

UC Irvine

UC Irvine Electronic Theses and Dissertations

Title

Nicotine's effects on auditory processing and the role of cortical interneurons

Permalink

<https://escholarship.org/uc/item/1br4j5zq>

Author

Askew, Caitlin

Publication Date

2018

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA,
IRVINE

Nicotine's Effects on Auditory Processing and the Role of Cortical Interneurons

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Biological Sciences

by

Caitlin Erika Askew

Dissertation Committee:
Professor Raju Metherate, Chair
Professor Karina S. Cramer
Professor Katumi Sumikawa

2018

DEDICATION

To Logan and Petra –

Logan, you have given more love and support than I could have ever dreamed. Thank you for giving meaning to everything I do.

Petra, your endless hugs and boundless enthusiasm never failed to ease the burden of difficult research days. Thank you for bringing light and joy into my work.

TABLE OF CONTENTS

	Page
LIST OF FIGURES	iv
ACKNOWLEDGMENTS	v
CURRICULUM VITAE	vi
ABSTRACT OF THE DISSERTATION	ix
INTRODUCTION	1
CHAPTER 1: Systemic nicotine increases gain and narrows receptive fields in A1 via integrated cortical and subcortical actions	18
CHAPTER 2: Nicotine Disinhibits Pyramidal Neurons in Auditory Cortex via VIP Interneurons	57
CONCLUSIONS	81
REFERENCES	85

LIST OF FIGURES

	Page
Introduction	
Figure 1 Schematic of how nicotine enhances sensory processing	2
Figure 2 Tonotopic organization in mouse auditory cortex	4
Figure 3 Schematic of how TINN is used to estimate perceptual filters in humans	6
Figure 4 Representation of $\alpha 7$ and $\alpha 4\beta 2$ nAChR receptors	7
Figure 5 Distribution of nAChRs in cortex	9
Figure 6 Representation of VIP-mediated disinhibition	16
Chapter 1	
Figure 7 TINN stimulus used to estimate RF widths and suppression of tone-evoked Responses	29
Figure 8 Systemic nicotine enhanced responses to narrow-notch stimuli and reduced responses to intermediate-notch stimuli in A1	31
Figure 9 A closer look at responses to intermediate-notch TINN stimuli	33
Figure 10 Systemic nicotine produced similar effects on TINN-evoked MUA	35
Figure 11 Systemic nicotine effects occurred despite the presence of intracortical muscimol	37
Figure 12 In ICc, systemic nicotine reduced responses to intermediate-notch NN stimuli but did not enhance any TINN-evoked response	39
Figure 13 In MGv, systemic nicotine reduced responses to intermediate-notch NN stimuli but did not enhance any TINN-evoked response	40
Figure 14 Simultaneous recordings in A1 and ICc or MGv confirm differential effects of systemic nicotine	42
Figure 15 Comparison of RFs and response suppression in A1, MGv, and ICc	44
Figure 16 Nicotine microinjection in STR enhanced TINN-evoked responses in A1	46
Figure 17 Cortical microinjection of positive allosteric modulator, NS9283, enhanced TINN-evoked responses in A1	49
Figure 18 Summary schematic depicting effects of systemic nicotine on RFs in the auditory lemniscal pathway	55
Chapter 2	
Figure 19 Nicotine selectively depolarized Pyr and VIP neurons	66
Figure 20 Nicotine directly depolarized VIP neurons via $\beta 2$ -containing receptors and indirectly depolarized Pyr neurons	68
Figure 21 Nicotine increased the frequency of sIPSCs in Pyr, SOM, and VIP neurons	70
Figure 22 Nicotine had no effect on EPSCs or evoked IPSCs in Pyr neurons	71
Figure 23 Nicotine depolarized and enhanced sIPSC frequency in Pyr neurons via VIP neurons	74

ACKNOWLEDGMENTS

Thank you to Raju Metherate, I could not have asked for a better mentor. He has been an incredible support, always willing and available to talk through the data and provide insightful feedback. He has clearly explained every difficult auditory or nicotinic acetylcholine receptor concept I've come across and provided a solid foundation for the breadth of knowledge I've accumulated in these 5 years. Beyond just the research, Raju has also taught me how to write, a life-long skill for any career I pursue.

Thank you to the members of the Metherate lab – especially Irakli Intskirveli who patiently spent many hours training me on every technique used for these experiments and Ronit Lazar whose work with breeding mice and making solutions helped relieve the workload of graduate school.

Thank you to my committee members for providing helpful suggestions and for my generous funding sources, the Center for Hearing Research and the National Science Foundation.

Thank you to my family – especially my parents, Suzy, Scott, and Carol, who always (and irrationally) believed that I could do anything, and supported even the craziest adventures and ideas. This dissertation would not have been possible without your sacrifices and love.

CURRICULUM VITAE

EDUCATION

- Ph.D. **Neurobiology and Behavior**, University of California Irvine, expected June 2018
Dissertation: Nicotine's effects on auditory processing and the role of cortical interneurons
- M.S. **Neurobiology and Behavior**, University of California Irvine, 2016
- B.S. **Neuroscience**, Brigham Young University, 2012

TEACHING EXPERIENCE AND TRAINING

Laboratory Instructor, Neurobiology N113L, UC Irvine, 2015-2016

- Created neuroscience course lectures and quizzes, graded and provided feedback for written abstracts and lab reports, taught all material and led students in lab sessions
- Participated in the development of a “flipped classroom” approach to teaching material

Discussion Course Leader, Introductory Biology Bio93, UC Irvine, 2014-2015

- Designed and conducted weekly review sessions, incorporated active learning techniques, prepared and graded quizzes and assignments

Neuroscience Tutor, Neurobiology Neuro205, Brigham Young University, 2009

- Worked one-on-one with introductory neuroscience students, explaining difficult concepts

Teaching Assistant Professional Development Program, UC Irvine, 2014

- Participated in pedagogy training, learned about different student learning styles and how to teach effectively for a diverse class, practiced teaching techniques and received feedback

Scientific Communications Course N292, UC Irvine, 2015

- Participated in a weekly course focused on effectively communicating scientific topics, prepared presentations and received feedback

Cub Scout STEM Leader, 2014-2015

- Prepared weekly lessons and activities for local Cub Scout group, focused on helping Scouts fulfill requirements for STEM awards

Service Mission, Rome, Italy, 2010-2011

- Organized and taught English courses, learned to speak Italian fluently

RESEARCH EXPERIENCE

University of California Irvine, 2013-2018

Auditory Cortex Lab, advisor: Raju Metharate

- Studied nicotinic acetylcholine receptors in auditory cortex
- Trained in *in vivo* local field potential recordings and *in vitro* whole-cell patch clamp, in mouse auditory cortex

Seizure Lab, advisor: Diane O'Dowd

- Studied sodium currents in a drosophila model of Dravet syndrome

- Trained in drosophila brain dissections, creating neuronal cultures, whole-cell patch clamp in culture

Huntington's Disease Lab, advisor: Leslie Thompson

- Studied methylation in stem cell progenitors from Huntington's disease patients
- Trained in Chromatin IP, stem cell culture, mouse behavioral tasks

Brigham Young University, 2007-2012

Drug Addiction Lab, advisor: Scott Steffensen

- Studied the role of ventral tegmental area GABA neurons in addiction
- Trained in whole-cell patch clamp in mouse brain slices

Biophysics Lab, advisor: John Bell

- Studied membrane changes in apoptosis and lipid vesicle phase properties
- Trained in fluorometry and lipid vesicle preparation

Hebrew University, 2012-2013

Olfaction and Audition Lab, advisor: Adi Mizrahi

- Studied the synaptic development of adult-born neurons in the olfactory bulb
- Trained in mouse surgery and virus injection, perfusion, cryostat brain slicing, immunohistochemistry

Motor Control Lab, advisor: Yifat Prut

- Analyzed data from primate motor cortex recordings using MATLAB, including isolating and characterizing single units

University of British Columbia, 2009

Huntington's Disease Lab, advisor: Michael Hayden

- Studied palmitoylation of GLT-1 in Huntington's disease
- Trained in biotin surface labeling, and creation of a stably transfected cell line

National Institutes of Health, 2008

Neurogenetics Lab, advisor: Kenneth Fischbeck

- Studied protein aggregation in Spinal and Bulbar Muscular Atrophy
- Trained in tissue culture and Western blot

FELLOWSHIPS AND AWARDS

National Science Foundation Graduate Research Fellowship, 2015-2018

- Very competitive national research award, 3 years full funding

Center for Hearing Research Fellowship, 2014

- UC Irvine merit-based fellowship, awarded to outstanding students conducting hearing research

Gordon Hein Scholarship, 2013

- UC Irvine merit-based scholarship, awarded to outstanding graduate students

National Institutes of Health Exceptional Summer Student Award, 2008

- Based on a nomination from the student's NIH research advisor, as well as a poster presentation

PUBLICATIONS

- Askew C**, Lopez A, Wood M, Metherate R (2018) Nicotine disinhibits Pyramidal Cells in Auditory Cortex via VIP Interneurons. *In preparation*
- Askew C**, Intskirveli I, Metherate R (2017) Systemic Nicotine Increases Gain and Narrows Receptive Fields in A1 via Integrated Cortical and Subcortical Actions. *eNeuro*
- Askew CE**, Metherate R (2016) Synaptic interactions and inhibitory regulation in auditory cortex. *Biol Psychol* 116:4–9
- Vaughn AR, Bell TA, Gibbons E, **Askew C**, Franchino H, Hirsche K, Kemsley L, Melchor S, Moulton E, Schwab M, Nelson J, Bell JD (2015) Biochimica et Biophysica Acta Relationships between membrane water molecules and Patman equilibration kinetics at temperatures far above the phosphatidylcholine melting point. *BBA - Biomembr* 1848:942–950
- Allison DW, Wilcox RS, Ellefsen KL, **Askew CE**, Hansen DM, Wilcox JD, Sandoval SS, Eggett DL, Yanagawa Y, Steffensen SC (2011) Mefloquine Effects on Ventral Tegmental Area Dopamine and GABA Neuron Inhibition : A Physiologic Role for Connexin-36 Gap Junctions. *Synapse* 813:804–813.
- Huang K, Kang MH, **Askew C**, Kang R, Sanders SS, Wan J, Davis NG, Hayden MR (2010) Palmitoylation and function of glial glutamate transporter-1 is reduced in the YAC128 mouse model of Huntington disease. *Neurobiol Dis* 40:207–215.
- Palazzolo I, Nedelsky NB, **Askew CE**, Harmison GG, Kasantsev AG, Taylor JP, Fischbeck KH, Pennuto M, *Endocrinologia I* (2010) B2 Attenuates Polyglutamine-Expanded Androgen Receptor Toxicity in Cell and Fly Models of Spinal and Bulbar Muscular Atrophy. *J Neurosci Res* 2216:2207–2216.

ABSTRACT OF THE DISSERTATION

Nicotine's Effects on Auditory Processing and the Role of Cortical Interneurons

By

Caitlin Erika Askew

Doctor of Philosophy in Biological Sciences

University of California, Irvine, 2018

Professor Raju Metherate, Chair

Nicotinic acetylcholine receptor (nAChR) activation has been shown to improve sensory-cognitive function. In the auditory system, nicotine (an agonist of nAChRs) enhances the brain's response to attended, relevant stimuli while reducing the response to distracters. Activation of nAChRs has a similar ability to improve stimulus detection in other sensory systems and while it is a remarkable and consistent finding, there is a lack of comprehensive studies on the neural activity behind this phenomenon. Using the mouse auditory system as a model, here we test the role of nAChRs in auditory processing, specifically spectral processing, and the underlying cortical circuitry driving nicotine's effects.

In Chapter 1, we used a tone-in-notched-noise stimulus to examine how nicotine influences the ability to filter incoming frequency information. Current-source density recordings in the primary auditory cortex of the anesthetized mouse revealed that systemic nicotine effectively narrows receptive fields, increases response gain in the center of the receptive field, and enhances responses to the tone. Subsequent manipulations demonstrated that modulation of cortical receptive fields and tone-evoked responses occurred at multiple levels in the ascending auditory pathway. These actions at nAChRs in cortical and subcortical

circuits, which mimic effects of auditory attention, likely contribute to the nicotinic enhancement of sensory and cognitive processing.

In Chapter 2, we performed whole-cell recordings in the auditory cortex of mouse brain slices to investigate the role of discrete cell types. Bath application of nicotine selectively depolarized pyramidal (Pyr) and vasoactive intestinal peptide (VIP)-containing neurons and enhanced spontaneous inhibitory post-synaptic currents (sIPSC) in Pyr, VIP, and SOM cells. We found that VIP cell activation is responsible for both the nicotinic depolarization and sIPSC enhancement observed in Pyr neurons, implicating a disinhibitory neural circuit in the nicotinic modulation of cortex. This disinhibition likely renders Pyr cells more excitable and responsive to incoming stimuli, thus providing a probable mechanism for nicotine's beneficial effects on cortical processing and function.

Together with the findings in Chapter 1, these results further reveal the neural basis of nicotine's effects and substantiate efforts to use nicotine as a therapeutic for those with cognitive or sensory processing disorders.

INTRODUCTION

Nicotine enhances sensory-cognitive function

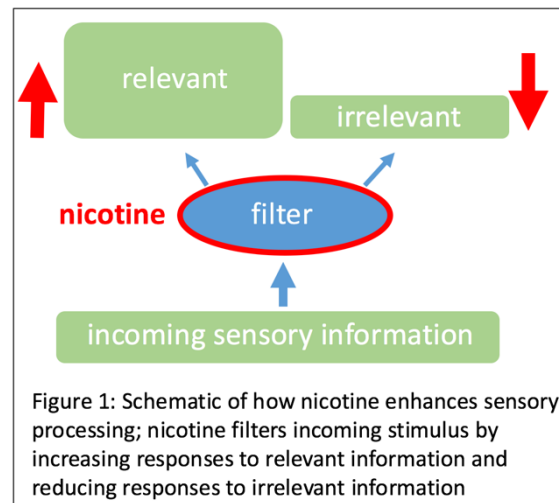
Nicotine is known to enhance cognitive and sensory function, demonstrated by a number of studies in both animals and humans. In animals, nicotine improves working memory and performance on tasks such as object recognition and avoidance training (see Rezvani and Levin, 2001 for a review), as well as visual signal detection (Rezvani et al., 2002). Yet the most compelling evidence for nicotine's beneficial effects arises from studies with humans. Although just a few examples, nicotine enhances accuracy on a working memory task (Kumari et al., 2003), improves performance on the Stroop test (Provost and Woodward, 1991), and increases detections and reaction time in a rapid information processing task (Wesnes and Warburton, 1984). Nicotine similarly improves performance for sensory processing, including for both visual and auditory tasks (Lawrence et al., 2002; Harkrider and Hedrick, 2005; Hong et al., 2011; Warbrick et al., 2012; Gupta and Mittal, 2014). In one task, subjects were presented random digits at a rate of 100 digits/min and asked to press a button when three consecutive odd numbers or three consecutive even numbers appeared; nicotine patch application increased the number of accurate hits in this task (Lawrence et al., 2002).

Some groups have also coupled human behavioral measures with physiological recordings to investigate the neural substrate of nicotinic task improvement. Nicotine administered through a patch increases the response magnitude and reduces onset latencies in the electrophysiological auditory phenomenon known as "mismatch negativity", which also correlated with enhanced performance on a consonant-vowel discrimination task (Harkrider and Hedrick, 2005; Inami et al., 2005). This suggests that nicotine may improve auditory

processing through auditory gating mechanisms as well as increasing processing speed.

Additionally, subjects given a nasal nicotine spray had both reduced reaction times in an odd-ball type visual task and more activation in certain brain regions, as detected by MRI (Warbrick 2012). Yet much remains unclear and the question persists: precisely how does nicotine improve cognitive and sensory processing?

One leading hypothesis is that nicotine enhances attentional filtering; i.e., nicotine increases neural responses to important, attended stimuli and reduces neural responses to unimportant, background stimuli (Kassel, 1997). This attentional filtering would effectively sort out relevant stimuli from irrelevant noise and



thereby improve processing through a similar manner as attention. Proposed in 1997, Kassel's stimulus-filter hypothesis is still influential today and more recent work has shown that nicotine increases neural responses to relevant stimuli and decreases neural responses to distracting stimuli. In primary visual cortex, several groups have demonstrated that nicotine or other nicotinic receptor agonists increase responses to visual stimuli (Disney et al., 2007; Soma et al., 2013; Alves-Pinto et al., 2016), while in human auditory cortex Knott et al (2009) found that nicotine decreased the amplitude of responses elicited by distractor stimuli.

Nevertheless, not all research corroborates the idea that nicotine enhances cognitive and sensory processing. A few recent examples specific to the auditory system find no nicotine effect on auditory attention task performance (Knott et al., 2009; Smucny et al., 2015).

Although perplexing, inconsistent results may be due to a number of uncontrolled factors, including but not limited to: smoker vs nonsmoker participants, healthy vs impaired participants, acute vs chronic nicotine application, and easy vs difficult task level. It has been suggested that beneficial nicotine effects may result merely from withdrawal relief, given that many studies are conducted in smokers asked to abstain from cigarette use for a period before nicotine administration (Heishman, 1998). Yet, a recent meta-analysis on nicotine studies with participants who are nonsmokers or minimally deprived smokers proposes that the nicotinic improvement in cognitive function persists even when participants do not suffer from withdrawal effects (Heishman et al., 2010).

Moreover, many studies use healthy participants and an acute nicotine administration, even though nicotine may be more beneficial as a long-term therapeutic for impaired individuals. Newhouse et al (2012) found that in subjects with mild cognitive impairment, daily transdermal nicotine administration improved attention, memory, and overall cognitive performance. Several other groups have demonstrated nicotine-induced cognitive improvement in participants with impairments such as ADHD and schizophrenia (Conners et al., 1996; Barr et al., 2008), further implying that nicotine may be especially useful for these individuals.

The majority of evidence suggests that nicotine or nicotinic agonists represent a possible therapeutic for those with cognitive or sensory processing deficits, yet nicotine's ability to enhance sensory function is not well characterized. The present dissertation seeks to clarify how nicotine enhances auditory processing, both by investigating nicotine's effects on spectral processing as well as probing the underlying cortical circuitry.

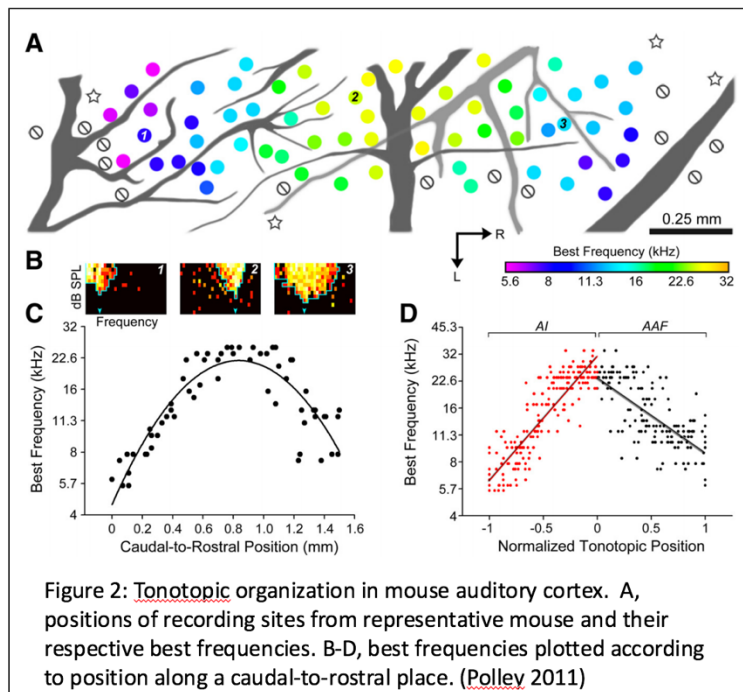
Auditory spectral processing

Auditory processing is a broad term referring to our ability to make sense of incoming auditory information. One type of auditory processing is spectral processing, which encompasses simple frequency discrimination as well as understanding more spectrally complex stimuli such as vowel sounds in speech and pitch perception (Wallace et al., 2011). Frequency discrimination is compromised in individuals with dyslexia and other language disorders (France et al., 2002; McArthur and Bishop, 2004; Mengler et al., 2005; Moore et al., 2010), demonstrating its crucial role in functional auditory processing.

An organizational feature in the auditory system known as tonotopy is vital for spectral processing. Tonotopy is a spatial map of frequencies, i.e., similar frequencies are represented in neighboring regions within an auditory nucleus, thus allowing neural populations to more easily

resolve the spectral content of a stimulus. Because of tonotopy, individual neurons and populations of neurons can be defined by their receptive fields (RF), which are the distinctive profiles of frequencies and intensities that activate that neuron/population of neurons.

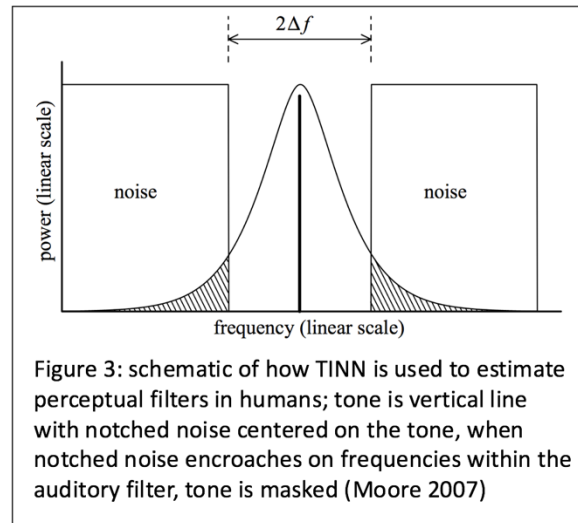
Each neuron or recording site within a tonotopic auditory region also has



a characteristic frequency (CF) – the frequency with the lowest threshold responses. That is, a local field potential recording in primary auditory cortex (A1) may have a CF of 10 kHz; when presenting tones at a low intensity such as 5dB, the recorded population of neurons may respond only to 10 kHz and no other frequencies, thus establishing 10 kHz as the frequency with the lowest threshold.

Receptive fields are one way in which spectral processing can be studied and RF widths may vary depending on behavioral state or auditory processing impairments. Attention has a remarkable ability to simultaneously narrow and increase gain in the center of RFs, presumably leading to finer tuning and more precise frequency selectivity. For example, when ferrets attend to a tone during a discrimination task, the responses in A1 to the target tone are enhanced while the responses to non-target sounds are reduced (Fritz et al., 2005). A similar phenomenon was seen in the primate, where attending to an auditory stimulus increases gain at the preferred stimulus in A1 and increases suppression at all other frequencies (O’Connell et al., 2014). These findings translate to humans as well, where attention to a tone enhances fMRI responses selectively at the cortical sites where that tone is represented (Costa et al., 2013). Additionally, individuals with impaired auditory function such as hearing loss or cochlear implants have wider perceptual filters (Glasberg and Moore, 1986; Dubno and Dirks, 1989; Summers and Leek, 1994). Together, these data validate the idea that narrowed RFs with increased central gain indicate a more selective, precise tuning of the auditory system and improved spectral processing.

