecamylamine and is not observed by maternal mecamylamine exposure alone, indicating that the effect is due to the maternal-nicotine-induced activation of nAChRs, and not to blocking endogenous ACh signals by inducing nAChR desensitization. Our results show that the complete functional loss of α2* nAChRs occurs despite the partial loss of α2 mRNA-expressing cells. It is possible that decreased cell-surface expression of functional

α2* nAChRs could occur by altered assembly, cellular trafficking, and/or degradation of receptors. Alternatively, developmental nicotine exposure selectively affects cells that express high levels of functional α2* nAChRs, leaving cells expressing low levels of functional α2* nAChRs intact. The highly sensitive method for in situ detection of α2 mRNA used in the current study could detect cells expressing low levels of functional α2* nAChRs. However, the exact mechanism remains elusive.

The majority of α2 nAChR-containing O/A interneurons are most likely activated by cholinergic inputs from the medium septum and, thus, in hippocampal slices activation of these receptors requires stimulation of cholinergic afferents or nicotine application. Thus, the contribution of these interneurons to baseline inhibition is most likely minimal and their contribution to inhibition of pyramidal cells becomes clear during nicotine application. Bath application of nicotine normally increases sIPSCs in CA1 pyramidal cells (Jia et al., 2009). However, this effect of nicotine is almost absent in maternal-nicotine-exposed adolescent rats. We have previously reported that the effect of bath-applied nicotine is blocked by the non-α7 nAChR antagonist DHbE, but not the α7 nAChR antagonist methyllycaconitine, and is mimicked by A85380 (a non-α7 nAChR-selective agonist with higher affinity for β2* nAChRs than β4*nAChRs), but not the α7 nAChR-selective agonist choline (Jia et al., 2009). These results suggest that the effect of nicotine is mediated by the activation of non-a7 nAChRs, but not a7 nAChRs. Furthermore, we have previously found that activation of nAChRs in the O/A region is responsible for the effect of nicotine (Jia et al., 2009). Although α7 and α4β2* nAChRs are also expressed in interneurons in the O/A region, only α2* nAChRs are non-desensitizing in the presence of nicotine (McQuiston and Madison, 1999; Sudweeks and Yakel, 2000; Yamazaki et al., 2005; Jia et al., 2009). Given the presence of α2* nAChR-containing interneurons in the O/A region that continuously fire action potentials during bath application of nicotine, triggering sIPSCs in postsynaptic pyramidal cells (Jia et al., 2009), α2 nAChR activation is clearly involved in the effect of bath-applied nicotine. Thus, the observed loss of α2* nAChR function in O/A interneurons could be involved in the absence of bath nicotine's effect on sIPSCs. However, because many O/A interneurons still express α2 mRNA in maternal-nicotine-exposed hippocampi, it is possible that the lack of effect of bath nicotine additionally involves the disruption of the normal development of α2* nAChR-containing interneuron connections. In rats, the axons of immature OLM cells already reach the stratum lacunosum moleculare at birth, when most pyramidal cells are quiescent with no synaptic connections (Gozlan and Ben-Ari, 2003). GABA released from interneurons, which is excitatory during the early postnatal period, has a critical role in the activity-dependent formation of GABAergic synapses (Ben-Ari, 2002; Gozlan and Ben-Ari, 2003; Ben-Ari et al., 2004) by increasing intracellular Ca^{2+} in silent pyramidal cells (Leinekugel et al., 1995), leading to the expression of functional $GABA_A$ receptors (Gubellini et al., 2001). During this critical postnatal period, maternal nicotine could cause inappropriate release of GABA from O/A interneurons via α2* nAChR activation, thereby affecting the normal development of inhibitory connections between O/A interneurons and pyramidal cells.

Several previous studies have demonstrated that exposure to nicotine during early development causes long-lasting deficits in learning and memory (Ankarberg et al., 2001; Eppolito and Smith, 2006; Sorenson et al., 1991; Vaglenova et al., 2004; Yanai et al., 1992;

Portugal et al., 2012). However, the outstanding question remains which cellular and molecular changes induced by nicotine underlie the resulting cognitive impairment. We have recently found that early postnatal exposure of mouse pups to nicotine via maternal milk impairs hippocampus-dependent memory during adolescence (Nakauchi et al., 2015). In these mice, LTP at the SC pathway is not diminished, but unexpectedly the threshold for LTP induction becomes lower, and VSD imaging shows enhanced CA1 depolarization after SC stimulation (Nakauchi et al., 2015). This raises the possibility that facilitated LTP can induce behavioral impairments by strengthening synapses that compete with those required for hippocampus-dependent memory. In the current study, we observed similar effects on LTP and hippocampal network activity in adolescent rats postnatally exposed to nicotine. Thus, it is possible that such a mechanism also impairs hippocampus-dependent memory in maternal-nicotine-exposed adolescent rats.

