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Nicotinic Cholinergic Signaling Determines the Fate of Adultborn Neurons in the
Dentate Gyrus of the Hippocampus

A dissertation submitted in partial satisfaction of the requirements for the degree
Doctor of Philosophy

in

Biology

by

Nolan Robert Campbell

Committee in charge:

Professor Darwin Berg, Chair
Professor Anirvan Ghosh
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2010

The Dissertation of Nolan Robert Campbell is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2010

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Andrew Halff, Catarina Fernandes, and Darwin Berg are co-authors on chapter one, which was published in the Journal of Neuroscience.

Darwin Berg is a co-author on chapter two, which is being prepared for publication in the Journal of Neuroscience.

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PUBLICATIONS

Spatiotemporal Characterization of Short Versus Long Duration Calcium Transients in Embryonic Muscle and Their Role in Myofibrillogenesis. NR Campbell, SP Podugu, and MB Ferrari Dev Biol, Apr 2006; 292(1): 253-64.

Endogenous Signaling Through α 7-containing Nicotinic Receptors Promotes Maturation and Integration of Adultborn Neurons in the Hippocampus. NR Campbell, CC Fernandes, AW Halff, and DK Berg J Neurosci, Feb 2010; 30:8734-8744.

ABSTRACT OF THE DISSERTATION

Nicotinic Cholinergic Signaling Determines the Fate of Adultborn Neurons in the Dentate Gyrus of the Hippocampus

by

Nolan Robert Campbell

Doctor of Philosophy in Biology

Professor Darwin Berg, Chair

While nAChRs are poised to regulate adult neurogenesis in the dentate gyrus of the hippocampus, a careful examination of the effects of nicotinic signaling on adult neurogenesis has not been performed. Here we examine, *in vivo*, the maturation and survival of adultborn neurons from nAChR knockout mice or mice receiving nAChR agonists. We find that endogenous, cell autonomous signaling through $\alpha 7$ -nAChRs is necessary for proper maturation, synaptic integration, and survival of adultborn neurons. Nicotine exposure at levels relevant for human smokers acts through the same receptor to kill young adultborn neurons, and increase survival of the more mature population. Independent of effects on survival, nicotine enhances dendritic growth and spine formation of adultborn neurons. DMXBA, a partial $\alpha 7$ -nAChR agonist, can be used to increase dendritic growth without eliciting detrimental effects on survival. These results demonstrate a profound role for nAChR signaling in controlling the fate of adultborn neurons in the dentate gyrus of the hippocampus.

INTRODUCTION

Neuronal Nicotinic Acetylcholine Receptors

Neuronal nicotinic acetylcholine receptors (nAChRs) comprise a diverse family of ionotropic membrane channels. Structurally they are all composed of five subunits, and each subunit has four transmembrane domains. Functionally they all respond to the endogenous ligand acetylcholine (ACh) or the non-endogenous ligand nicotine by opening a cationic-selective pore. The family can be divided into heteromeric and homomeric subtypes. Heteromeric receptors are composed of variable combinations of α_{2-6} and β_{2-4} subunits. Homomeric receptors are made from 5 identical subunits of α_{7-9} (see Taly et al., 2009, and Albuquerque et al., 2009, for reviews). Expression of nAChR genes can vary by brain region and neuron type, and a single cell can display multiple combinations of nAChR subtypes (see Millar and Gotti, 2009 for review). Both $\alpha\beta$ composition and stoichiometry affect receptor function (Alkondon and Albuquerque, 1993; Zwart and Vijverberg, 1998; Nelson et al., 2003).

For this dissertation I focus on homomeric $\alpha 7$ -nAChRs and heteromeric $\beta 2$ -containing nAChRs, of which $\alpha 4\beta 2$ is the most prevalent. These nAChR subtypes are the best studied and most abundant in the CNS. Functionally, they differ substantially in activation and desensitization kinetics, agonist affinities, and ion permeability. Compared with $\alpha 4\beta 2$ -nAChRs, the $\alpha 7$ -nAChRs have 1) lower affinity for ACh- and nicotine-mediated activation, 2) faster activation and desensitization kinetics, 3) higher

conductance, and 4) far higher calcium permeability relative to sodium (Alkondon and Albuquerque, 1993; Castro and Albuquerque, 1993; Castro and Albuquerque, 1995).

Cholinergic innervation of both receptor subtypes occurs broadly throughout the adult mammalian brain where, generally, the receptors exert regulation over excitatory and inhibitory circuitry. In the interests of brevity, I confine detailed discussion of receptor localization and function to the relevant circuitry of the hippocampus.

Robust cholinergic input to the hippocampus is provided by the medial septum (Woolf, 1991; Duter et al., 1995). Cholinergic terminals synapse on hilar and granule neurons in the dentate gyrus, and pyramidal and interneurons in the CA1 and CA3 fields (Frotscher and Leranth, 1995). Ultrastructural images of hippocampal sections labeled with α -bungarotoxin (α -Bgt), a highly selective and potent peptide toxin antagonist of α 7-nAChRs, revealed the presence of these receptors at most pre- and postsynaptic contacts in the hippocampus, as well as many non-synaptic sites (Fabian-Fine et al., 2001). While similar ultrastructural studies are not available for α 4 β 2-nAChRs, electrophysiological studies suggest that α 4 β 2-nAChRs in the hippocampus are also broadly expressed and are located at both pre- and postsynaptic sites (Jones and Yakel, 1997; Alkondon et al., 1999; Ji and Dani, 2000; Ji et al., 2001; Fayuk and Yakel, 2005).

This broad distribution permits nAChRs to regulate glutamatergic and GABAergic signaling in the hippocampus by a variety of mechanisms. Presynaptic nAChRs can facilitate or induce release of GABA and glutamate from presynaptic terminals (Gray et al., 1996; Alkondon et al., 1997; Radcliffe and Dani, 1998;

Yamamoto et al., 2005). Postsynaptic nAChRs on interneurons provide sufficient depolarization to elicit action potentials that can inhibit or dis-inhibit pyramidal neurons via monosynaptic or multi-synaptic circuits (Alkondon et al., 1997; Ji and Dani, 2000; Fujii et al., 2000; Sudweeks and Yakel, 2000; Sher et al., 2004). Receptor activation also regulates synaptic plasticity in the hippocampus; it can induce either long-term depression or long-term potentiation via presynaptic facilitation, postsynaptic depolarization, or indirectly through other neuromodulators (Ge and Dani, 2005; Fujii et al., 1999; Tang and Dani, 2009).

Adult Neurogenesis

The dentate gyrus of the hippocampus displays another, less common form of plasticity, the process of adult neurogenesis. Contrary to long-held beliefs, it is now recognized that in two regions of the mammalian brain new neurons are added throughout adult life. A large body of work in recent years has shed light on the adultborn neuron developmental process, the factors that regulate it, and its importance.

In the hilus of the dentate, rapidly amplifying neural precursor cells are born from a relatively quiescent set of adult stem cells (Seri et al., 2004; Ahn and Joyner, 2005). Following terminal mitosis these cells mature with a predictable time-course into dentate granule neurons (Fig. 1). Briefly, I will highlight key points in adultborn neuron development.

Regarding morphology, the cells are recognizable as granule neurons at one week. Spines are first seen at week 2 and dendritic arbors become fully mature by

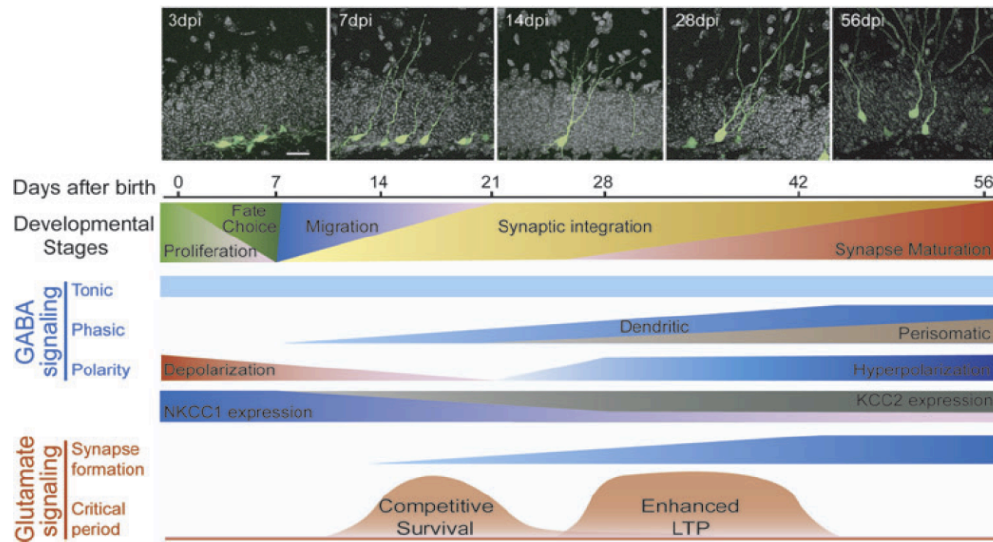


Fig.1 Adultborn Neuron Development in the Dentate Gyrus (Ge et al., 2008)

week four. Spine growth and maturation continue for several months (Zhao et al., 2006).

Regarding synaptic input, the neurons display the following developmental sequence for acquisition: 1) tonic GABAergic, 2) synaptic dendritic GABAergic, 3) synaptic glutamatergic, and 4) synaptic somatic GABAergic. GABAergic currents change from depolarizing to hyperpolarizing between weeks 2 and 4 (Esposito et al., 2005; Overstreet Wadiche et al., 2005, 2006; Tozuka et al., 2005; Wang et al., 2005; Ge et al., 2006; Karten et al., 2006). These events mimic those that occur in early postnatal development, albeit with a slower progression.

Regarding survival, many more cells are initially generated than are integrated into the network. Massive cell loss occurs immediately following proliferation as cells make their fate choice. In addition to this early loss, glutamatergic activity-dependent competitive survival occurs in a critical period from weeks 2 to 4, when the neurons

are first integrating into glutamatergic networks. Cells that survive this period persist long-term (Kempermann et al., 2003; Tashiro et al., 2006; Tashiro et al., 2007).

Ultimately, the surviving cells mature to become indistinguishable from other granule neurons (Laplagne et al., 2006; Ge et al., 2007; Laplagne et al., 2007). They receive glutamatergic synaptic input from the entorhinal cortex via the perforant path and extend mossy fiber axons to the CA3 region of the hippocampus (Bruehl-Jungerman et al., 2006; Hastings and Gould, 1999).

Factors influencing adultborn neuron development include external environment, behavior, and emotional state, and at the cellular level are mediated by growth factor and synaptic signaling. Increases in adult neurogenesis occur when animals are placed in enriched environments, when exercised, or given hippocampal-dependent learning tasks (Kempermann et al., 1997; Van Praag et al., 1999; Gould et al., 1999). Conversely, stress and depression decrease adult neurogenesis (Pham et al., 2003; Alonso et al., 2004). Blockade of the growth factors VEGF and BDNF reduce increases in adult neurogenesis caused by exercise and enriched environment (Cao et al., 2004; Fabel et al., 2003; Rossi et al., 2006). BDNF concentrations correlate with stress- and depression-induced decreases in neurogenesis (Duman and Monteggia, 2006), and blocking BDNF signaling prevents antidepressant-induced neurogenic increases (Li et al., 2008; Sairanen et al., 2005). Depolarizing GABAergic currents enhance the growth and synaptic integration of adultborn neurons (Ge et al., 2006), and glutamatergic signaling through NMDA receptors on adultborn neurons is necessary for survival between 2 and 4 weeks post-birth (Tashiro et al., 2006). Both dopamine and serotonin signaling can also influence adult neurogenesis, but their

actions are likely through indirect mechanisms (Banasr et al., 2004; Malberg et al., 2000; Santarelli et al., 2003; Hoglinger et al., 2004).

Hippocampal function depends on adult neurogenesis. The importance of neurogenesis varies considerably depending on the tasks examined and the specific parameters measured in the experiments. Ablation of adultborn neurons has been achieved by focal irradiation, anti-mitotic drugs, or genetically induced cell death. Under conditions lacking neurogenesis, impairments have been observed in hippocampal-dependent behavioral tasks including the Morris water maze, Barnes maze, contextual fear conditioning, trace fear conditioning, and trace eye blink conditioning (see Deng et al., 2010, for review). In addition to these classical assays, research suggests adult neurogenesis is involved in pattern separation (Clelland et al., 2009), memory extinction (Deng et al. 2009), transition of memories from the hippocampus to the cortex (Kitamura et al., 2009), and resistance to addictive behaviors (Noonan et al., 2009). In the absence of neurogenesis, no impairments are observed in functions that do not require the hippocampus. This indicates that adultborn neuron loss selectively impairs hippocampal-dependent performance (see Deng et al., 2010, for review).

That neurogenesis is necessary for proper hippocampal function is not without controversy. Some have observed no effects of adultborn neuron ablation, often in the same tasks that others have reported to be negatively affected by blockade of adult neurogenesis. Differences in the timing and extent of ablation and in the difficulty of the task may account for some or all of these discrepancies (Deng et al., 2010).

Nicotinic Signaling and Adult Neurogenesis

The $\alpha 7$ - and $\alpha 4\beta 2$ nAChRs are well positioned to regulate the process of adult neurogenesis. As previously mentioned, nicotinic signaling through these receptors can alter glutamatergic and GABAergic signaling in the hippocampus, which in turn can alter adultborn neuron maturation and survival. In addition, early in development adultborn neurons express both $\alpha 7$ - and $\alpha 4\beta 2$ nAChRs and receive direct cholinergic innervation (Kaneko et al., 2006; Ide et al., 2008).

Previous studies support a role for nAChR signaling in adult neurogenesis. Nicotine exposure kills neural precursor cells in vitro prior to neuronal differentiation and in vivo kills young adultborn neurons (Berger et al., 1998; Abrous et al., 2002). Cholinergic forebrain lesion decreases survival of adultborn neurons; conversely, increasing cholinergic signaling by administration of an acetylcholinesterase inhibitor enhances adultborn neuron survival (Cooper-Kuhn et al., 2004; Kaneko et al., 2006). Finally, in mice lacking $\alpha 4\beta 2$ -nAChRs, age-dependent decreases in adultborn neuron proliferation occur (Harrist et al., 2004).

For my dissertation research I examine the effects of nAChR signaling on adultborn neuron maturation and survival. In Chapter 1, we demonstrate that cell-autonomous, endogenous signaling through $\alpha 7$ -nAChRs encourages dendritic growth, synaptic connectivity, and survival of adultborn neurons. In Chapter 2 we show that nicotine, acting through $\alpha 7$ -nAChRs, produces radically different outcomes depending on the timing of administration; it co-opts endogenous signaling pathways to enhance dendritic growth and survival in more mature adultborn neurons, but kills the immature population.

CHAPTER 1

ENDOGENOUS SIGNALING THROUGH α 7-CONTAINING NICOTINIC RECEPTORS PROMOTES MATURATION AND INTEGRATION OF ADULTBORN NEURONS IN THE HIPPOCAMPUS

by

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ABSTRACT

Neurogenesis in the dentate gyrus occurs throughout adult mammalian life and is essential for proper hippocampal function. Early in their development, adultborn neurons express homomeric $\alpha 7$ -containing nicotinic acetylcholine receptors ($\alpha 7$ -nAChRs) and receive direct cholinergic innervation. We show here that functional $\alpha 7$ -nAChRs are necessary for normal survival, maturation, and integration of adultborn neurons in the dentate gyrus. Stereotaxic retroviral injection into the dentate gyrus of wildtype and $\alpha 7$ -knockout ($\alpha 7$ KO) male and female mice was used to label and birthdate adultborn neurons for morphological and electrophysiological measures; BrdU injections were used to quantify cell survival. In $\alpha 7$ KO mice, we find that adultborn neurons develop with truncated, less complex dendritic arbors, and display GABAergic postsynaptic currents with immature kinetics. The neurons also have a prolonged period of GABAergic depolarization characteristic of an immature state. In this condition they receive fewer spontaneous synaptic currents and are more prone to die during the critical period when adultborn neurons are normally integrated into behaviorally relevant networks. Even those adultborn neurons that survive the critical

period retain long-term dendritic abnormalities in $\alpha 7$ KO mice. Interestingly, local infection with retroviral constructs to knockdown $\alpha 7$ -mRNA mimics the $\alpha 7$ KO phenotype, demonstrating that the relevant $\alpha 7$ -nAChR signaling is cell-autonomous. The results indicate a profound role for $\alpha 7$ -nAChRs in adult neurogenesis and predict that $\alpha 7$ -nAChR loss will cause progressive impairment in hippocampal circuitry and function over time as fewer neurons are added to the dentate gyrus and those that are added integrate less well.

INTRODUCTION

Continuing neurogenesis in the adult dentate gyrus is necessary for hippocampal function (Shors et al., 2001, 2002; Rola et al., 2004; Snyder et al., 2005; Winocur et al., 2006). Abnormalities in adult neurogenesis are likely to exacerbate major neurological disorders, including Alzheimer's disease and depression (Gough, 2007; Sahay and Hen, 2007; Verret et al., 2007; Kotani et al., 2008; Perera et al., 2008) and contribute to addiction and relapse behavior (Noonan et al., 2009). Understanding how adultborn neurons develop and integrate into preformed neural networks may suggest targets for therapeutic intervention and assist in developing stem cell therapies for repairing damaged neuronal tissue (Sohur et al., 2006; Okano and Sawamoto, 2008).

Pioneering work on adult neurogenesis indicates that ionotropic receptor signaling plays important roles. Depolarizing GABAergic activity alters precursor proliferation and is necessary for dendritic growth of adultborn neurons (Liu et al.,

2005; Ge et al., 2006). Glutamatergic activity through NMDA receptors encourages survival of adultborn neurons during a critical period when the neurons are first assimilated into behaviorally relevant networks (Tashiro et al., 2006; Tashiro et al., 2007). Nicotinic cholinergic input is also positioned well to influence adult neurogenesis. Early on, the neurons receive cholinergic innervation and express two major types of ionotropic nicotinic acetylcholine receptors (nAChRs): homopentameric $\alpha 7$ -containing receptors ($\alpha 7$ -nAChRs) and heteropentameric $\beta 2$ -containing receptors ($\beta 2^*$ -nAChRs; Kaneko et al., 2006; Ide et al. 2008).

Substantial evidence indicates that cholinergic signaling regulates adult neurogenesis. Cholinergic forebrain lesion decreases adultborn neuron survival, and enhancing cholinergic activity increases survival (Cooper-Kuhn et al., 2004; Kaneko et al., 2006). Chronic nicotine exposure in vivo reduces adultborn neuron proliferation (Abrous et al., 2002; Scerri et al., 2005; Shingo and Kito, 2005), while $\beta 2$ -nAChR knockout (KO) mice show decreased proliferation, but normal survival, of hippocampal adultborn neurons (Harrist et al., 2004; Mechawar et al., 2004). Contributions of $\alpha 7$ -nAChRs to adult neurogenesis are unknown.

In early postnates, $\alpha 7$ -nAChRs contribute importantly to hippocampal development. Young hippocampal neurons in $\alpha 7$ KO mice have a prolonged period of GABAergic excitation due to delayed appearance of the mature chloride transporter KCC2 and extended presence of the immature chloride transporter NKCC1 (Liu et al., 2006). Further, $\alpha 7$ -nAChR signaling helps drive giant depolarizing potentials that shape network development and contribute to synaptic plasticity (Maggi et al., 2001,

2003; Le Magueresse et al., 2006). Subsequent $\alpha 7$ -nAChR activity enhances both GABA and glutamate release (Gray et al., 1996; Alkondon et al., 1997A; Radcliff and Dani, 1998; Alkondon and Albuquerque, 2001).