Perceptual filters, the behavioral correlate of receptive fields, also reflect the tuning of the auditory system, and are usually derived from responses to a tone-in-notched-noise (TINN) stimulus (Glasberg and Moore, 1990). This TINN stimulus consists of a pure tone played simultaneously with notched noise of varying notch sizes. The notch is a gap in which some of the frequencies of the noise have been



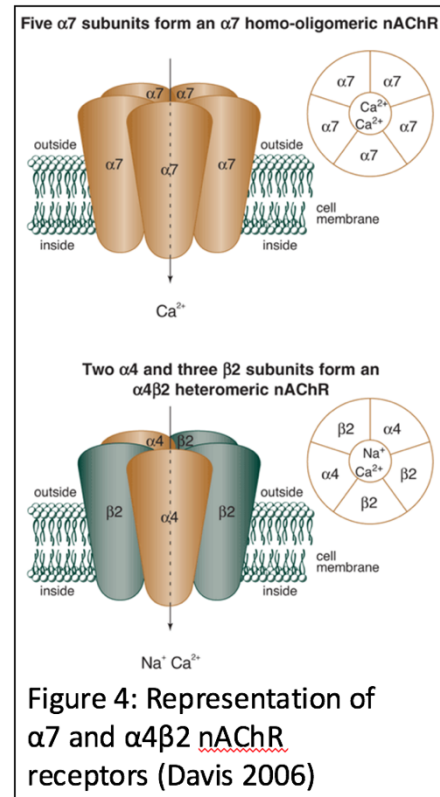
eliminated and it is centered on the tone frequency. At narrow notch sizes, the tone is well-masked (i.e.; the listener cannot hear the tone in the midst of the noise) and requires a high intensity in order to be perceived. At wider notch sizes, the threshold for tone perception drops, likely because less noise is passing through the filter centered on the tone frequency. These tone thresholds are then used to estimate the width of the auditory filter. Although a common method for estimating perceptual filters, TINN is rarely used in animal electrophysiology. Rather, a pure tone stimulus set has been traditionally utilized to determine receptive field shapes. Nevertheless, TINN can be a useful stimulus to probe spectral processing and specifically receptive field shapes, especially because it more accurately simulates a natural stimulus by activating multiple frequency channels simultaneously as compared to pure tones.

There is some evidence that nicotine or nicotinic acetylcholine receptor agonists produce a similar effect on RFs as attention (Disney et al., 2007; Gilbert et al., 2007; Knott et al., 2009; Pinto et al., 2013). Work from our lab has shown that nicotine enhances the responses to CF and reduces the response to a spectrally distant, non-CF, stimulus in A1, implying but not

directly showing RF narrowing with increased gain (Liang et al., 2008; Kawai et al., 2011; Intskirveli and Metherate, 2012). Since these RF changes appear to be beneficial to auditory spectral processing, Chapter 1 of this dissertation examines more thoroughly the nicotinic modulation of RFs using a TINN stimulus.

Nicotinic acetylcholine receptors in cortex

The previously described studies on nicotine are fundamental in our understanding of how this drug may improve sensory processing. Yet, to effectively utilize nicotine, it is also crucial to have a basic knowledge of its underlying mechanism. Nicotine is a selective agonist for nicotinic acetylcholine receptors (nAChRs) which are ligand-gated ionotropic channels distributed throughout the central nervous system. The nAChR is a pentamer, composed of five subunits including $\alpha 2$ - $\alpha 10$



and $\beta 2$ - $\beta 4$, arranged in varied combinations. Specific combinations are more common than others, and in cortex the $\alpha 7$ homomeric receptor (all 5 subunits in receptor are $\alpha 7$) and the $\alpha 4\beta 2^*$ (receptor includes combination of $\alpha 4$ and $\beta 2$ subunits, * indicates that other subunits may contribute to the composition of this subtype) are the most commonly found. The kinetics of nAChR also vary depending on receptor type. The $\alpha 7$ nAChR receptor has a low affinity for agonists and is rapidly desensitized while the $\alpha 4\beta 2^*$ receptor has a high affinity for nicotine and is more slowly desensitized. Thus, various receptor subtype combinations may differentially

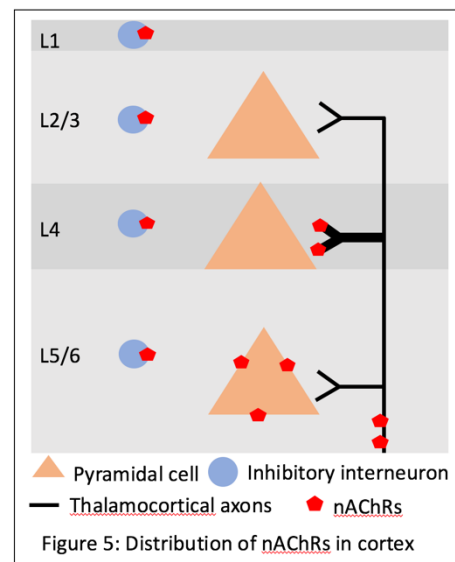
contribute to a given nicotine effect depending not only on the expression level of the receptor type but also the concentration of agonist (see Dani and Bertrand, 2007 for a review).

Significant research has been conducted on nAChRs in the neocortex, given that cortical regions are traditionally considered regions of higher-order processing. Nicotinic receptors are endogenously activated by acetylcholine and the most substantial source of cholinergic input originates from the nucleus basalis in the basal forebrain (BF). These cholinergic projections terminate in all cortical layers, although are especially dense in layer 5 (Umbriaco et al., 1994). Historically thought to release acetylcholine non-selectively and via volume transmission, recent studies have called this assumption into question; it now appears that cholinergic axons may also act through classical, fast synaptic transmission (Turrini et al., 2001; Lendvai and Vizi, 2008; Sarter et al., 2009). Related to nicotine's effects on attention, the release of ACh in cortex is critical in attentional mechanisms. For example, ACh from BF projections is released during tasks that require attention (Himmelheber et al., 2000) and BF stimulation has been shown to improve sensory coding by increasing reliability of responses to visual stimuli (Pinto et al., 2013).

To understand how nicotine modulates cortical circuitry, it is important to begin with the localization of nAChRs. Hybridization and radioligand binding studies find nAChRs distributed in all cortical layers, although function may vary by layer (Clarke et al., 1985; Wada et al., 1989). For instance, in visual cortex Disney et al (2007) observed that nicotine enhanced responsiveness in layer 4 (L4) while suppressing responsiveness outside of L4. Nicotinic receptors are also preferentially expressed on particular cell types as well specific subcellular compartments (soma, dendrites, presynaptic, postsynaptic, etc.). Some studies show a

presynaptic locus for nAChRs, where their activation can most effectively modulate neurotransmitter release. More specifically, nAChRs are found on thalamocortical presynapses; that is on the axon terminals of thalamic projections to L4 (input layer) of the cortex (Sahin et al., 1992; Gil et al., 1997; Lavine et al., 1997; Clarke, 2004). These thalamocortical presynaptic nAChRs increase glutamate release, causing enhanced depolarization and activation of cortical L4 cells. Nicotinic receptors are additionally associated with thalamocortical axons themselves, rather than just the axon terminals, and can increase excitability of the axons and also facilitate neurotransmitter release (Ding et al., 2004; Kawai et al., 2011; Bieszczad et al., 2012; Butt et al., 2014).

Nicotinic receptors are not expressed solely on axons and axon terminals, but also on postsynaptic locations such as the cell soma and dendrites. Concerning postsynaptic expression of nAChRs on cortical cells, it appears that functional nAChRs are found almost exclusively on inhibitory interneurons and not on pyramidal cells (Porter et al., 1999; Disney et al., 2007). In whole cell recordings from rat brain slices, nicotinic agonists had no effect on pyramidal cells, yet selectively activated specific inhibitory interneuron types (Porter et al., 1999). In primate visual cortex, Disney et al (2007) discovered that nAChRs are rarely present on pyramidal cells, but instead are expressed on inhibitory interneurons. Not all studies support this generalization and some demonstrate direct pyramidal cell activation by nAChRs. It appears that functional postsynaptic nAChRs



may be present on pyramidal cells, albeit preferentially on pyramidal cells in infragranular layer 5 and 6 and this may depend on the cortical region (Kassam et al., 2008; Zolles et al., 2009).

Although ionotropic in function, nAChR effects have also been recently considered neuromodulatory for a number of reasons. First, effects from nicotine can endure for 10s of minutes, producing long-lasting changes in cortical responsiveness as compared to classical fast synaptic transmission (Intskirveli and Metherate, 2012; Arroyo et al., 2014). Also, nAChRs have been shown to activate intracellular pathways, another feature typical of neuromodulation (Intskirveli and Metherate, 2012). Understanding that nAChR activation can produce neuromodulatory effects gives rise to the idea that nicotine can impact cortical circuitry beyond simple depolarization/hyperpolarization, and can additionally induce changes in plasticity and long-term function. Indeed, activation of nAChRs is critical for cortical function and is a necessary component for proper cortical processing. Cortical nAChR expression has been shown to be altered in several disease states including Alzheimer's disease, schizophrenia, and autism and dysfunctional or sparse nAChRs may be a main contributor to the circuitry dysfunction observed in these conditions (Guan et al., 1999, 2000; Martin-Ruiz et al., 2004). These observations are a significant rationale for investigative research into nicotine as a potential therapy.

Although some research has already been done, it is crucial to continue to work out the specific role of nAChRs in cortex. Not only do we learn more about basic function of cortical networks, but it also informs us about how nicotine as a therapeutic can best improve cortical processing.

Cortical interneurons

The neural circuits intrinsic to neocortex include excitatory pyramidal neurons that release glutamate, and inhibitory non-pyramidal interneurons that release GABA. These circuits respond to a variety of inputs from thalamic relay nuclei and other cortical regions to process cortical information, the result of which underlies basic perception and cognition. As knowledge in this area has exploded in recent years with the introduction of new molecular and genetic tools, it has quickly become clear that GABAergic interneurons are an essential and diverse regulatory component in cortical circuitry.

GABAergic inhibitory interneurons comprise about 15-25% of all neurons in cortex (Rudy et al., 2011; DeFelipe et al., 2013). They are found in all cortical layers, where they form a minority of cells in each layer with the exception of layer 1; although layer 1 is cell-sparse, containing mostly the axons and dendrites of excitatory neurons whose cell bodies lie elsewhere, nearly all cells intrinsic to layer 1 are GABAergic. Inhibitory interneurons can be directly activated by thalamocortical axons or inputs from other cortical areas, but the pattern of responding to inputs varies according to interneuron subtype (Kepecs and Fishell 2014). Interneuron outputs are similarly specific; notably, they can target either the soma or dendrites of other neurons, which is an important determinant of function. Direct inhibitory projections to the soma or axon initial segment exert powerful inhibition of neuron output, while projections to distal dendrites are presumably more modulatory. Projection patterns can vary according to interneuron subtype, or be mixed within groups, depending on the method of interneuron classification (Kepecs and Fishell, 2014).

Inhibitory interneurons can be categorized in non-overlapping groups based on molecular markers, notably the calcium binding protein parvalbumin (PV interneurons), and the peptides somatostatin (SOM interneurons) and vasoactive intestinal peptide (VIP interneurons). The majority of cortical interneurons fall into one of these three groups, although a remaining ~20% do not express any of these markers (Xu et al., 2010). It is also important to note that there exist other methods of classification, often using morphological or physiological characteristics, and these other classification methods overlap with the currently described method. Yet, the molecular markers are becoming an increasingly popular schema for inhibitory interneurons.

These three interneuron classes (PV, SOM, VIP) appear to have distinct functions and connectivity. For example, PV interneurons mediate feed-forward inhibition and can enhance functional connectivity in cortical (vertical) columns, but not lateral or feedback connections within (horizontal) layers (Hamilton et al., 2013). Activation of PV neurons strongly suppresses spontaneous firing and weakly suppresses stimulus-evoked firing, producing an overall enhancement of afferent input “signal to noise” ratios. Conversely, SOM neurons in the superficial layers of visual cortex are excited by horizontal projections in the same layer, contributing to surround suppression in layer 2/3 pyramidal neurons (Adesnik et al., 2013). PV cells also have broader frequency receptive fields and faster response onset than SOM cells, while the narrower tuning of SOM cells is more similar to that of excitatory pyramidal neurons (Li et al., 2014). This differential tuning and latency are consistent with the notion that PV neurons mediate robust feed-forward inhibition. Anatomical evidence agrees with this idea, as PV cells project more locally than do SOM cells and often target neuronal somata, allowing for

powerful and temporally precise effects (Yuan et al., 2011). In contrast, SOM cells more often target distal dendrites of pyramidal neurons, and their slower time course allows for integration of inputs over a broader time frame (Pouille and Scanziani, 2001; Di Cristo et al., 2004).

Interestingly, VIP neurons have been recently shown to have a disinhibitory role in cortex, although this will be explored in greater detail in the next section.

As mentioned previously, nicotine may preferentially affect inhibitory interneurons. In hippocampus, a region with similar circuitry to cortex, nicotine can selectively excite interneurons but not pyramidal neurons (Frazier et al., 1998). Similarly, in prefrontal cortex, nAChRs are found on both inhibitory interneurons and pyramidal cells, but nicotine-induced neuronal firing occurs only in interneurons (Poorthuis et al., 2013). Nicotinic receptor agonists selectively affect specific interneuron subtypes. In one study in motor cortex, agonists produce excitation of VIP interneurons but no effect on PV or SOM interneurons (Porter et al., 1999), and in another study, nAChR selectively activated non-fast-spiking interneurons in layers 2/3 and 5 in several neocortical regions (Gulledge et al., 2007)

Several groups demonstrate the existence of nAChRs on PV cells but most show no direct activation, thus the receptors may not be functional. Any nicotinic changes to PV cell activity are usually shown to be indirect; i.e. other interneurons excited by nicotine that then synapse onto PV cells. In contrast to Porter et al's study, recent studies have found that SOM are activated by nAChRs in visual cortex and hippocampus (Jia et al., 2010; Chen et al., 2015). Perhaps most interestingly, VIP cells are consistently demonstrated to be responsive to nAChR activation (Porter et al., 1999; Lee et al., 2010; Alitto and Dan, 2013; Fu et al., 2014; Chen et al., 2015).

VIP interneurons

In recent years, there has been considerable attention devoted to the VIP interneurons. Although constituting a small percentage (10-15%) of the cortical inhibitory interneuron population, VIP interneurons have been demonstrated to play a crucial role in cortical circuitry.

These interneurons are defined by their expression of VIP, a small polypeptide that was originally discovered to be present in the gut. Here, VIP regulates intestinal functions such as inhibiting gastric acid secretion and relaxing smooth muscles. In the 1970's however, it was discovered that VIP was also present in the central nervous system, inspiring further examination into potential functions of VIP and VIP-containing cells outside of the intestinal system (Bryant et al., 1976). VIP-expressing neurons were subsequently found in diverse brain regions, including the amygdala, suprachiasmatic nucleus, midbrain, and cerebral cortex (Fuxe et al., 1977; Sims et al., 1980). Within these brain regions, it has been shown that VIP neurons also have a wide range of functions. For example, numerous studies delineate the role of VIP neurons in the suprachiasmatic nucleus, demonstrating that in this region they regulate circadian rhythms (Aton et al., 2005). Just in the last few years, cortical VIP interneurons have additionally gained attention and their role in cortical circuitry is beginning to emerge.

In cortex, VIP neurons are exclusively inhibitory interneurons (Miyoshi et al., 2010; Taniguchi et al., 2011). That is, they project to local neuron populations and release GABA, which produces inhibition of neighboring neurons. VIP interneurons are dispersed throughout all cortical layers, although especially concentrated in layers 2/3 (60% of all cortical VIP interneurons) (McDonald et al., 1982; Obata-Tsuto et al., 1983; Morrison et al., 1984; Prönneke

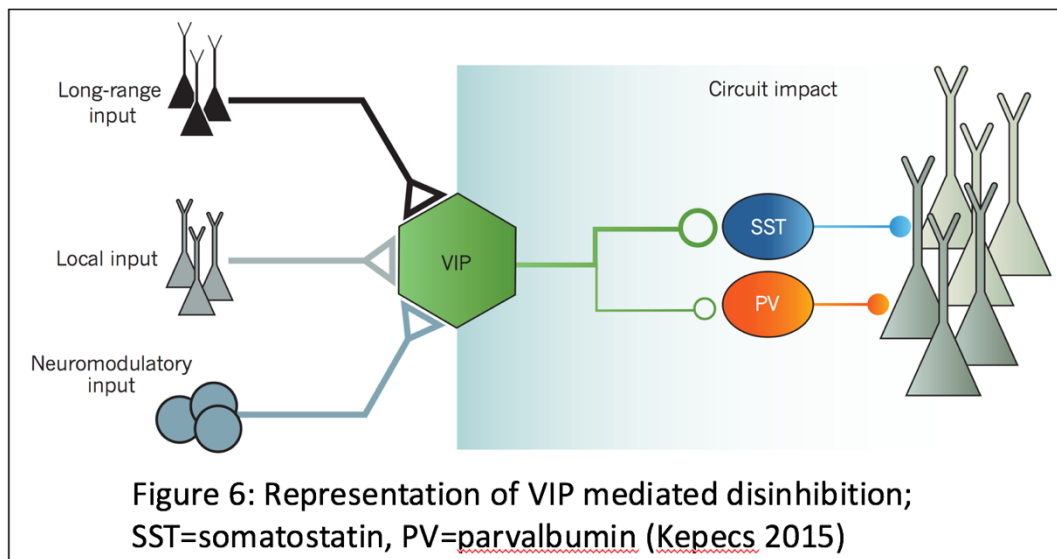
et al., 2015). While always expressing VIP, these interneurons cannot be considered entirely homogenous; rather VIP interneurons themselves can have varied morphological and physiological properties. Most commonly, VIP interneurons are either bipolar or bitufted, often with a long axon extending vertically and towards deeper cortical layers (McDonald et al., 1982; Miyoshi et al., 2010; Karnani et al., 2016). There appears to be a spatial restriction in the spread of VIP interneuron processes compared to other interneuron subtypes, suggesting that VIP cells may be specialized for unusually localized modulation (Karnani et al., 2016). Additionally, many VIP interneurons appear to have either an irregular or fast adapting firing pattern in response to whole-cell current injection (Miyoshi et al., 2010). Even though many VIP interneurons exhibit these traits, others have reported different properties, including tripolar and basket morphologies, as well as non-adapting or delayed non-fast-spiking patterns (Miyoshi et al., 2010). Even within the “irregular-spiking” VIP neurons, several subgroups have been discovered (Porter et al., 1998). Taken as a whole, these studies describe a heterogeneous population of cortical VIP interneurons. It is likely that diverse groups within VIP interneurons also have discrete roles in cortical circuitry, although this has yet to be explored.

Despite the heterogeneity, two important functional traits have emerged for this cell population: 1) VIP interneurons preferentially innervate other interneurons and 2) VIP interneurons facilitate excitatory responses in pyramidal cells through disinhibition. David et al (2007) demonstrated that in rat somatosensory cortex, almost all PV cells are contacted by VIP interneurons, despite the proportionately low number of VIP cells. Several other groups found that VIP cells preferentially contact somatostatin (SOM) neurons, and this remains the dominant hypothesis. In somatosensory cortex VIP interneurons provide much more inhibition

to SOM cells as compared to pyramidal cells or fast-spiking interneurons (Lee et al., 2013).

Three additional studies show that this holds true in visual cortex and auditory cortex as well (Pfeffer et al., 2013; Pi et al., 2013; Karnani et al., 2016).

Furthermore, preferential innervation of other inhibitory interneurons gives rise to a functional role of VIP interneurons: disinhibition. If cortical VIP neurons inhibit interneurons that in turn inhibit pyramidal cells, the end result is disinhibition of pyramidal cells. Several studies demonstrate that this indeed occurs. Pi et al revealed that optogenetic activation of VIP cells *in vivo* increases tone-evoked firing of pyramidal cells, but found that this VIP neuron-induced enhancement occurs in only a subset of pyramidal cells. Optogenetic activation of VIP interneurons also increases stimulus-evoked pyramidal cell responses in primary visual cortex (V1) (Fu et al., 2014). Also in V1, projections from frontal cortex to visual cortex enhance visually-evoked responses in V1, and this facilitation is mediated through V1 VIP cells (Mesik et



al., 2015). Finally, VIP cell activation reduces inhibitory post-synaptic potentials in pyramidal cells in mouse visual cortex, directly showing that the pyramidal cell facilitation seen previously

is a result of disinhibition (Karnani et al., 2016). Together, these studies establish that cortical VIP cells preferentially contact other inhibitory interneurons, resulting in pyramidal cell disinhibition.

Another consistent characteristic of VIP interneurons is that they are responsive to nAChR agonists, including nicotine. Porter et al. found that of all the cortical interneuron subtypes, nAChR agonists exclusively activate VIP cells and several others revealed that VIP cells are responsive to ACh via nAChR activation (Alitto and Dan, 2013; Lee et al., 2013; Fu et al., 2014; Bell et al., 2015). Additionally, nAChR activation has been shown to mediate pyramidal cell disinhibition in cortex, although none of these studies have examined the interneuron molecular subtypes involved (Ji and Dani, 2000; Christophe et al., 2002; Arroyo et al., 2012). As a group, these studies circumstantially suggest that the nicotinic enhancement of stimulus-evoked responses demonstrated in sensory cortex may be mediated through VIP-induced disinhibition, yet this link has not been directly addressed. Therefore, this dissertation investigates this potential relationship in Chapter 2.

CHAPTER 1

Systemic nicotine increases gain and narrows receptive fields in A1 via integrated cortical and subcortical actions

Abstract

Nicotine enhances sensory and cognitive processing via actions at nicotinic acetylcholine receptors (nAChRs), yet the precise circuit- and systems-level mechanisms remain unclear. In sensory cortex, nicotinic modulation of receptive fields (RFs) provides a model to probe mechanisms by which nAChRs regulate cortical circuits. Here we examine RF modulation in mouse primary auditory cortex (A1) using a novel electrophysiological approach: current-source density (CSD) analysis of responses to tone-in-notched-noise (TINN) acoustic stimuli. TINN stimuli consist of a tone at the characteristic frequency (CF) of the recording site embedded within a white noise stimulus filtered to create a spectral “notch” of variable width centered on CF. Systemic nicotine (2.1 mg/kg) enhanced responses to the CF tone and to narrow-notch stimuli, yet reduced the response to wider-notch stimuli, indicating increased response gain within a narrowed RF. Subsequent manipulations showed that modulation of cortical RFs by systemic nicotine reflected effects at several levels in the auditory pathway: nicotine suppressed responses in the auditory midbrain and thalamus, with suppression varying with spectral distance from CF so that RFs became narrower, and facilitated responses in the thalamocortical pathway, while nicotinic actions within A1 further contributed to both suppression and facilitation. Thus, multiple effects of systemic nicotine integrate along the ascending auditory pathway. These actions at nAChRs in cortical and subcortical circuits, which

mimic effects of auditory attention, likely contribute to nicotinic enhancement of sensory and cognitive processing.