Our previous studies showed that bath application of nicotine facilitates LTP induction and enhances circuit excitability via α2* nAChR activation (Nakauchi et al., 2007). In the current study, we found that these effects of nicotine were completely lost by maternal nicotine exposure, confirming the functional loss of α2* nAChRs. Additionally, this functional loss was accompanied by facilitated LTP and enhanced circuit excitability in rats as is observed with maternal-nicotine-exposed adolescent mice (Nakauchi et al., 2015). However, unlike maternal-nicotine-treated rats or mice, α2 knockout mice did not show facilitated LTP and enhanced circuit excitability (Nakauchi et al., 2007). These findings indicate that facilitated LTP and enhanced circuit excitability are not due to the down-regulation of α2* nAChRs. We have recently found that facilitated LTP and enhanced circuit excitability are absent in maternal nicotine-exposed α2 knockout mice (unpublished results), suggesting that the effects are likely adaptive changes caused by activation of α2* nAChRs during nicotine exposure. However, the observations do not provide a clue to the mechanisms underlying facilitated LTP and enhanced circuit excitability.

α2*-nAChR-expressing OLM cells appear to contribute to hippocampal-dependent behavior via gating information flow through the hippocampus (Lovett-Barron et al., 2014). OLM cells receive direct cholinergic inputs from the medial septum (Leao et al., 2012; Lovett-Barron et al., 2014) and this cholinergic activation is required for encoding hippocampus-dependent contextual fear conditioning (Lovett-Barron et al., 2014). The present study demonstrates that the nicotinic cholinergic control of OLM cells via α2* nAChRs is lost in maternal-nicotine-exposed adolescent rats, indicating uncontrolled sensory information flow into CA1 pyramidal cells from the entorhinal cortex (Fig. 6). Thus, this loss might underlie maternal-nicotine-induced impairment of hippocampus-dependent memory. However, OLM cells also express α7 nAChRs and muscarinic receptors (McQuiston and Madison, 1999; Sudweeks and Yakel, 2000; Leao et al., 2012, Lawrence et al., 2006; Lovett-Barron, 2014), through which cholinergic inputs can activate OLM cells. Although it is known that maternal nicotine exposure causes the altered expression of these receptors in the hippocampus (Tizabi et al., 2000; Yanai et al., 1992), it remains unknown whether maternal nicotine exposure affects the expression of these receptors in OLM cells. If the downregulation of these receptors occurs, maternal nicotine exposure would totally prevent the cholinergic control of OLM cells. Our current study demonstrates that maternal nicotine exposure alters the function of α2*-nAChR-containing O/A interneurons and causes a long-

lasting disturbance in the control of CA1 circuits and LTP. These changes might be linked to maternal-nicotine-induced hippocampal memory impairments.

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Abbreviations

ACSF	artificial cerebrospinal fluid
APV	2-amino-5-phosphonovaleric acid
DHβE	dihydro-β-erythroidine
DNQX	6,7-dinitroquinoxaline-2,3-dione
fEPSPs	field excitatory postsynaptic potentials
IR-DIC	infrared differential interference contrast
LTP	long-term potentiation
nAChR	nicotinic acetylcholine receptors
O/A	oriens/alveus
OLM	oriens-lacunosum moleculare
SC	Schaffer collateral
sIPSC	spontaneous inhibitory postsynaptic current
TA	temporoammonic
TBS	theta burst stimulation
VSD	voltage-sensitive dye

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- Early postnatal nicotine exposure decreases α2* nAChR function in OLM cells
- Early postnatal nicotine exposure disrupts the inhibition of CA1 pyramidal cells by OLM cells
- Early postnatal nicotine exposure affects CA1 circuit activity and LT P induction
- Early postnatal nicotine exposure alters the nicotinic control of CA1 circuit activity and LTP induction