Here we show that adultborn neurons in $\alpha 7$ KO mice receive fewer spontaneous synaptic currents and are disadvantaged for survival during the critical period in vivo. The neurons develop with severely truncated dendritic arbors, a prolonged depolarizing chloride gradient, and GABAergic currents with immature kinetics. These results indicate that $\alpha 7$ -nAChR signaling modulates the tempo of adultborn neuron physiological and morphological development. We further demonstrate that this function results from cell-autonomous $\alpha 7$ -nAChR signaling and is necessary to prevent persistent morphological abnormalities of granule cells in the dentate gyrus.

MATERIALS AND METHODS

Transgenic Animals. All mice were C57Bl/6 background and used at 1-3 months of age. Control and experimental mice were age-matched in all experiments. Heterozygous $\alpha 7$ KO mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Their offspring were genotyped by PCR, and homozygous $\alpha 7$ KO mice were used for experiments. GAD67-GFP mice, generated and provided by Guoping Feng (Duke University), were crossed with the $\alpha 7$ KO mouse line. To ensure that only GAD67-GFP heterozygous mice were used in experiments, homozygous GAD67-GFP mice were bred with nulls to produce test animals.

RNAi Constructs. Lentiviral vectors were constructed to reduce $\alpha 7$ -mRNA levels in vivo by RNA interference ($\alpha 7$ RNAi). A Genscript algorithm was used to design sequences uniquely homologous to mouse $\alpha 7$ -mRNA. A control scrambled sequence was obtained with Genscript sequence scrambler and shown not to be homologous with any sequence in the mouse genome. The sequences were inserted into lentiviral vectors (FG12; Addgene plasmid 14884) under an H1 promoter along with GFP under a ubiquitin promoter and linked to their reverse complement by the loop sequence TCTCTTGAA to form short-hairpin RNAs. Their compositions are: 5'-AGGCAGATATCAGCAGCTATA-3', 5'-ACCACCAACATTTGGCTACAA-3', and 5'-GAGAGTACGCTAAGATCCTAA-3' for $\alpha 7$ RNAi-1, $\alpha 7$ RNAi-2, and scrambled RNAi, respectively. The effectiveness of the lentiviral $\alpha 7$ RNAi constructs was tested by infecting 16-day-old mouse (E18 C57B1/6) hippocampal 3.5 cm cultures (plated at 62,500 cells/cm²) with 5 μ l virus stock (1×10^8 PFU/ml), and collecting the cells 1 week later (Supplementary Fig. 1). Solubilized $\alpha 7$ -nAChRs were immunoprecipitated with monoclonal antibody (mAb) 319 and radiolabeled with I¹²⁵- α -bungarotoxin (I¹²⁵- α Bgt; Conroy et al., 2003). Total protein was measured using the Bradford assay (Bio-Rad Protein Assay). The $\alpha 7$ RNAi-1 construct was used for all experiments reported here.

Tissue Preparation. Mice were anesthetized by intraperitoneal injection of 10 mg/ml ketamine and 1 mg/ml xylazine in sterile 0.9% NaCl at a volume of 0.01 ml/g body weight. Toe pinch was used to determine effectiveness of anesthesia. Animals were transcardially perfused with 4% paraformaldehyde in phosphate-buffered saline

(PBS). Brains were removed and post-fixed in 4% paraformaldehyde in PBS overnight, then equilibrated in 30% sucrose. Next, the tissue was frozen, and coronal slices (40 μm) were cut using a cryostat microtome. Sections were dried on superfrost plus slides (Fisher) and processed immediately for immunostaining.

Immunostaining. Slide mounted slices were blocked in 5% normal donkey serum in PBS with 0.5% triton X-100 (PBS-TX) for 30 minutes. Primary antibodies were diluted in the same blocking solution and applied overnight at 4°C. Primary antibodies included anti-NKCC1 mAb (1:1000 dilution, Developmental Studies Hybridoma Bank, University of Iowa), anti-5-Bromo-2-deoxyuridine (BrdU) mAb BU1/75 (1:200 dilution, Abcam), and anti-GFP mAb 3E6 (1:1000 dilution, Molecular Probes). Slides were then washed three times in PBS-TX for 10 minutes and incubated with secondary antibodies at 1:500 in PBS-TX for 2-4 hours at room temperature. After washing three times in PBS-TX for 10 minutes, slides were mounted with coverslips using Vectashield mounting medium containing dapi and stored in the dark at 4°C until imaged.

Imaging and Quantification. Imaging was performed within 48 hours of immunostaining using a Zeiss axiovert microscope with 3I deconvolution software for image analysis. For morphological measurements neurons were imaged at 63x magnification, and a z-stack was compiled by acquiring images every 0.5 μm through the section. Dendritic measurements were made in ImageJ using the NeuronJ tracing application. Spine counts were taken over a 20 μm segment of dendrite located within 100 μm of the granule cell layer boundary. NKCC1 fluorescence measurements were

made in a 2 μm -thick ring surrounding the adultborn granule cell nucleus, defined by dapi and BrdU staining. Cell selection and quantification were performed blind to genotype.

Stereotaxic Viral Injection. A Moloney's Murine Leukemia Virus construct expressing green fluorescent protein (MMLV-GFP) was provided by Fred Gage (Salk Institute) and modified to express mcherry (MMLV-cherry). Lentiviral constructs were purchased (FG12; Addgene plasmid 14884) and modified to express $\alpha 7\text{RNAi}$ and scrambled sequences as described (see RNAi Constructs). Viruses were generated by transfecting the constructs into HEK293T cells. Harvest and concentration by ultra-centrifugation generated viral titers of 10^8 pfu/ml in sterile PBS. The viral suspensions were stereotaxically delivered as described (Van Praag et al., 2002) at the following position from Bregma: anteroposterior, -2mm; lateral, 1.7mm; ventral, -2mm. For electrophysiological experiments a second injection was made from Bregma: anteroposterior, -2.5mm; lateral, 2mm; ventral -2.2mm. Animal body temperature was maintained throughout the surgery until anesthesia wore off. After surgery, animals were housed singly and monitored to ensure no signs of infection, pain, or distress.

BrdU Labeling. BrdU was injected intraperitoneally at 10 mg/ml in sterile 0.9% NaCl to yield a single dose of 50 $\mu\text{g/g}$ body weight on each of four consecutive days. Mice were singly housed for 2 or 4 weeks following the initial injection until tissue preparation. Following perfusion all steps were performed blind to genotype. After cryostat sectioning, slices were dipped in 2N HCl for 30 minutes at 37°C, followed by 0.1 M borate buffer for 10 minutes at room temperature. After

immunostaining, counts were made of BrdU-positive cells in the first third of the granule cell layer in every fourth section through the entire hippocampus. Adultborn neurons are largely confined to the first third of the granule cell layer (Zhao et al., 2006).

Electrophysiology. Following anesthesia (see tissue preparation), mice were decapitated. Brains were quickly removed from the skull and placed in ice-cold solution containing (in mM): sucrose 75, NaCl 87, KCl 2.5, CaCl₂ 0.5, MgCl₂ 7, NaHCO₃ 25, NaH₂PO₄ 1.25, glucose 20, and bubbled with 95% O₂/5% CO₂, pH 7.4. Transverse, 250 µm thick hippocampal slices were cut using a vibratome (series 1000 Plus, Technical Products International Inc., St Louis, USA) and initially stored at 30°C in artificial cerebrospinal fluid containing (ACSF, in mM): NaCl 119, KCl 2.5; NaH₂PO₄ 1, NaHCO₃ 26, MgCl₂ 1.3, CaCl₂ 2.5, glucose 10, pH 7.4, and equilibrated with 95% O₂/5% CO₂. Slices were allowed to recover at least 1 hour in oxygenated ACSF at 24°C prior to recording and then continuously perfused with the same solution at a rate of 2–3 ml/min while recording.

The whole-cell patch-clamp configuration was employed both in voltage-clamp and current-clamp modes. Microelectrodes (5–8 MΩ) were pulled from borosilicate glass capillaries (Sutter Instruments, Novato, CA, USA) with a P-97 pipette puller (Sutter Instruments).

To record spontaneous postsynaptic currents (PSCs), the electrodes were filled with an internal solution containing (in mM): CsCl 135, MgCl₂ 4, EGTA 0.1, HEPES 10, MgATP 2, NaGTP 0.3 and Na₂phosphocreatine 10, pH 7.4, adjusted with CsOH to 280–290 mOsm. Cells were held at a membrane potential of -80 mV, and currents

were recorded for five continuous minutes. The resting membrane potential was determined in current-clamp mode immediately after establishing the whole-cell configuration. The internal solution consisted of (in mM): K-gluconate 125, KCl 15, NaCl 8, EGTA 2, HEPES 10, MgATP 4, NaGTP 0.3, and Na₂phosphocreatine 10, pH 7.3, adjusted with KOH to 280-290 mOsm. Cells with absolute leak current >100 pA at the holding potential (V_{Hold}) were discarded.

For perforated-patch recordings, gramicidin was diluted in the pipette solution (in mM: 135 CsCl, 4 MgCl₂, 0.1 EGTA, 10 HEPES, pH 7.4, 300 mOsm) to a final concentration of 50 µg/ml immediately prior to use. Pipettes had resistances of 5-8 MΩ. Small voltage-steps (10 mV, 50 ms) were evoked prior to extracellular stimulation to monitor membrane and access resistance. If the holding current or either resistance changed significantly, the experiment was discarded. Extracellular stimulation (80-240 µA and 0.2 ms duration, 0.1 Hz) was performed using a concentric bipolar electrode (125 µm diameter; Frederick Haer Company, Bowdoin, ME), with a pulse generator (Master-8, A.M.P.I., Jerusalem, Israel) coupled through a stimulus isolator (S.I.U. 90; Neuro Data Instruments, New York, NY). The stimulation electrode was placed on the granule cell layer 300 µm away from the recorded cell. During the recording of evoked GABAergic PSCs, the cells were perfused with oxygenated ACSF containing CNQX (20 µM) and APV (20 µM) to block glutamatergic PSCs and isolate monosynaptic GABAergic PSCs. Recordings were performed under voltage-clamp at multiple holding potentials. Peak current amplitude and holding potential were plotted to yield the chloride equilibrium potential (E_{Cl}) from a linear fit of the I-V curve for each cell. As a control for

perforated-patch integrity, in some cells E_{Cl} was redetermined after establishing a whole-cell configuration and adjusting the intracellular chloride concentration to yield a slightly positive E_{Cl} (2.7 mV). The measured values in these cases were in reasonable agreement with predicted values (Supplementary Fig. 2A). To ensure PSCs were GABAergic, gabazine was used to block GABA_A receptors after E_{Cl} acquisition and demonstrate loss of the evoked events (Supplementary Fig. 2B). Current kinetic measurements were made from ≥ 5 averaged traces acquired at -80 mV holding potential. Current rise time was determined between 40% and 100% of peak amplitude using a single exponential best fit. Current decay was determined between 10% and 90% of peak amplitude. Since the best fit for decay was either one or two exponentials, weighted decay was calculated using the equation $A_1\tau_1 + A_2\tau_2$ where A is the relative amplitude and τ is the decay constant for each component.

All recordings were collected using a MultiClamp 700A amplifier (Molecular Devices, Sunnyvale, CA, USA), filtered at 2 kHz, and digitized at 5 kHz with pCLAMP 9 software (Molecular Devices); analysis was performed with Clampfit 9.2 software.

Statistical analysis. Student's t test (t test) was used for comparing means if single pairs were involved. One-way ANOVA with Bonferroni's post hoc test was used for comparing means for ≥ 3 groups. Kolmogorov-Smirnov (KS) statistical analysis was used to compare cumulative frequency distributions. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Materials. All reagents were purchased from Sigma (St. Louis, MO) unless otherwise indicated.

RESULTS

Reduced Survival of Adultborn $\alpha 7$ KO Neurons through the Critical Period

To determine if $\alpha 7$ -nAChRs contribute to cholinergic regulation of adultborn neuron survival (Cooper-Kuhn et al., 2004; Kaneko et al., 2006), we birthdated adultborn neurons in WT and $\alpha 7$ KO young adult mice by injecting BrdU and following their fate. Hippocampal slices were taken for BrdU immunostaining 2 and 4 weeks later, to allow quantification before and after the critical period for activity-dependent survival (Tashiro et al., 2006). No difference was seen in the number of BrdU-labeled cells in the dentate gyrus of WT and $\alpha 7$ KO mice at 2 weeks (Fig. 1). A clear difference was found, however, at 4 weeks. At this time $\alpha 7$ KO mice had significantly fewer BrdU-labeled granule neurons than did WT mice (Fig. 1). The results indicate that $\alpha 7$ -nAChR signaling is necessary for optimal adultborn neuron survival through the critical period occurring between 2 and 4 weeks post neuronal birth.

Because survival through the critical period depends on glutamatergic signaling (Tashiro et al., 2006), we tested whether adultborn $\alpha 7$ KO neurons receive reduced synaptic input. This was done by first labeling adultborn neurons in vivo using stereotaxic injection of MMLV-GFP, which can express only in dividing cells. Labeling is largely confined to neurons born within a 3-day window following virus

injection (Zhao et al., 2006). Three weeks later we prepared fresh hippocampal slices, identified GFP-expressing neurons in the dentate gyrus region, and performed patch-clamp recording in voltage-clamp mode (Fig. 2A). Adultborn $\alpha 7$ KO neurons had significantly fewer spontaneous postsynaptic currents (PSCs) than did WT neurons, and their PSCs were smaller in size (Fig. 2B). (Pharmacological blockade of glutamatergic and GABAergic transmission eliminated both spontaneous and evoked PSCs – see below and Ge et al., 2006). The results indicate that in the absence of $\alpha 7$ -nAChRs, adultborn neurons receive less synaptic activity, offering a possible explanation for their reduced chances of surviving through the critical period.

Reduced Dendritic Arbors on Adultborn $\alpha 7$ KO Neurons

To determine whether the reduced synaptic input to adultborn $\alpha 7$ KO neurons possibly reflected fewer synapses being present on the cells, we carried out a morphological analysis. Young adult WT and $\alpha 7$ KO mice were injected with MMLV-GFP stereotaxically into the dentate gyrus, and 3 weeks later taken for dendritic measurements of GFP-expressing neurons. Reductions in both the total dendritic length and in the number of dendritic branch points were found for adultborn $\alpha 7$ KO neurons compared to their age-matched WT counterparts (Fig. 3). Because no differences were found in the number of spines per unit length of dendrite (15 ± 2 and 13 ± 2 spines/20 μm for WT and $\alpha 7$ KO dendrites, respectively), we calculate that adultborn $\alpha 7$ KO neurons are likely to have substantially fewer spines in aggregate than do WTs and therefore may have proportionately fewer synapses (though this

would require other techniques to confirm). A reduction in synapses would account for the reduced number of PSCs, and, further, would suggest that $\alpha 7$ -nAChR signaling may be necessary for normal dendritic growth on adultborn neurons.

To corroborate an $\alpha 7$ -nAChR effect on dendritic growth in larger populations of adultborn neurons, we crossed $\alpha 7$ KO mice with a GFP-reporter mouse line that expresses GFP under a partial GAD67 promoter. In the mature dentate gyrus of this line, GFP is expressed only in adultborn neurons 1-3 weeks post-neurogenesis (G. Feng, personal communication), prior to their assuming a glutamatergic fate (Toni et al., 2008). Examining 40 individual neurons from each of 3 WT/GFP and 3 $\alpha 7$ KO/GFP mice revealed clear differences (Fig. 4A). Comparing either the most complex neurons (Fig. 4B) or the population histograms of dendritic branch points (Fig. 4C) showed that adultborn neurons in $\alpha 7$ KO mice have a reduced dendritic complexity compared to WTs. KS statistical analysis of the dendritic branch point cumulative frequency histogram confirmed that the morphological differences were highly significant (Fig. 4D). BrdU-birthdating of adultborn neurons demonstrated that GFP expression occurred at comparable neuronal ages in the two mouse lines (data not shown). These results confirm that $\alpha 7$ -nAChRs are required for normal dendritic growth in adultborn neurons of the dentate gyrus.

Compromised Maturation of Adultborn $\alpha 7$ KO Neurons

The finding of reduced dendritic arbors on adultborn $\alpha 7$ KO neurons raised the question of whether the deficit was specific or might be part of a more global failure

of the neurons to mature on schedule. One indicator of maturation is the time during development when the chloride gradient acquires an equilibrium potential (E_{Cl}) sufficiently negative as to support inhibitory GABA_A-mediated currents. Prior to this, GABA is more depolarizing (and often excitatory) due to a reversed chloride gradient caused by early expression of the chloride transporter (importer) NKCC1. Subsequently, NKCC1 expression decreases and the chloride transporter (exporter) KCC2 appears. This inverts the chloride gradient, lowering E_{Cl} below threshold for action potentials and thereby rendering GABA inhibitory as required for adult function. Importantly, the initial period of depolarizing/excitatory GABAergic signaling is necessary both for early postnatal and adultborn neurons to develop properly and integrate into circuits (Rivera, et al., 1999; Ben-Ari Y 2002; Payne et al., 2003; Ge et al., 2006).

To determine if maturation of the chloride gradient is perturbed in adultborn neurons of $\alpha 7$ KO mice, we performed gramicidin-induced perforated-patch-clamp recording on 3-week-old adultborn neurons. Stereotaxic injection of MMLV-GFP was used to label the neurons in vivo 3 weeks before preparing hippocampal slices. Recordings were then obtained from GFP-expressing cells. By measuring the amplitude of the evoked GABAergic PSC as a function of holding potential, we were able to construct I-V plots and calculate the mean reversal potential, this being E_{Cl} . Pharmacological blockers eliminated contributions from glutamatergic PSCs, and the GABA_A receptor blocker gabazine confirmed that the PSCs being measured under these conditions were GABAergic (Supplementary Fig. 2B). The results reveal that 3-week-old adultborn $\alpha 7$ KO neurons retain an E_{Cl} that is significantly more positive than

that of age-matched WT neurons (Fig. 5A-C). No change was found in the mean resting membrane potential of adultborn $\alpha 7$ KO and WT neurons at 3 weeks of age (Fig. 5C). Comparing E_{Cl} to the resting membrane potential reveals that GABA_A receptor activation remains depolarizing in adultborn neurons from $\alpha 7$ KO mice after it has switched to hyperpolarizing in WT mice.

Maturation of the chloride gradient usually involves loss of NKCC1 and appearance of KCC2 as noted above. To compare NKCC1 levels in $\alpha 7$ KO and WT neurons, we birthdated the neurons by BrdU injection in vivo, and then prepared slices 3 weeks later for BrdU and NKCC1 immunostaining. Three-week-old adultborn $\alpha 7$ KO neurons in the dentate gyrus displayed substantially higher levels of NKCC1 than did equivalent neurons in WT mice (Fig. 5D,E). NKCC1 immunostaining in mature neurons in the outer third of the granule cell layer, which contains few adultborn neurons, revealed no significant differences between WT and $\alpha 7$ KOs. Loss of $\alpha 7$ -nAChR signaling, therefore, delays the reduction in NKCC1 levels in adultborn neurons but does not permanently prevent the reduction from occurring in the broader population of mature granule cells. Taken together, the results indicate that endogenous signaling through $\alpha 7$ -nAChRs is required to generate the normal maturation rate of the chloride gradient in adultborn neurons.

Another marker for maturation in adultborn neurons is the appearance of GABA_A receptors containing $\alpha 1$ subunits. Early appearing forms of the receptor lack the $\alpha 1$ subunit and therefore lack the fast rise and decay kinetics associated with the mature form (Overstreet-Wadiche et al., 2005; Markwardt et al., 2009). We recorded

evoked GABAergic PSCs using the perforated patch-clamp configuration on 3-week-old adultborn neurons in fresh slices previously labeled in vivo with MMLV-GFP as described above (Fig. 6A). Measuring the rise and decay kinetics of the events in adultborn neurons revealed significantly longer times in $\alpha 7$ KO neurons compared to WT (Fig. 6B). This suggests that adultborn $\alpha 7$ KO neurons retain expression of immature GABA_A receptor subunits over a longer developmental period than do WT. Delayed appearance of $\alpha 1$ -containing GABA_A receptors, a prolonged presence of NKCC1 and depolarizing chloride gradients, and a retarded dendritic arborization in adultborn $\alpha 7$ KO neurons indicate that $\alpha 7$ -nAChR signaling normally helps drive neuronal maturation following neurogenesis in the adult dentate gyrus.