Introduction

Nicotine is known to enhance cognitive and sensory processing (Rezvani et al., 2002; Levin and McClernon, 2006; Warbrick et al., 2012; Gupta and Mittal, 2014), including auditory processing (Knott et al., 2009; Smucny et al., 2015). Nicotine activates nicotinic acetylcholine (ACh) receptors (nAChRs), which are present throughout the auditory system (Clarke et al., 1985; Morley and Happe, 2000; Dani and Bertrand, 2007; Bieszczad et al., 2012). These nAChRs normally are activated by endogenous ACh, which is a key neuromodulator of cognitive and sensory processes (Himmelheber et al., 2000; Hasselmo and Sarter, 2011; Klinkenberg et al., 2011). Similarly, nicotine is hypothesized to enhance sensory processing through increased attentional filtering, i.e., an increased ability to attend to task-relevant stimuli and ignore distractors (Kassel, 1997; Gilbert et al., 2007; Behler et al., 2015; Smucny et al., 2015). In sensory cortex, activation of nAChRs most often enhances responses evoked by optimal sensory stimuli, but also can produce response suppression to non-optimal stimuli (Liang et al., 2006; Disney et al., 2007; Kawai et al., 2011; Intskirveli and Metherate, 2012). Conversely, loss of cortical nAChRs during aging or disease states is associated with diminished cognitive processing (Whitehouse et al., 1986; Albuquerque et al., 2009), and as a result, nicotine and other nAChR agonists are being considered for therapeutic use (Taly et al., 2009; Hurst et al., 2012; Newhouse et al., 2012). However, beyond its ability to enhance sensory-cognitive function, including sensory-evoked responses, little is known about the circuit-level

mechanisms by which nicotine acts. Such an understanding will help to direct development of therapeutic treatments for specific disorders, including central auditory processing disorders.

Here we investigate physiological effects of nicotine that are relevant to auditory processing, a broad term encompassing tasks ranging from simple tone detection to speech comprehension (Wallace et al., 2011). In psychoacoustics, a common approach used to examine perceptual filters engaged in auditory processing is to study detection of a tone in notched noise (TINN) (Patterson, 1976). That is, a listener is required to detect a tone in the presence of a notched-noise (NN) masker, i.e., a white noise stimulus filtered to create a spectral “notch” of variable width centered at the tone frequency. As the notch is progressively narrowed, the width of the hypothetical perceptual filter used to detect the tone is estimated by the notch width at which the tone-detection threshold begins to rise. The physiological equivalent of a perceptual filter is the frequency receptive field (RF), which traditionally is measured using pure tones (Sutter et al., 1999). However, since TINN stimuli more closely approximate real-life stimuli by activating multiple frequency channels simultaneously, TINN-evoked electrophysiological responses may be more informative for understanding auditory processing. Additionally, delaying the onset of the tone embedded within the TINN stimulus can provide information about temporal, as well as spectral, processing. For these reasons, we have adopted the TINN stimulus in a novel approach to investigate neurophysiological mechanisms of auditory processing.

Previously, we have shown that systemic nicotine enhances the response to characteristic frequency (CF) stimuli, and reduces the response to a spectrally distant stimulus in rat and mouse primary auditory cortex (A1) (Liang et al., 2008; Kawai et al., 2011; Intskirveli

and Metherate, 2012). These results imply, but do not show directly, that nicotine narrows RFs in A1 and increases gain within the narrowed RF. Here we used TINN-evoked responses to measure RF characteristics and directly show nicotine-induced increased gain within narrowed RFs. Moreover, modulation of RFs in A1 by systemic nicotine is the result of distinct nicotinic effects at several levels of the auditory pathway—including midbrain, thalamus, the thalamocortical pathway and cortex—that integrate to produce the overall effect observed in A1.

Materials and Methods

Animals

Adult (60-90 day old) male FVB mice were used for all procedures in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and as approved by the University of California, Irvine Institutional Animal Care and Use Committee (IACUC). Mice were anesthetized with urethane (0.7 g/kg ip; Sigma) and xylazine (13 mg/kg ip; Phoenix Pharmaceuticals), placed in a sound-attenuating chamber (AC-3; IAC) and maintained at 37°C. Anesthesia was supplemented as necessary with urethane (0.13 g/kg) and xylazine (1.3 mg/kg) via an intraperitoneal catheter to avoid movement of mice. Note that urethane anesthesia does not suppress nAChR function, unlike anesthetics such as barbiturates and ketamine (Hara and Harris, 2002; Tassonyi et al., 2002). The head was secured in a stereotaxic frame (model 923; Kopf Instruments) with blunt earbars. After a midline incision, the skull was cleared and secured to a custom head holder. A craniotomy was performed in the appropriate region for electrode placement or microinjections and the exposed brain was kept moist with

warmed saline. After the craniotomy, the blunt earbars were removed to permit acoustic stimulation.

Electrophysiology and Acoustic Stimulation

Stimulus-evoked local field potentials (LFPs) were recorded with a glass micropipette filled with 1 M NaCl (~1 M Ω at 1 kHz) for locating auditory regions, or a 16-channel silicon multiprobe (~2–3 M Ω at 1 kHz for each 177- μm^2 recording site, 100 μm separation between recording sites; NeuroNexus Technologies). Recordings were filtered and amplified (1 Hz to 1 kHz, AI-401 or AI-405, CyberAmp 380; Axon Instruments), digitized (5 kHz), and stored on a computer (Apple Macintosh running AxoGraph software). Acoustic stimuli were digitally synthesized and controlled with custom MATLAB software (Dr. Tom Lu, Center for Hearing Research Computing and Engineering Core) and delivered through an open-field speaker (ES-1 or FF-1 with ED-1 driver; Tucker-Davis Technologies) positioned ~3 cm in front of the left ear. For calibration [sound pressure level (SPL), in dB re: 20 μPa] a microphone (model 4939 and Nexus amplifier; Brüel and Kjaer) was positioned in place of the animal at the tip of the left earbar. TINN stimuli (Fig. 7A) consisted of a tone at the CF of the recording site, embedded within notched noise (notch centered at CF). CF tones were 100 ms duration (5 ms linear rise and fall ramps), frequency range 10-20 kHz, 15 dB above threshold and onset 50 ms after NN onset. NN component was 200 ms in duration with 5 ms linear rise and fall ramps, variable notch size (0.1-2.5 octaves), fixed amplitude (set at 25 dB above threshold for white noise) and overall frequency range 1-50 kHz. For data collection, TINN stimuli were delivered at a rate of 0.5/s in sets of 25 trials.

Determination of recording sites

A1: The craniotomy was centered approximately 3 mm posterior, 4 mm lateral and 2 mm ventral to bregma. To identify A1, we recorded tone-evoked responses from multiple sites ~250 μm apart along the anterior-posterior (AP) axis in auditory cortex, using a glass micropipette inserted into layer 4 (~400- μm depth) and orthogonal to the cortical surface. Based on responses to a standard set of tones (1-40 kHz in 2.5 kHz steps, -10 dB to 70 dB SPL in 5 dB steps), we determined CF (frequency with the lowest threshold) for each recording site. CF maps were constructed to identify the tonotopy expected for A1, including a reversal of tonotopy at the border with the anterior auditory field (Stiebler et al. 1997). We then chose a site within A1 having a CF of 10–20 kHz (so that TINN stimuli could be constructed with the spectral notch centered near the middle (in octaves) of our frequency range of 1-50 kHz), and mapped along the dorsal-ventral (DV) axis of the presumed isofrequency region to find the site with the shortest-latency, largest-amplitude surface LFP (i.e., isofrequency region mapped using a micropipette placed on the cortical surface). This site was used for all subsequent procedures. We inserted the 16-channel multiprobe perpendicular to the cortical surface to record LFPs throughout the cortical depth, and re-determined CF more precisely (steps of 1 kHz and 5 dB) based on the initial slope and onset latency of LFPs recorded 300–400 μm below the surface. Tone-evoked LFPs were considered threshold responses when their amplitude exceeded 3 standard deviations of the mean baseline (determined over the 100 ms preceding the tone).

Medial Geniculate, ventral division (MGv): We mapped the MG body with a glass micropipette angled 20 degrees from horizontal and inserted through auditory cortex, starting

~3 mm posterior to bregma and mapping in the AP, DV and medial-lateral (ML) planes until the expected tonotopy for the MGv was identified (Hackett et al., 2011). We then inserted a 16-channel multiprobe at the same angle so that several channels would span MGv, selected a channel with clear tone-evoked responses, and re-determined CF using similar methods as for A1.

Inferior Colliculus, central nucleus (ICc): A glass micropipette was inserted vertically ~1 mm lateral and ~1 mm posterior to lambda, and we mapped along AP, DV, and ML axes to identify the tonotopy expected for the ICc (Stiebler and Ehret, 1985). We then inserted a 16-channel multiprobe vertically so that multiple channels would span the IC, selected a channel with clear tone-evoked responses, and re-determined CF, as above.

To confirm placement of the multiprobe in MGv or ICc, after each experiment the animal was perfused with 4% paraformaldehyde, the brain was removed and sectioned in the “thalamocortical” (MGv) (Cruikshank and Rose, 2002) or transverse (ICc) plane, and the multiprobe track visualized and confirmed to pass through the appropriate structure.

Drug Administration

For systemic injections, nicotine ditartrate (Tocris) was dissolved in saline (2.1 mg/kg free base), and delivered subcutaneously. Since the effects of systemic nicotine on tone-evoked responses in A1 last 30 min or longer (Kawai et al., 2011; Intskirveli and Metherate, 2012), all post-nicotine data were obtained within 20 min. For intracerebral microinjections, nicotine was dissolved in artificial cerebrospinal fluid (ACSF; in mM: 125 NaCl, 2.5 KCl, 25 NaHCO₃, 1.25 KH₂PO₄, 1.2 MgSO₄, 2.0 CaCl₂, 10 dextrose) to a final concentration of 10 μM. Similarly, NS9283

(3-[3-(3-Pyridinyl)-1,2,4-oxadiazol-5-yl]benzotrile, Tocris) was dissolved in dimethyl sulfoxide (DMSO) for a stock concentration of 10 mM, with a final dilution in ACSF to 10 μ M (0.1% DMSO). Vehicle control injections were performed with either ACSF or 0.1% DMSO. All microinjection solutions also contained 2% tetramethylrhodamine dextran (10 kD, Molecular Probes or Invitrogen) or fluorescein dextran (10 kD, Molecular Probes) to mark injection sites. Muscimol (5-Aminomethyl-3-155 hydroxyisoxazole, Sigma) was dissolved in ACSF (100-200 μ M, 1 μ l) and applied to the cortical surface near the entry point of the multiprobe using a 1 μ l Hamilton syringe. For intracerebral injections, we used a 0.5 μ l Hamilton syringe fitted with a micropipette (~20 μ m tip). Intracortical injections were within 100 μ m of the multiprobe, and injections in the superior thalamic radiation (STR) targeted a location 1.6 mm posterior, 2.3 mm lateral and 2.8 mm ventral to bregma. To confirm injection sites, after experiments and animal perfusion the brain was removed and sectioned in the thalamocortical (STR injections) or coronal (cortical injections) plane. The brightest (center) region of fluorescence was designated the injection site.

Data Analysis

Stimulus-evoked responses were the average of 25 trials. CSD profiles were constructed off-line as described previously (Intskirveli and Metherate 2012). One dimensional CSD profiles are the second spatial derivative of the LFP laminar profile (Muller-Preuss and Mitzdorf, 1984); conventionally, a current sink implies the location, timing and magnitude of underlying synaptic excitation. The response onset was defined as the time at which the CSD trace crossed a threshold 2x SD above baseline. The middle-layer current sink with shortest onset latency was

designated layer 4 (L4), and selected for subsequent analysis. For both CSD and LFP traces, the initial slope was measured over the 10 ms following response onset (50 data points). The L4 current sink reflects monosynaptic thalamocortical input as well as intracortical activity, as demonstrated recently using a titrated dose of the GABA agonist muscimol to suppress intracortical activity but not monosynaptic inputs (Intskirveli et al., 2016); as a result, our 10 ms analysis window includes both response types. Slope data were analyzed and plotted using GraphPad Prism, with slopes normalized to the plateau value of a sigmoidal curve fit to the data from each animal. Group RF data were compared using repeated-measures 2-way ANOVA ($\alpha = 0.05$) and sorted into bins of 0.3 octaves for plotting. Mean values are presented \pm SEM and “n” values represent number of mice. Multiunit activity (MUA) was estimated by high-pass filtering LFP data at 500 Hz, rectifying and averaging responses across 25 trials, and smoothing the result using a Gaussian filter width of 5 ms.

Results

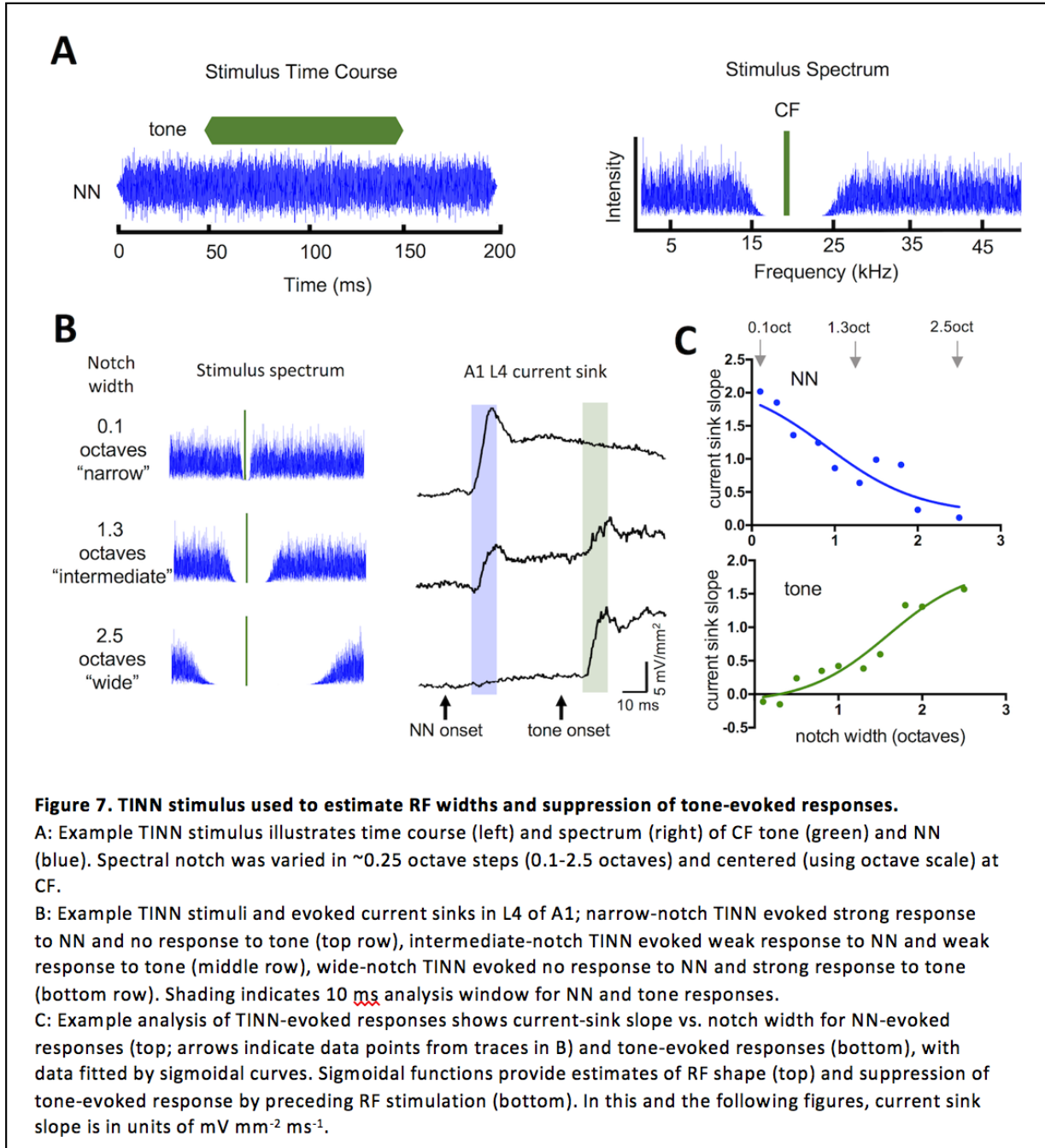
Tone-in-notched-noise (TINN) -evoked responses in A1

TINN stimuli traditionally are used in psychoacoustics to estimate perceptual filters (Patterson, 1976), but are used here to provide an electrophysiological measure of RF structure and dynamics (Fig. 7A). Our TINN stimulus has two components: a tone set to the CF of the recording site (15 dB above CF threshold), and a NN component with the spectral notch centered on CF (Fig. 7A, right; noise range 1-50 kHz, fixed amplitude, notch range 0.1-2.5 octaves). Tone and NN onsets are asynchronous, with the tone beginning 50 ms after the NN (Fig. 7A, left). This arrangement provides two advantages over simultaneous onset: first, the NN evokes a response that precedes tone onset (Fig. 7B, right), and therefore can be attributed solely to the NN stimulus; and second, the 50 ms delay allows for development of NN-evoked inhibition, potentially including both feedforward and lateral inhibition (Semple, 1995; Sutter et al., 1999; Wehr and Zador, 2003, 2005). Thus, use of the TINN stimulus allowed us to simultaneously assess important spectral and temporal characteristics of the RF.

We inserted a 16-channel linear multiprobe into A1 to record stimulus-evoked local field potentials (LFPs) throughout the cortical depth at 100 μm intervals, and subsequently derived CSD profiles offline, as previously described (Kawai et al., 2011; Intskirveli and Metherate, 2012). For this study, we identified and focused on the shortest-latency current sink in the middle layers, which we refer to as the layer 4 (L4) current sink. This current sink reflects monosynaptic thalamocortical input from the medial geniculate body as well as subsequent intracortical activity, and our 10 ms analysis window includes both response types (see Materials and Methods). We quantified TINN-evoked responses by measuring the slope of the

L4 current sink over the first 10 ms after response onset, separately for the NN component (Fig. 7B, right, blue shaded area in example traces), and for the CF tone (Fig. 7B, green shaded area). We then plotted response slope vs. notch width, separately for each component (Fig. 7C).

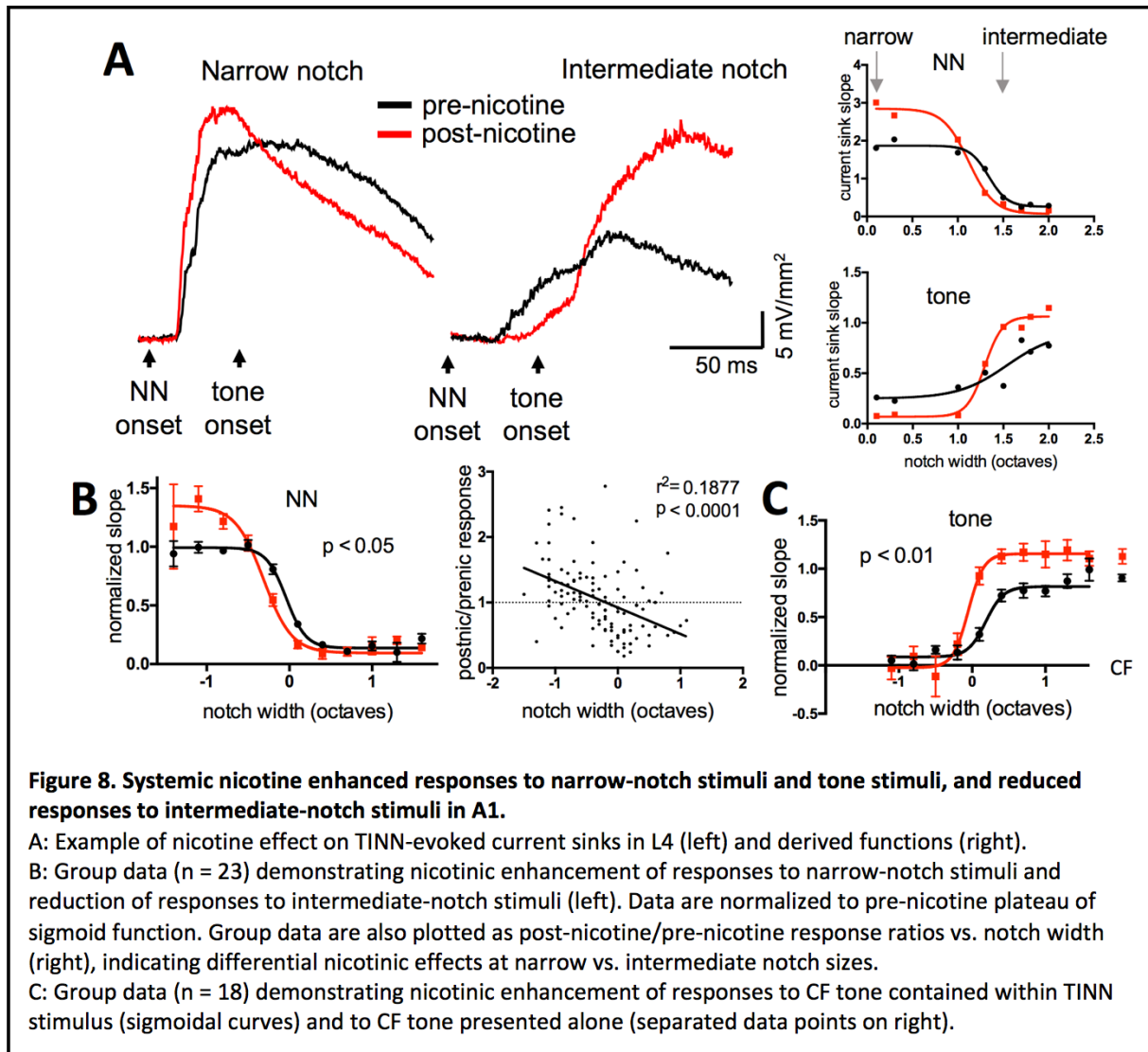
The function obtained by plotting NN-evoked responses vs. notch width (Fig. 7C, top) provides an estimate of the RF for a recording site, analogous to that obtained using a sequence of pure tones but with the advantage that NN stimuli activate multiple frequency channels simultaneously, i.e., a more naturalistic stimulus. Then, the response to the CF tone provides information about RF dynamics (Fig. 7C, bottom). For example, at narrow notch widths, the strong NN-evoked response is followed by little or no response to the tone (Fig 7B, top row), presumably because both the NN and tone stimuli activate largely overlapping frequency channels. At intermediate notch widths, the NN-evoked response is reduced and the tone-evoked response begins to emerge (Fig 7B, middle row). At wide notch widths, the NN-evoked response is weak or nonexistent and exerts little effect on the tone-evoked response (Fig 7B, bottom row). Plotting the magnitude of the tone-evoked response vs. notch width (Fig 7C, bottom) provides a quantitative estimate of suppression by the NN stimulus, which complements the RF measure (Fig. 7C, top).