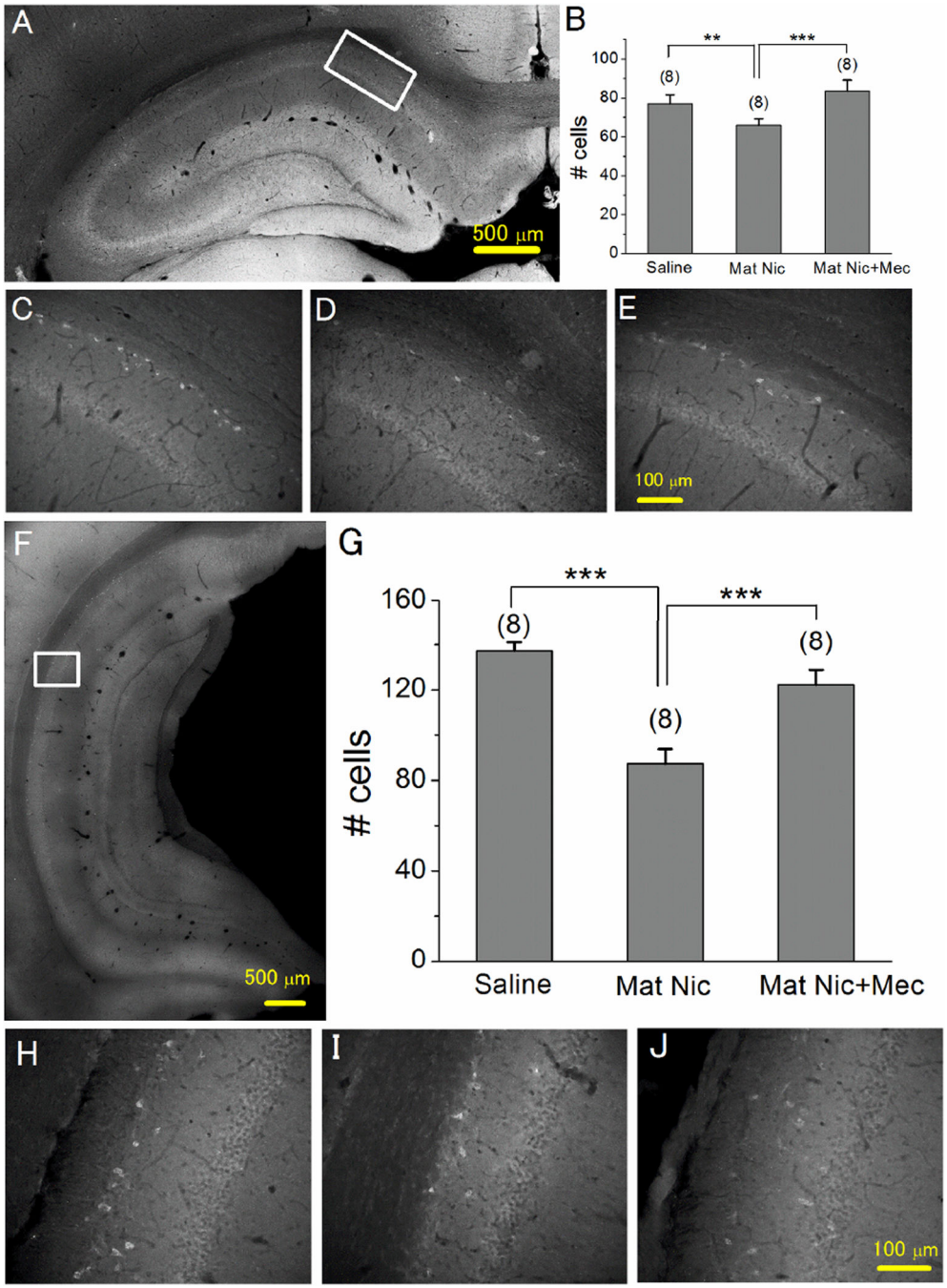

Figure 1. Maternal nicotine-exposure decreases numbers of detectable α2 mRNA-positive O/A interneurons

In situ hybridization with an antisense α2 RNA probe. Enlarged images of the dorsal and ventral hippocampus region indicated by the white box in (A) and (F) are shown in (C, H; control), (D, I; maternal-nicotine-exposed), and (E, J; maternal-nicotine- and mecamylamine-exposed). Numbers of α2 mRNA-positive O/A interneurons in the dorsal (B) and ventral (G) hippocampus were counted. ** $p < 0.01$, *** $p < 0.001$