Extended Vulnerability of the Adultborn Granule Cell Population to Loss of $\alpha 7$ -nAChRs

Adultborn $\alpha 7$ KO neurons develop behind schedule and have a greater chance of dying during the critical period, but some do survive. The question remains as to whether adultborn $\alpha 7$ KO neurons that survive the critical period continue to display deficits after the normal period of development. To examine this, we stereotaxically injected MMLV-GFP in vivo, birthdating adultborn neurons and filling them with fluor for subsequent visualization. After 6 weeks, hippocampal slices were prepared and examined for GFP-expressing cells. The images revealed that adultborn $\alpha 7$ KO neurons retain deficits in dendritic length and complexity even after 6 weeks (Fig. 7). This is long after dendritic development has been completed in adultborn WT neurons

(Zhao et al., 2006). The results indicate that even those adultborn $\alpha 7$ KO neurons that survive the critical period retain morphological defects.

Cell-Autonomous Action of $\alpha 7$ -nAChRs to Regulate Adultborn Maturation

To gain insight into the mechanism by which $\alpha 7$ -nAChR signaling regulates adultborn neuron maturation, we tested the hypothesis that the relevant $\alpha 7$ -nAChRs act in a cell-autonomous manner, i.e. must be present on the adultborn neurons themselves. To address this we generated lentiviral constructs encoding $\alpha 7$ RNAi with GFP, then stereotaxically co-injected this virus with MMLV expressing the red fluorescent protein mcherry into WT mice. This dual viral approach allowed us to compare the dendritic growth of $\alpha 7$ RNAi-expressing (red and green = yellow) and $\alpha 7$ RNAi-lacking (red) adultborn neurons in the same animal (Fig. 8A). Other WT mice were co-injected with lentivirus expressing a scrambled RNAi construct and MMLV-mcherry as an additional control. Adultborn neurons expressing $\alpha 7$ RNAi showed significantly reduced dendritic complexity and length compared to either RNAi-lacking adultborn neurons in the same animal or scrambled RNAi-expressing adultborn neuron controls (Fig. 8B,C). In fact, the values obtained for adultborn neurons expressing $\alpha 7$ RNAi were indistinguishable from those obtained for adultborn $\alpha 7$ KO neurons of the same age, and both clearly reduced compared to adultborn WT (see Fig. 3C).

Lenti- $\alpha 7$ RNAi often infected granule neurons in the outer third of the granule cell layer, which were likely to be fully mature prior to infection. Quantification of

the dendritic arbors of such neurons showed no deficits (Supplementary Fig. 3), suggesting that the ability of $\alpha 7$ RNAi to affect dendritic morphology is developmentally constrained. To control for potential off-target effects of the $\alpha 7$ RNAi, we repeated this experiment in $\alpha 7$ KO mice. Adultborn neurons in $\alpha 7$ KO mice that received $\alpha 7$ RNAi were not significantly different from RNAi-lacking adultborn neurons in the same animals (Fig. 8D,E). This indicates that the dendritic arbor defects caused by $\alpha 7$ RNAi are dependent on the presence of $\alpha 7$ -nAChRs in those neurons. Taken together these results demonstrate that $\alpha 7$ -nAChR signaling acts in a cell-autonomous manner to regulate dendritic arborization of adultborn neurons, a key feature of their maturation.

DISCUSSION

We show here for the first time that endogenous nicotinic cholinergic signaling through $\alpha 7$ -nAChRs in vivo enhances the survival and integration of adultborn neurons in the dentate gyrus. In the absence of functional $\alpha 7$ -nAChRs, adultborn neurons have truncated, less complex dendritic arbors, receive fewer PSCs, display GABAergic PSCs with slower kinetics, and maintain an extended period of GABAergic depolarization due to an immature chloride gradient. Together this indicates that the neurons are in an immature state. Fewer neurons survive the critical period under these conditions, and the survivors have persistent deficiencies in dendritic arborization. As a result, fewer neurons are added to the adult dentate gyrus,

thereby over time compromising renewal of the mossy fiber pathway relaying entorhinal input to the hippocampus.

Our results demonstrate that dendritic maturation of adultborn neurons is regulated by cell-autonomous $\alpha 7$ -nAChR signaling. This excludes the possibility that the $\alpha 7$ -nAChR effects are indirect, e.g. resulting from $\alpha 7$ -nAChR-mediated increases in network activity impinging on the neurons. In other systems neurotransmitter-mediated regulation of dendritic morphology involves calcium signaling and subsequent changes in gene expression (Buttery et al., 2006; Aizawa et al., 2004; Gaudilliere et al., 2004; Borodinsky et al., 2003). Since $\alpha 7$ -nAChRs have a high relative permeability to calcium (Bertrand et al., 1993; Seguela et al., 1993), can generate calcium events in hippocampal neurons even in the absence of detectable currents (Szabo et al., 2008; Fayuk and Yakel, 2007), and drive calcium-dependent gene transcription (Hu et al., 2002), this is the most plausible mechanism at present. Transcriptional regulation may also explain $\alpha 7$ -nAChR-dependent changes in physiological maturation (Liu et al., 2006).

The deficits reported here resulted from loss of $\alpha 7$ -nAChRs. None involved $\beta 2^*$ -nAChRs, the other major class of nicotinic receptors in the hippocampus. Both are expressed at early stages by adultborn neurons, and the neurons receive direct cholinergic innervation (Kaneko et al., 2006; Ide et al. 2008). In contrast to $\alpha 7$ -nAChRs, however, $\beta 2^*$ -nAChRs appear to be essential primarily for initial generation of the cells, and only during middle age (Harrist et al., 2004; Mechawar et al., 2004). Differences in activation kinetics, ion permeabilities, surface locations, and tethering

to intracellular signaling cascades provide candidate mechanisms enabling $\alpha 7$ -nAChRs and $\beta 2^*$ -nAChRs to exert distinct regulatory controls (Vernino et al., 1992; Seguela et al., 1993; Alkondon et al. 1997B; Zoli et al., 1998; Fenster et al., 1999; Chang and Berg, 2001; Wooltorton et al., 2003; Alkondon and Albuquerque 2005; Xu et al., 2006). Interestingly, the $\alpha 7$ -nAChR dependence is also different from that of NMDA receptors. The latter are required by adultborn neurons for optimal survival but also appear to mediate competitive interactions when functional on other neurons (Tashiro et la., 2006). In contrast, $\alpha 7$ -nAChRs are required whether or not other neurons receive input via $\alpha 7$ -nAChRs.

Adultborn neurons examined here underwent their final mitosis in mice that were at least one month old. At this time the granule cell layer is fully formed, and neurogenesis is confined to the subgranular layer as it is throughout adulthood (Altman and Bayer, 1990; Esposito et al., 2005). Adultborn neurons generated in young and old animals appear to have similar fates with respect to differentiation and morphological end state (Morgenstern et al., 2008; Ahlenius et al., 2009). This suggests that the results obtained here are likely to apply broadly across the population of adultborn neurons in the dentate gyrus, though it should be noted that aged adults may differ from young adults in some respects. Importantly, results from adultborn $\alpha 7$ KO neurons here were always compared to age-matched adultborn WT neurons.

The $\alpha 7$ -nAChR requirement for timely maturation of the chloride gradient in adultborn neurons is similar to that reported previously for developing neurons in early postnatal mouse hippocampus and embryonic chick spinal cord (Liu et al.,

2006). The mechanisms, however, may differ. Adult neurogenesis proceeds more slowly than in the embryo (Esposito et al., 2005; Overstreet-Wadiche et al., 2006), and adultborn neurons replace pre-existing synapses rather than increase the total as seen in the early postnate (Toni et al., 2007). The biggest difference, however, is likely to be the presence of spontaneous waves of excitation seen in much of the developing nervous system, including the dentate gyrus (Ben Ari et al., 1989; Kasyanov et al., 2004; Overstreet-Wadiche et al., 2006). In embryonic spinal cord and early postnatal hippocampus, nicotinic activity initiates or enhances these waves (Hanson and Landmesser, 2003; Le Magueresse et al., 2006). The waves may enable nicotinic activity to act ubiquitously (though indirectly) to excite large populations and coordinate their maturation. The adult dentate gyrus has no comparable waves of excitation (Overstreet-Wadiche et al., 2006), and may instead rely on direct cholinergic input to guide the development and integration of adultborn neurons. Nonetheless, $\alpha 7$ -nAChRs appear to have some common effects in the early postnatal and adult dentate gyrus on neuronal development: adultborn and early postnatal neurons rely on $\alpha 7$ -nAChR signaling to terminate the initial period of GABAergic excitation/depolarization.

A period in which GABA currents are depolarizing (and likely excitatory) is widely thought essential for proper neuronal development and integration for both early postnatal and adultborn neurons (Ben-Ari, 2002; Represa and Ben-Ari, 2005; Tozuka et al., 2005; Ge et al., 2006; Cancedda et al., 2007). Extended periods of depolarizing/excitatory GABA signaling, as found in $\alpha 7$ KOs, might therefore be expected to correlate with increased dendritic arborization and innervation. The

opposite was found: adultborn $\alpha 7$ KO neurons have (1) shorter, less complex dendritic arbors, (2) reduced synaptic input, (3) an immature form of GABA_A receptors apparently lacking $\alpha 1$ subunits (Overstreet-Wadiche et al., 2005), and 4) increased likelihood of dying during the critical 2-4 weeks post-neurogenesis (Tashiro et al., 2006). The results indicate that adultborn neurons require $\alpha 7$ -nAChR signaling for normal development and integration, advancing them to some trigger point that enables the chloride gradient to mature and render GABA currents inhibitory.

Adult neurogenesis is essential for proper hippocampal function. Adultborn neurons integrate into functional hippocampal networks and are activated by hippocampal-dependent learning tasks (Ramirez-Amaya et al., 2006; Tashiro et al., 2007). Ablation of the precursor cells that normally generate adultborn neurons impairs certain types of hippocampal-dependent learning and memory (Shors et al., 2001, 2002; Rola et al., 2004; Snyder et al., 2005; Winocur et al., 2006). Key functions for adultborn neurons include coding time and place integration (Aimone et al., 2006), spatial pattern separation (Clelland et al., 2009), reinforcement of preexisting memories (Trouche et al., 2009), and transition of memories from hippocampal to cortical circuits (Kitamura et al., 2009). Recently adultborn neurons have been shown to play a role in diminishing addictive behavior and reducing the incidence of relapse (Noonan et al., 2009). Loss of $\alpha 7$ -nAChR signaling will cause cumulative deficits to accrue over time in hippocampal circuitry as fewer adultborn neurons survive and those that do are less likely to be appropriately integrated into functioning circuits. The expected outcome would be a pronounced decline in

hippocampal function, perhaps most acute for memories that are space- and time-dependent (Aimone et al., 2006; Clelland et al., 2009; Trouche et al., 2009).

Early deficits in Alzheimer's disease involve loss of cholinergic neurons and a diminution of cholinergic signaling (Whitehouse et al., 1982; Francis et al., 1999; Nordberg, 2001; Lyness et al., 2003; O'Neil et al., 2007). The β -amyloid peptide, which accumulates during the disease (Selkoe, 1994), impairs choline uptake and acetylcholine release, further compromising cholinergic signaling (Auld et al., 1998; Kar et al., 1998). Moreover, β -amyloid peptide has been reported to inhibit α 7-nAChR function either directly or indirectly (Wang et al., 2000a,b; Liu et al., 2001; Pettit et al., 2001; Dougherty et al., 2003; Grassi et al., 2003; Lee and Wang, 2003; Pym et al., 2005), though it has also been reported to have α 7-nAChR agonist activity at low concentrations (Dineley et al., 2001, 2002; Dougherty et al., 2003; Grassi et al., 2003; Wang et al., 2003). Several studies have reported specific decrements in α 7-nAChRs associated with Alzheimer's disease (Hellstrom-Lindahl et al., 1999; Guan et al., 2000; Lee et al., 2000; but see Reid et al., 2000). The present studies predict that the loss or blockade of α 7-nAChRs in Alzheimer's disease would exacerbate the symptoms by decreasing the incorporation of adultborn neurons. Supporting this idea is the observation that donepezil, an acetylcholinesterase inhibitor approved as a drug for treatment of Alzheimer's disease, has been shown to promote adultborn neuron survival during the critical period (Kaneko et al., 2006).

Our results identify α 7-nAChRs as potential pharmacological targets for amplifying adultborn neuron integration and survival. An impediment to prescribing

nicotinic agonists, however, is the observation that prolonged nicotine exposure at concentrations encountered by smokers can have detrimental effects on the survival of adultborn neurons (Abrous et al., 2002; Scerri et al., 2005; Shingo and Kito, 2005). The nicotine-mediated death of adultborn neurons occurs early in their development whereas the beneficial effects of endogenous $\alpha 7$ -nAChR signaling seen here become apparent 2-4 weeks after neurogenesis. Either additional nAChR subtypes are involved or the manner of receptor activation is critical. This motivates further examination of mechanisms controlling nicotinic regulation of adult neurogenesis.

FIGURE LEGENDS

Fig. 1 Absence of $\alpha 7$ -nAChRs decreases the chance of survival for adultborn neurons during the critical period. **(A)** Immunofluorescent images of the dentate gyrus showing BrdU (red) and dapi (blue) staining 4 weeks after injection of BrdU to WT (top) and $\alpha 7$ KO (bottom) mice. **(B)** Quantification of BrdU-immunopositive cells at 2 weeks (left, 2 WPI) and 4 weeks (right, 4 WPI) after BrdU injection (mean \pm SEM; n = 4 mice per condition). * $p < 0.05$, Student's *t*-test. Scale bar: 40 μ m.

Fig. 2 Adultborn neurons lacking $\alpha 7$ -nAChRs receive less synaptic activity than normal. **(A)** PSCs recorded in 3-week-old adultborn neurons at -80 mV holding potential, identified by GFP-labeling from MMLV-GFP injection in vivo 3 weeks earlier: WT (top); $\alpha 7$ KO (bottom). **(B)** PSC frequency (left) and amplitude (right) for WT and $\alpha 7$ KO neurons (mean \pm SEM; n = 6 WT and 5 $\alpha 7$ KO neurons). * $p < 0.05$, *** $p < 0.001$, Student's *t*-test.

Fig. 3 Reduced dendritic arbors are found for 3-week-old adultborn neurons in mice lacking $\alpha 7$ -nAChRs when analyzed by MMLV-GFP labeling in vivo. **(A)** Deconvolved z-stack image of an adultborn granule neuron 3 weeks after the mouse was stereotaxically injected with MMLV-GFP in the dentate gyrus. Digitally magnified dendritic segments (inset) were used for measurement of spine numbers. **(B)** Traces of dendrites from granule neurons in WT (left) and $\alpha 7$ KO (right) mice 3

weeks after labeling with MMLV-GFP. **(C)** Quantification of dendritic properties in terms of dendritic branch points (left) and dendritic length (right) of cells as in **B** (mean \pm SEM; n = 3 mice per condition with > 4 cells per animal). *p<0.05, one-way ANOVA with Bonferroni's post-hoc test for multiple comparisons. Scale bars: 10 μ m.

Fig. 4 Absence of α 7-nAChRs causes adultborn neurons to have decreased dendritic complexity as visualized with GAD67 reporter mice. **(A)** GFP expression in adultborn neurons in the dentate gyrus of GAD67-GFP reporter mice viewed at 10X (left) and 63X (right) magnification. **(B)** Dendritic arbor traces of the most complex neurons observed in WT (left) and α 7KO (right) mice. **(C)** Dendritic branch point histograms (mean \pm SEM; n = 3 mice per condition; 40 neurons per mouse). **(D)** Cumulative frequency plot of dendritic branch points (n = 120 granule neurons each for WT and α 7KO mice). ***p<0.001, KS test.

Fig. 5 Delayed maturation of the chloride gradient in adultborn α 7KO neurons extends the period of depolarizing GABAergic responses. **(A)** Superimposed perforated patch-clamp recordings of GABAergic PSCs evoked in 3-week-old adultborn WT (left) and α 7KO (right) neurons at the indicated holding potentials. The neurons were labeled in vivo with MMLV-GFP and visualized in freshly prepared slices at the time of recording. **(B)** Peak amplitude of the evoked GABAergic PSC as a function of voltage in a WT (black) and an α 7KO (red) neuron as in **A**. **(C)**

Interpolated reversal potentials (left; E_{Cl} ; $n = 6$ WT and 5 $\alpha 7$ KO) and resting membrane potentials (right; V_{REST} ; $n = 6$ WT and 8 $\alpha 7$ KO) for WT and $\alpha 7$ KO neurons. **(D)** NKCC1 immunostaining (green) of BrdU-labeled (red) 3-week-old adultborn neurons from a WT (top) and $\alpha 7$ KO (bottom) dentate gyrus, mounted in dapi-containing media to reveal nuclei (blue). **(E)** Quantification of NKCC1 levels in neurons as in **D** (3-Week-Old) or from neurons in the outer third of the granule cell layer (mature) from the same mice (mean \pm SEM; $n = 3$ animals per condition; ≥ 10 neurons per mouse). * $p < 0.05$; ** $p < 0.01$, Student's *t*-test.

Fig. 6 In the absence of $\alpha 7$ -nAChRs, GABAergic PSCs display immature kinetics. **(A)** Averaged and normalized evoked GABAergic PSCs from a WT (gray) and an $\alpha 7$ KO (black) 3-week-old adultborn neuron. **(B)** Quantification of rise time (left) and weighted decay (τ ; right) of evoked GABAergic PSCs as in **A** (mean \pm SEM; $n = 5$ WT and 4 $\alpha 7$ KO). ** $p < 0.01$, *** $p < 0.001$ Student's *t*-test.

Fig. 7 The dendritic deficits found in adultborn $\alpha 7$ KO neurons persist long after normal dendritic development is complete in WT. **(A)** Deconvolved z-stack image of a 6-week-old adultborn neuron from an $\alpha 7$ KO mouse labeled in vivo by injection of MMLV-GFP at the time of cell division. **(B)** Dendritic arbor traces of 6-week-old adultborn neurons from WT (left) and $\alpha 7$ KO (right) mice. **(C)** Dendritic branch points (left) and dendritic length (right) of 6-week-old adultborn neurons visualized by

MMLV-GFP labeling (mean \pm SEM; n = 4 mice per condition with > 4 cells per animal). *p<0.05, Student's *t*-test. Scale bars: 10 μ m.

Fig. 8 Cell-autonomous signaling through α 7-nAChRs supports dendritic maturation of adultborn granule neurons. **(A)** Stereotaxic coinjection of lenti- α 7RNAi (green) and MMLV-mcherry (red) yields both adultborn neurons expressing α 7RNAi (top row, yellow cell) and adultborn neurons lacking RNAi expression (bottom row, red cells) in the same animal. Images are shown at 10x (left) and magnified at the region of interest (white box) to 63x (middle and right). **(B)** Dendritic arbor traces of 3-week-old adultborn neurons expressing α 7RNAi (top), scrambled RNAi (middle), or lacking RNAi expression in animals injected with lenti- α 7RNAi (bottom). **(C)** Dendritic branch points (left) and dendritic length (right) of 3-week-old adultborn neurons infected as in **A** and **B** (mean \pm SEM; n = 3 mice per condition with > 4 cells per animal). **(D)** Dendritic traces of 3-week-old α 7KO adultborn neurons expressing α 7RNAi (top) or lacking RNAi expression in animals injected with lenti- α 7RNAi (bottom). **(E)** Dendritic branch points (left) and dendritic length (right) of 3-week-old α 7KO adultborn neurons (mean \pm SEM; n = 4 mice per condition). *p<0.05, one-way ANOVA with Bonferroni's post-hoc test for multiple comparisons.