Nicotinic modulation of TINN-evoked responses

We recorded TINN-evoked current sinks in L4 before and after systemic administration of nicotine (2.1 mg/kg, s.c.), with post-nicotine responses obtained within 20 min, i.e., before nicotine effects dissipated (Kawai et al., 2011). We analyzed NN-evoked responses (prior to tone onset), fitting the data with a sigmoid function, and found a drug effect that varied with notch width: nicotine enhanced responses to narrow-notch stimuli and reduced responses to intermediate-notch stimuli (example in Fig. 8A, group data for 23 animals in Fig. 2B, left). To obtain the average RF across animals (Fig. 8B), individual sigmoid functions were aligned using the notch width corresponding to the half-maximal, pre-nicotine response (e.g., notch width of ~1.3 octaves in Fig. 8A); this reference notch width is plotted as “0 octaves” in Fig. 8B. A repeated-measures 2-way ANOVA showed a main effect of notch width ($n = 23$, $F_{30,152} = 35.66$, $p < 0.0001$), a main effect of nicotine ($F_{1,152} = 5.288$, $p = 0.023$), and an interaction term reflecting different effects of nicotine at narrow vs. intermediate notches ($F_{30,152} = 2.646$, $p < 0.0001$). In contrast, control injections of saline had no effect (data not shown; $n = 11$, saline main effect $F_{1,53} = 1.834$, $p = 0.18$). Nicotine’s opposite effects for narrow-notch vs. intermediate-notch stimuli shifted the sigmoidal RF function to narrower widths and higher slope plateau values, indicating increased gain within a narrowed RF (Fig. 8B; sigmoid function fitted to mean data shifted 0.27 octaves to left (at 50% max), and to 36% higher slope plateau value).

In keeping with the descriptions above, we will refer to notch widths as “narrow” (eliciting the maximal plateau response), “intermediate” (near the reference notch width) and “wide” (eliciting no response). Thus, NN stimuli with intermediate notch widths stimulate only



the RF edges, and NN stimuli with wide notch widths stimulate outside the RF (evidenced by no effect on tone-evoked responses, which reach their maximal plateau level; see below, Fig. 8C).

A related analysis of nicotine's effects, shown in Fig. 8B (right), presents NN-evoked responses as post-nicotine / pre-nicotine ratios. These are the same data used for average RFs (Fig. 8B, left), but with individual pre- and post-nicotine data expressed as a ratio (to avoid meaningless ratios, only responses with pre-drug values > 2 SD above noise levels are included).

Consistent with the results shown for average RFs (Fig. 8B, left), this analysis reveals a tendency for normalized slope responses to narrow-notch stimuli to be enhanced (ratio >1) and responses to intermediate-notch stimuli to be reduced (ratio <1; $r^2 = 0.1850$, $p < 0.0001$).

We next analyzed NN-evoked suppression of responses to CF tones, and found that nicotine reduced suppression of, and/or overtly enhanced, tone-evoked responses (Fig. 8A, C). Nicotine did not change the near-complete suppression of tone-evoked responses for narrow-notch stimuli, but enhanced tone-evoked responses for intermediate- and wide-notch stimuli (Fig 8C) (repeated-measures 2-way ANOVA, $n = 18$, nicotine main effect $F_{1,113} = 7.954$, $p < 0.01$, interaction $F_{30,113} = 1.210$, $p < 0.05$). Again, saline injections had no effect (not shown, $n = 10$, $F_{1,45} = 1.953$, $p = 0.17$). Figure 8C also shows the response to the CF tone presented by itself (without NN stimulation), demonstrating that the “plateau” response to the tone presented within NN is unaffected by wide-notch stimuli; nicotine enhanced responses to CF tone alone, indicating that nicotinic effects on tone for wide-notch stimuli result from an overt enhancement rather than reduced suppression (Fig. 8C, right) (paired t-test, $n = 23$, pre-nic mean = 0.93 ± 0.04 , post-nic mean = 1.18 ± 0.08 , $t_{18} = 2.893$ $p = 0.01$). The overall results from TINN-evoked responses indicate that nicotine increased gain within a narrowed RF (Fig. 8B), which in turn reduced suppression of CF-evoked responses by intermediate-notch stimuli, and enhanced CF-evoked responses following wide-notch stimuli (Fig. 8C).

Because nicotine’s effects at intermediate notch widths were complex (reduced response to NN stimulus, enhanced response to tone), we examined this more closely. Figure 9A superimposes pre-nicotine functions for NN- and tone-evoked responses (same functions as in Fig. 8B and 8C). The graph shows that NN stimulation of the RF edges produced relatively

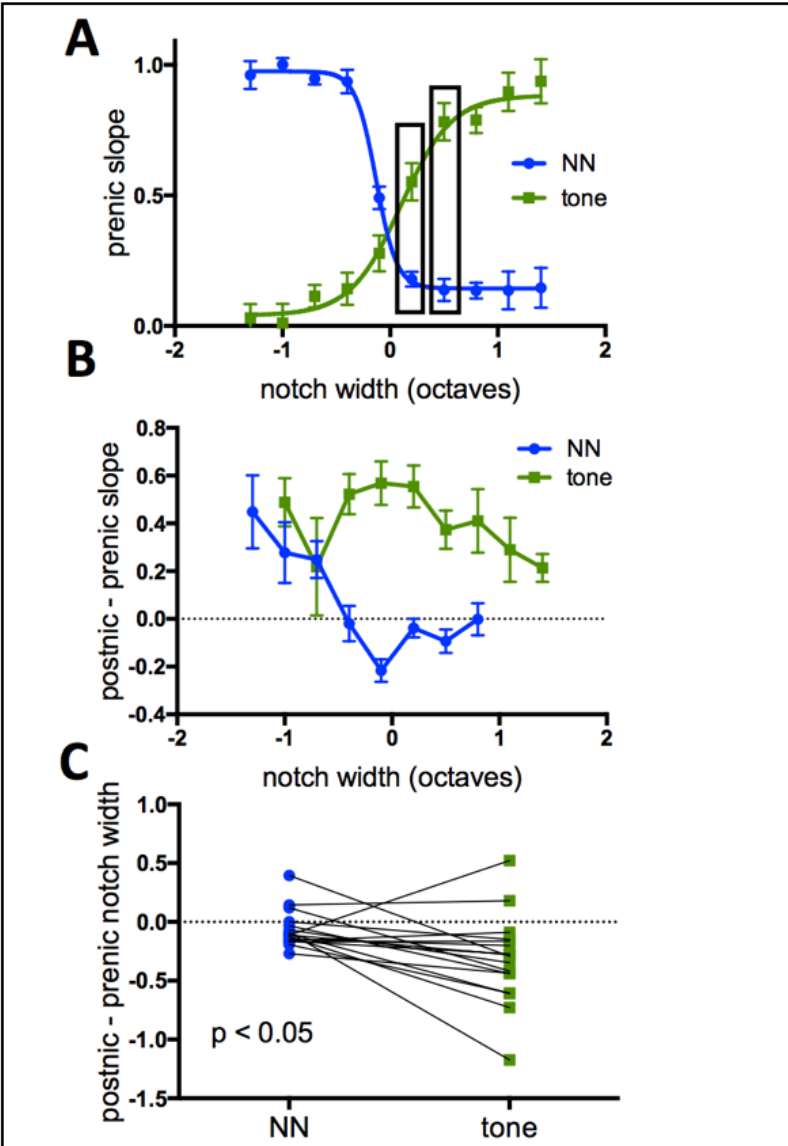


Figure 9. A closer look at responses to intermediate-notch TINN stimuli.

A: Superimposed group data for pre-nicotine responses emphasize substantial suppression of tone-evoked responses, even by relatively weak NN-evoked responses (boxed data points).

B: Effects of nicotine on normalized response values (post-nicotine minus pre-nicotine slope) showing reduction of NN-evoked responses at intermediate notch widths and enhancement of tone-evoked responses over a wider range of notch sizes.

C: Nicotine-induced shift in width of sigmoid functions (in octaves, measured at 50% max), i.e., RF and tone-suppression functions, reveal larger shift in tone-suppression function.

weak responses, as might be expected, yet substantial reduction of the tone-evoked response 50 ms later (data highlighted by boxes in Fig. 9A). The net change produced by nicotine is shown in Fig. 9B, separately for NN-evoked responses (blue data points) and tone-evoked responses (green) (same data as in Figs. 8B and 8C, but expressed as difference functions). Reduction of the NN-evoked response is greatest near the RF edge, whereas enhancement of the tone-evoked response occurs over a wider range of notch widths, including stimulation outside the RF. Thus, the altered tone-evoked response likely results

from overt enhancement (increased gain), as well as reduced suppression due to a narrowed RF and, possibly, altered lateral inhibition (see Discussion).

The nicotine-induced shift in RF width (Fig. 8B) and tone-suppression function (Fig. 8C) for each animal are quantified in Fig. 9C (measured at half-maximal values). Nicotine produced a greater shift of the tone-suppression function, suggesting that the change in tone-evoked response is not fully accounted for by RF narrowing (RF shift 0.06 ± 0.04 octaves, tone-suppression shift 0.32 ± 0.09 octaves, paired t-test, $n = 18$, $t_{16} = 2.818$, $p = 0.012$).

We also examined the effect of nicotine on TINN-evoked multiunit activity (MUA), since the L4 current sink largely reflects synaptic activity (neural input), whereas MUA reflects neural output. MUA was estimated by high-pass filtering (>500 Hz) and rectifying the evoked LFP response in L4, and then integrating the resulting trace over the 50 ms following either NN or tone onset. As with the L4 current sink, nicotine enhanced NN-evoked MUA for narrow-notch stimuli and reduced MUA for intermediate-notch stimuli (Fig. 10A, B; $n = 21$, repeated-measures 2-way ANOVA, main notch effect $F_{30,130} = 25.14$, $p < 0.0001$, main nicotine effect $F_{1,130} = 8.427$, $p = 0.004$, interaction $F_{30,130} = 2.764$, $p < 0.0001$). Nicotine also appeared to enhance tone-evoked MUA for intermediate-notch stimuli (Fig 10A, right), however this effect was more difficult to discern for MUA activity integrated over 50 ms than for current-sink initial slopes (measured over 10 ms). We therefore measured, for each animal, tone-evoked MUA at the notch width associated with the greatest enhancement of current-sink slope, and found MUA enhancement as well (Fig. 10C, paired t-test, $t_{15} = 3.495$, $p = 0.003$). These results show that nicotinic regulation of TINN-evoked responses is similar for both CSD and MUA measures.

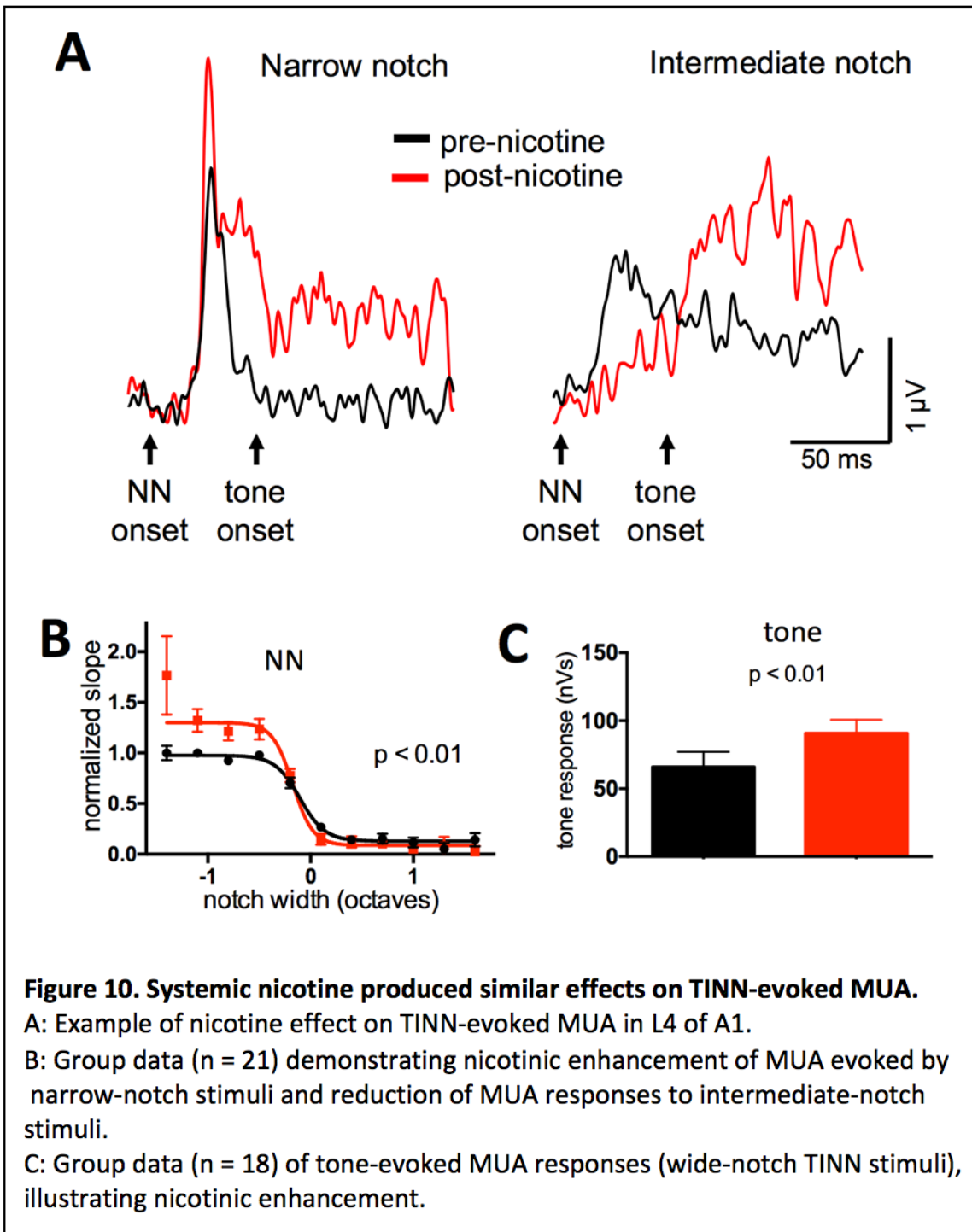


Figure 10. Systemic nicotine produced similar effects on TINN-evoked MUA.

A: Example of nicotine effect on TINN-evoked MUA in L4 of A1.

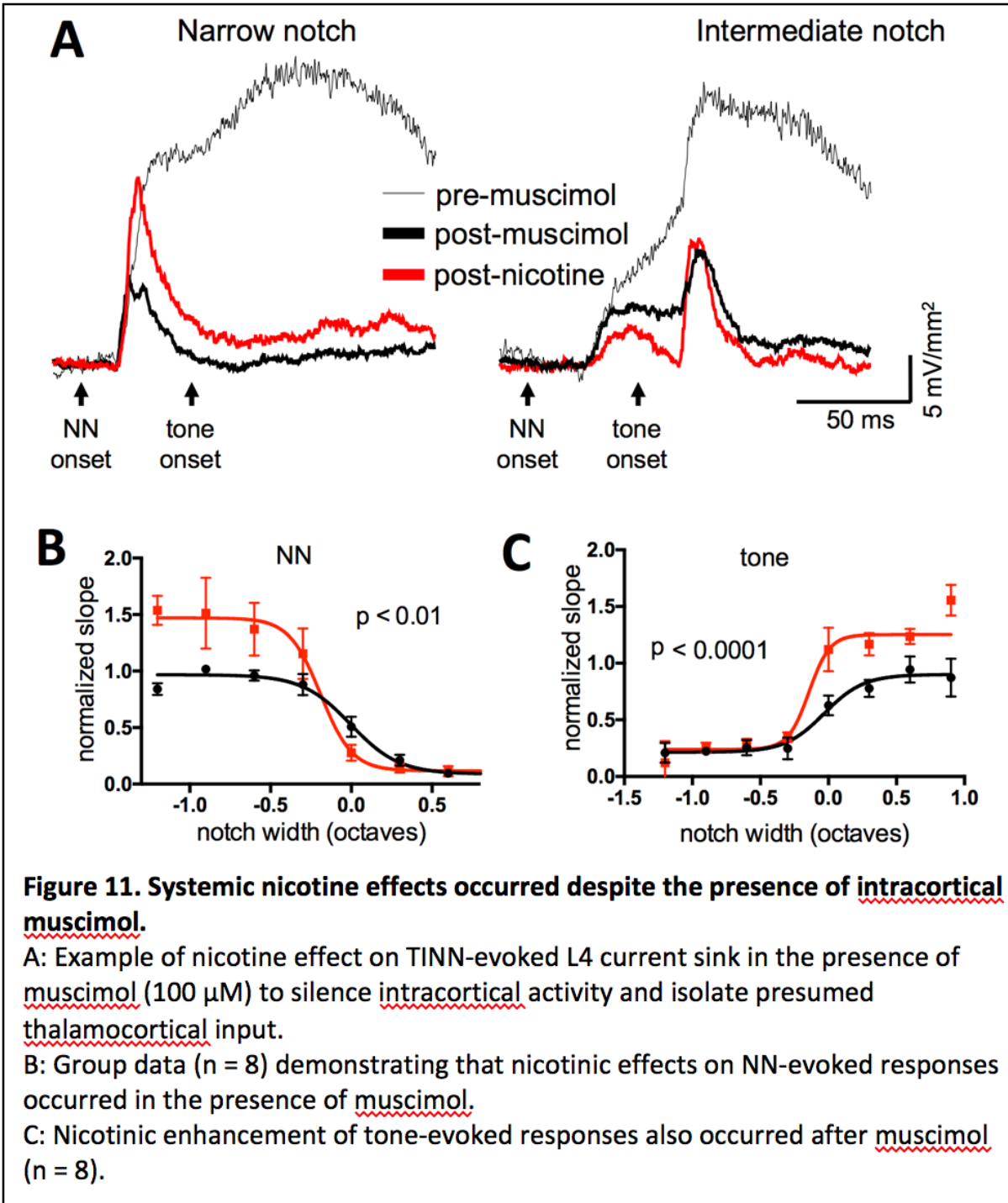
B: Group data ($n = 21$) demonstrating nicotinic enhancement of MUA evoked by narrow-notch stimuli and reduction of MUA responses to intermediate-notch stimuli.

C: Group data ($n = 18$) of tone-evoked MUA responses (wide-notch TINN stimuli), illustrating nicotinic enhancement.

Nicotine modulation of TINN-evoked responses in subcortical regions

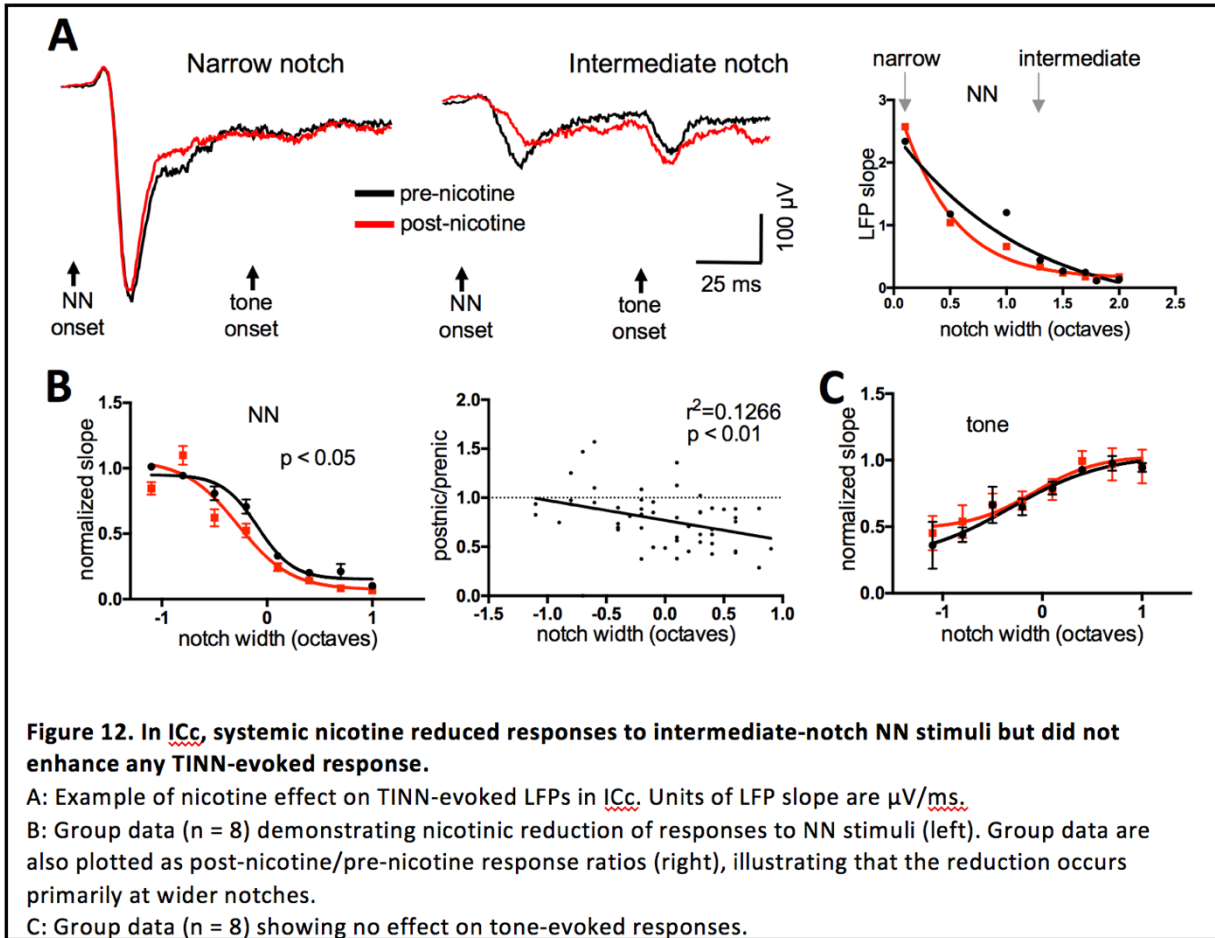
Since nicotine was delivered systemically, its effects recorded in A1 could originate outside the cortex. Thus, we sought to determine if effects in A1 were inherited from subcortical regions. We applied the GABA-A receptor agonist muscimol (100-200 μ M) to the cortical surface and recorded L4 current sinks evoked by TINN stimuli. This dose of muscimol was recently shown to be optimal for silencing intracortical activity while preserving L4 responses to monosynaptic thalamocortical input (Intskirveli et al., 2016). Our rationale was that if effects recorded in A1 depend on nicotinic actions on intracortical circuits, then applying muscimol prior to nicotine would preclude those effects.