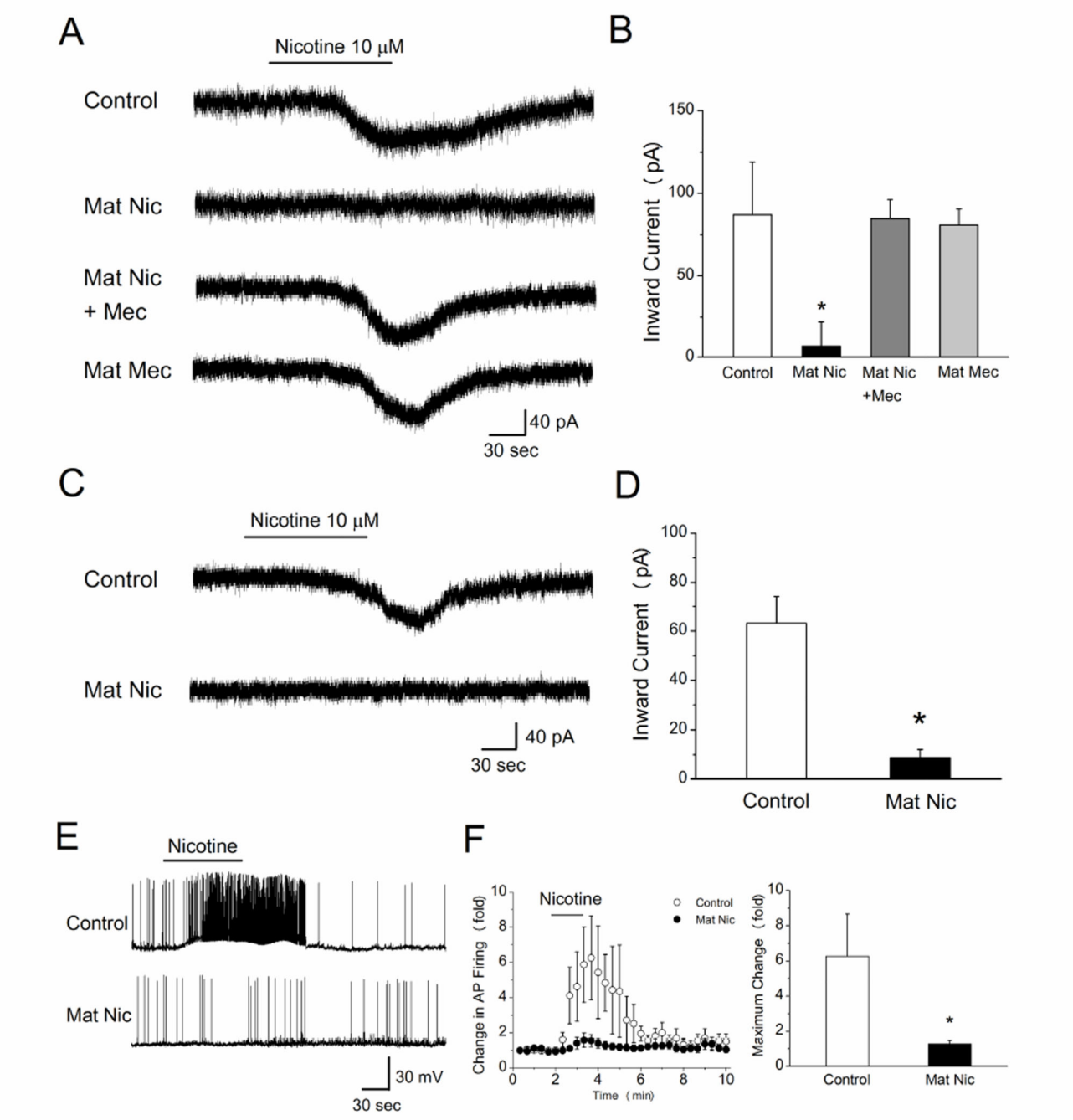

Figure 2. Maternal nicotine exposure reduced acute nicotine-induced responses in O/A interneurons during adolescence and adulthood