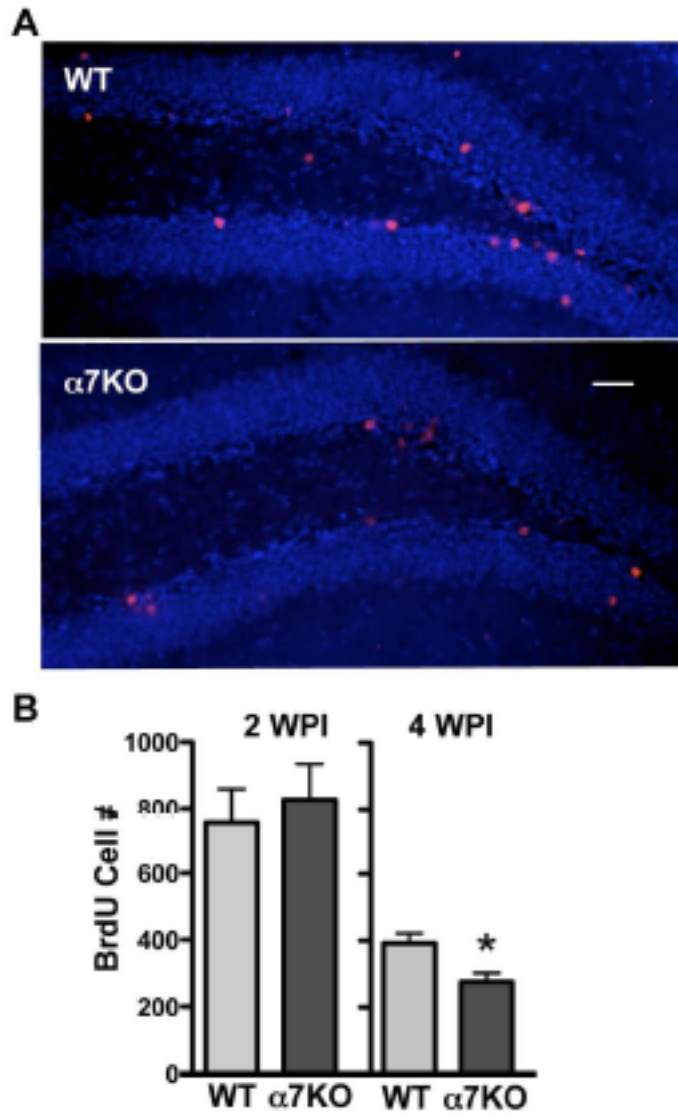


Fig. 1 Absence of $\alpha 7$ -nAChRs decreases the chance of adultborn neuron survival.

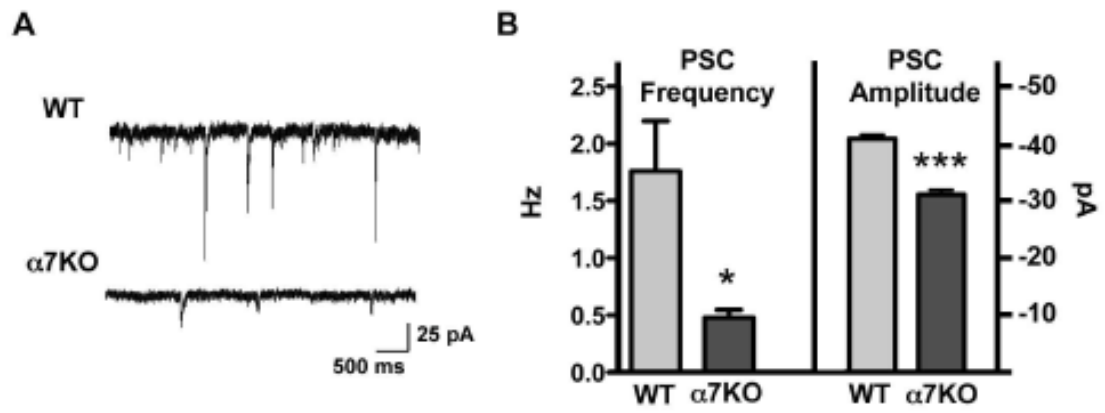


Fig. 2 Adultborn neurons lacking $\alpha 7$ -nAChRs receive less synaptic activity.

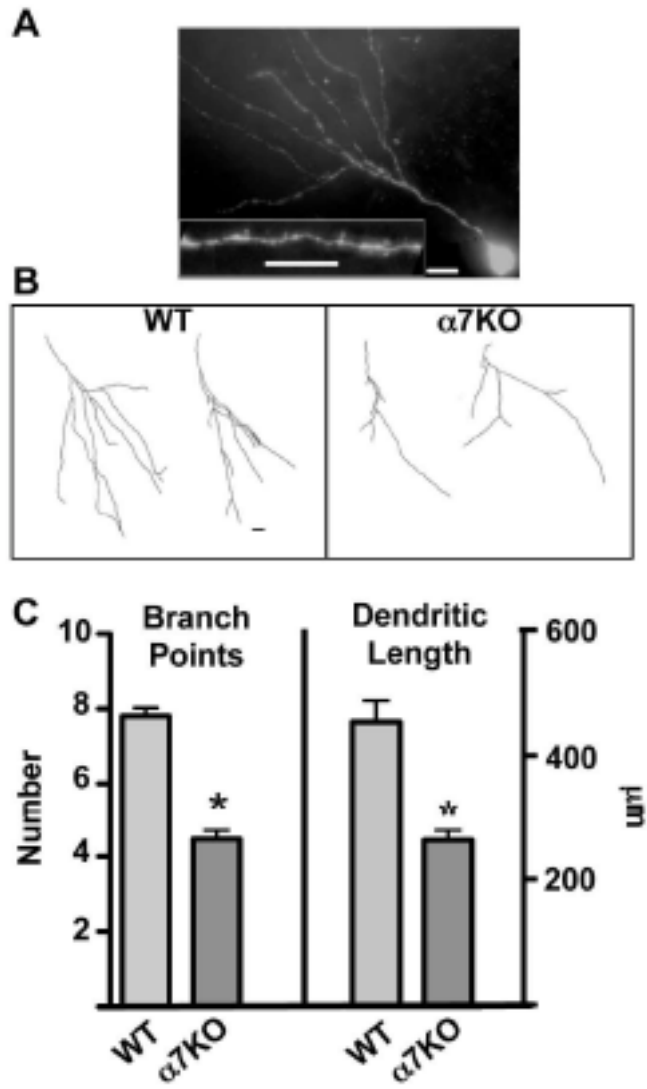


Fig. 3 Reduced dendritic arbors in mice lacking $\alpha 7$ -nAChRs.

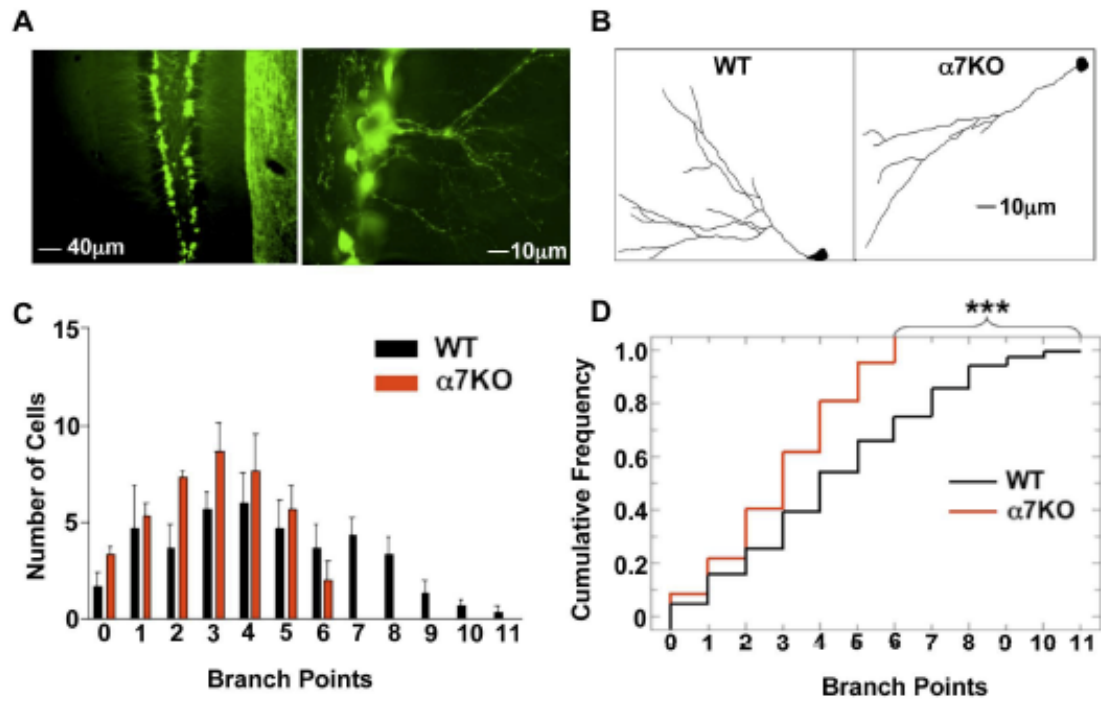


Fig. 4 Absence of $\alpha 7$ -nAChRs decreases dendritic complexity in GAD67-GFP mice.

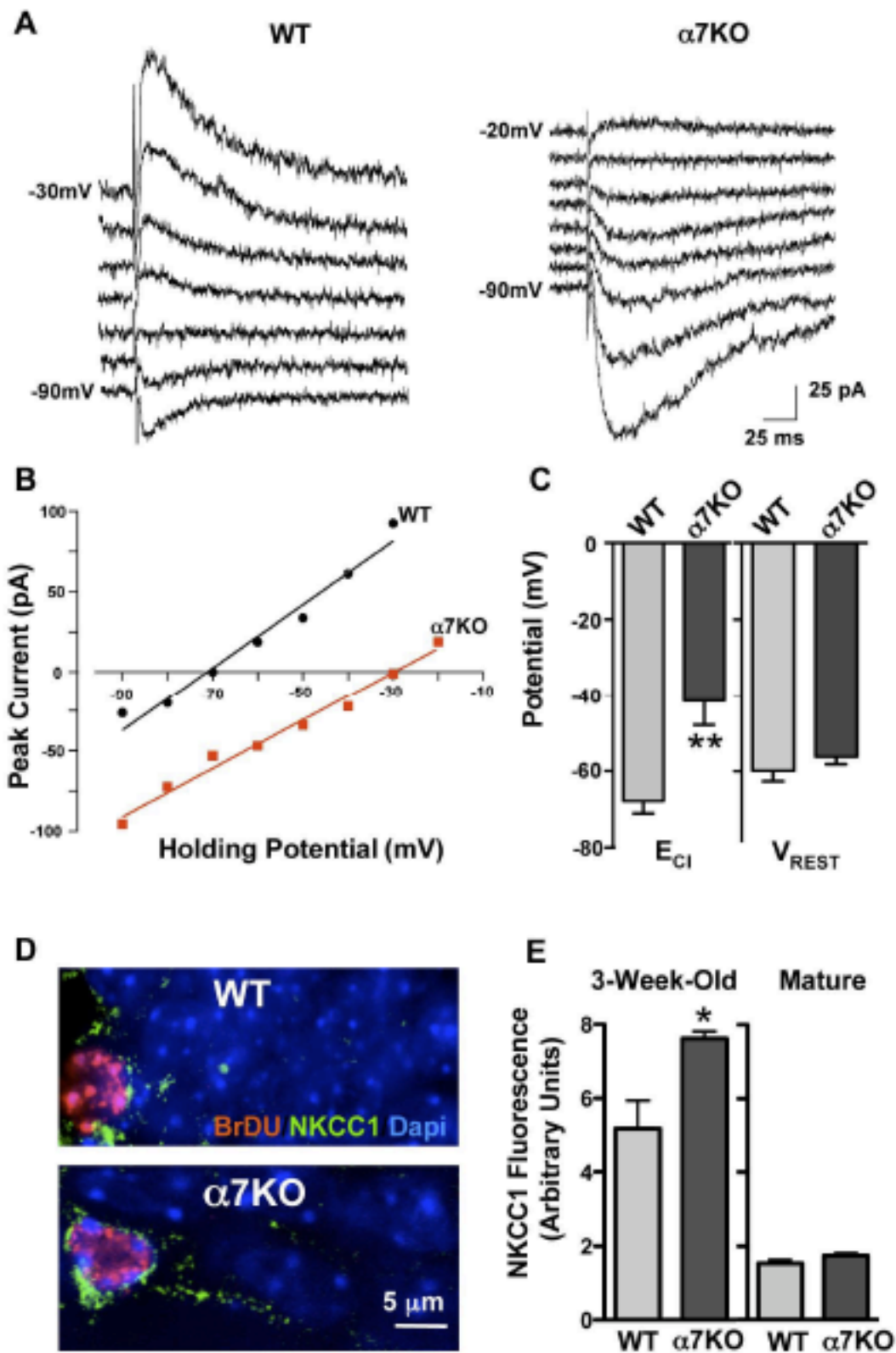


Fig. 5 Delayed maturation of the chloride gradient in adultborn $\alpha 7$ KO neurons.

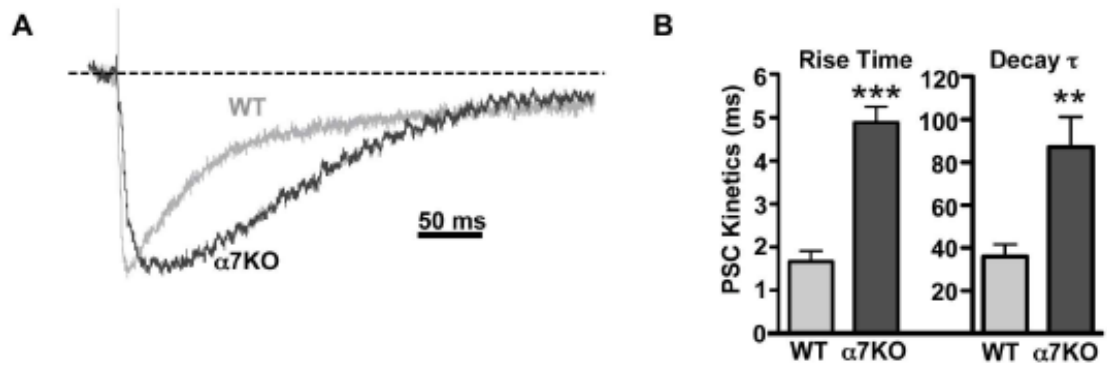


Fig. 6 In the absence of $\alpha 7$ -nAChRs, GABAergic PSCs display immature kinetics.

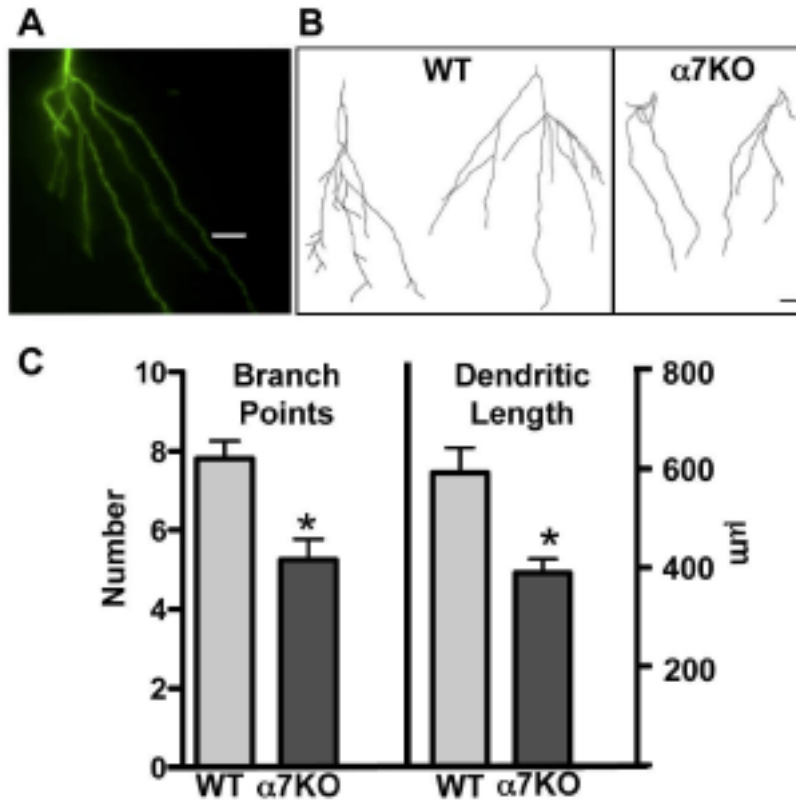


Fig. 7 The dendritic deficits found in adultborn $\alpha 7$ KO neurons persist.

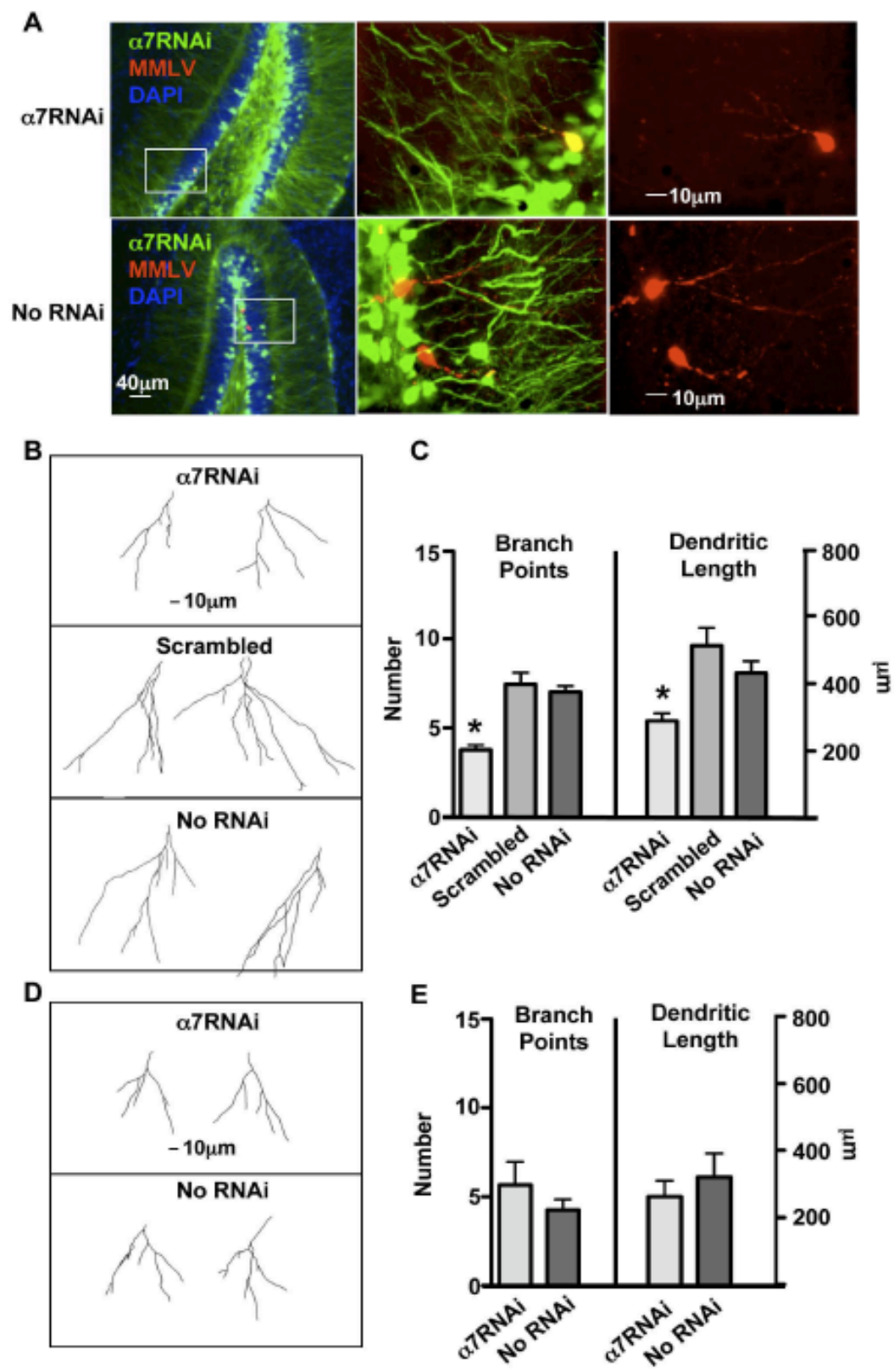


Fig. 8 Cell-autonomous signaling through $\alpha 7$ -nAChRs supports dendritic maturation.