Muscimol did not affect the initial slope of NN-evoked current sinks, but strongly reduced longer-latency components (Fig. 11A, paired t-test, $n = 8$; initial slope $t_7 = 1.665$, $p = 0.139$; amplitude at 100 ms $t_7 = 2.304$, $p = 0.0052$), consistent with our expectation that muscimol can suppress intracortical activity without affecting thalamocortical inputs (Intskirveli et al., 2016). However, the subsequent administration of systemic nicotine produced changes to the NN-evoked responses similar to those observed in the absence of muscimol (Fig 11A, B; $n = 8$, repeated-measures 2-way ANOVA, main notch effect $F_{22,35} = 9.379$, $p < 0.0001$, main nicotine effect $F_{1,35} = 8.751$, $p = 0.006$, interaction effect $F_{22,35} = 1.446$, $p = 0.16$). Similarly, nicotine enhanced tone-evoked responses following intermediate- and wide-notch stimuli (Fig. 11C: main nicotine effect $F_{1,34} = 28.43$, $p < 0.0001$, interaction effect $F_{23,34} = 2.661$, $p = 0.005$). Thus, the qualitatively similar effects indicated that some nicotinic effects do not require intracortical activity.



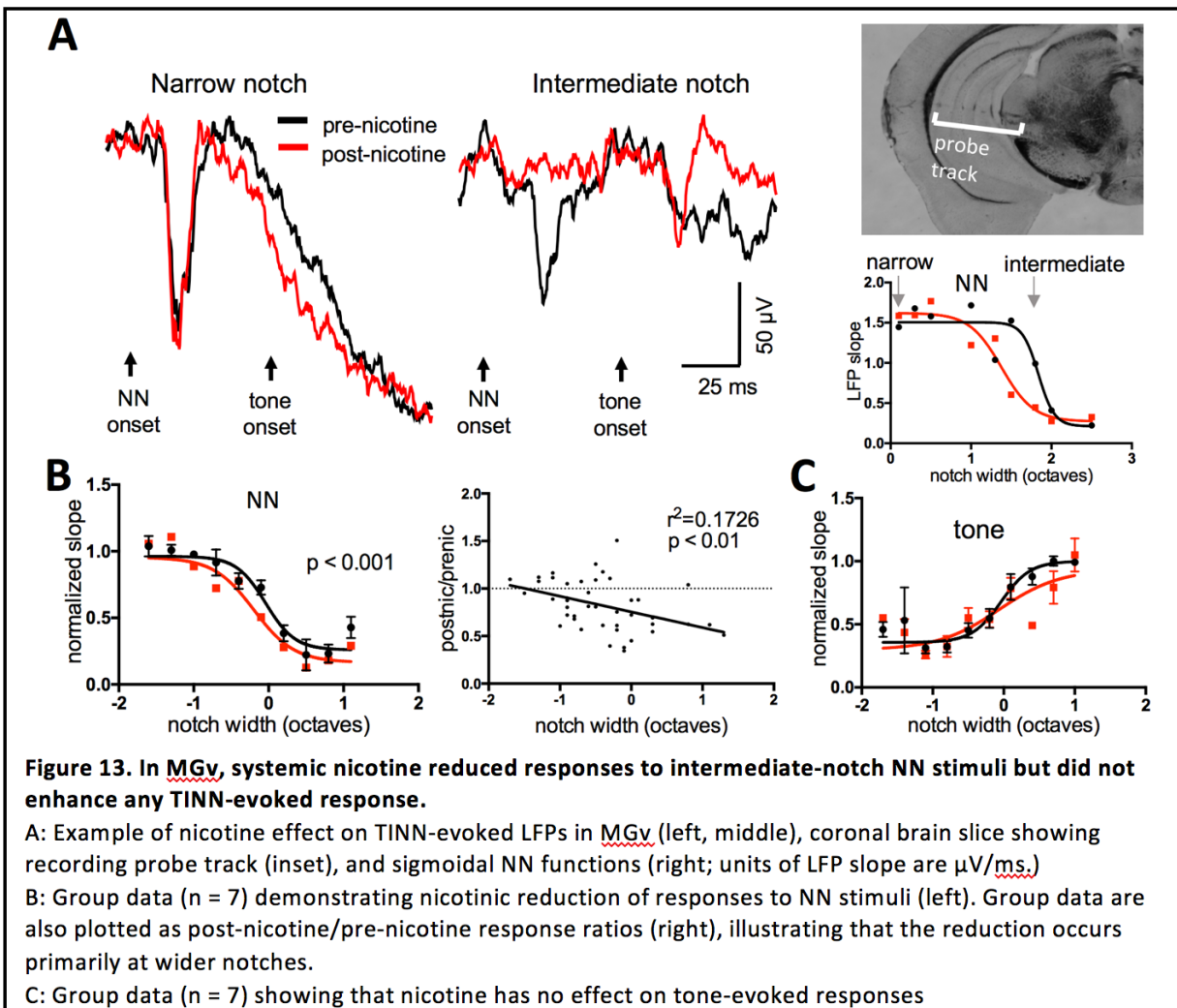
To identify potential subcortical loci of nicotinic actions, we recorded from the central nucleus of the inferior colliculus (ICc). Following an initial microelectrode mapping to identify

the ICc based on the expected tonotopic progression of CFs (Stiebler and Ehret, 1985), we inserted a linear multiprobe into the ICc from the dorsal surface and selected a recording site with a clear CF and strong evoked responses (Fig. 12A). ICc location was confirmed with *post hoc* histology to reconstruct the multiprobe track. We analyzed LFP recordings, since the assumptions for 1-dimensional CSD analysis may not hold true for subcortical structures, but still measured response slope over the first 10 ms after response onset. For NN-evoked responses, systemic nicotine had little effect on the response to narrow-notch stimuli, but reduced the response to intermediate-notch stimuli; notably, nicotine did not produce clear enhancement of any NN-evoked response (Fig 12A, B; repeated-measures 2-way ANOVA, $n = 8$, main notch effect $F_{22,38} = 40.67$, $p < 0.0001$, main nicotine effect $F_{1,38} = 5.970$, $p = 0.019$, interaction $p = 0.12$). Examination of post-/pre-nicotine response ratios confirmed that nicotine's suppressive effect varied with notch width (Fig 12B, right; $r^2 = 0.1266$, $p = 0.009$). However, nicotine had no effect on tone-evoked responses (Fig. 12A, C; main nicotine effect $p = 0.46$). Finally, saline injections produced no effect on any TINN-evoked response (not shown, $n = 3$, $F_{1,10} = 3.723$, $p = 0.09$). Thus, the effects of systemic nicotinic in ICc were largely suppressive, varied with notch width, and, notably, did not facilitate any TINN-evoked response component.



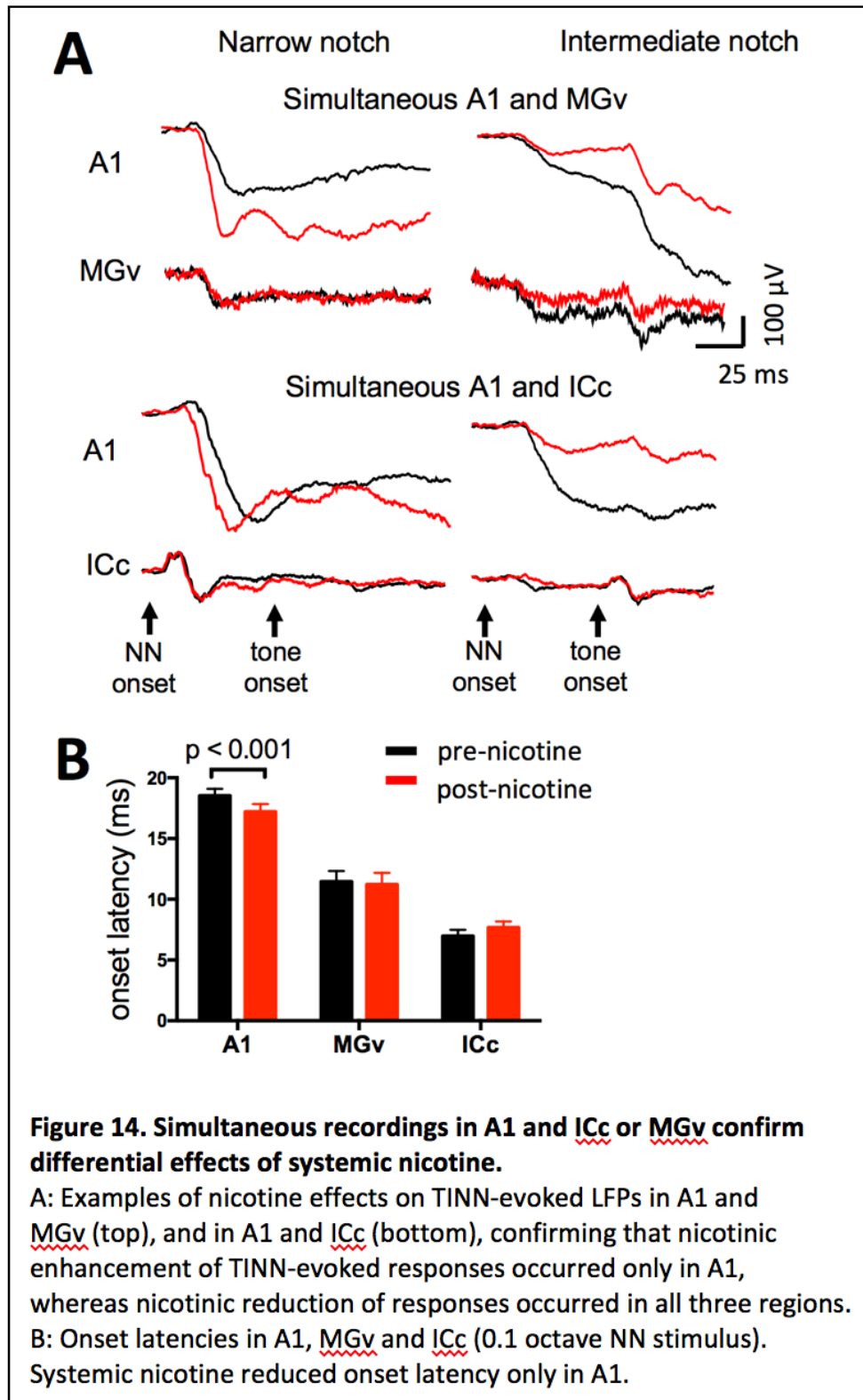
Since ICc recordings revealed no evidence for nicotinic facilitation or altered tone-evoked responses, we next recorded “downstream” to the ICc in the ventral division of the medial geniculate body (MGv). As with IC experiments, following microelectrode mapping we inserted a multiprobe from the lateral surface of the brain into the MG to record LFPs, and confirmed recording sites based on the expected progression of CFs (Hackett et al., 2011) and *post hoc* visualization of the multiprobe track (Fig. 13A). The effects of systemic nicotine were similar to those seen in the IC: nicotine had little effect on narrow-notch NN-evoked responses, reduced responses to intermediate-notch stimuli, and had no effect on tone-evoked responses

(Fig 13A-C; NN-evoked responses: $n = 7$, repeated-measures 2-way ANOVA, main notch effect $F_{24,30} = 13.83$, $p < 0.0001$, main nicotine effect $F_{1,30} = 14.29$, $p = 0.0007$, interaction effect $p = 0.7$; tone-evoked responses: main nicotine effect $p = 0.32$). The post-/pre-nicotine response ratio again showed that the drug effect increased with notch width (Fig 13B, right; $n = 7$, $r^2 = 0.1726$, $p = 0.006$). Saline controls showed no effect (not shown, $n = 5$, main saline effect $F_{1,29} = 0.4884$, $p = 0.30$). The effects of systemic nicotine in MGv therefore resembled those seen in ICc in that they were primarily suppressive, varied with notch width, and did not facilitate any response component.



The apparent differential effects of systemic nicotine in A1 vs. subcortical regions—i.e., only in A1 was there enhancement of responses to narrow-notch stimuli and CF tones—was reinforced in 5 animals with simultaneous recordings in A1 and either MGv ($n = 2$, Fig 14A, top) or ICc ($n = 3$, Fig 14A, bottom). For this direct comparison, we examined only LFPs in each region (rather than converting to CSDs in A1). In each case, nicotine enhanced the response to narrow-notch NN stimuli in A1, but not in subcortical regions. For intermediate-notch stimuli, nicotinic reduction of responses was seen at all recording sites. These simultaneous recordings reinforce the conclusion that nicotinic facilitation occurs downstream to processing in ICc and MGv.

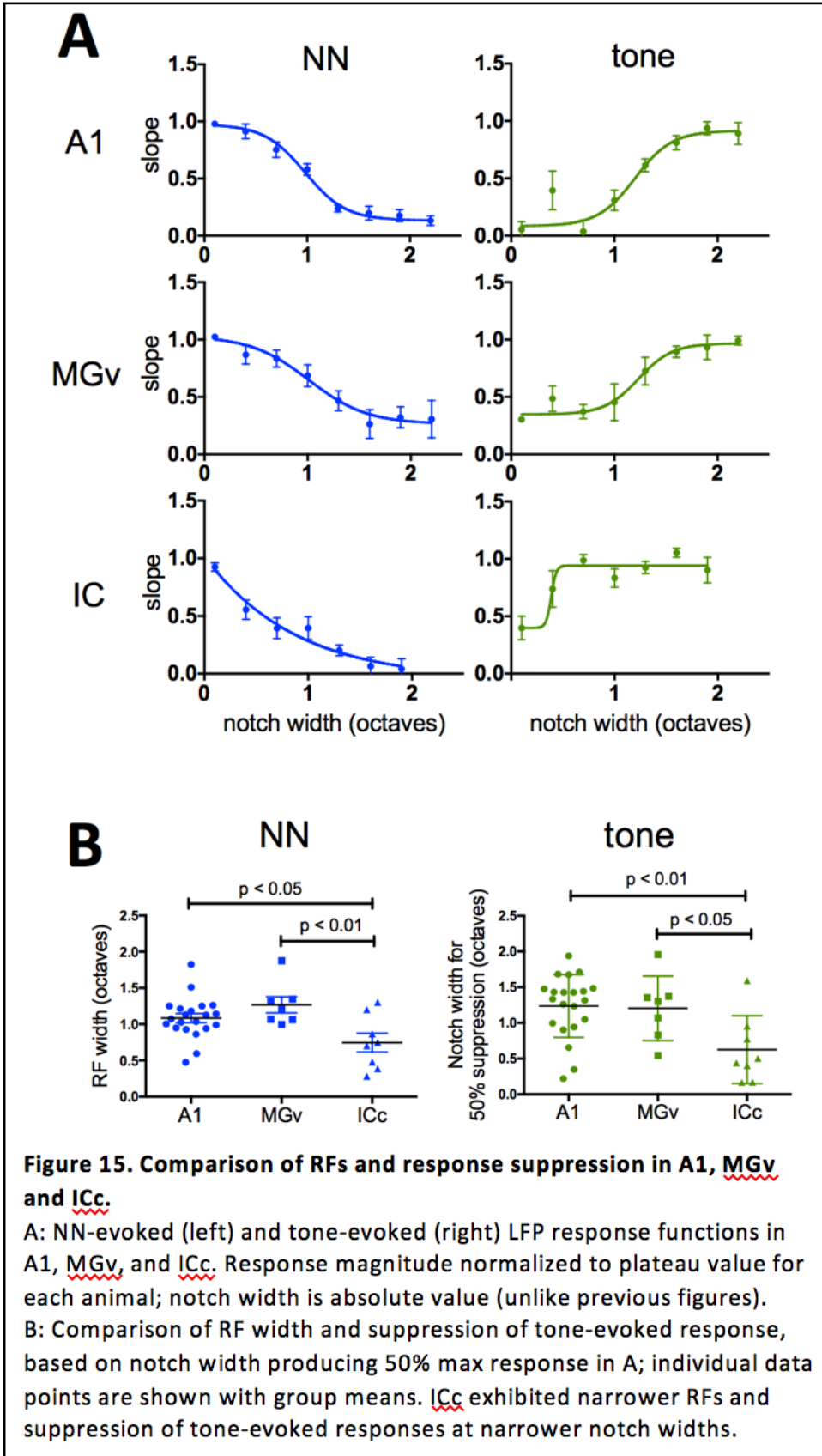
An additional comparison across auditory regions concerned the onset latency of NN-evoked responses. Prior studies have noted nicotinic reduction of onset latency in A1 for tone-evoked responses (Liang et al., 2006; Kawai et al., 2011; Intskirveli and Metherate, 2012) and isolation of the thalamocortical pathway *in vitro* demonstrated nicotinic reduction of spike latency for MG-evoked axon spikes (Kawai et al., 2007). In the present study, onset latency was determined in each region for the narrowest-notch (0.1 octave) NN-evoked response. Systemic nicotine reduced onset latency in A1 (Fig. 14B, $n = 23$, paired t-test, $t_{22} = 3.977$, $p = 0.0006$), but had no effect in MGv or ICc ($n = 7$, $t_6 = 0.7035$, $p = 0.51$, and $n = 8$, $t_7 = 1.543$, $p = 0.16$, respectively). Again, these results are consistent with the notion that nicotinic facilitation occurs downstream to processing in ICc and MGv.



Overall, the data indicate that the subcortical effects of systemic nicotine are largely suppressive and act to narrow RFs in auditory relay nuclei. We conclude that at least a portion of nicotinic narrowing of RFs in A1 is inherited from subcortical regions. However, we did not observe nicotinic modulation of tone-evoked responses in subcortical regions, nor facilitation of any TINN-evoked response; these issues will be addressed further, below, after a comparison of response characteristics across auditory regions.

Comparison of RFs and TINN-evoked response features across A1, MGv and ICc

The use of similar techniques to record TINN-evoked responses in A1, MGv and ICc provides an opportunity to compare response features across the three regions (as for onset latency, above). We therefore compared RF functions derived from NN-evoked responses, as well as NN-evoked suppression of tone-evoked responses. The results in Fig. 15 consist of the same data represented in previous figures but with two differences to facilitate comparison: first, response magnitudes are plotted as a function of absolute notch width (rather than aligned to a reference notch width), and second, A1 data are derived from LFP recordings, rather than current sinks (note that RF widths derived from LFPs (Fig. 15) do not differ from RF widths derived from CSDs for the same recording sites (Fig. 8B); paired t-test, $n = 10$, $p = 0.68$).

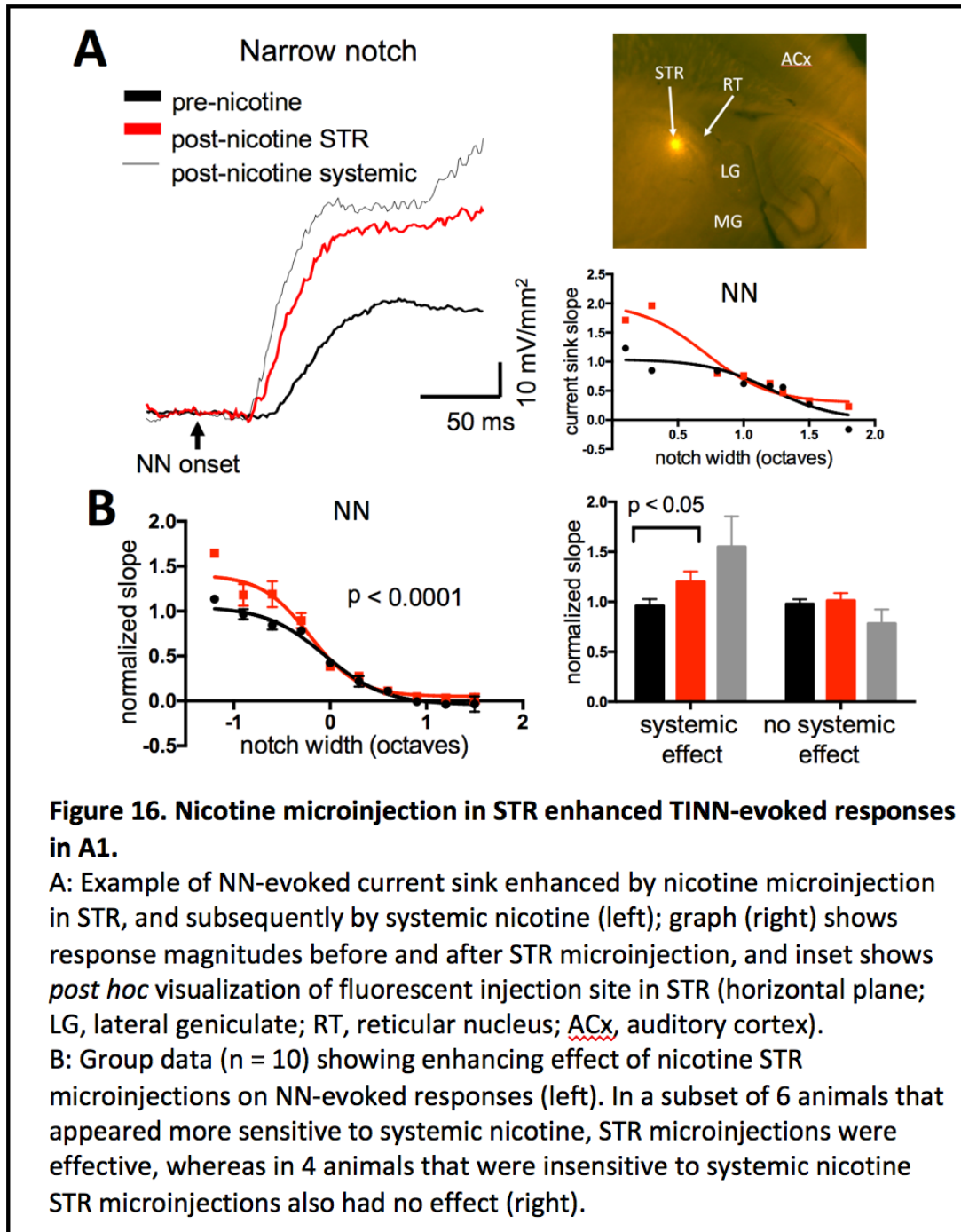


To compare RF width across the three regions, for each animal we determined the notch width that produced the half-maximal, NN-evoked response (i.e., the reference notch width, per Fig. 8B). Individual RF widths are plotted in Fig. 15B (left), grouped by region. RF widths in A1 and MGv were similar, but wider than in ICc (one-way ANOVA with Tukey's multiple comparison test: A1 vs MGv, $p = 0.36$; A1 vs ICc, $p = 0.03$; MGv vs ICc $p = 0.006$). We used a similar approach to compare NN-evoked suppression of tone-evoked responses, and determined the notch width for each animal that produced 50% suppression (half-maximal response for the sigmoid functions in Fig. 15A, right). The results are in Fig. 15B (right). Again, the notch width was similar in A1 and MGv, and narrower in ICc (one-way ANOVA with Tukey's multiple comparison test: A1 vs MGv, $p = 0.98$; A1 vs ICc, $p = 0.007$; MGv vs ICc, $p = 0.046$). Overall, these results show that RF widths in A1 and MGv are similar, averaging just over one octave, and wider than in ICc.