Whole-cell recordings were made from interneurons located at the O/A border. A. Under voltage clamp at −60 mV, bath application of nicotine produced a slow inward current in O/A interneurons of adolescent rats exposed to saline (control), maternal nicotine + mecamylamine (Mat Nic + Mec), or maternal mecamylamine (Mat Mec), but not maternal nicotine (Mat Nic). B. Summary plot of the peak inward current recorded from adolescent rats exposed to saline, Mat Nic, Mat Nic + Mec, and Mat Mec. C. Under voltage clamp at −60 mV, bath application of nicotine produced a slow inward current in O/A interneurons of

adult rats exposed to saline (control), but not Mat Nic. D. Summary plot of the peak inward current recorded from adult rats exposed to saline and Mat Nic. E. Under current clamp, bath application of nicotine (10 μM) depolarized O/A interneurons and induced a discharge of action potentials in the hippocampi of adolescent rats exposed to saline, but not Mat Nic. F. Left, Summary plot of the fold change in action potential firing in response to bath application of nicotine (10 μM) over time, recorded from adolescent rats exposed to saline or Mat Nic. Right, Summary plot of the fold change in maximum action potential firing by bath application of nicotine. *$p < 0.05$

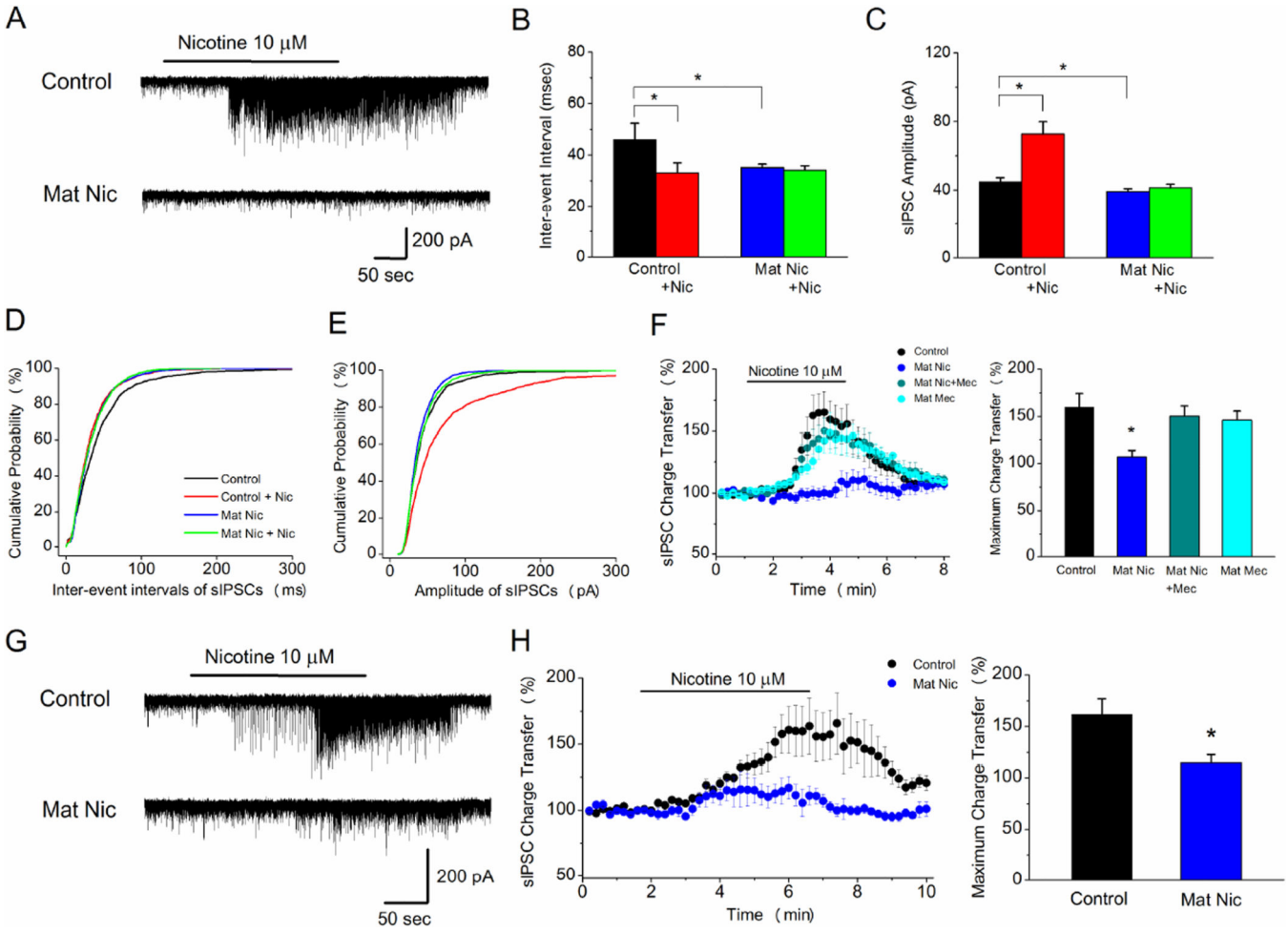

Figure 3. Maternal nicotine exposure alters inhibitory control of pyramidal cells by O/A interneurons