Andrew Halff, Catarina Fernandes, and Darwin Berg are co-authors on chapter one, which was published in the Journal of Neuroscience. The dissertation author was the primary investigator and author of this paper.

CHAPTER 2

**NICOTINE HAS OPPOSING EFFECTS ON THE FATE OF ADULTBORN
NEURONS DEPENDING ON EXPOSURE TIMING**

by

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ABSTRACT

Adultborn neurons in the hippocampus aid memory formation and provide protection against addictive behavior. Nicotinic cholinergic input promotes survival and maturation of adultborn neurons, but paradoxically nicotine exposure kills them. Here we show that nicotine, acting through a single class of nicotinic receptors, produces radically different outcomes depending on timing of administration. Chronic nicotine exposure initiated prior to final mitosis kills most adultborn neurons in their first week; conversely, chronic nicotine exposure initiated one week after final mitosis increases the number of adultborn neurons surviving through the critical period 2-4 weeks post-mitosis. Both treatments cause the surviving adultborn neurons to achieve greater dendritic arborization via the same class of nicotinic receptors. Survival and dendritic arborization are regulated separately: chronic DMXBBA, a partial agonist of the receptors, enhances arborization without influencing survival even when initiated prior to final mitosis. The results demonstrate multiple nicotinic effects on the fate of adultborn neurons and indicate risks for therapeutic strategies targeting even a single class of nicotinic receptors indiscriminately in space and time.

INTRODUCTION

New neurons are generated throughout adult life in the dentate gyrus of the hippocampus. Ablating adult neurogenesis in the hippocampus impairs trace and spatial memory (Shors et al., 2001; Snyder et al., 2005), pattern separation (Clelland et al., 2009), memory extinction (Deng et al. 2009), and memory transition from hippocampal to cortical areas (Kitamura et al., 2009). Recent evidence suggests a

causal role for decreased neurogenesis in addiction behavior; ablation of adultborn neurons enhances drug seeking and impairs extinction following cocaine administration (Noonan et al., 2009).

Adultborn neurons early on receive cholinergic innervation (Kaneko et al., 2006; Ide et al. 2008) and express both major classes of neuronal nicotinic acetylcholine receptors: $\beta 2$ -containing heteropentameric receptors ($\beta 2^*$ -nAChRs) and $\alpha 7$ -containing homopentameric receptors ($\alpha 7$ -nAChRs; see Dani and Bertrand, 2007, for review). Deletion of $\beta 2^*$ -nAChRs through β -gene knockout ($\beta 2$ KO) causes fewer adultborn neurons to be generated (Harrist et al., 2004). Knockout of $\alpha 7$ -nAChRs ($\alpha 7$ KO) reduces the ability of adultborn neurons to survive through the critical period 2-4 weeks post-mitosis (Campbell et al., 2010) when they normally become functionally integrated into networks and dependent on glutamatergic activity for survival (Tashiro et al., 2006, 2007). Adultborn neurons that do survive in $\alpha 7$ KOs have reduced dendritic arborization and network integration (Campbell et al., 2010). Despite this dependence on nicotinic cholinergic input, chronic exposure to nicotine reduces the likelihood that adultborn neurons survive at least one week (Abrous et al., 2002; Scerri et al., 2005).

Here we examine the effects of nicotine on the fate of adultborn neurons. We find that early exposure to nicotine is detrimental for survival while subsequent exposure rescues neurons and promotes dendritic development, in both cases via $\alpha 7$ -nAChRs. Moreover, effects on survival and development can be separately manipulated depending on the type of $\alpha 7$ -nAChR agonist employed. Stimulation of

$\beta 2^*$ -nAChRs promotes spine accumulation. The results indicate multiple dimensions for nicotinic regulation of adultborn neuron survival and integration.

MATERIALS AND METHODS

Transgenic mice. Male and female mice (1-3 months old) had C57Bl/6 background. The $\alpha 7$ KOs were purchased (Jackson Laboratories, Bar Harbor, ME), bred as heterozygotes, and genotyped by PCR. Homozygous offspring were used for experiments. The $\beta 2$ KOs were bred as homozygotes (provided by Marla Feller, University of California, Berkeley, CA). All experiments were performed with age-matched controls.

Anesthesia. Mice were anesthetized by intraperitoneal injection with a sterile solution of 10 mg/ml ketamine and 1 mg/ml xylazine in 0.9% NaCl at 0.01 ml/g body weight. Toe pinch was used to verify anesthesia.

Osmotic minipumps. Osmotic minipumps (Alzet model 1004 for nicotine or 2001 and 2004 for DMXBA) were loaded as instructed by the manufacturer to deliver nicotine or DMXBA in sterile 0.9% NaCl at 2 mg/kg body weight per hour or 4 mg/kg body weight for 2X DMXBA. Prior to insertion, pumps were placed in sterile 0.9% NaCl at 37°C for 24 hours. For implantation, a single incision was made in the dorsal skin between the scapuli, and the pump was inserted into the subcutaneous tissue. When treatments required five weeks of drug delivery, the partially used pump was replaced with a new pump after one week of delivery. Pump weight and volume measurements were used to verify amount of drug delivered.

Labeling and imaging. BrdU and retroviral labeling, as well as BrdU counts and quantification of dendritic morphology, were performed as described (Campbell et al., 2010). Aberrant migration was interpreted as a labeled neuron found outside the inner half of the granule layer in the dentate gyrus.

Statistical analysis. Student's t-test was used for comparing two values. One-way ANOVA with Bonferroni's post hoc test was used when comparing three or more values. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

RESULTS

Nicotinic effects on survival of adultborn neurons depend on $\alpha 7$ -nAChRs and timing

To test the effects of nicotine on survival of adultborn neurons, we implanted nicotine-containing osmotic minipumps designed to deliver nicotine at a blood concentration of 0.6 μM throughout the experiment. This concentration represents the upper range found in human smokers (Matta et al., 2007). Adultborn neurons were birth-dated by injecting BrdU 1 week after implanting the minipumps. Cell counts performed one week after birth dating demonstrated a significant reduction in the number of surviving neurons, compared to saline-infused controls (Fig. 1A,B). Importantly, nicotine under this regimen had no effect in $\alpha 7$ KO mice, indicating that the detrimental effects seen depended entirely on $\alpha 7$ -nAChRs (Fig. 1A,B).

Initiating nicotine infusion at a later time, however, had the opposite outcome. In this case adultborn neurons were birth-dated by injecting BrdU, and then the nicotine minipumps were implanted one week later. Cell counts were performed 4

weeks after birth dating, a time when the glutamatergic-dependent critical period has ended (Tashiro et al., 2006, 2007). In this case the nicotine treatment increased the number of surviving neurons, and the effect was obliterated in $\alpha 7$ KOs (Fig. 1C). The results indicate that nicotine supplied after the first week acts through $\alpha 7$ -nAChRs to enhance adultborn neuronal survival during the critical period (Campbell et al., 2010).

To assess the cumulative effects of nicotine, we implanted the pumps one week prior to birth-dating and continued infusion up through week 4 when cell counts were performed. Nicotine produced a substantial decrement in the number of neurons surviving through the critical period, and, again, had no effect in $\alpha 7$ KOs (Fig. 1D). Taken together, the results show that nicotine has opposing effects depending on the time when exposure is initiated, and that chronic nicotine through the full developmental period substantially reduces the number of surviving adultborn neurons.

Nicotine effects on dendrite and spine development are mediated by different nAChRs

The contribution of adultborn neurons to behavior depends not only on their survival but also on their functional incorporation into networks. Functional incorporation is enhanced by activity and learning (Ge et al. 2006; Tronel et al., 2010). To determine if nicotine affects this aspect of adultborn neuron fate, we injected MMLV-GFP retrovirus, which both birth dates adultborn neurons and allows their dendrites and spines to be imaged (Campbell et al., 2010).

Nicotine or saline pumps were surgically implanted one week after viral injection; dendritic arborization and spines were quantified 4 weeks after viral injection. Nicotine significantly enhanced dendritic growth as reflected by total dendritic branch length in WT and $\beta 2$ KO, but not $\alpha 7$ KO, mice (Fig. 2A,B). It significantly enhanced spine density in WT and $\alpha 7$ KO, but not $\beta 2$ KO, mice (Fig. 2C,D). Dendritic complexity, reflected in the number of branch points per cell, did not differ between nicotine- and saline-treated animals in any genotype (WT saline: 7.81 ± 0.21 , WT nicotine: 8 ± 0.29 branches/cell). The results demonstrate that nicotine treatment enhances morphological parameters of adultborn neurons using two pathways: stimulation of $\alpha 7$ -nAChRs enhances dendritic growth while stimulation of $\beta 2^*$ -nAChRs increases spine number.

Adultborn neurons surviving chronic nicotine have more dendrites but aberrant migration

To determine whether adultborn neurons surviving chronic nicotine, i.e. nicotine initiated prior to their birth, were developmentally handicapped, we birth dated them with MMLV-GFP one week after implanting the pumps. Morphological measurements 4 weeks after viral injection showed that neurons that survived the nicotine treatment had larger and more complex dendritic arbors than did saline-treated control neurons (Fig. 3A-C). They also had greater numbers of spines (Fig. B). The results indicate that nicotinic regulation of survival is distinct from regulation of development; the latter can be augmented in those adultborn neurons surviving chronic nicotine exposure.

Unexpectedly, nicotine exposure initiated prior to final mitosis, caused a substantial number of adultborn neurons to migrate incorrectly, ending in locations outside the dentate gyrus inner granule layer at 4 weeks post-birth (Fig. 3C). This was never the case for saline-treated animals but occurred for $26 \pm 6\%$ of adultborn neurons in nicotine-treated animals ($n = 188$ cells, 3 animals). This result, together with the finding that early nicotine exposure increases dendritic complexity (Fig. 3B) while subsequent nicotine exposure does not (see above), indicates that the first week of life for adultborn neurons places them at special risk for manipulation by nicotine.

Nicotinic effects on survival and dendritic growth can be separated by a partial agonist

The fact that chronic nicotine exposure can have both detrimental effects on neuronal survival and yet positive effects on dendritic growth raises the question of whether these outcomes can be separated by the mode or extent of chronic stimulation. One strategy we tested was to limit stimulation by replacing nicotine with DMXBBA, a selective partial agonist for $\alpha 7$ -nAChRs. DMXBBA can improve cognition and alleviate some gating deficits in mouse models of schizophrenia (see Olincy and Stevens, 2007 for review).

DMXBBA was supplied via osmotic minipump at a concentration previously shown to induce cognitive enhancement (Stevens et al., 1998). One week after initiating infusion, adultborn neurons were birth date labeled either by MMLV-GFP or BrdU injection. Survival and morphological quantification were assessed 4 weeks after injection. Remarkably, DMXBBA significantly enhanced the dendritic length of

adultborn neurons while having no effect on survival, compared to saline-treated controls (Fig 4A-C). No abnormal migration was observed in DMXBBA-treated animals, and no changes were seen in dendritic complexity (saline: 5.33 ± 0.26 , DMXBBA: 6.45 ± 0.48 ; $n = 16$ cells, 4 animals). Thus, DMXBBA achieved a subset of the nicotinic effects; it promoted dendritic growth but did not influence survival, migration, or dendritic complexity. Nor could it protect against chronic nicotinic effects. Co-administration of nicotine and DMXBBA via separate pumps, either at normal or double DMXBBA strength, did not prevent nicotine from reducing the number of surviving adultborn neurons (Fig. 4C). Though DMXBBA competes with nicotine for binding to $\alpha 7$ -nAChRs (De Fiebre et al., 1994), it cannot protect against the negative effects of nicotine in vivo at the concentrations used here.

DISCUSSION

Our results indicate that nicotine acts through $\alpha 7$ -nAChRs to kill young adultborn neurons, while encouraging survival of the more mature population. Under chronic nicotine exposure the net effect is robust cell loss, moreover a significant fraction of neurons that survive display abnormal localization. Nicotine exposure also amplifies adultborn neuron dendritic growth and spine formation, the former by acting through $\alpha 7$ -nAChRs and the latter by acting through $\beta 2$ -nAChRs. Survival and growth are independently regulated, and can be pharmacologically separated with the partial $\alpha 7$ -nAChR agonist DMXBBA.

Of particular interest is the observation that nicotine has opposing effects on the fate of adultborn neurons depending on their developmental age at the time of

exposure. This is in sharp contrast to endogenous signaling through the receptors, which only promotes enhancement of adultborn neurons. The ability of nicotine to induce adultborn neuron death raises novel health concerns for tobacco usage. It also highlights the difficulties in manipulating nAChRs without incurring detrimental effects. Numerous modifiers of nAChR signaling are being developed and tested for treating a range of neurological disorders (see Taly et al., 2009 for review). Unfortunately, either blocking $\alpha 7$ -nAChRs or amplifying their signaling can negatively impact adultborn neuron survival and also risks changes to their connectivity and migration. Conversely, nAChRs may provide useful targets for manipulating and studying specific aspects of adult neurogenesis, such as spine or dendritic arbor growth.

It is known that competition occurs between adultborn neurons of different ages (Dupret et al., 2007), thus one might predict that nicotine induced death of young neurons and increased survival of older neurons are mechanistically linked to one another. This model, however, cannot explain our results. Under chronic nicotine exposure both young and old adultborn neuron survival is decreased, and endogenous signaling enhances the older population without affecting the younger (Campbell et al. 2010). It appears that nicotine co-opts the endogenous $\alpha 7$ -nAChR signaling pathways to enhance survival and, through a separate mechanism, induces young adultborn neuron death. A likely mechanism for nicotine induced cell death is provided by a prior study where nicotine killed cultured neural progenitor cells prior to but not after differentiation. It was found that prior to differentiation the neurons lack sufficient

calcium buffering proteins to prevent neurotoxicity from calcium flux through $\alpha 7$ -nAChRs (Berger et al., 1998).

To our knowledge we provide the first evidence that $\beta 2$ -nAChR signaling can alter adultborn neuron connectivity. Previous studies of adult neurogenesis in $\beta 2$ KO mice did not examine morphology (Harrist et al., 2004; Mechawar et al., 2004). Comparisons of cortical pyramidal neurons in WT and $\beta 2$ KO mice recently demonstrated that $\beta 2$ -nAChRs are necessary for normal dendritic morphology and spine density in certain brain regions (Ballesteros-Yanez et al., 2010). Our results show that nicotine acts through $\beta 2$ -nAChRs to enhance spine density without altering the dendritic morphology of adultborn neurons. Interestingly, endogenous signaling through $\beta 2$ -nAChRs does not appear necessary for normal spine formation of the neurons; see Fig 2B WT and $\beta 2$ KO columns for comparison. This, again, shows novel functions for nicotine beyond endogenous receptor signaling. The ability of nicotine to act as a pharmacological chaperone of $\beta 2$ -nAChRs to alter receptor number, signaling intensity, stoichiometry, and mobility may account for this result (see Lester et al., 2009 for review). That nicotine signaling has different effects when acting through $\beta 2$ - and $\alpha 7$ -nAChRs is not surprising as these receptors have different localizations, ion permeability, nicotine binding efficacies, and activation kinetics (See Albuquerque et al., 2009 for review).

An unexpected finding is that nicotinic regulation of survival and morphological maturation are independently controlled. This is supported by two results. 1) Nicotine increases dendritic growth and spine density both when it kills and

saves adultborn neurons, and 2) DMXBBA treatment increases dendritic growth without affecting survival. Hippocampal dependent learning uniformly increases adultborn neuron survival, dendritic growth and spine density (Gould et al., 1999; Tronel et al., 2010). This argues against the effects of nicotine reported here arising as a secondary consequence of nicotine induced increases in learning. Further, it suggests that different signaling pathways may regulate growth and survival. Functionally, adultborn neuron survival and enhanced connectivity are both predicted to amplify the processing power of the dentate (London and Hausser, 2005; Deng et al. 2010). Thus, it seems both nicotine and DMXBBA may have positive effects on dentate function. It is, however, difficult to predict what the net effect for hippocampal function would be in conditions of chronic nicotine usage, which would both enhance dendritic growth and spine density and decrease adultborn neuron survival.

Adultborn neurons integrate into behaviorally relevant neural networks between 2 and 4 weeks post neuronal birth, and during this time they contribute unique properties to the hippocampal network (Tashiro et al., 2007; Deng et al., 2009). Viewed in conjunction with these results our findings suggest that hippocampal response to tobacco will be altered depending on usage duration. During early usage nicotine will increase the growth and survival of the relevant adultborn neurons. During prolonged usage fewer neurons will integrate into the network but those that do will have enhanced dendritic arbors and spine density. Stopping nicotine after prolonged use will further impair adultborn neuron integration, as those few survivors will have smaller dendritic arbors and fewer spines. Decreased integration of adultborn neurons has been shown to lower performance on a number of hippocampal

dependent tasks (see introduction). Our results predict that the lowest hippocampal performance would occur during initial cessation of chronic nicotine use. Consistent with this model, in hippocampal dependent fear conditioning acute nicotine enhances task performance, chronic nicotine usage blocks this enhancement and withdrawal from chronic nicotine impairs performance (Davis et al., 2005). Decreased neurogenesis has also been shown to increase drug-seeking behavior (Noonan et al., 2009). Since chronic exposure decreases adultborn neuron survival, nicotine could enhance drug-seeking behavior in part through $\alpha 7$ -nAChR mediated loss of the adultborn neuron population. Consistent with this model $\alpha 7$ KO mice have decreased drug-seeking compared to WT after but not before one week of nicotine usage. More work is needed to understand the contribution of adult neurogenesis to nicotine-induced addictive behaviors.

FIGURE LEGENDS

Fig. 1 Nicotinic effects on survival of adultborn neurons depend on $\alpha 7$ -nAChRs and timing. **A)** BrdU staining in the dentate gyrus of WT (left) and $\alpha 7$ KO (right) mice implanted with nicotine pumps one week before BrdU injection and perfused one week after. **B-C)** Survival of adultborn neurons in WT and $\alpha 7$ KO mice receiving saline or nicotine pumps. **B)** BrdU injection one week after pump implantation, perfusion one week later. **C)** BrdU injection one week before pump implantation, perfusion four weeks later. **D)** BrdU injection one week after pump implantation, perfusion four weeks later (mean \pm SEM; n = 3-5 with ≥ 4 cells per n).

Fig. 2 Nicotine effects on dendrite and spine development are mediated by different nAChRs. Nicotine or saline containing pumps were implanted into WT, $\alpha 7$ KO, or $\beta 2$ KO mice one week after stereotaxic injection with MMLV-GFP retrovirus and perfused four weeks after injection. **A)** Sample dendritic arbors of WT mice receiving saline (left) or nicotine (right). **B)** Quantification of Dendritic Length. **C)** Sample dendritic regions for spine counts from WT (left), $\alpha 7$ KO (middle), or $\beta 2$ KO (right) mice receiving saline (top) or nicotine (bottom). **D)** Quantification of spine density (mean \pm SEM; n = 4-6 with ≥ 4 cells per n).

Fig. 3 Adultborn neurons surviving chronic nicotine have more dendrites but aberrant migration. **A)** Sample dendritic traces of adultborn neurons receiving chronic saline

(top) or nicotine (bottom) as in **Fig 1D**. **B**) Dendritic length, branch points, and spine density in WT mice receiving chronic saline or nicotine. **C**) Neurons displaying normal migration (closed arrows) or aberrant migration (open arrows) in chronic saline (left) or nicotine (right) treated WT mice (mean \pm SEM; n = 3 with \geq 4 cells per n).