Origin of nicotinic enhancement of TINN-evoked responses

The results thus far show that systemic nicotine can reduce NN-evoked responses in subcortical auditory regions, yet the origin of nicotinic enhancement remains unclear. Nicotinic enhancement in A1 persisted in the presence of muscimol-induced silencing of intracortical circuits, yet was not observed in MGv (or ICc). Since a previous *in vitro* study found that nicotine increased the excitability of thalamocortical axons, but did not affect transmitter release at thalamocortical terminals (Kawai et al., 2007), we tested the involvement of the thalamocortical pathway in facilitating responses. To do so, we recorded L4 current sinks in A1 before and after microinjecting nicotine into the superior thalamic radiation (STR), a distinct

white matter tract within the thalamus through which myelinated axons from MGv course on their way to A1 (Fig. 16A, inset). Nicotine microinjections were delivered using a micropipette attached to a Hamilton syringe, with each injection site visualized using a fluorescent dye (Fig. 16A); only data from injection sites centered within STR were considered further.



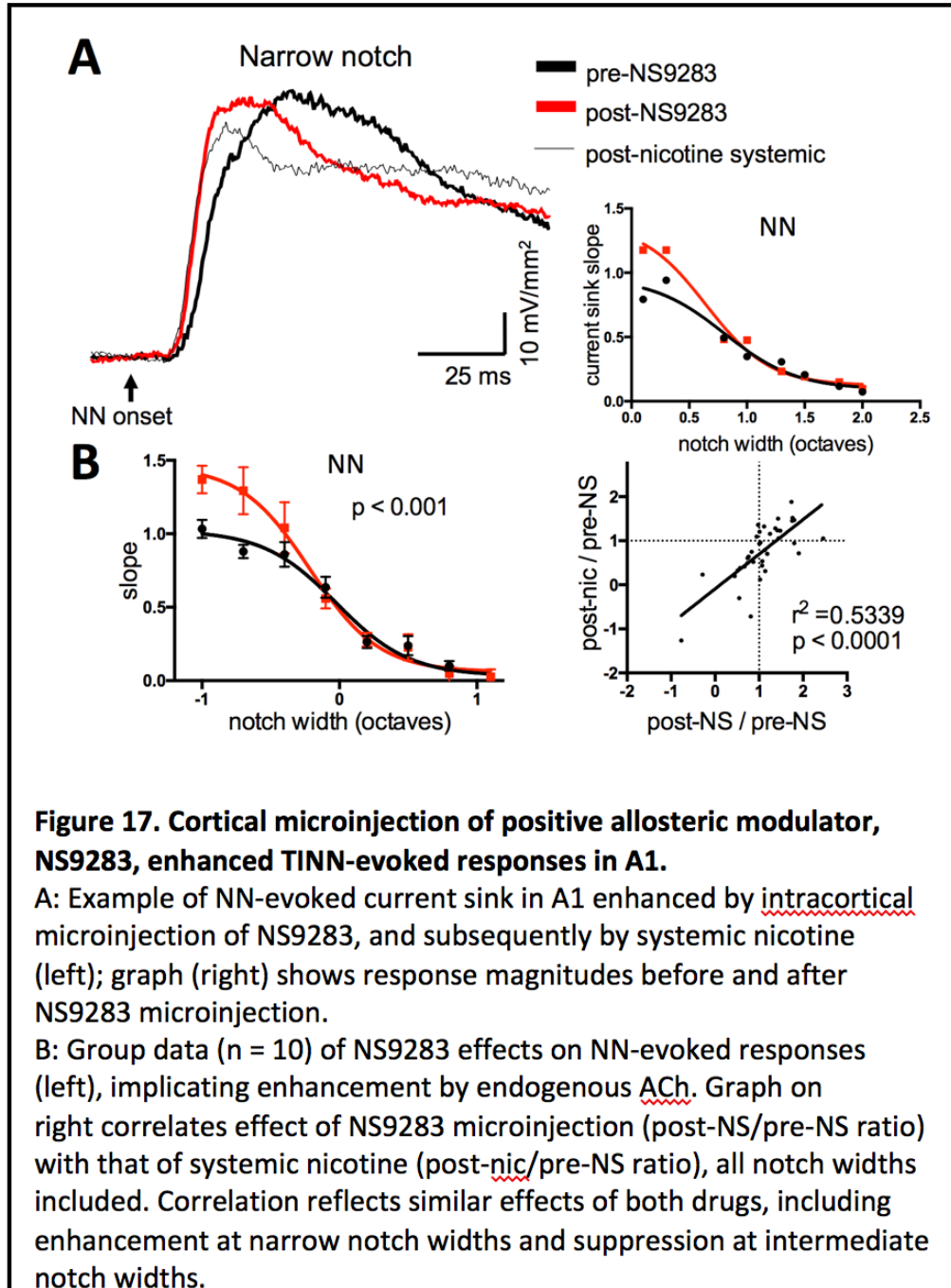
Microinjection of nicotine (10 μ M, 50-100 μ l) in STR enhanced A1 responses to narrow-notch NN stimuli, with little effect on responses to wider-notch stimuli (Fig. 16A, B; n = 10, repeated-measures 2-way ANOVA, main notch effect $F_{26,55} = 32.25$, $p < 0.0001$, main nicotine effect $F_{1,55} = 19.37$, $p < 0.0001$, interaction effect $F_{26,55} = 1.51$, $p = 0.10$). Nicotine in STR also enhanced tone-evoked responses, similar to systemic effects recorded in A1 (not shown, repeated measures 2-way ANOVA, n = 10, $F_{1,41} = 4.593$, $p = 0.038$) These data indicate that nicotinic actions in the thalamocortical pathway can enhance acoustic-evoked responses in A1, and explain, at least partly, how systemic nicotine can enhance responses in A1, but not MGv.

Even though, on average, nicotine injected into STR enhanced the cortical response to narrow-notch stimuli, there was substantial individual variability. We minimized the impact of misplaced injections by including only data from injection sites centered in STR, but other factors likely played a role, notably the variable density of nAChRs within the thalamocortical pathway (Bieszczad et al., 2012). To control for inter-animal variability in sensitivity to nicotine, we followed each STR microinjection after 30 min with systemic nicotine, for comparison (Fig. 16A). Notably, STR nicotine microinjection effects appeared to correlate with systemic nicotine effects in each experiment, and accordingly we visually sorted STR injection experiments into two groups: those with an apparent effect of systemic nicotine vs. those without. Animals that visually showed systemic nicotine enhancement of the narrowest-notch (0.1 octave) NN-evoked response (paired t-test, n = 6, $t_5 = 2.229$, $p = 0.076$) also exhibited enhanced responses after STR microinjection (Fig. 16B, right; paired t-test, n = 6, $t_5 = 3.198$, $p = 0.02$). In contrast, animals with little effect of systemic nicotine (paired t-test, n = 4, $t_3 = 1.421$, $p = 0.25$) exhibited

no effect of STR injection (Fig. 16B; paired t-test, $n = 4$, $t_3 = 0.3944$, $p = 0.7$). Thus, the variable effects of nicotine microinjection into STR were at least partly due to inter-animal variability in sensitivity to nicotine. Note that the lack of microinjection effect in the subset of animals that was insensitive to nicotine, despite verified injection sites within STR, also serves as a control that microinjections *per se* do not alter cortical responses.

The results thus far implicate auditory subcortical nuclei and the thalamocortical pathway in systemic nicotine-induced response suppression and facilitation, respectively. However, prior studies using intracortical microinjection of antagonists to block effects of systemic nicotine have suggested that both effects can arise within A1 (Kawai et al., 2011; Intskirveli and Metherate, 2012). This raises the possibility that the overall effects of systemic nicotine may depend on independent nicotinic actions in subcortical regions (nicotinic suppression), the thalamocortical pathway (enhancement) and A1 (both suppression and enhancement). As a final manipulation, therefore, we investigated the effects of local microinjection in A1. Initially, we attempted to inject nicotine itself, but were unable to obtain consistent results. We then tried a different approach, to inject a positive allosteric modulator of nAChRs, NS9283. This drug does not activate nAChRs on its own, but does amplify nicotine- or ACh-evoked responses for nAChRs containing $\alpha 2$ or $\alpha 4$ subunits (Timmermann et al., 2012). Since nicotinic effects in A1 are thought to depend on $\alpha 4\beta 2$ nAChRs (Kawai et al., 2011), NS9283 should enhance effects of endogenous ACh or exogenous nicotine acting at these receptors.

Microinjection of NS9283 (10 μ M, 50-100 μ l) in A1 resulted in enhanced NN-evoked responses to narrow-notch stimuli, and in some cases reduced responses to intermediate-notch



stimuli (Fig. 17A, B; $n = 10$, repeated-measures 2-way ANOVA, main notch effect $F_{21,45} = 14.2$, $p < 0.0001$, main NS9283 effect $F_{1,45} = 15.42$, $p = 0.003$, interaction $F_{21,45} = 2.896$, $p = 0.0014$).

Microinjection of vehicle (DMSO) had no effect (not shown, $n = 4$, $F_{1,10} = 1.932$, $p = 0.19$).

Surprisingly, NS9283 injections had no effect on tone-evoked responses (not shown, repeated

measures 2-way ANOVA, $p = 0.75$). Although the main effect of NS9283 appears to be enhanced responses to narrow-notch stimuli, in individual cases we also saw reduced responses to intermediate-notch stimuli, i.e., dual effects resembling those of systemic nicotine (Fig. 8). Since we followed NS9283 microinjections with systemic nicotine after 15 min, we were able to compare directly in each animal the effects of NS9283 with any further effect of systemic nicotine. The results are in Fig. 17B (right) which plots post-/pre-drug ratios for NS2983 (x-axis) vs. systemic nicotine (y-axis), including data for all notch widths. Notably, both drugs tended to produce similar effects, either enhancement (at narrower notches) or suppression (at intermediate notches) ($r^2 = 0.5339$, $p < 0.0001$). These data suggest that both nicotinic enhancement and suppression of responses can arise within A1.

Discussion

We have examined the effects of systemic nicotine on auditory processing, using CSD analysis of TINN-evoked responses. In A1, systemic nicotine enhanced responses to narrow-notch NN stimuli, reduced responses to intermediate-notch stimuli, and enhanced responses to CF tones (presented either alone, or within wide-notch stimuli); these results demonstrate increased response gain within narrowed RFs. Modulation of RFs in A1 reflected nicotine effects at several levels in the auditory pathway, including response suppression that varied with notch width (narrower RFs) in ICc and MGv, facilitation in the thalamocortical pathway, and both suppression and facilitation within A1. These effects of systemic nicotine, integrated and relayed up the lemniscal auditory pathway, produce increased gain within narrowed RFs in A1 (Fig. 18, discussed below).

Use of TINN stimuli and CSD analysis to probe auditory processing

TINN stimuli are widely used in psychoacoustics to estimate perceptual filters (Patterson, 1976). Here we use TINN stimuli and CSD analysis to derive a novel measure of RFs—the physiological analogue of perceptual filters—with several advantages over RFs created using tone stimuli and unit recordings. TINN stimuli activate multiple frequency channels simultaneously to better approximate naturalistic stimuli, and the 50 ms stimulus onset asynchrony (NN vs. tone) provides a snapshot of spectrotemporal dynamics. CSD analysis is based on LFPs, which capture subthreshold synaptic activity, and the resulting RFs are broader than spike-based RFs (Galván et al., 2002; Norena and Eggermont, 2002; Kaur et al., 2004). Three features emerge from this analysis (Figs. 7, 8): first, the response to NN

stimulation alone (measured before presentation of the CF tone) is used to derive a RF; second, the response to the CF tone when it follows the widest-notch stimuli (i.e., NN stimulation outside the RF) is similar to that following presentation of a CF tone alone; and third, the 50 ms delay before tone presentation permits assessment of spectrotemporal processes, including response adaptation as well as feedforward and lateral inhibition. That is, the 50 ms delay is short enough so that responses are adapted when there is overlap between the neural populations excited by the NN and tone stimuli, and long enough for development of cortical IPSPs (Metherate and Ashe, 1994; Wehr and Zador, 2005). A fixed delay will not capture all temporal features; however, intermediate-width NN stimuli that stimulate the RF edges—as evidenced by weak excitation at the recording site—nonetheless produced strong reduction of tone-evoked response, likely demonstrating the presence of lateral inhibition similar to that produced by two-tone stimulus protocols (Semple, 1995). Overall, use of a TINN stimulus provides a useful snapshot of RF dynamics.

Nicotinic modulation of responses in A1

Our conclusion that nicotine increases response gain within narrowed RFs extends findings that nicotine enhanced responses to CF stimuli and reduced responses to non-CF stimuli (1-2 octaves distant from CF) (Liang et al., 2008; Intskirveli and Metherate, 2012). Nicotinic enhancement of CF-evoked responses can be blocked by intracortical infusion of dihydro- β -erythroidine (DH β E), an antagonist of α 4 β 2-containing nAChRs (Kawai et al., 2011), or by inhibition of MAP kinase activated by nAChRs (Intskirveli and Metherate, 2012). Importantly, nicotinic enhancement of inputs to L4, or subsequent intracortical activity, was prevented by

inhibition of MAP kinase in the thalamocortical pathway, or A1, respectively. The present findings that microinjection of nicotine into the thalamocortical pathway enhanced the L4 current sink further support the notion that nAChRs in the auditory thalamocortical pathway enhance thalamocortical inputs (Kawai et al., 2007).

Nicotine reduced the cortical response to intermediate-notch NN stimuli, indicating a narrowed RF. This effect reflects, in part, RF narrowing in afferent pathways since similar effects were observed in ICc and MGv, and in L4 when intracortical activity was silenced by muscimol. A logical consequence of narrower RFs could be reduced adaptation following stimulation of RF edges, consistent with our observation of CF-evoked responses being less suppressed by intermediate-width stimuli (Fig. 8C). That is, reduced excitation in A1 following stimulation of RF edges could, in turn, reduce the adaptation of tone-evoked responses. However, we cannot distinguish between reduced suppression and overt facilitation of tone-evoked responses, especially since the latter is apparent with wide-notch stimuli as well as stimulation with CF tones presented alone (Fig. 8C); either or both mechanisms would enhance response magnitude, and may contribute to the effect observed with intermediate-notch stimuli. Moreover, since reduction of cortical responses may involve an intracortical mechanism (effects of NS9283), narrowing of RFs could result from enhanced intracortical inhibition. In visual cortex, lateral inhibition has been attributed to GABAergic interneurons that express somatostatin (SOM) (Adesnik et al., 2013), and SOM interneurons are excited by nicotine (Jia et al., 2009; Leão et al., 2012). Alternatively, parvalbumin (PV)-expressing interneurons are implicated in feedforward and lateral inhibition (PV neurons have broader RFs than excitatory neurons to which they project) (Wu et al., 2008), and PV interneurons are excited by nicotine in

some studies (Poorthuis et al., 2013), but not others (Porter et al., 1999). Thus, nicotinic enhancement of PV-interneurons may enhance both kinds of inhibition. Other interneurons expressing vasoactive intestinal peptide (VIP) may contribute to the facilitatory effects of nicotine via disinhibition, e.g., inhibition of PV interneurons (Porter et al., 1999; Alitto and Dan, 2013; Fu et al., 2014; Bell et al., 2015). Thus, multiple nicotinic mechanisms may contribute to narrowing of RFs, reduced suppression and/or overt facilitation of tone-evoked responses in A1.

Nicotine effects integrate across levels of the ascending auditory pathway

Our hypothesis that effects of systemic nicotine originate largely in cortex was refuted by silencing cortex using muscimol. At the dose employed, intracortical activity was largely silenced, but the remaining activity—monosynaptic thalamocortical input (Intskirveli et al., 2016)—clearly exhibited increased gain and narrowed RFs after systemic nicotine. These effects arose from different loci in the auditory pathway, including narrowed RFs in ICc and MGv, and increased gain in the thalamocortical pathway. Note that the exact nicotinic actions responsible for response suppression (narrowed RFs) in ICc and MGv are not known, though both structures exhibit a high density of nAChRs (Morley and Happe, 2000; Bieszczad et al., 2012). Also, RF narrowing in ICc and MGv was not associated with subcortical facilitation, which occurred only after nicotine microinjection into the thalamocortical pathway, demonstrating the locus of enhanced cortical inputs. This finding is consistent with increased excitability of thalamocortical axons and the presence of nAChRs in the thalamocortical white matter (Ding et al., 2004; Kawai et al., 2007; Bieszczad et al., 2012). Finally, nAChR-mediated suppression and

facilitation also occur within A1, as demonstrated by effects of NS9283, as well as the effects of intracortical DH β E in previous studies (Kawai et al., 2011; Intskirveli and Metherate, 2012). Importantly, since NS9283 is a positive allosteric modulator, its effects imply similar actions of endogenous ACh.

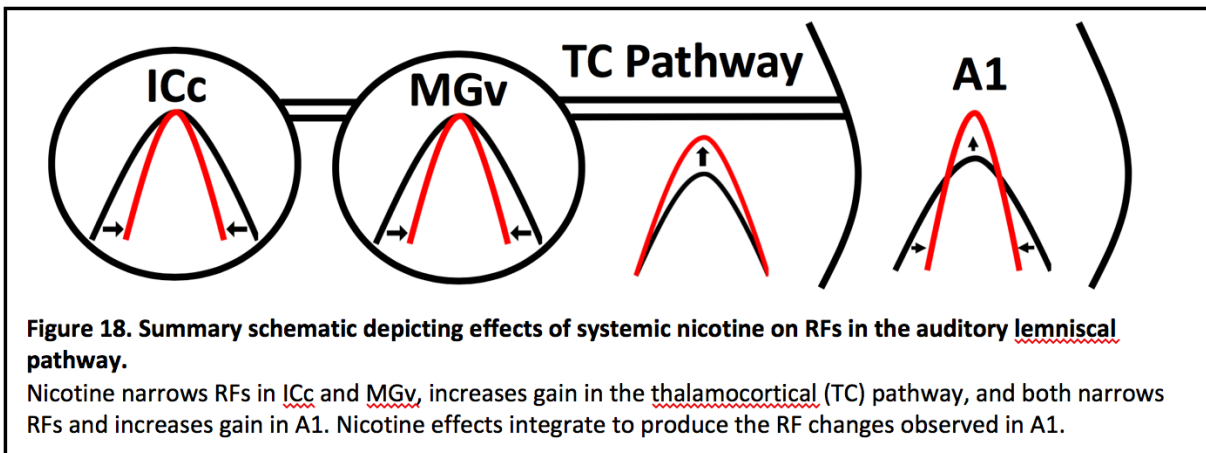


Figure 18 summarizes our main findings, using a framework for understanding the effects of systemic nicotine on auditory processing. Nicotinic effects in ICc and MGv are solely suppressive, yet vary with spectral distance from CF to narrow RFs, whereas effects in the thalamocortical pathway are solely facilitatory. Suppression and facilitation also occur within A1, and the integrated effects of systemic nicotine produce increased gain within narrowed RFs. Although nAChRs gate excitatory currents, suppressive effects of nicotine occur widely due to nAChRs located on inhibitory neuron somata to cause overt excitation, or on presynaptic terminals to enhance GABA release (Wonnacott, 1997; Albuquerque et al., 2009). As described above, the facilitatory effect of nicotine in the thalamocortical pathway likely results from increased axon excitability, and facilitatory effects within A1 may arise from excitation or disinhibition. Detailed cellular analyses in each region will be needed to understand these actions, but the strength of the CSD approach is to reveal the overall effect in each region.

Relevance of results to auditory-cognitive function

An important question is to what extent the results relate to auditory-cognitive function, given the anesthetized preparation. Anesthesia permits a relatively stable brain state, and urethane specifically does not depress nAChRs (see Methods). Evoked responses in the anesthetized auditory cortex resemble responses in some, but not all, waking states (e.g., passive, aroused or attentive) (Clementz et al., 2002; Kato et al., 2015; Reinhold et al., 2015). The nicotinic increased gain observed here resembles that seen for some sensory-evoked responses in awake animals and nonsmoking humans (wearing a nicotine patch) (Guha and Pradhan, 1972; Bringmann, 1994; Harkrider and Champlin, 2001). Thus, the effects are likely relevant for some, but not all, waking states in humans.

Intriguingly, the main effects of systemic nicotine—increased gain within narrowed RFs—also occur during auditory attention in humans and nonhuman primates (Okamoto et al., 2007; Lakatos et al., 2013; O’Connell et al., 2014). These effects may underlie the dual perceptual consequences of nicotine, i.e., increased processing capacity and narrowed attention (Friedman et al., 1974; Kassel, 1997; Knott et al., 2009). The similarity of effects (nicotine vs. attention) may reflect the involvement of the cholinergic system in attention (Levin and McClernon, 2006; Albuquerque et al., 2009; Hasselmo and Sarter, 2011; Miwa et al., 2011). Consequently, the findings also suggest the possible therapeutic use of nicotine to treat disorders involving diminished attention, which are increasingly being recognized as a subset of central auditory processing disorders (Moore, 2015).

CHAPTER 2

Nicotine Disinhibits Pyramidal Neurons in Auditory Cortex via VIP Interneurons

Abstract

Nicotine activates nicotinic acetylcholine receptors and improves cognitive and sensory function, in part by its actions in cortical regions. Previous studies show that nicotine amplifies stimulus-evoked responses in sensory cortex, potentially contributing to nicotine's enhancement of sensory processing. However, the role of specific cell types in the nicotinic modulation of sensory cortex remains unclear. Here, we performed whole-cell recordings from pyramidal (Pyr), parvalbumin- (PV), somatostatin- (SOM), and vasoactive intestinal peptide- (VIP) expressing neurons in the auditory cortex of mouse brain slices and found that bath application of nicotine selectively depolarizes Pyr and VIP neurons, with no effect on the membrane potential of SOM or PV neurons. Nicotine also enhanced the frequency of spontaneous inhibitory post-synaptic potentials in Pyr, VIP, and SOM cells, but not PV cells. Using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), we show that the activation of VIP neurons is responsible for the nicotinic depolarization and sIPSC frequency enhancement observed in Pyr neurons. Thus, nicotine drives VIP cell firing which then disinhibits Pyr cells, likely making them more excitable and responsive to incoming auditory stimuli. These results present a probable mechanism for nicotine's beneficial effects on cognitive and sensory function.