Spontaneous IPSCs (sIPSCs) were recorded in pyramidal cells before and after bath application of nicotine in the presence of the glutamate receptor antagonists APV and DNQX. A. Representative recordings from pyramidal cells in the hippocampus of adolescent rats exposed to saline (control) or maternal nicotine (Mat Nic). Bath-nicotine-induced sIPSCs were significantly reduced in maternal-nicotine-exposed pyramidal cells. Average inter-event intervals (B) and amplitudes (C) of sIPSCs recorded in control and maternal-nicotine-exposed pyramidal cells before and after bath application of nicotine. D, E. Cumulative probability distributions for inter-event intervals and amplitudes for the cells shown in A. Note that the inter-event interval of sIPSCs was shorter (i.e., sIPSC frequency was higher) and their amplitude was smaller in maternal-nicotine-exposed pyramidal cells, compared to controls. Also note that perfusion of nicotine caused a significant increase in both the frequency and amplitude of sIPSCs in control, but not in maternal-nicotine-exposed pyramidal cells. F, left. Summary plot of % change in sIPSC charge transfer in response to bath application of nicotine over time, recorded from adolescent rats exposed to saline, Mat Nic, Mat Nic + Mec or Mat Mec. Right. Summary plot of % change in maximum sIPSC charge transfer elicited by bath application of nicotine. sIPSC charge transfer was binned at 0.2 min. G. Representative recordings from a pyramidal cell in the hippocampus of adult rats

exposed to saline (control) or Mat Nic. H. Left, Summary plot of % change in sIPSC charge transfer in response to bath application of nicotine over time, recorded from adult rats exposed to saline or Mat Nic. Right, Summary plot of % change in maximum sIPSC charge transfer caused by bath application of nicotine. *$p < 0.05$

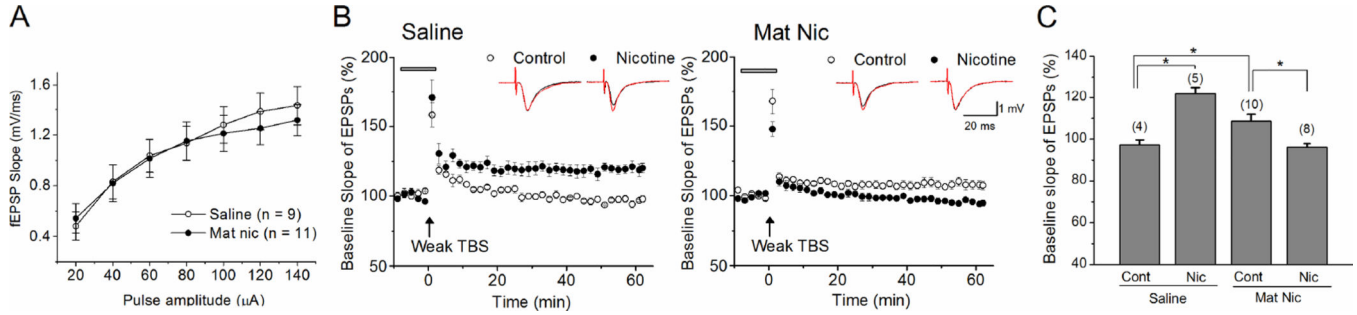

Figure 4. Maternal nicotine exposure alters LTP induction and α2*-nAChR-expressing O/A interneuron-mediated control of LTP

A. Field EPSPs were recorded in the stratum radiatum of CA1 by stimulating the SC pathway. There was no significant difference between slices from control and maternal nicotine-exposed rats in the stimulus-response relationship. B. Weak TBS, which did not induce LTP in control hippocampal slices (left), induced small LTP in maternal nicotine-exposed slices (right). Weak TBS induced LTP in slices from saline-treated control slices when in the presence of bath nicotine (left). However, in maternal nicotine-exposed slices (right), weak TBS-induced LTP was blocked when in the presence of bath nicotine. Traces above each graph are representative waveforms recorded before and 50–55 min after LTP-inducing stimulation in the absence and presence of bath nicotine. LTP-inducing stimulation was delivered at the time indicated by the arrow, and the horizontal bar indicates bath application of nicotine (1 μM). C. Summary plot of % change in in the slope of field EPSPs, measured in saline and maternal-nicotine (Mat Nic) hippocampi in the absence and presence of acute nicotine 50–55 min after weak TBS. *$p < 0.05$

