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

Elise Kleeman [1], Sakura Nakauchi [1], Hailing Su, Richard Dang, Marcelo A. Wood, Katumi Sumikawa *

Department of Neurobiology and Behavior, University of California Irvine, Irvine, CA 92697-4550, USA

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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.

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 (Batstra, Hadders-Algra, & Neeleman, 2003; Bruin, Gerstein, & Holloway, 2010; Fried, Watkinson, & Gray, 2003; Thompson, Levitt, & Stanwood, 2009). Although cigarette smoke contains more than 8000 chemicals, nicotine is thought to be the leading cause of these impairments (Pauly & 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 (Ankarberg, Fredriksson, & Eriksson, 2001; Eppolito & Smith, 2006; Nakauchi et al., 2015; Sorenson, Raskin, & Suh, 1991; Vaglenova, Birru, Pandiella, & Breese, 2004; Yanai, Pick, Rogel-Fuchs, & Zahalka, 1992). 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 & 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, Nakauchi, Su, Tanimoto, & Sumikawa, 2016). In particular, we found that early postnatal nico-

* Corresponding author.
E-mail address: ksumikaw@uci.edu (K. Sumikawa).
[1] E.K., S.N. contributed equally to this work.

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tine 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 & Buzsáki, 1996). These interneurons receive cholinergic inputs from the medial septum, and can facilitate LTP at SC-CA1synapses and block LTP at temporoammonic (TA) pathway synapses (Leão et al., 2012; Nakauchi, Brennan, Boulter, & Sumikawa, 2007). 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 (Gilbert & Brushfield, 2009; Kesner, 2007). Thus, OLM cells are thought to be critical mediators of the formation of spatial memories (Leão 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 (Chen et al., 2016; Damborsky, Griffith, & Winzer-Serhan, 2012, 2015; Nakauchi et al., 2015). 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, Le Magueresse, Changeux, & Cherubini, 2003; Maggi et al., 2004). Our previous study also showed that early nicotine exposure resulted in facilitated LTP (Chen et al., 2016; Nakauchi et al., 2015). 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, Parker, Matta, & Sharp, 2005) and 207 ± 40 ng/ml in mouse dams (Eugenín 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 & 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 min, whereas in rats it is about 54 min (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 electrophysiological 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 (Intlekofer et al., 2013; Okuda, Roozendaal, & McGaugh, 2004).

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 & Wood, 2014) by experimenters blind to the treatment group. Briefly, before training the mice were handled 2 min 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 min. During the retention test 24 h 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 lx. 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.