Introduction

Nicotine is known to enhance cognitive function and has been shown to improve performance on a variety of attentional, memory, and sensory tasks (Rezvani and Levin, 2001; Lawrence et al., 2002; Levin and McClernon, 2006; Swan and Lessov-schlaggar, 2007). Consequently, nicotine may be a promising therapeutic drug to those with cognitive or sensory processing disorders (Kumari and Postma, 2005; Newhouse et al., 2012). Nicotine primarily acts via nicotinic acetylcholine receptors (nAChR), which are distributed throughout the brain (Clarke et al., 1985; Dani and Bertrand, 2007). However, neocortex in particular has been implicated as a region critical to the performance enhancement observed with nicotine, and nAChR activation markedly increases neuronal responsiveness in cortical areas associated with attention and sensory processing (Lawrence et al., 2002; Disney et al., 2007; Sun et al., 2017).

Nicotinic receptors are not equally distributed across cell types in cortex, and this selectivity may be key to understanding the mechanism of nicotine's pro-cognitive effects (Porter et al., 1999; Gullledge et al., 2007; Arroyo et al., 2014). Functional nAChRs in pyramidal (Pyr) neurons appear to be sparse and mostly in deeper layers, while inhibitory interneurons are far more responsive to nAChR activation (Porter et al., 1999; Disney et al., 2007; Zolles et al., 2009; Poorthuis et al., 2013). Among the main classes of cortical inhibitory interneurons, most parvalbumin - (PV) and somatostatin (SOM) –expressing neurons are not sensitive to nAChR activation (Porter et al., 1999; Gullledge et al., 2007). Rather, sensitive cells often express other markers such as vasoactive intestinal peptide (VIP), cholecystokinin, or calretinin (Porter et al., 1999; Gullledge et al., 2007). VIP cells especially have been shown to be responsive to nAChR activation in several recent studies (Lee et al., 2010; Alitto and Dan, 2013; Fu et al., 2014; Bell et

al., 2015), yet nicotine's effects on VIP cells is not well characterized, especially with low-level concentrations more relevant to a therapeutic administration of nicotine such as the patch or gum (Rezvani and Levin, 2001; Newhouse et al., 2012). Additionally, it is still unclear how nicotinic activation of VIP interneurons alters cortical network dynamics.

Notably, VIP cells have become increasingly implicated in cortical disinhibition of Pyr cells. It appears that VIP cells preferentially innervate other interneurons, especially SOM cells, which in turn inhibit Pyr cells (Lee et al., 2013; Pfeffer et al., 2013). This VIP cell-mediated disinhibition has been found to occur during multiple behavioral states such as locomotion and task performance, and ultimately increases Pyr cell excitability and firing (Pi et al., 2013; Fu et al., 2014; Jackson et al., 2016). If VIP interneurons express functional nAChRs, nicotine might activate this disinhibitory microcircuit, yet no one has investigated this possibility.

Here, we performed whole-cell recordings from Pyr, VIP, SOM, and PV cells in acute brain slices from the mouse auditory cortex to further examine the specificity and intensity of nicotinic modulation across cell types. We found that nicotine weakly depolarizes Pyr cells, while potently depolarizing and exciting VIP cells. Additionally, using Designer Receptors Activated by Designer Drugs (DREADDs) to silence VIP cell activity, we discovered that VIP neurons mediate the nicotinic depolarization of Pyr cells. Thus, nicotine disinhibits Pyr cells, likely making them more excitable and responsive to incoming auditory stimuli. This disinhibition via VIP cells supports the neuronal firing increase observed in other studies and may enhance cortical processing, thus providing a probable mechanism for nicotine's beneficial effects on cognitive and sensory function.

Methods

Animals

Male and female mice, 25-50 days old, were used for all experiments. The care and use of mice was approved by the University of California, Irvine Institutional Animal Care and Use Committee. To identify interneuron subtypes for recording, we used three different mouse lines that expressed the fluorescent protein tdTomato under interneuron-specific promoters. For VIP, SOM, and PV cells we crossed the respective homozygous mice VIP-ires-cre ($VIP^{tm1(cre)Zjh}$), SOM-ires-cre ($Sst^{tm2.1(cre)Zjh}$), or PV-ires-cre ($Pvalb^{tm1(cre)Arb}$) with the homozygous tdTomato reporter mouse Ai9 (B6.Cg-Gt(*ROSA*)26Sor^{tm9(CAG-tdTomato)/Hze}). All mice were obtained from The Jackson Laboratory. To generate mice for injection of DREADDs, we crossed homozygous VIP-ires-cre mice with FVB mice. Recordings from Pyr cells were performed in either FVB mice or the offspring of VIP-ires-cre/FVB mice.

Slice preparation

Mice were anesthetized with isoflurane and decapitated. Brains were quickly removed into cold ACSF containing 125 mM NaCl, 2.5 mM KCl, 25 mM NaHCO₃, 1.25 KH₂PO₄, 1.2 mM MgSO₄, 2.0 mM CaCl₂ and 10 mM dextrose, bubbled with 95% O₂/5% CO₂. Auditory thalamocortical slices (~400 μm for stimulation experiments and ~250 μm for all other experiments) were prepared using a vibroslicer (Leica VT1000) as described previously

(Cruikshank et al., 2002). Slices were placed in a holding chamber containing oxygenated ACSF at room temperature for ~1 hr before recording.

Electrophysiology

Slices were transferred to a submersion chamber and maintained in continuous bath flow of ACSF (~2.5–3 ml/min) at room temperature. Whole-cell recordings were obtained with patch pipettes (1.5–5 M Ω) filled with either a K⁺-based internal solution containing (in mM) 135 K-gluconate, 1 KCl, 2 MgCl₂, 1 Na-ATP, 0.5 Na-GTP, 1 EGTA, 10 HEPES, or a Cs⁺-based internal solution containing (in mM) 135 CsMeSO₄, 5 CsCl, 2 MgCl₂, 1 Na-ATP, 0.5 Na-GTP, 1 EGTA, 10 HEPES (pH 7.3 and 270 mOsm). Responses were acquired in voltage-clamp or current-clamp mode with the MultiClamp 700B amplifier (Molecular Devices) and AxoGraph software. Signals were amplified and low-pass filtered at 2 kHz and digitally sampled at 10 kHz. Series resistance (6–15 M Ω) was continuously monitored, and data were discarded if the resistances changed more than 30%. Voltages were not adjusted to compensate for the liquid junction potential (~10 mV). Neurons were visualized using infrared differential interference contrast (IR-DIC) and fluorescence with Zeiss Axioskop 2. Auditory cortex was identified based off previous studies in the mouse thalamocortical slice (Cruikshank et al., 2002) and confirmed in some cell recordings by a short-latency response to thalamocortical pathway stimulation.

All drugs were added to ACSF and bath-applied to the slice: 1 μ M nicotine (Sigma), 50–100 μ M picrotoxin (PTX, Sigma), 10–20 μ M 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Sigma), 10 μ M D-2-amino-5-phosphonovalerate (AP5, Tocris), 100 nM clozapine *N*-oxide (CNO, abcam), 10 nM methyllycaconitine citrate (MLA, Sigma), 0.5–1 μ M dihydro- β -erythroidine (DH β E,

Sigma). To stimulate thalamocortical afferents, a bipolar concentric electrode (125 μm outer diameter, Frederick Haer) was placed in visually-identified superior thalamic radiation (STR) in the thalamocortical pathway. Stimulus pulses (100-400 μA) were given every 10 seconds and evoked responses were averaged from 5-10 repetitions.

For current-clamp recordings, neurons were selected only if the resting membrane potential was negative to -50 mV and experiments were conducted at resting membrane potential. For voltage-clamp, the reversal potential for excitatory post-synaptic currents (EPSC) was presumed to be around 0 mV. Recordings at 0 mV contained small negative amplitude spontaneous currents in addition to the large positive amplitude currents; these negative currents are likely spontaneous EPSCs and confirm that the positive amplitude currents are exclusively inhibitory post-synaptic currents (IPSC). Because EPSCs are more difficult to isolate, we first estimated stimulus-evoked IPSC reversal potential in 1 mV steps in a subset of neurons and obtained a value of ~ 52 mV; this clamp potential was then used for EPSC measurements.

Viral infusion

Three-week old male and female hemizygous VIP-Cre mice received 2 x 0.5- μL unilateral infusions to auditory cortex (From Bregma: M/L +4.0 mm, A/P -2.55/-2.85 mm; from cortical surface D/V -1.1/-0.8mm) of either AAV2.8-hSyn-DIO-mCherry or AAV2.8-hSyn-DIO-HM4D-mCherry. Viruses were infused at a rate of 6 $\mu\text{L}/\text{h}$ by using a 30 gauge Neuros Hamilton syringe (product #65456-01) mounted to a Leica Biosystems Nanoinjector Motorized f/Stereotaxics pump (Product #39462901). All infusions used the Leica Microsystem Angle Two Stereotaxic System. For all experiments, animals were allowed to recover for a minimum of 3 weeks before

tissue harvesting. All viruses were purchased from UNC Vector Core (mCherry Lot: AV4981CD 2014; HM4D AV4980B) or Addgene (mCherry #44362, Lot: v4330; HM4D #44362, Lot: v4331). Viral purity was confirmed via Sanger Sequencing (Genewiz) as previously described (Lopez 2018).

Immunohistochemistry

Mice were anesthetized with 50 mg/kg sodium pentobarbital and perfused with ice-cold 0.1M PBS and 4% paraformaldehyde. Brains were harvested, soaked in 4% paraformaldehyde for 24 hours at 4°C, and cryoprotected in 30% sucrose at 4°C until completely submerged. Tissue was then flash frozen in dry ice-chilled isopentane and 40 µm histological sections containing auditory cortex were collected using a Leica CM 1850 cryostat at -20°C. To confirm expression of Cre-dependent vector in VIP+ neurons of the auditory cortex, free-floating sections were washed three times for 5 minutes in 0.1M PBS. Slices were then blocked in blocking serum (10% Normal Goat Serum, 0.5% Triton X-100, in 0.1M PBS; 1 hour) and incubated at 4°C overnight in primary solution (10% Normal Goat Serum, 0.5% Triton X-100; anti-DsRed [1:500], Clontech #1408015; anti-VIP [1:500], Santa Cruz #sc-25347). Slices were washed in 0.1% PBS-Tween 20 and incubated in secondary solution (10% Normal Goat Serum, 0.5% Triton X-100; DsRed/mCherry, Alexa Fluor goat anti-rabbit 555 [1:1000]; VIP, Alexa Fluor 488 goat anti-mouse [1:1000], in PBS). Following secondary incubation, tissue was washed in 0.1% PBS-Tween20 and incubated in DAPI [1:15000] in 0.1M PBS. Sections were then slide mounted and cover slipped using VectaShield Mounting Medium (product #H-1000).

Analysis

All recordings were analyzed in AxoGraph and all statistical tests were performed in GraphPad Prism ($\alpha = 0.05$). Data are expressed as the mean \pm standard error of the mean. The laminar location of each recorded neuron was determined by percent of full cortical width, estimated based off previous studies in mouse auditory cortex (Cruikshank et al., 2001): L1 0-13%, L2/3 14-33%, L4 34-49%, L5 50-72%, L6 73-100%.

Membrane potential, spontaneous IPSC/EPSC frequency and amplitude, and firing rate were all determined from a 1-minute recording span; pre-nicotine data were measured from the 1 minute immediately prior to nicotine application and nicotine data were measured from 4 to 5 minutes after the start of nicotine application. Statistical tests compared pre-nicotine to nicotine data. Membrane potential was the averaged membrane potential over the 1 minute. Nicotinic effects on membrane potential were expressed in the figures as “Depolarization”; i.e., nicotine membrane potential – pre-nicotine membrane potential. Frequency (in Hertz) was determined from the number of events during the 1 minute. Amplitude for spontaneous IPSCs/EPSCs was determined by measuring peak amplitude for each event, then averaging all event amplitudes over the 1 minute. The amplitude for evoked IPSCs/EPSCs was measured as peak amplitude 0-100 ms after stimulation, averaged from 5-10 repetitions.

Results

Distinct cell types each have a unique functional role in the cortical network (Kawaguchi and Kubota, 1997; Pfeffer et al., 2013; Kepecs and Fishell, 2014). There is some evidence that nicotine selectively modulates specific cell types, a feature that is likely critical to understanding

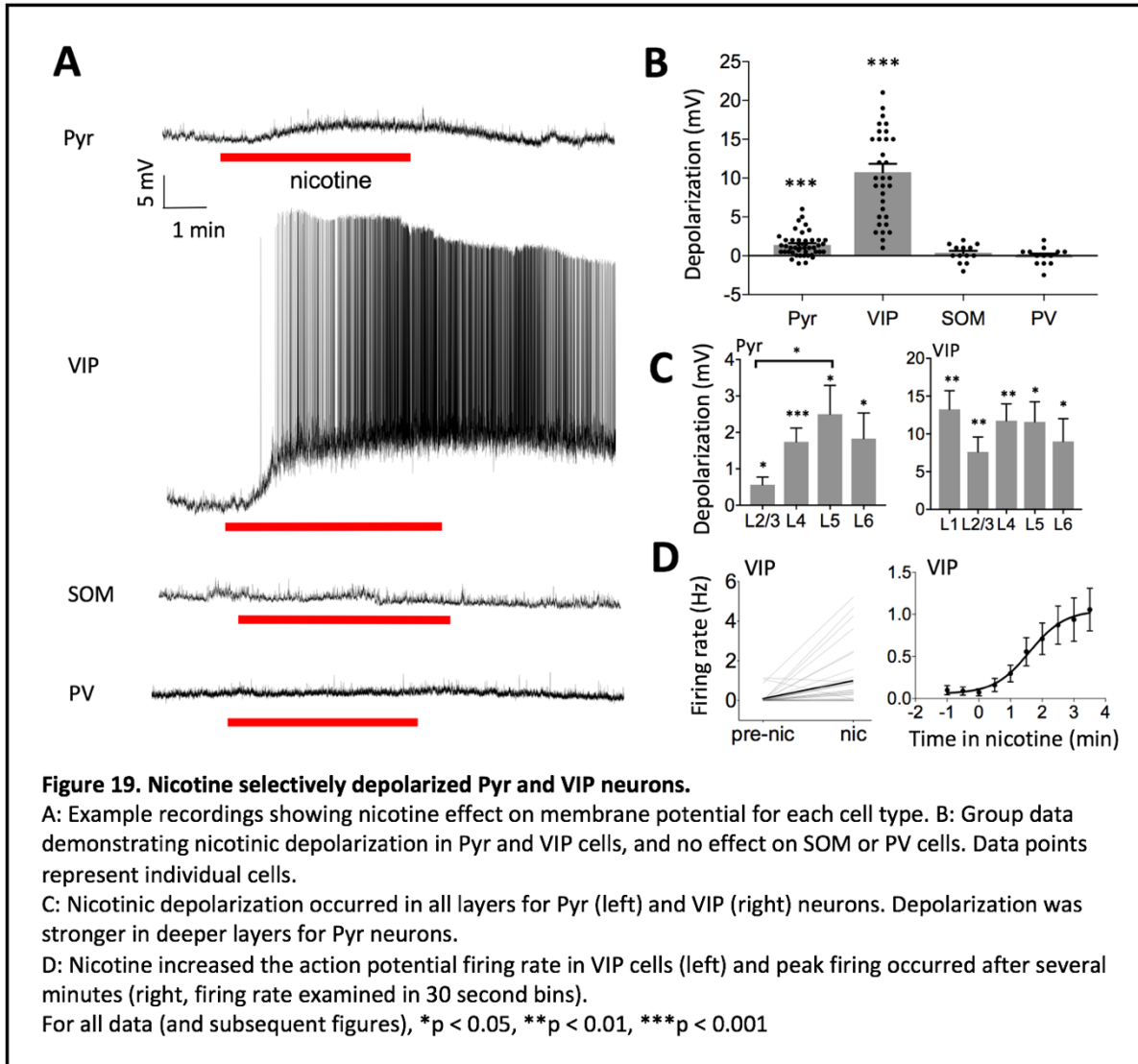
the neural basis of nicotinic effects (Porter et al., 1999; Gullledge et al., 2007). To further determine the specificity of nicotine, we obtained whole-cell recordings from four non-overlapping classes of cells that constitute the majority of cortical neurons: Pyr, VIP, SOM, and PV neurons (Rudy et al., 2011). Pyr cells were identified morphologically by their pyramidal-shaped cell body and large apical dendrite. VIP, SOM, and PV cells were identified by crossing VIP-Cre, SOM-Cre, or PV-Cre mice with the Cre reporter mouse Ai9, thus conferring tdTomato fluorescence to each respective cell type.

Slices were taken from 25-50 day-old mice and although P25-P50 represents a time of continuing cortical and nAChR development (Slotkin, 2002; Kawai et al., 2011), we found no correlation of major nicotine effects with age (details below). All recordings were performed in the auditory cortex in a thalamocortical slice preparation, and each data set includes cells from across cortical layers; nicotine was bath-applied (1 μ M).

Nicotine selectively depolarizes VIP and Pyr neurons

First, we examined how nicotine alters the membrane potential of each cell type using current-clamp whole-cell recordings and a K⁺-based internal solution. We found that nicotine weakly but consistently depolarized Pyr cells an average of 1.39 ± 0.22 mV (Fig. 19A,B; paired *t* test: *n* = 46 cells, 22 mice, $t_{(45)} = 6.17$, *p* < 0.0001). This effect was observed in each cortical layer (Fig. 19C; paired *t* test: L2/3 *n* = 18 cells, 11 mice, $t_{(17)} = 2.71$, *p* = 0.015. L4 *n* = 15 cells, 11 mice, $t_{(14)} = 4.60$, *p* = 0.0004. L5 *n* = 6 cells, 5 mice, $t_{(5)} = 3.14$, *p* = 0.026. L6 *n* = 6 cells, 5 mice, $t_{(5)} = 2.61$, *p* = 0.048), although it appeared stronger in deeper layers (Fig. 19C; One-way ANOVA

comparing layer effects: $F_{(3,41)} = 4.002$, $p = 0.014$, with Tukey's post hoc test L2/3 vs. L5 $p = 0.024$).



Furthermore, nicotine strongly depolarized VIP interneurons an average of 10.76 ± 1.08 mV (Fig. 19A,B; paired t test: $n = 33$ cells, 14 mice, $t_{(32)} = 9.94$, $p < 0.0001$). This potent response caused a majority (22/33) of VIP cells to fire action potentials, as reflected in spike frequency measures before and after nicotine application (Fig. 19D, left; paired t test: $n = 33$ cells, 14 mice, $t_{(32)} = 3.64$, $p = 0.0009$). Depolarization and spiking began soon after bath application of

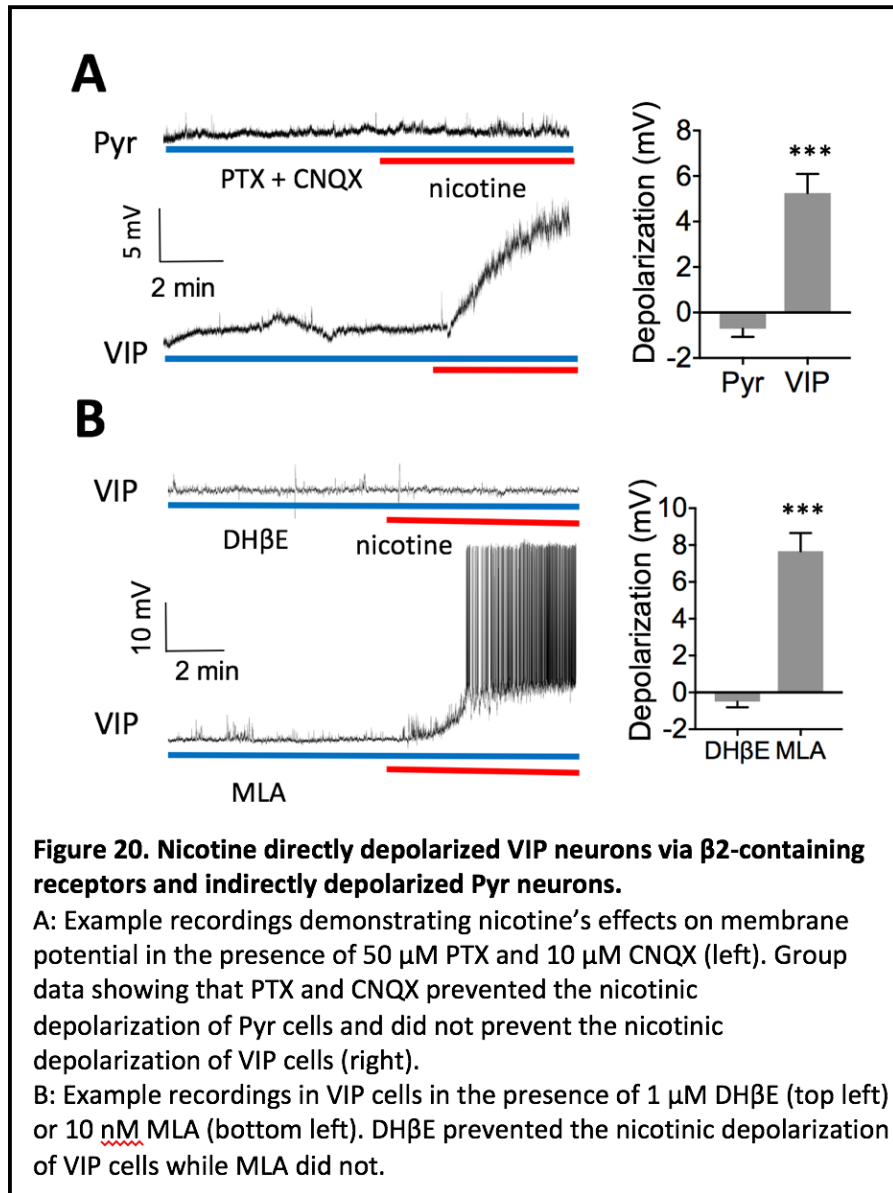
nicotine and took several minutes to reach peak firing (Fig. 19D, right; One-way ANOVA comparing firing rate across time bins: $F_{(32,288)} = 9.84$, $p < 0.0001$, with Tukey's post hoc test comparing bins vs -1 min: -0.5 $p = 0.96$, 0 $p = 0.91$, 0.5 $p = 0.97$, 1 $p = 0.14$, 1.5 $p = 0.048$, 2 $p = 0.015$, 2.5 $p = 0.012$, 3 $p = 0.020$, 3.5 $p = 0.005$).