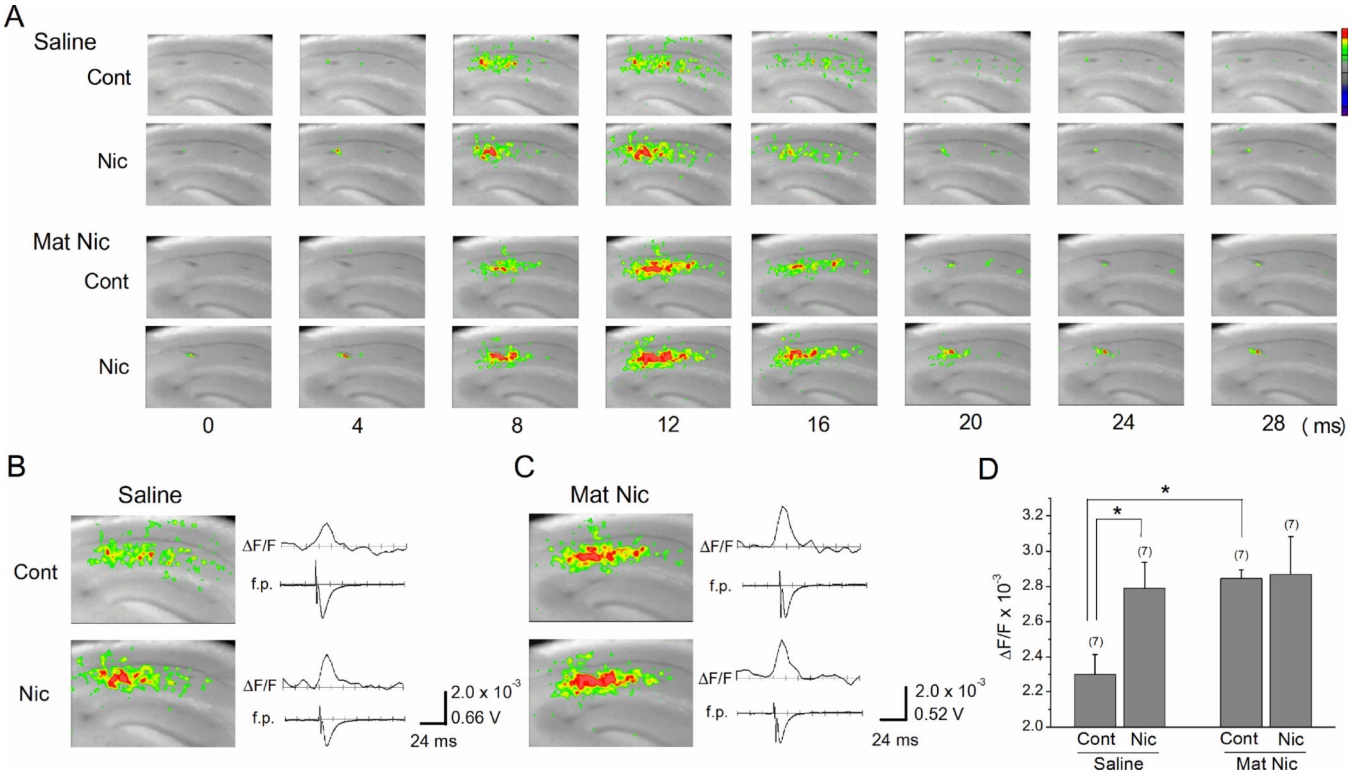

Figure 5. Maternal nicotine exposure alters circuit activity and nicotinic control of depolarizing neuronal activity at the SC pathway

Voltage-sensitive dye imaging, which can detect changes in neuronal activity and is not restricted to synapses, showed that maternal nicotine exposure increased depolarization in the CA1 after SC stimulation. A. Pseudocolor representations of the time course of voltage changes in response to a single stimulus at SC pathway in saline- and maternal nicotine (Mat Nic)-treated rats, recorded first in the absence and then in the presence of nicotine. B, C. Left, sample pseudocolor representations of maximum optical signal from voltage sensitive dye after SC stimulation, recorded first in the absence (control) and then in the presence of nicotine, for saline- and maternal-nicotine-exposed slices. Right, sample simultaneous optical (ΔF/F) and fEPSP traces comparing responses to SC stimulation in the absence and presence of nicotine for saline- and maternal-nicotine-exposed slices. ΔF/F increased in the presence of nicotine in saline but not maternal-nicotine slices, while fEPSP amplitude showed no change. D. Averages of maximum optical responses in saline and maternal-nicotine slices show that, although maternal-nicotine hippocampi show higher baseline activity than saline hippocampi, they show no change in response to bath application of nicotine, unlike saline slices. *$p < 0.05$