Fig. 4 Nicotinic effects on survival and dendritic growth can be separated by a partial agonist. **A**) Sample dendritic traces of adultborn neurons from mice receiving chronic saline (top) or DMXBBA (bottom). **B**) Dendritic length of neurons as in **A**. **C**) Survival of adultborn neurons in WT mice chronically receiving various combinations of saline, nicotine, and/or DMXBBA (mean \pm SEM; n = 3-4 with \geq 4 cells per n)

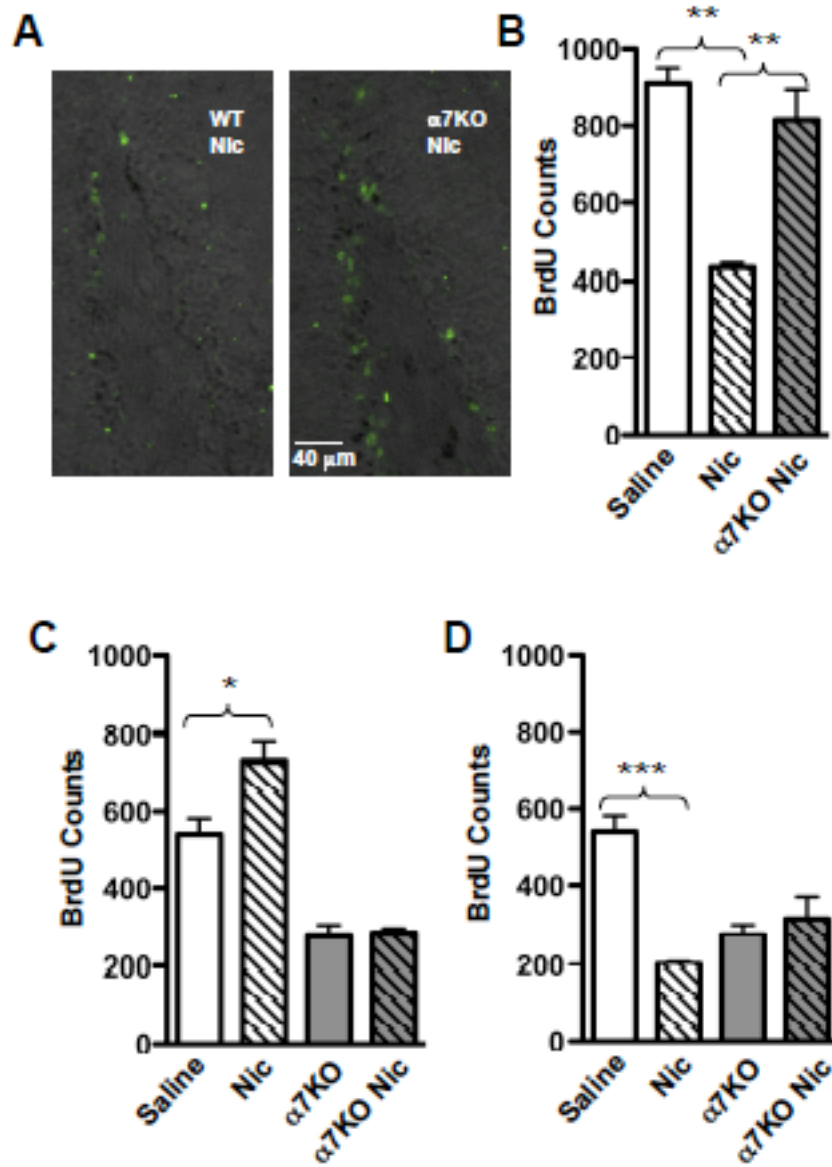


Fig. 1 Nicotinic effects on survival depend on $\alpha 7$ -nAChRs and timing.

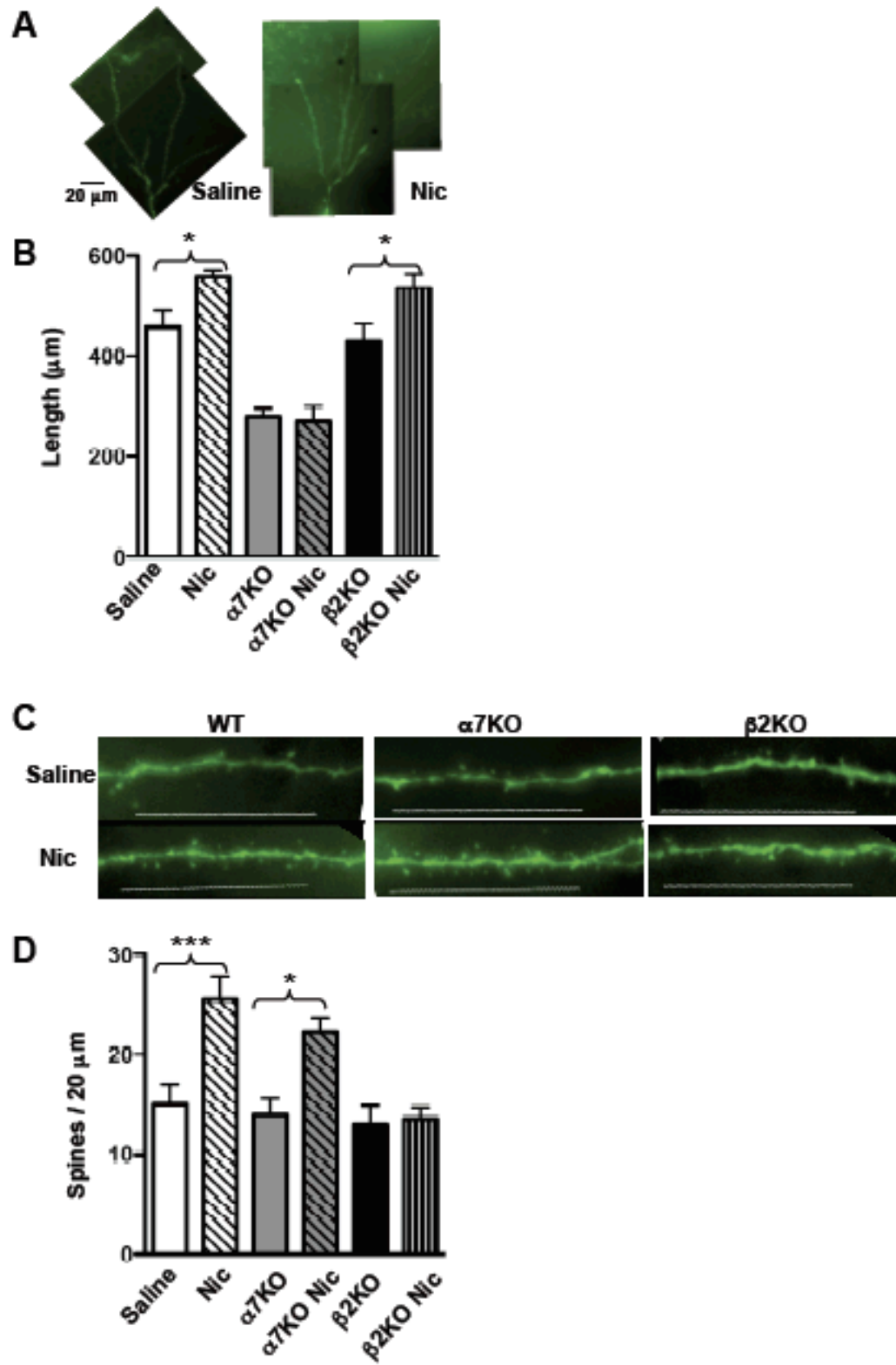


Fig. 2 Nicotine effects on dendrites and spines are mediated by different nAChRs.

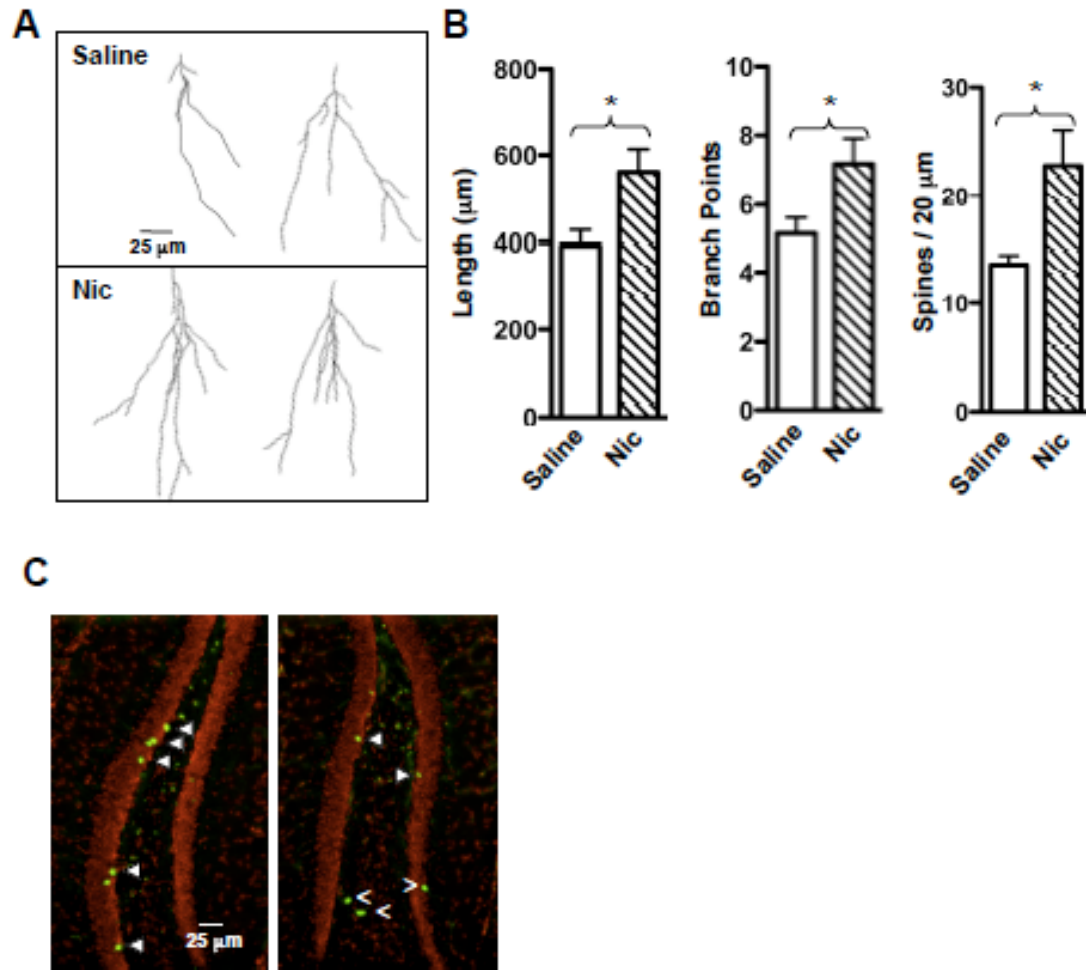


Fig. 3 Chronic nicotine causes more dendrites but aberrant migration.

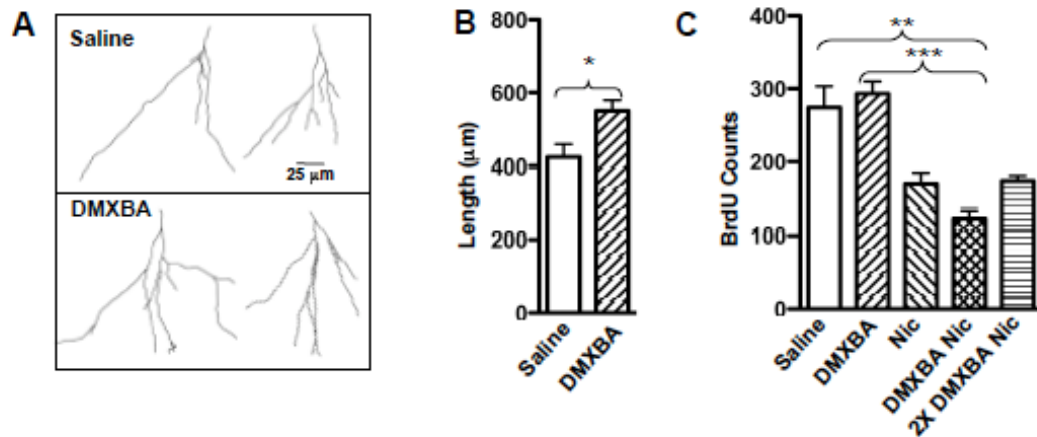


Fig. 4 Nicotinic effects can be separated by a partial agonist.

Darwin Berg is a co-author on chapter two, which is being prepared for publication in the Journal of Neuroscience. The dissertation author was the primary investigator and author of this paper.

DISCUSSION

The results reported here demonstrate that nAChRs exert powerful control over adult neurogenesis. Endogenous nAChR signaling enhances adultborn neuron growth, electrophysiological maturation, and survival, and nicotinic agonists can elicit variable effects on adult neurogenesis depending on the agonist used and the timing of administration. Distinct receptor subtypes provide unique contributions to this process. These results further our understanding of how nAChRs contribute to neuronal development and hippocampal function, and have implications for neuropathologies including Alzheimer's disease and addiction.

A recurring theme through this dissertation work is the finding that signaling through $\alpha 7$ -nAChRs affects dendritic maturation of adultborn neurons. Loss of $\alpha 7$ -nAChRs decreases dendritic growth, while activating $\alpha 7$ -nAChRs with nicotine or DMXBBA increases growth. Interestingly, the relevant population of $\alpha 7$ -nAChRs appears to be localized on the adultborn neurons themselves.

The effects of altering the length and complexity of dendrites can be profound for information flow through a neural network. Since the primary input for glutamatergic synapses onto granule neurons occurs through the perforant path in the molecular layer of the dentate gyrus (Scharfman, 2007), shortened dendritic arbors may limit the total number of glutamatergic inputs a cell receives. Indeed granule neurons with truncated dendritic arbors receive fewer spontaneous synaptic currents (Ge et al., 2006; Campbell et al., 2010). In addition to the decreased total input, changes in dendritic length and complexity are likely to alter the computational

properties of neurons (London and Hausser, 2005), the effects of which can be difficult to predict.

Related findings on the connection of nAChR signaling to dendritic structure have been reported by others. Blocking nicotinic signaling in retinal ganglion cells causes their dendritic arbors to collapse (Lohmann et al., 2002). Nicotine exposure amplifies dendritic complexity and growth of medium spiny neurons in the nucleus accumbens (McDonald et al., 2005), and a large scale comparison of dendrites between WT and nAChR knockout mice revealed nAChR dependent dendritic changes in various cortical regions (Ballesteros-Yáñez et al., 2010). The molecular mechanisms underlying each of these findings are poorly understood. Since we observe that the relevant signaling occurs cell-autonomously, defining a molecular mechanism is a tractable problem and is a logical goal for future work on adult neurogenesis.

Another interesting issue raised by this work is the question of whether the effects seen here are unique to the process of adult neurogenesis or might also occur in prenatal or early postnatal development. Some of the results suggest conserved mechanisms across hippocampal development. Signaling through $\alpha 7$ -nAChRs determines the period in which GABA is depolarizing in both early postnatal and adultborn neuron development (Liu et al., 2006). Also, nicotine is capable of inducing spine growth through $\beta 2$ -containing nAChRs in each case (unpublished results). Thus, it seems likely that nAChR-dependent dendritic growth may be conserved as well. Comparisons of the mature population of granule neurons, however, revealed no significant differences between WT and $\alpha 7$ KO mice. It remains a possibility that

nAChR signaling affects the timing of postnatal dendritic development, but that compensatory signaling allows the neurons to eventually reach the same level of length and complexity. Nicotine exposure in prenatal or perinatal periods has dramatic consequences for brain development (Roy et al., 1998; Paz et al., 2007). It may be informative to test whether a common mechanism for nicotine-induced cytotoxicity is shared between adultborn neurons and neurons at these ages. These possibilities can be addressed in future work.

Our results provide a novel mechanism through which nAChR signaling can influence learning and memory function in the hippocampus. Cholinergic signaling has long been recognized as a contributor to hippocampal-dependent learning and memory (See Hasselmo, 2006 for review), but the role of nicotinic signaling has commonly been thought to be restricted to LTP induction or facilitation of presynaptic neurotransmitter release (Gray et al., 1996; Alkondon et al., 1997; Radcliffe and Dani, 1998; Yamamoto et al., 2005; Ge and Dani, 2005; Fujii et al., 1999; Tang and Dani, 2009). We demonstrate that nAChR signaling up-regulates adult neurogenesis, a form of plasticity necessary for proper hippocampal learning and memory function (see Deng et al., 2010, for review). As this regulation occurs on the time-course of weeks, it is unlikely to contribute to task-specific neuronal integration. It could, however, serve as a mechanism for amplifying the computational power of the dentate depending on hippocampal demand. Micro-dialysis experiments have demonstrated that ACh release in the hippocampus depends on hippocampal demand (Yamamuro et al., 1995; Fadda et al., 1996, 2000; Orsetti et al., 1996; Ragozzino et al., 1996; Stancampiano et al., 1999; Nail-Boucherie et al., 2000; Stefani and Gold, 2001;

McIntyre et al., 2003; Chang and Gold, 2003). Greater amounts of ACh are released during tasks requiring hippocampal function; this in turn would increase the synaptic connectivity and survival of the adultborn neuron population. It is interesting to note that nicotine usage would initially mimic the endogenous amplification of adult neurogenesis, but due to its lethal effects on young adultborn neurons, under long-term usage adultborn neuron survival would, instead, be decreased.

Given that nAChRs appear necessary for proper adult neurogenesis and that adult neurogenesis has been demonstrated to be necessary for hippocampal-dependent learning and memory, a logical prediction would be that $\alpha 7$ KO mice will display deficits in these processes. Indeed $\alpha 7$ KO mice show decreased performance on working/episodic-like memory and spatial learning and memory tasks (Levin et al., 2009; Fernandes et al., 2006). In another study, however, $\alpha 7$ KO mice showed no impairment on latency to find a hidden platform on the Morris Water Maze (Paylor et al., 1998). Performance on this task may not depend on the dentate gyrus (Nakashiba et al., 2008), and experiments eliminating NMDA receptor signaling that decreased adult neurogenesis also showed no impairment on this task (McHugh et al., 2007). Many of the behavioral tasks that are impaired in adultborn neuron ablation studies have not been performed on $\alpha 7$ KO mice. Additionally, our model predicts that cumulative deficits will arise in the $\alpha 7$ KO dentate gyrus over time, but age-dependent studies of hippocampal function in $\alpha 7$ KO mice remain to be performed. These could provide interesting directions for further study.

The cumulative deficits we predict in the absence of signaling through $\alpha 7$ -nAChRs have implications for Alzheimer's disease. Cholinergic neurons are lost early in the progression of the disease, and $\alpha 7$ -nAChR signaling is highly impaired (Whitehouse et al., 1982; Francis et al., 1999; Nordberg, 2001; Lyness et al., 2003; O'Neil et al., 2007; Wang et al., 2000a,b; Liu et al., 2001; Pettit et al., 2001; Dougherty et al., 2003; Grassi et al., 2003; Lee and Wang, 2003; Pym et al., 2005; Hellstrom-Lindahl et al., 1999; Guan et al., 2000; Lee et al., 2000; but see Reid et al., 2000). Further loss of adultborn neurons and their impaired synaptic integration in the absence of cholinergic signaling is expected to exacerbate losses that occur in hippocampal connectivity. Amplification of the survival or integration of adultborn neurons might permit recovery of lost local circuitry. Indeed donepezil, an acetylcholinesterase inhibitor and a current treatment for Alzheimer's disease, increases adultborn neuron survival (Kaneko et al., 2006). DMXBA may be a candidate for enhancing adultborn neuron growth and integration or activating other important $\alpha 7$ -nAChR signaling pathways impaired in the disease without incurring negative effects risked by other nAChR agonists such as nicotine.