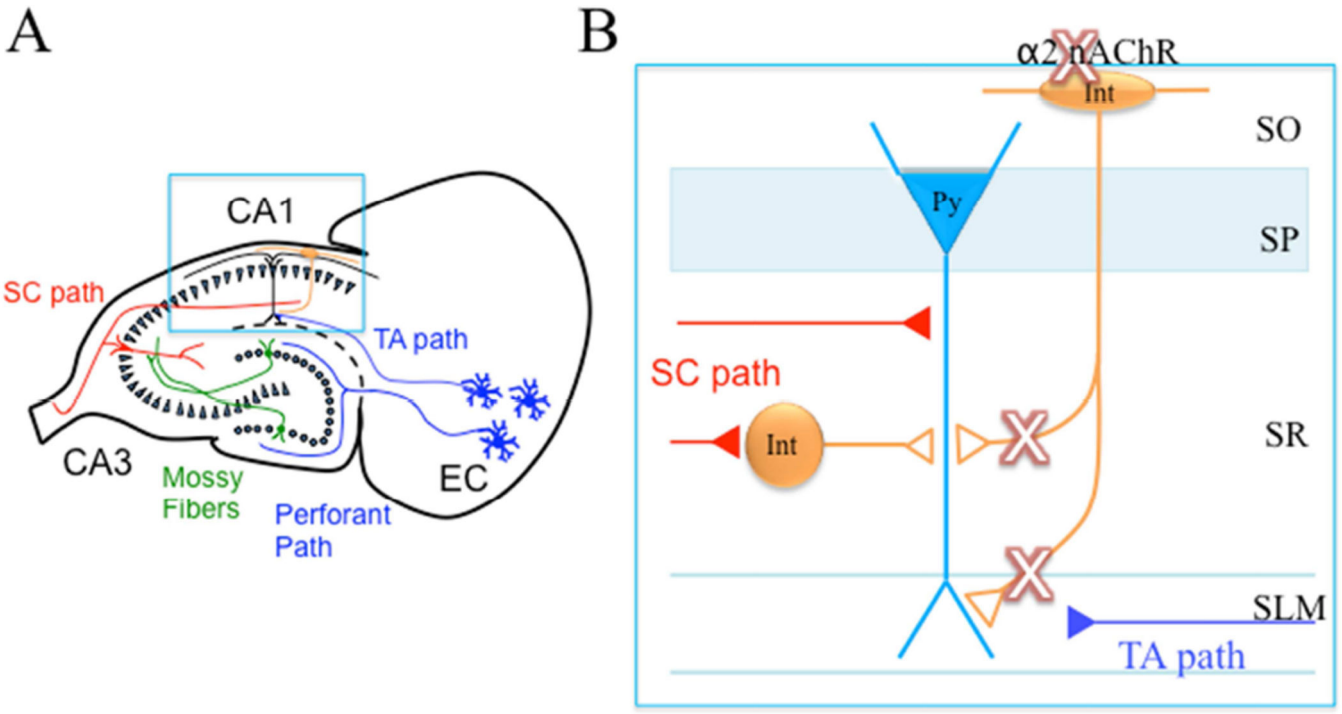

Figure 6. Simplified CA1 circuits showing how the loss of α2 nAChR function on O/A interneurons affects the operation of CA1 circuits

(A) A simplified circuit diagram of entorhinal/hippocampal synaptic pathways. (B) Enlarged view of the CA1 circuit indicated by the blue box in A. Activation of α2* nAChRs on O/A interneurons inhibits TA inputs while disinhibiting SC inputs. The loss of α2* nAChR function can therefore affect the relative strength of inputs to CA1 pyramidal cells from the TA pathway, which provides direct sensory information from entorhinal cortex layer III, and from the SC pathway, which conveys internal representations of the multisensory context (entorhinal cortex layer II → dentate gyrus → CA3 → CA1). SO, stratum oriens; SP, stratum pyrimidale; SR, stratum radiatum; SLM, stratum lacunosum moleculare; Int, interneuron; Py, pyramidal cell