It is interesting to speculate how our results may also impact an understanding of the neuropathological condition of nicotine addiction. Human studies have indicated that enhanced cognition may contribute to the initial reward that encourages further nicotine use, and human smokers have reported that cognitive impairments following nicotine cessation contribute to the decision to relapse (Baker et al., 2004; Davis and Gould, 2008). While many neurological pathways are likely to be altered by nicotine usage and cessation, our results indicate that nicotine effects on adult

neurogenesis correlate with cognitive performance. During early usage nicotine increases the growth and survival of adultborn neurons, and following prolonged use, stopping nicotine further impairs adultborn neuron integration. Whether this in anyway effects addiction behavior remains to be tested. Recent work indicated, however, that loss of adult neurogenesis increases drug-seeking behavior following cocaine administration (Noonan et al., 2009). Since the net effect of nicotine is to kill adultborn neurons, nicotine could enhance addictive behavior in part through loss of the adultborn neuron population. Consistent with this idea, when nicotine is made available, WT and $\alpha 7$ KO mice display equivalent consumption during the first week of testing, but $\alpha 7$ KO mice consume significantly less when availability persists longer than one week (Levin et al., 2009). Our results motivate a deeper inspection of the contribution of adult neurogenesis to nicotine-induced addictive behaviors.

REFERENCES

- Abrous DN, Adriani W, Montaron MF, Aurousseau C, Rougon G, Le Moal M, Piazza PV (2002) Nicotine self-administration impairs hippocampal plasticity. *J Neurosci* 22:3656–3662.
- Ahlenius H, Visan V, Kokaia M, Lindvall O, Kokaia Z (2009) Neural stem and progenitor cells retain their potential for proliferation and differentiation into functional neurons despite lower number in aged brain. *J Neurosci* 29:4408–4419.
- Ahn S, Joyner AL (2005) In vivo analysis of quiescent adult neural stem cells responding to Sonic hedgehog. *Nature* 437:894–97.
- Aimone JB, Wiles J, Gage FH (2006) Potential role for adult neurogenesis in the encoding of time in new memories. *Nat Neurosci* 9:723–727.
- Aizawa H, Hu SC, Bobb K, Balakrishnan K, Ince G, Gurevich I, Cowan M, Ghosh A (2004) Dendritic development regulated by CREST, a calcium-regulated transcriptional activator. *Science* 303:197–202.
- Albuquerque EX, Pereira EF, Alkondon M, Rogers SW (2009) Mammalian nicotinic acetylcholine receptors: from structure to function. *Physiol Rev* 89:73–120.
- Alkondon M, Albuquerque EX (1993) Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. I. Pharmacological and functional evidence for distinct structural subtypes. *J Pharmacol Exp Ther* 265: 1455–1473.
- Alkondon M, Albuquerque EX (2001) Nicotinic acetylcholine receptors $\alpha 7$ and $\alpha 4\beta 2$ subtypes differentially control GABAergic input to CA1 neurons in rat hippocampus. *J Neurophysiol* 86:3043–3055.
- Alkondon M, Albuquerque EX (2005) Nicotinic receptor subtypes in rat hippocampal slices are differentially sensitive to desensitization and early in vivo functional up-regulation by nicotine and to block by bupropion. *J Pharmacol Exp Ther* 313: 740–750.
- Alkondon M, Pereira EF, Barbosa CT, Albuquerque EX (1997) Neuronal nicotinic acetylcholine receptor activation modulates gamma-aminobutyric acid release from CA1 neurons of rat hippocampal slices. *J Pharmacol Exp Ther* 283:1396–411.
- Alkondon M, Pereira EF, Eisenberg HM, Albuquerque EX (1999) Choline and selective antagonists identify two subtypes of nicotinic acetylcholine receptors that

- modulate GABA release from CA1 interneurons in rat hippocampal slices. *J Neurosci* 19:2693–705.
- Alkondon M, Pereira EFR, Barbosa CTF, Albuquerque EX (1997) Neuronal nicotinic acetylcholine receptor activation modulates γ -aminobutyric acid release from CA1 neurons of rat hippocampal slices. *J Pharmacol Exp Ther* 283:1396-1411.
- Alkondon M, Pereira EFR, Cortes WS, Maelicke A, Albuquerque EX (1997) Choline is a selective agonist of α 7 nicotinic acetylcholine receptors in the rat brain neurons. *Eur J Neurosci* 9: 2734–2742.
- Alonso R, Griebel G, Pavone G, Stemmelin J, Le Fur G, and Soubrie P (2004) Blockade of CRF1 or V1B receptors reverses stress-induced suppression of neurogenesis in a mouse model of depression. *Mol Psychiatry* 9: 224.
- Altman J, Bayer SA (1990) Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. *J Comp Neurol* 301:365-381.
- and pathological aging. *Psychoneuroendocrinology* 32 Suppl 1: S26-30.
- Auld DS, Kar S, Quirion R (1998) β -Amyloid peptides as direct cholinergic neuromodulators: a missing link? *TINS* 21:43-49.
- Baker TB, Brandon TH, Chassin L (2004) Motivational influences on cigarette smoking. *Annu Rev Psychol* 55:463–491.
- Ballesteros-Yáñez I, Benavides-Piccione R, Bourgeois JP, Changeux JP, Defelipe J (2010) Alterations of cortical pyramidal neurons in mice lacking high-affinity nicotinic receptors. *Proc Natl Acad Sci U S A*. [Epub ahead of print]
- Banasr M, Hery M, Printemps R, Daszuta A (2004) Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. *Neuropsychopharmacology* 29:450–60.
- Ben-Ari Y (2002) Excitatory actions of GABA during development: the nature of the nurture. *Nat Rev Neurosci* 3:728-739.
- Ben-Ari Y, Cherubini E, Corradetti R, Gaiarsa JL (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J Physiol (Lond)* 416:303–325.
- Berger F, Gage FH, Vijayaraghavan S (1998) Nicotinic receptor-induced apoptotic cell death of hippocampal progenitor cells. *J Neurosci* 18:6871–6881.

- Bertrand D, Galzi JL, Devillers-Thiery A, Bertrand S, Changeux JP (1993) Mutations at two distinct sites within the channel domain M2 alter calcium permeability of neuronal $\alpha 7$ nicotinic receptor. *Proc Natl Acad Sci (USA)* 90:6971-6975.
- Borodinsky LN, O'Leary D, Neale JH, Vicini S, Coso OA, Fiszman ML (2003) GABA-induced neurite outgrowth of cerebellar granule cells is mediated by GABA(A) receptor activation, calcium influx and CaMKII and erk1/2 pathways. *J Neurochem* 84:1411-1420.
- Bruel-Jungerman E, Davis S, Rampon C, Laroche S (2006) Long-term potentiation enhances neurogenesis in the adult dentate gyrus. *J Neurosci* 26(22):5888-93.
- Buttery P, Beg AA, Chih B, Broder A, Mason CA, Scheiffele P (2006) The diacylglycerol-binding protein $\alpha 1$ -chimaerin regulates dendritic morphology. *Proc Natl Acad Sci (USA)* 103:1924-1929.
- Campbell NR, Fernandes CC, Halff AW, Berg DK (2010) Endogenous Signaling through $\alpha 7$ -Containing Nicotinic Receptors Promotes Maturation and Integration of Adult-Born Neurons in the Hippocampus. *J Neurosci* 30:8734-8744
- Cancedda L, Fiumelli H, Chen K, and Poo M (2007) Excitatory GABA Action Is Essential for Morphological Maturation of Cortical Neurons In Vivo. *J. Neurosci* 27: 5224 - 5235.
- Cao L, Jiao X, Zuzga DS, Liu Y, Fong DM, et al. (2004) VEGF links hippocampal activity with neurogenesis, learning and memory. *Nat Genet* 36:827-35.
- Castro NG, Albuquerque EX (1993) Brief-lifetime, fast-inactivating ion channels account for the α -bungarotoxin-sensitive nicotinic response in hippocampal neurons. *Neurosci Lett* 164: 137-140.
- Castro NG, Albuquerque EX (1995) α -Bungarotoxin-sensitive hippocampal nicotinic receptor channel has a high calcium permeability. *Biophys J* 68: 516-524.
- Chang K, Berg DK (2001) Voltage-gated channels block nicotinic regulation of CREB phosphorylation and gene expression in neurons. *Neuron* 32:855-865.
- Chang Q and Gold PE (2003) Switching memory systems during learning: Changes in patterns of brain acetylcholine release in the hippocampus and striatum in rats. *J Neurosci* 23:3001-3005.
- Clelland CD, Choi M, Romberg C, Clemenson GD Jr, Fragniere A, Tyers P, Jessberger S, Saksida LM, Barker RA, Gage FH, Bussey TJ (2009) A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science* 325:210-213.

- Conroy WG, Liu Z, Nai Q, Coggan JS, Berg DK (2003) PDZ-containing proteins provide a functional postsynaptic scaffold for nicotinic receptors in neurons. *Neuron* 38:759–771.
- Cooper-Kuhn CM, Winkler J, Kuhn GH (2004) Decreased neurogenesis after cholinergic forebrain lesion in the adult rat. *J Neurosci Res* 77:155-165.
- Dani JA, Bertrand D (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu Rev Pharmacol Toxicol* 47:699–729
- Davis JA, Gould TJ (2008) Associative learning, the hippocampus, and nicotine addiction. *Current Drug Abuse Reviews* 1:9–19.
- Davis JA, James JR, Siegel SJ, Gould TJ (2005) Withdrawal from chronic nicotine administration impairs contextual fear conditioning in C57BL/6 mice. *J Neurosci* 25:8708–8713.
- De Fiebre CM, Meyer EM, Zoltewicz J, Henry JC, Muraskin S, Kem WR, Papke RL (1995) Characterization of a family of anabaseine-derived compounds reveals that the 3-(4)-dimethylaminocinnamylidene derivative (DMAC) is a selective agonist at neuronal nicotinic $\alpha 7$ /[125I] α -bungarotoxin receptor subtypes. *Mol Pharmacology* 47:164–171.
- Deng W, Aimone JB, Gage FH (2010) New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nat Rev Neurosci* 11:339–350.
- Deng W, Saxe MD, Gallina IS, Gage FH (2009) Adult-born hippocampal dentate granule cells undergoing maturation modulate learning and memory in the brain. *J Neurosci* 29:13532–13542.
dentate granule neuron maturation in the adult mouse hippocampus. *J Neurosci* 26:3–11.
- Dineley KT, Bell KA, Bui D, Sweatt JD (2002) β -Amyloid peptide activates $\alpha 7$ nicotinic acetylcholine receptors expressed in *Xenopus* oocytes. *J Biol Chem* 277:25056-25061.
- Dineley KT, Westerman M, Bui D, Bell K, Ashe KH, Sweatt JD (2001) β -Amyloid activates mitogen-activated protein kinase cascade via hippocampal $\alpha 7$ nicotinic acetylcholine receptors: in vitro and in vivo mechanisms related to Alzheimer's disease. *J Neurosci* 21:4125-4133.

- Dougherty JJ, Wu J, Nichols RA (2003) β -Amyloid regulation of presynaptic nicotinic receptors in rat hippocampus and neocortex. *J Neurosci* 23:6740–6747.
- Duman RS, Monteggia LM (2006) A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* 59:1116–27.
- Dupret D, Fabre A, Döbrössy MD, Panatier A, Rodríguez JJ, Lamarque S, Lemaire V, Olier SH, Piazza PV, Abrous DN (2007) Spatial learning depends on both the addition and removal of new hippocampal neurons. *PLoS Biol* 5:e214.
- Dutar P, Bassant MH, Senut MC, Lamour Y (1995) The septohippocampal pathway: structure and function of a central cholinergic system. *Physiol Rev* 75:393–427.
- Espósito MS, Piatti VC, Laplagne DA, Morgenstern NA, Ferrari CC, Pitossi FJ, Schinder AF (2005) Neuronal differentiation in the adult hippocampus recapitulates embryonic development. *J Neurosci* 25:10074–10086.
- Fabel K, Tam B, Kaufer D, Baiker A, Simmons N et al. (2003) VEGF is necessary for exercise-induced adult hippocampal neurogenesis. *Eur J Neurosci* 18:2803–2812.
- Fabian-Fine R, Skehel P, Errington ML, Davies HA, Sher E, Stewart MG, Fine A (2001) Ultrastructural distribution of the α 7 nicotinic acetylcholine receptor subunit in rat hippocampus. *J Neurosci* 21:7993–8003.
- Fadda F, Cocco S, Stancampiano R (2000) Hippocampal acetylcholine release correlates with spatial learning performance in freely moving rats. *NeuroReport* 11:2265–2269.
- Fadda F, Melis F, Stancampiano R (1996) Increased hippocampal acetylcholine release during a working memory task. *Eur J Pharmacol* 307:R1–R2.
- Fayuk D, Yakel JL (2005) Ca^{2+} permeability of nicotinic acetylcholine receptors in rat hippocampal CA1 interneurons. *J Physiol* 566:759–68.
- Fayuk D, Yakel JL (2007) Dendritic Ca^{2+} signaling due to activation of α 7-containing nicotinic acetylcholine receptors in rat hippocampal neurons. *J Physiol (Lond)* 585.2:597–611.
- Fenster CP, Beckman ML, Parker JC, Sheffield EB, Whitworth TL, Quick MW, Lester RA (1999) Regulation of α 4 β 2 nicotinic receptor desensitization by calcium and protein kinase C. *Mol Pharmacol* 55: 432–443.
- Fernandes C, Hoyle E, Dempster E, Schalkwyk LC, Collier DA (2006) Performance deficit of α 7 nicotinic receptor knockout mice in a delayed matching-to-place

- task suggests a mild impairment of working/episodic-like memory. *Genes Brain Behav* 5:433–440.
- Francis PT, Palmer AM, Snape M, Wilcock GK (1999) The cholinergic hypothesis of Alzheimer's disease: a review of progress. *J Neurol Neurosurg Psychiatry* 66: 137-147.
- Frotscher M, Leranth C (1985) Cholinergic innervation of the rat hippocampus as revealed by choline acetyltransferase immunocytochemistry: a combined light and electron microscopic study. *J Comp Neurol* 239:237–46.
- Fujii S, Ji Z, Morita N, Sumikawa K (1999) Acute and chronic nicotine exposure differentially facilitate the induction of LTP. *Brain Res* 1999; 846: 137-43.
- Fujii S, Jia Y, Yang A, Sumikawa K (2000) Nicotine reverses GABAergic inhibition of long-term potentiation induction in the hippocampal CA1 region. *Brain Res* 863:259–65.
- Gaudilliere B, Konishi Y, Iglesia N de la, Yao G, Bonni A (2004) A CamKII-NeuroD signaling pathway specifies dendritic morphogenesis. *Neuron* 41:229-241.
- Ge S, Dani JA (2005) Nicotinic acetylcholine receptors at glutamate synapses facilitate long-term depression or potentiation. *J Neurosci* 25:6084–91.
- Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, Song H (2006) GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature* 439:589-593.
- Ge S, Sailor KA, Ming GL, Song H (2008) Synaptic integration and plasticity of new neurons in the adult hippocampus. *J Physiol* 586:3759–3765.
- Ge S, Yang CH, Hsu KS, Ming GL & Song H (2007) A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. *Neuron* 54:559–566.
- Gough NR (2007) Stimulating neurogenesis to treat Alzheimer's disease. *Sci STKE* 391: tw 213.
- Gould E, Beylin A, Tanapat P, Reeves A, and Shors TJ (1999) Learning enhances adult neurogenesis in the hippocampal formation. *Nat Neurosci* 2:260–265.
- Grassi F, Palma E, Tonini R, Amici M, Ballivet M, Eusebi F (2003) Amyloid β 1-42 peptide alters the gating of human and mouse α -bungarotoxin-sensitive nicotinic receptors. *J Physiol (Lond)* 547:147-157.

- Gray R, Rajan AS, Radcliffe KA, Yakehiro M, Dani JA (1996) Hippocampal synaptic transmission enhanced by low concentrations of nicotine. *Nature* 383:713-716.
- Guan ZZ, Zhang X, Ravid R, Nordberg A (2000) Decreased protein levels of nicotinic receptor subunits in the hippocampus and temporal cortex of patients with Alzheimer's disease. *J Neurochem* 74:237-243.
- Hanson MG, Landmesser LT (2003) Characterization of the circuits that generate spontaneous episodes of activity in the early embryonic mouse spinal cord. *J Neurosci* 23:587-600.
- Harrist A, Beech RD, King SL, Zanardi A, Cleary MA, Caldarone BJ, Eisch A, Zoli M, Picciotto MR (2004) Alteration of hippocampal cell proliferation in mice lacking the beta 2 subunit of the neuronal nicotinic acetylcholine receptor. *Synapse* 54:200-206.
- Hasselmo ME (2006) The role of acetylcholine in learning and memory. *Curr Opin Neurobiol* 16:710-715.
- Hastings NB, Gould E (1999) Rapid extension of axons into the CA3 region by adult-generated granule cells. *J Comp Neurol* 413(1):146-54.
- Hellstrom-Lindahl E, Mousavi M, Zhang X, Ravid R, Nordberg A (1999) Regional distribution of nicotinic receptor subunit mRNAs in human brain: comparison between Alzheimer and normal brain. *Molec Brain Res* 66:94-103.
- Höglinger GU, Rizk P, Muriel MP, Duyckaerts C, Oertel WH, et al. (2004) Dopamine depletion impairs precursor cell proliferation in Parkinson disease. *Nat Neurosci* 7:726-35.
- Hu M, Liu Q, Chang KT, Berg DK (2002) Nicotinic regulation of CREB activation in hippocampal neurons by glutamatergic and nonglutamatergic pathways. *Mol Cell Neurosci* 21:616-625.
- Ide Y, Fujiyama F, Okamoto-Furuta K, Tamamaki N, Kaneko T, Hisatsune T (2008) Rapid integration of young newborn dentate gyrus granule cells in the adult hippocampal circuitry. *Eur J Neurosci* 28(12): 2381-2392.
- Ji D, Dani JA (2000) Inhibition and disinhibition of pyramidal neurons by activation of nicotinic receptors on hippocampal interneurons. *J Neurophysiol* 83:2682-90.
- Ji D, Lape R, Dani JA (2001) Timing and location of nicotinic activity enhances or depresses hippocampal synaptic plasticity. *Neuron* 31:131-41.

- Jones S, Yakel JL (1997) Functional nicotinic ACh receptors on interneurons in the rat hippocampus. *J Physiol* 504(Pt. 3):603–10.
- Kaneko N, Okano H, Sawamoto K (2006) Role of the cholinergic system in regulating survival of newborn neurons in the adult mouse dentate gyrus and olfactory bulb. *Genes Cells* 11:1145-59.
- Kar S, Issa AM, Seto D, Auld DS, Collier B, Quirion R (1998) Amyloid β -peptide inhibits high-affinity choline uptake and acetylcholine release in rat hippocampal slices. *J Neurochem* 70:2179–2187.
- Karten YJ, Jones MA, Jeurling SI, Cameron HA (2006) GABAergic signaling in young granule cells in the adult rat and mouse dentate gyrus. *Hippocampus* 16:312–320.
- Kasyanov AM, Safiulina VF, Voronin LL, Cherubini E (2004) GABA-mediated giant depolarizing potentials as coincidence detectors for enhancing synaptic efficacy in the developing hippocampus. *Proc Nat Acad Sci (USA)* 101:3967-3972.
- Kempermann G, Gast D, Kronenberg G, Yamaguchi M, Gage FH (2003) Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development* 130:391–399.
- Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. *Nature* 386:493–495.
- Kitamura T, Saitoh Y, Takashima N, Murayama A, Niibori Y, Ageta H, Sekiguchi M, Sugiyama H, Inokuchi K (2009) Adult neurogenesis modulates the hippocampus-dependent period of associative fear memory. *Cell* 139:814-827.
- Kotani S, Yamauchi T, Teramoto T, Ogura H (2008) Donepezil, an acetylcholinesterase inhibitor, enhances adult hippocampal neurogenesis. *Chem Biol Interact* 175(1-3): 227-230.
- Laplagne DA, Esposito MS, Piatti VC, Morgenstern NA, Zhao C, van Praag H, Gage FH & Schinder AF (2006) Functional convergence of neurons generated in the developing and adult hippocampus. *PLoS Biol* 4, e409.
- Laplagne DA, Kamienkowski JE, Esposito MS, Piatti VC, Zhao C, Gage FH, Schinder AF (2007) Similar GABAergic inputs in dentate granule cells born during embryonic and adult neurogenesis. *Eur J Neurosci* 25, 2973–2981.
- Le Magueresse C, Safiulina V, Changeux JP, Cherubini E (2006) Nicotinic modulation of network and synaptic transmission in the immature hippocampus investigated with genetically modified mice. *J Physiol (Lond)* 576:533-546.

- Lee DHS, D'Andrea MR, Plata-Salaman CR, Wang H-Y (2000) Decreased $\alpha 7$ nicotinic acetylcholine receptor protein levels in sporadic Alzheimer's disease hippocampus. *Alzheimer's Reports* 3:217-220.
- Lee DHS, Wang H-Y (2003) Differential physiologic responses of $\alpha 7$ nicotinic acetylcholine receptors to β -amyloid1-40 and β -amyloid1-42. *J Neurobiol* 55:25-30.
- Lester HA, Xiao C, Srinivasan R, Son CD, Miwa J, Pantoja R, Banghart MR, Dougherty DA, Goate AM, Wang JC (2009) Nicotine is a selective pharmacological chaperone of acetylcholine receptor number and stoichiometry. Implications for drug discovery. *AAPS J* 11:167-177.
- Levin ED, Petro A, Rezvani AH, Pollard N, Christopher NC, Strauss M, Avery J, Nicholson J, Rose JE (2009) Nicotinic alpha7- or beta2-containing receptor knockout: effects on radial-arm maze learning and long-term nicotine consumption in mice. *Behav Brain Res* 196(2):207-13.
- Li Y, Luikart BW, Birnbaum S, Chen J, Kwon CH, et al. (2008) TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. *Neuron* 59:399-412.
- Liu Q-s, Kawai H, Berg DK (2001) β -Amyloid peptide blocks the response of $\alpha 7$ -containing nicotinic receptors on hippocampal neurons. *Proc Natl Acad Sci (USA)* 98:4734-4739.
- Liu X, Wang Q, Haydar TF, Bordey A (2005) Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. *Nat Neurosci* 8:1179-87.
- Liu Z, Neff RA, Berg DK (2006) Sequential interplay of nicotinic and GABAergic signaling guides neuronal development. *Science* 314:1610-1613.
- Lohmann C, Myhr KL, Wong RO (2002) Transmitter-evoked local calcium release stabilizes developing dendrites. *Nature* 418:177-181.
- London M, Hausser M (2005) Dendritic computation. *Annu Rev Neurosci* 28:503-532.
- Lyness SA, Zarow C, Chui HC (2003) Neuron Loss in key cholinergic and aminergic nuclei in Alzheimer's disease: a meta-analysis. *Neurobiol Aging* 24:1-23.

- Maggi L, Le Magueresse C, Changeux J-P, Cherubini E (2003) Nicotine activates immature “silent” connections in the developing hippocampus. *Proc Natl Acad Sci (USA)* 100:2059-2064.
- Maggi L, Sher E, Cherubini E (2001) Regulation of GABA release by nicotinic acetylcholine receptors in the neonatal rat hippocampus. *J Physiol (Lond)* 536:89-100.
- Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* 20:9104–10.
- Markwardt SJ, Wadiche JI, Overstreet-Wadiche LS (2009) Input-specific GABAergic signaling to newborn neurons in adult dentate gyrus. *J Neurosci* 29:15063-15072.
- Matta SG, Balfour DJ, Benowitz NL, Boyd RT, Buccafusco JJ, Caggiula AR, Craig CR, Collins AC, Damaj MI, Donny EC, Gardiner PS, Grady SR, Heberlein U, Leonard SS, Levin ED, Lukas RJ, Markou A, Marks MJ, McCallum SE, Parameswaran N, Perkins KA, Picciotto MR, Quik M, Rose JE, Rothenfluh A, Schafer WR, Stolerman IP, Tyndale RF, Wehner JM, Zirger JM (2007) Guidelines on nicotine dose selection for in vivo research. *Psychopharmacology (Berl)* 190(3):269-319.
- McDonald CG, Dailey VK, Bergstrom HC, Wheeler TL, Eppolito AK, Smith LN, Smith RF (2005) Periadolescent nicotine administration produces enduring changes in dendritic morphology of medium spiny neurons from nucleus accumbens. *Neurosci Lett* 385:163–167.
- McHugh TJ, Jones MW, Quinn JJ, Balthasar N, Coppari R, Elmquist JK, Lowell BB, Fanselow MS, Wilson MA, Tonegawa S (2007) Dentate gyrus nmda receptors mediate rapid pattern separation in the hippocampal network. *Science* 317:94–99.
- McIntyre CK, Marriott LK, Gold PE (2003) Patterns of brain acetylcholine release predict individual differences in preferred learning strategies in rats. *Neurobiol Learn Mem* 79:177–183.
- Mechawar N, Saghatelian A, Grailhe R, Scoriels L, Gheusi G, Gabellec MM, Lledo PM, Changeux JP (2004) Nicotinic receptors regulate the survival of newborn neurons in the adult olfactory bulb. *Proc Natl Acad Sci (USA)* 101:9822-9826.
- Millar NS and Gotti C (2009) Diversity of vertebrate nicotinic acetylcholine receptors. *Neuropharmacology* 56 237-246.
- Morgenstern NA, Lombardi G, Schinder AF (2008) Newborn granule cells in the ageing dentate gyrus. *J Physiol (Lond)* 586:3751-3757.

- Nail-Boucherie K, Dourmap N, Jaffard R, Costentin J (2000) Contextual fear conditioning is associated with an increase of acetylcholine release in the hippocampus of rat. *Cog. Brain Res* 9:193–197.
- Nakashiba T, Young JZ, McHugh TJ, Buhl DL, Tonegawa S (2008) Transgenic inhibition of synaptic transmission reveals role of CA3 output in hippocampal learning. *Science* 319:1260–1264.
- Nelson ME, Kuryatov A, Choi CH, Zhou Y, Lindstrom J (2003) Alternate stoichiometries of $\alpha 4\beta 2$ nicotinic acetylcholine receptors. *Mol Pharmacol* 63:332–341.
- Noonan MA, Bulin SE, Fuller DC, Eisch AJ (2009) Reduction of adult hippocampal neurogenesis confers vulnerability in an animal model of cocaine addiction. *J Neurosci* 30:304-315.
- Nordberg A (2001) Nicotinic receptor abnormalities of Alzheimer's disease: therapeutic implications. *Biol Psychiatry* 49:200-210.
- O'Neil JN, Mouton PR, Tizabi Y, Ottinger MA, Lei DL, Ingram DK, Manaye KF (2007) Catecholaminergic neuronal loss in locus coeruleus of aged female dtg APP/PS1 mice. *J Chem Neuroanat* 34:102-107.
- Okano H, Sawamoto K (2008) Neural stem cells: involvement in adult neurogenesis and CNS repair. *Phil Trans R Soc B* 363: 2111-2122.
- Olinic A, Stevens KE (2007) Treating schizophrenia symptoms with an alpha7 nicotinic agonist, from mice to men. *Biochem Pharmacol* 74:1192–1201.
- Orsetti M, Casamenti F, Pepeu G (1996) Enhanced acetylcholine release in hippocampus and cortex during acquisition of an operant behavior. *Brain Res* 724:89–96.
- Overstreet-Wadiche L, Bromberg DA, Bensen AL, Westbrook GL (2005) GABAergic signaling to newborn neurons in dentate gyrus. *J Neurophysiol* 94: 4528 - 4532.
- Overstreet-Wadiche LS, Bensen AL, Westbrook GL (2006) Delayed development of adult-generated granule cells in dentate gyrus. *J Neurosci* 26:2326–2334.
- Overstreet-Wadiche LS, Bromberg DA, Bensen AL & Westbrook GL (2006) Seizures accelerate functional integration of adult-generated granule cells. *J Neurosci* 26:4095–4103.
- Paylor R, Nguyen M, Crawley JN, Patrick J, Beaudet A, Orr-Urtreger A (1998) Alpha7 nicotinic receptor subunits are not necessary for hippocampal-dependent

- learning or sensorimotor gating: a behavioral characterization of *Acra7*-deficient mice. *Learn Mem* 5:302–316.
- Payne JA, Rivera C, Voipio J, Kaila K (2003) Cation-chloride co-transporters in neuronal communication, development, and trauma. *Trends Neurosci* 26:199-206.
- Paz R, Barsness B, Martenson T, Tanner D, Allan AM (2007) Behavioral teratogenicity induced by nonforced maternal nicotine consumption. *Neuropsychopharmacology* 32:693-699.
- Perera TD, Park S, Nemirovskaya Y, (2008) Cognitive role of neurogenesis in depression and antidepressant treatment. *Neuroscientist* 14: 326-338.
- Pettit DL, Shao Z, Yakel JL (2001) β -Amyloid1-42 peptide directly modulates nicotinic receptors in the rat hippocampal slice. *J Neurosci* 21:RC120, 1-5.
- Pham K, Nacher J, Hof PR, McEwen BS (2003) Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. *Eur J Neurosci* 17:879–886.
- Pym L, Kemp M, Raymond-Delpech B, Buckingham S, Boyd CA, Sattelle D (2005) Subtype-specific actions of β -amyloid peptides on recombinant human neuronal nicotinic acetylcholine receptors ($\alpha 7$, $\alpha 4\beta 2$, $\alpha 3\beta 4$) expressed in *Xenopus laevis* oocytes. *Br J Pharmacol* 146:964-971.
- Radcliffe KA, Dani JA (1998) Nicotinic stimulation produces multiple forms of increased glutamatergic synaptic transmission. *J Neurosci* 18:7075-7083.
- Ragozzino ME, Unick KE, Gold PE (1996) Hippocampal acetylcholine release during memory testing in rats: Augmentation by glucose. *Proc Natl Acad Sci* 93:4693–4698.
- Ramirez-Amaya V, Marrone DF, Gage FH, Worley PF, Barnes CA (2006) Integration of new neurons into functional neural networks. *J Neurosci* 26:12237–12241.
- Reid RT, Sabbagh MN, Corey-Bloom J, Tiraboschi P, Thal LJ (2000) Nicotinic receptor losses in dementia with Lewy bodies: comparisons with Alzheimer's disease. *Neurobiol Aging* 21:741-746.
- Represa A, Ben-Ari Y (2005) Trophic actions of GABA on neuronal development. *Trends Neurosci* 28:278-283.
- Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K (1999) The K^+/Cl^- co-transporter *KCC2* renders GABA hyperpolarizing during neuronal maturation. *Nature* 397:251-255.

- Rola R, Raber J, Rizk A, Otsuka S, VandenBerg SR, Morhardt DR, Fike JR (2004) Radiation-induced impairment of hippocampal neurogenesis is associated with cognitive deficits in young mice. *Exp Neurol* 188:316-330.
- Rossi C, Angelucci A, Costantin L, Braschi C, Mazzantini M, et al. (2006) Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. *Eur J Neurosci* 24:1850-56.
- Roy TS, Andrews JE, Seidler FJ, Slotkin TA (1998) Nicotine evokes cell death in embryonic rat brain during neurulation. *J Pharmacol Exp Ther* 287:1136-1144.
- Sahay A, Hen R (2007) Adult hippocampal neurogenesis in depression. *Nat Neurosci* 10:1110-1115.
- Sairanen M, Lucas G, Ernfors P, Castren M, Castren E (2005) Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J Neurosci* 25:1089-94.
- Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, et al. (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301:805-9.
- Scerri C, Stewart CA, Breen KC, Balfour DJK (2005) The effects of chronic nicotine on spatial learning and bromodeoxyuridine incorporation into the dentate gyrus of the rat. *Psychopharmacology* 184:540-546.
- Scharfman HE, ed (2007) *The Dentate Gyrus: A comprehensive guide to structure, function, and clinical implications*. 163:1-840.
- Seguela P, Wadiche J, Dineley-Miller K, Dani JA, Patrick JW (1993) Molecular cloning, functional properties, and distribution of rat brain $\alpha 7$: a nicotinic cation channel highly permeable to calcium. *J Neurosci* 13:596-604.
- Selkow DJ (1994) Normal and abnormal biology of the β -amyloid precursor protein. *Annu Rev Neurosci* 17:489-517.
- Seri B, García-Verdugo JM, Collado-Morente L, McEwen BS, Alvarez-Buylla A (2004) Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus. *J Comp Neurol* 478:359-78.
- Sher E, Chen Y, Sharples TJ, Broad LM, Benedetti G, et al. (2004) Physiological roles of neuronal nicotinic receptor subtypes: new insights on the nicotinic modulation

- of neurotransmitter release, synaptic transmission and plasticity. *Curr Top Med Chem* 4:283–97.
- Shingo AS, Kito S (2005) Effects of nicotine on neurogenesis and plasticity of hippocampal neurons. *J Neural Transm* 112:1475-1478.
- Shors TJ, Miesagae G, Beylin A, Zhao M, Rydel T, Gould E (2001) Neurogenesis in the adult rat is involved in the formation of trace memories. *Nature* 410:372-376.
- Shors TJ, Townsend DA, Zhao M, Kozorovitskiy Y, Gould E (2002) Neurogenesis may relate to some but not all types of hippocampal dependent learning. *Hippocampus* 12:578-584.
- Snyder JS, Hong NS, McDonald R, Wojtowicz JM (2005) A role for adult neurogenesis in spatial long-term memory. *Neuroscience* 130:843-852.
- Sohur US, Emsley JG, Mitchell BD, Macklis JD (2006) Adult neurogenesis and cellular brain repair with neural progenitors, precursors and stem cells. *Philos Trans R Soc Lond B Biol Sci.* 361:1477–1497.
- Stancampiano R, Cocco S, Cugusi C, Sarais L, Fadda F (1999) Serotonin and acetylcholine release response in the rat hippocampus during a spatial memory task. *Neuroscience* 89:1135 –1143.
- Stefani MR, Gold PE (2001) Intrahippocampal infusions of K-ATP channel modulators influence spontaneous alternation performance: Relationships to acetylcholine release in the hippocampus. *J Neurosci* 21:609–614.
- Stevens KE, Kem WR, Mahnir VM, Freedman R (1998) Selective alpha7-nicotinic agonists normalize inhibition of auditory response in DBA mice. *Psychopharmacology* 136:320–327.
- Sudweeks SN, Yakel JL (2000) Functional and molecular characterization of neuronal nicotinic ACh receptors in rat CA1 hippocampal neurons. *J Physiol* 527(Pt. 3):515–28.
- Szabo SI, Zelles T, Lendvai B (2008) Intracellular Ca⁺² dynamics of hippocampal interneurons following nicotinic acetylcholine receptor activation. *Neurochem Int* 52:135-141.
- Taly A, Corringier PJ, Guedin D, Lestage P, Changeux JP (2009) Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. *Nat Rev Drug Discov* 8(9):733-50.

- Tang J, Dani JA (2009) Dopamine enables in vivo synaptic plasticity associated with the addictive drug nicotine. *Neuron* 63(5):673-82.
- Tashiro A, Makino H, Gage FH (2007) Experience-specific functional modification of the dentate gyrus through adult neurogenesis: a critical period during an immature stage. *J Neurosci* 27:3252–3259.
- Tashiro A, Sandler VM, Toni N, Zhao C, Gage FH (2006) NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. *Nature* 442:929-932.
- Toni N, Laplagne DA, Zhao C, Lombardi G, Ribak CD, Gage FH, Schinder AF (2008) Neurons born in the adult dentate gyrus form functional synapses with target cells. *Nat Neurosci* 11:901-907.
- Toni N, Teng EM, Bushong EA, Aimone JB, Zhao C, Consiglio A, van Praag H, Martone ME, Ellisman MH, Gage FH (2007) Synapse formation on neurons born in the adult hippocampus. *Nat Neurosci* 10:727–734.
- Tozuka Y, Fukuda S, Namba T, Seki T, Hisatsune T (2005) GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. *Neuron* 47:803–815.
- Tronel S, Fabre A, Charrier V, Oliet SH, Gage FH, Abrous DN (2010) Spatial learning sculpts the dendritic arbor of adult-born hippocampal neurons. *PNAS* 107:7963-8.
- Trouche S, Bontempi B, Roulet P, Rampon C (2009) Recruitment of adult-generated neurons into functional hippocampal networks contributes to updating and strengthening of spatial memory. *Proc Natl Acad Sci (USA)* 106: 5919-5924.
- Van Praag H, Kempermann G, Gage FH (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 2: 266–270.
- Van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH (2002) Functional neurogenesis in the adult hippocampus. *Nature* 415:1030–1034.
- Vernino S, Amador M, Luetje CW, Patrick J, Dani JA (1992) Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. *Neuron* 8:127–134.
- Verret L, Trouche S, Zerwas M, Rampon C (2007) Hippocampal neurogenesis during normal

- Wang H-Y, Lee DHS, D'Andrea MR, Peterson PA, Shank RP Reitz AB (2000a) β -Amyloid1-42 binds to α 7 nicotinic acetylcholine receptor with high affinity. *J Biol Chem* 275:5626-5632.
- Wang H-Y, Lee DHS, Davis CB, Shank RP (2000b) A β 1-42 binds selectively and with picomolar affinity to α 7 nicotinic acetylcholine receptors. *J Neurochem* 75:1155-1161.
- Wang H-Y, Li W, Benedetti NJ, Lee DHS (2003) α 7 Nicotinic acetylcholine receptors mediate β -amyloid peptide-induced tau protein phosphorylation. *J Biol Chem* 278:31547-31553.
- Wang LP, Kempermann G & Kettenmann H (2005). A subpopulation of precursor cells in the mouse dentate gyrus receives synaptic GABAergic input. *Mol Cell Neurosci* 29, 181–189.
- Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, DeLong MR (1982) Heterogeneity in epidermal basal keratinocytes: morphological and functional correlations. *Science* 215:1237-1239.
- Winocur G, Wojtowicz JM, Sekeres M, Snyder JS, Wang S (2006) Inhibition of neurogenesis interferes with hippocampus-dependent memory function. *Hippocampus* 16:296-304.
- Woolf NJ (1991) Cholinergic systems in mammalian brain and spinal cord. *Prog Neurobiol* 37:475–524.
- Wooltorton JR, Pidoplichko VI, Broide RS, Dani JA (2003) Differential desensitization and distribution of nicotinic acetylcholine receptor subtypes in midbrain dopamine areas. *J Neurosci* 23: 3176–3185.
- Xu J, Zhu Y, Heinemann SF (2006) Identification of sequence motifs that target neuronal nicotinic receptors to dendrites and axons. *J Neurosci* 26:9780–9793.
- Yamamoto S, Kanno T, Nagata T, Yaguchi T, Tanaka A, Nishizaki T (2005) The linoleic acid derivative FR236924 facilitates hippocampal synaptic transmission by enhancing activity of presynaptic α 7 acetylcholine receptors on the glutamatergic terminals. *Neuroscience* 130:207–13.
- Yamamuro Y, Hori K, Tanaka K, Iwano H, Nomura M (1995) Septo-hippocampal cholinergic system under the discrimination learning task in the rat: A microdialysis study with the dual-probe approach. *Brain Res* 684:1–7.

- Zhao C, Teng EM, Summers RG Jr, Ming GL, Gage FH (2006) Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J Neurosci* 26:3–11.
- Zoli M, Lena C, Picciotto MR, Changeux JP (1998) Identification of four classes of brain nicotinic receptors using $\beta 2$ mutant mice. *J Neurosci* 18: 4461–4472.
- Zwart R, Vijverberg HPM (1998) Four pharmacologically distinct subtypes of $\alpha 4\beta 2$ nicotinic acetylcholine receptor expressed in *Xenopus laevis* oocytes. *Mol Pharmacol* 54:1124–1131.