Nicotine's depolarization of VIP cells also occurred in all layers (Fig. 19C; paired t test: L1 $n = 8$ cells, 4 mice, $t_{(7)} = 5.38$, $p = 0.001$. L2/3 $n = 8$ cells, 7 mice, $t_{(7)} = 3.87$, $p = 0.0062$. L4 $n = 8$ cells, 6 mice, $t_{(7)} = 5.28$, $p = 0.0012$. L5 $n = 5$ cells, 4 mice, $t_{(4)} = 4.37$, $p = 0.012$. L6 $n = 4$ cells, 2 mice, $t_{(3)} = 2.97$, $p = 0.048$). The degree of nicotinic depolarization in Pyr and VIP cells did not correlate with age (not shown; Pearson correlation for age [days] vs. depolarization: Pyr $n = 46$ cells, 22 mice, $r^2 = 0.015$, $p = 0.42$. VIP $n = 33$ cells, 14 mice, $r^2 = 0.010$, $p = 0.58$). In contrast to its effects on Pyr and VIP cells, nicotine did not alter the membrane potential of SOM or PV neurons (Fig. 19A,B; paired t test: SOM $n = 13$ cells, 5 mice, $t_{(12)} = 0.95$, $p = 0.36$. PV $n = 13$ cells, 5 mice, $t_{(12)} = 0.12$, $p = 0.90$). Therefore, nicotine selectively depolarizes VIP and Pyr cells across cortical layers, with the most powerful effect on VIP cells.

Nicotine directly depolarizes VIP neurons via $\beta 2$ -containing receptors and indirectly depolarizes Pyr cells

To determine if the depolarization of Pyr and VIP cells resulted from direct activation of nicotinic receptors located on these cell types, we applied nicotine after blocking synaptic activity. We bath-applied 10 μM CNQX and 50 μM PTX for 7-10 minutes prior to nicotine, to block AMPA and GABA-A receptors, respectively. CNQX and PTX reduced nicotinic depolarization of Pyr cells (Fig. 20A; paired t test: $n = 7$ cells, 2 mice, $t_{(6)} = 1.99$, $p = 0.09$),

suggesting that nicotine's effects occurred indirectly. However, the nicotinic depolarization of VIP cells persisted in the presence of CNQX and PTX, implying direct nAChR activation (Fig. 20A; paired t test: $n = 12$ cells, 6 mice, $t_{(11)} = 6.21$, $p < 0.0001$).



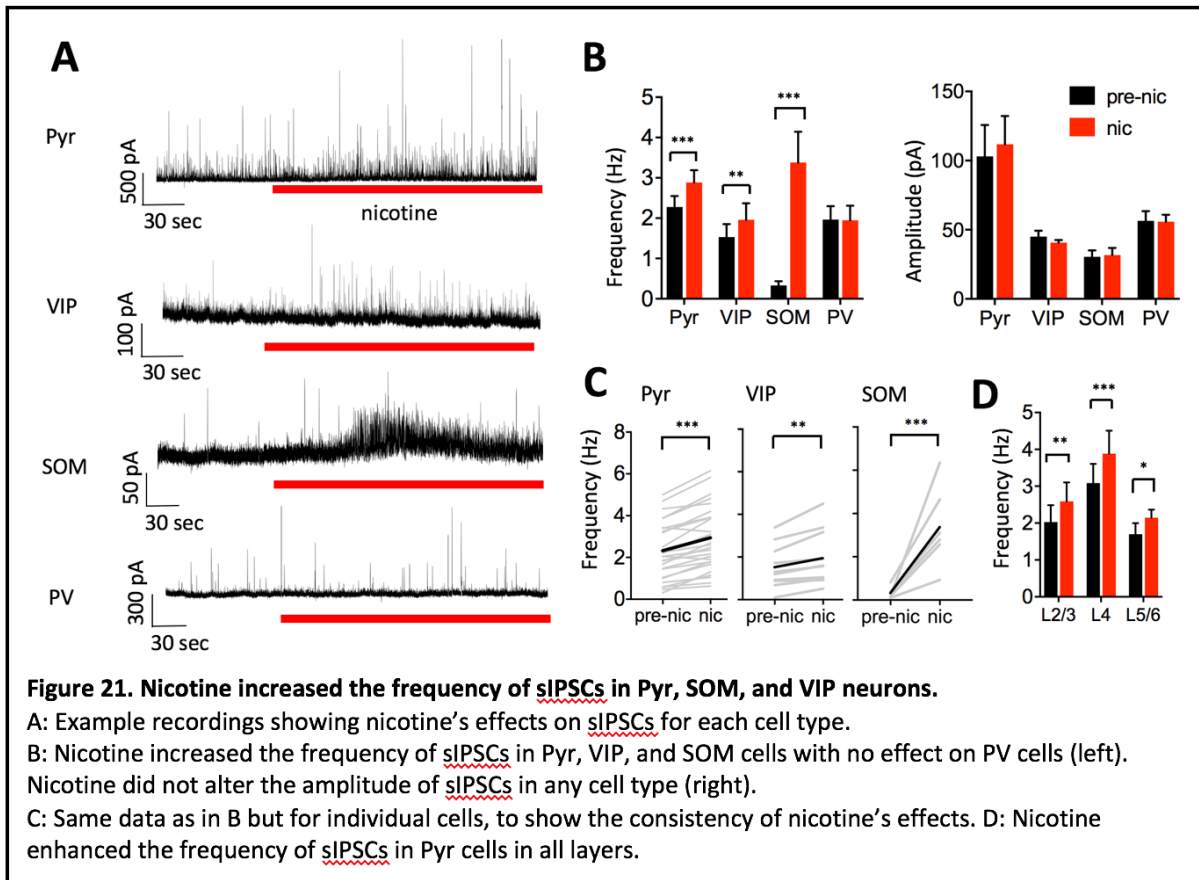
We then sought to identify the receptor subtype mediating the activation of VIP cells.

The two main types of nAChRs in cortical regions are the homomeric $\alpha 7$ receptor and the heteromeric $\alpha 4\beta 2$ receptor (Dani and Bertrand, 2007; Arroyo et al., 2014). We bath-applied 10

nM MLA, an $\alpha 7$ receptor antagonist, prior to nicotine application, yet nicotine continued to depolarize VIP cells (Fig. 20B; paired t test: $n = 6$ cells, 2 mice, $t_{(5)} = 7.75$, $p = 0.0006$). In another group of cells, we bath-applied 0.5-1 μM DH β E, an antagonist of $\beta 2$ -containing nAChRs (Xiao and Kellar, 2004). DH β E completely prevented the nicotinic depolarization, demonstrating that nicotine's actions on VIP cells are mediated by $\beta 2$ -containing receptors (Fig. 20B; paired t test: $n = 10$ cells, 4 mice, $t_{(9)} = 1.63$, $p = 0.14$). These results indicate the likely involvement of $\alpha 4\beta 2$ receptors, but do not preclude the involvement of other subunits such as $\alpha 2$ or $\alpha 5$ (Kleeman et al., 2016; Koukouli et al., 2017). Overall, the results indicate that nicotine indirectly depolarizes Pyr cells and directly depolarizes VIP neurons via $\beta 2$ -containing, but not $\alpha 7$, nAChRs.

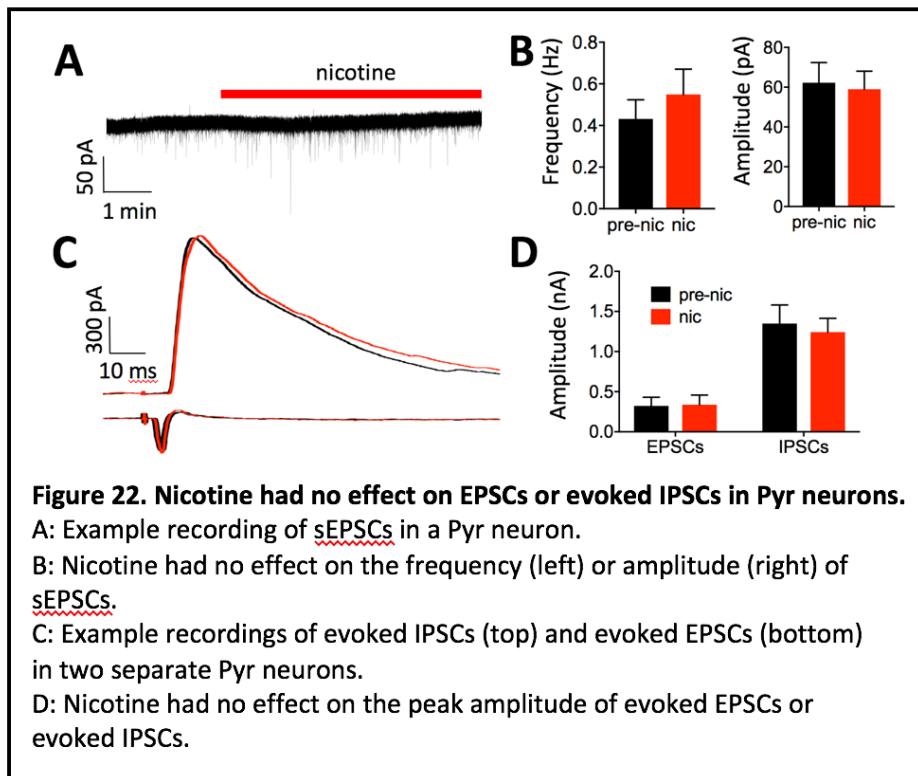
Nicotine enhances sIPSCs in Pyr, VIP, and SOM neurons

Given nicotine's potent effect on VIP interneurons, we next assessed nicotinic modulation of inhibition by recording spontaneous inhibitory post-synaptic currents (sIPSC). Recordings were performed in voltage-clamp with a Cs^+ -based internal solution and cells were clamped at 0 mV to isolate sIPSCs. We found that nicotine weakly, but consistently, increased the frequency of sIPSCs in Pyr cells (Fig. 21A,B; paired t test: $n = 26$ cells, 10 mice, $t_{(25)} = 7.44$, $p < 0.0001$) and that this enhancement occurred in each cortical layer (Fig. 21D; paired t test: L2/3 $n = 8$ cells, 4 mice, $t_{(7)} = 3.96$, $p = 0.006$. L4 $n = 9$ cells, 6 mice, $t_{(8)} = 6.41$, $p = 0.0002$. L5/6 $n = 9$ cells, 5 mice, $t_{(8)} = 3.13$, $p = 0.014$). Nicotine also weakly, but consistently, increased the frequency of sIPSCs in VIP cells (Fig. 21A,B; paired t test: $n = 10$ cells, 3 mice, $t_{(9)} = 3.94$, $p = 0.0034$).



Notably, nicotine strongly increased the frequency of sIPSCs in SOM cells (Fig. 21A,B; paired t test: $n = 6$ cells, 3 mice, $t_{(5)} = 4.19$, $p = 0.0086$). Prior to nicotine application, SOM neurons had dramatically fewer sIPSCs than Pyr cells (Fig. 21A,B; unpaired t test comparing frequency: $t_{(30)} = 3.42$, $p = 0.0019$). Although the degree of nicotinic effects on sIPSCs varied considerably among Pyr, VIP, and SOM cells, the frequency enhancement occurred in almost all of these cells (Fig. 21C). Nicotine had no effect on sIPSC frequency in PV cells (Fig. 21A,B; paired t test: $n = 8$ cells, 2 mice, $t_{(7)} = 0.11$, $p = 0.91$), or the amplitude of sIPSCs in any cell type (Fig. 21B; paired t test: Pyr $t_{(25)} = 1.09$, $p = 0.29$. VIP $t_{(9)} = 1.39$, $p = 0.20$. SOM $t_{(5)} = 0.46$, $p = 0.67$. PV $t_{(7)} = 0.24$, $p = 0.82$), although we did observe in some cells that the largest-amplitude responses appeared only with nicotine application.

We additionally examined spontaneous excitatory post-synaptic currents (sEPSC) in Pyr cells by clamping the membrane potential at -52mV (the approximate reversal potential of stimulus-evoked IPSCs). Nicotine had no effect on frequency or amplitude of sEPSCs (Fig. 22A,B; paired *t* test: *n* = 9 cells, 5 mice, frequency $t_{(8)} = 1.23$, *p* = 0.25, amplitude $t_{(8)} = 0.96$, *p* = 0.37).



We also recorded stimulus-evoked currents by stimulating with a bipolar concentric electrode placed in the thalamocortical pathway. Similar to spontaneous currents, evoked IPSCs were recorded at 0mV and evoked EPSCs at -52mV. Nicotine did not alter the amplitude of either evoked IPSCs or evoked EPSCs (Fig. 22D,C; paired *t* test: IPSCs *n* = 10 cells, 6 mice, $t_{(9)} = 1.07$, *p* = 0.31. EPSCs *n* = 6 cells, 4 mice, $t_{(5)} = 0.85$, *p* = 0.43). Consequently, nicotine appears to modulate sIPSCs, likely due to VIP neuron activation, but not sEPSCs or stimulus-evoked synaptic responses.

Nicotine disinhibits Pyr neurons via VIP interneurons

Recent studies have shown that VIP interneurons preferentially inhibit other inhibitory interneurons that, in turn, inhibit Pyr cells. Consequently, VIP cell activation suppresses other interneurons and results in the disinhibition of Pyr cells (Lee et al., 2013; Pfeffer et al., 2013). Since nicotine directly activates VIP cells and indirectly depolarizes Pyr cells in our experiments, it is possible that the nicotinic depolarization of Pyr cells depends on activation of VIP interneurons. To address this, we silenced VIP interneurons using DREADDs that primarily prevent synaptic release of neurotransmitter from infected cells (Stachniak et al., 2014; Amat et al., 2017; Lichtenberg et al., 2017). Cre-inducible AAV hM4D viruses were injected into the auditory cortex of VIP-Cre mice (Fig. 5A). These inhibitory DREADDs expressed in VIP neurons were activated by the agonist CNO (100 nM), allowing us to functionally silence VIP cells. We initially used higher concentrations of CNO (1-10 μ M) common to DREADDs electrophysiology studies (Alexander et al., 2009; Krashes et al., 2011; Urban et al., 2016), but found that these higher concentrations depolarized about half of pyramidal cells 1-2 mV.

To ensure that DREADD expression *per se* did not alter nicotinic effects in Pyr cells, we first confirmed that nicotine continued to depolarize Pyr cells and enhance sIPSCs (Fig. 23C,D; paired *t* test: depolarization $n = 5$ cells, 2 mice, $t_{(4)} = 4.71$, $p = 0.0093$. sIPSC frequency $n = 7$ cells, 3 mice, $t_{(6)} = 3.32$, $p = 0.02$). Similarly, we confirmed that 100 nM CNO application in the absence of DREADDs did not affect Pyr cells (not shown; paired *t* test: depolarization $n = 5$ cells, 2 mice, $t_{(4)} = 0.18$, $p = 0.87$. sIPSC frequency $n = 6$ cells, 2 mice, $t_{(5)} = 0.97$, $p = 0.38$).

However, in DREADD-infected mice, CNO prevented the nicotinic depolarization and sIPSC frequency enhancement previously observed (Fig. 23B,C,D; paired *t* test: depolarization $n = 9$ cells, 3 mice, $t_{(8)} = 0.60$, $p = 0.56$. sIPSC frequency $n = 7$ cells, 3 mice, $t_{(6)} = 0.76$, $p = 0.47$). Thus, silencing VIP neurons prevents nicotine's effects in Pyr cells and provides evidence that nicotinic activation of VIP neurons leads to disinhibition of Pyr cells.

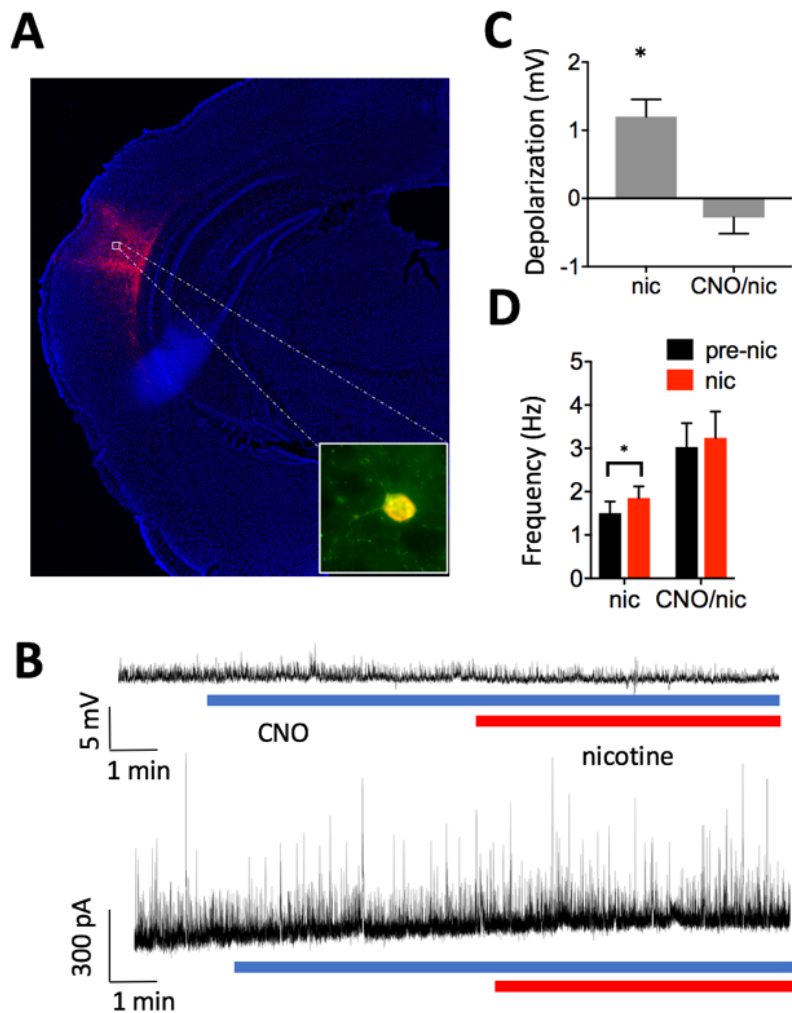


Figure 23. Nicotine depolarized and enhanced sIPSC frequency in Pyr neurons via VIP neurons.

A: Coronal section with immunohistochemistry against DAPI (blue), DREADDs viral construct (red), and VIP (green) in DREADDs-infused auditory cortex. Inset shows co-labeling between DREADDs and VIP in an example cell.

B: Example recordings from Pyr neurons in DREADD-infected mice demonstrating that CNO application prevents nicotine's effects on membrane potential (top) and sIPSCs (bottom).

C: All data are from Pyr cells in DREADDs-infected mice; nicotine depolarized Pyr cells and CNO prevented the nicotinic depolarization of Pyr cells.

D: All data are from Pyr cells in DREADDs-infected mice; nicotine enhanced the frequency of sIPSCs and CNO prevented the nicotinic enhancement of sIPSC frequency.

Discussion

In this study, we examined the cell-type specificity of nicotinic effects in auditory cortex and revealed three key findings: 1) Nicotine selectively affects the membrane potential of discrete cell types. That is, nicotine weakly depolarizes Pyr cells, strongly depolarizes and excites VIP cells, and does not alter membrane potential of SOM or PV cells. 2) Nicotine selectively enhances sIPSCs in specific cell types. That is, nicotine weakly enhances sIPSC frequency in Pyr and VIP cells, strongly enhances sIPSC frequency in SOM cells and does not alter sIPSCs in Pyr cells; and 3) Nicotinic depolarization and enhanced sIPSC frequency in Pyr cells is indirect and mediated by VIP neurons, implicating nicotinic activation of a disinhibitory neural circuit.

It is important to note that our nicotine bath application lasts several minutes, whereas many studies use rapid and brief application to avoid desensitizing nAChRs. Thus, our results reflect non-desensitizing effects of nicotine and may be most relevant to *in vivo* administration of systemic nicotine or other agonists. Conversely, endogenous ACh activation of nAChRs presents a more complex picture, with both phasic and tonic actions contributing to its pro-cognitive effects (Sarter et al., 2009; Klinkenberg et al., 2011).

Nicotine's effects on Pyr cells

We observed that nicotine weakly and indirectly depolarizes Pyr cells in all cortical layers. Overall, most studies find little evidence of direct nAChR activation on Pyr cells (Christophe et al., 2002; Disney et al., 2007; Gullledge et al., 2007). A few exceptions include direct nAChR activation of L5 and L6 Pyr cells, although these studies used higher

concentrations of nicotine (10 μ M – 1 mM) or ACh (1 mM) and were performed in regions other than auditory cortex (Kassam et al., 2008; Zolles et al., 2009). It is likely that at least some Pyr cells in our preparation express nAChRs, but these cells may exclusively respond to higher concentrations or a rapid application of nicotine. Although we saw no nicotinic depolarization of Pyr cells in the presence of glutamate and GABA receptor antagonists, it is possible that if we recorded from a larger population of L5/6 Pyr cells and/or used rapid application of higher concentrations, some evidence for direct nAChR activation might begin to emerge.

We also found that nicotine enhances the frequency of sIPSCs in Pyr cells, consistent with prior studies (Couey et al., 2007). Our experiments additionally show that the Pyr cell sIPSC enhancement is mediated through VIP cells, presumably by a weak but direct VIP to Pyr cell projection such as is seen in previous studies (Lee et al., 2013; Pfeffer et al., 2013).

Although we saw no change in the amplitude of stimulus-evoked responses, this doesn't preclude the ability of nicotine to modify cortical responses to sensory stimuli as seen previously (Intskirveli and Metherate, 2012; Askew et al., 2017). Rather, the nicotinic depolarization of Pyr cells would result in heightened responsiveness, thus altering and presumably improving the cortical processing of stimuli. Also, effects on interneurons will likely have complex outcomes on intracortical processing of sensory stimuli, potentially contributing to the narrowing of acoustic receptive fields (Askew et al., 2017).

Nicotine's effects on interneurons

We found that nicotine has distinct effects on specific interneuron types. Nicotine strongly depolarizes and excites VIP cells via β 2-containing nAChRs, while having no effect on

SOM or PV cell membrane potential. Also, nicotine potently enhanced the frequency of sIPSCs in SOM neurons, weakly enhanced the frequency of sIPSCs in VIP cells, and had no effect on sIPSCs in PV cells.

In support of our data, there are several studies that show direct nAChR activation of VIP cells. It appears that the majority of cells responsive to nAChR agonists express VIP and ACh activates VIP neurons via $\alpha 4\beta 2$ receptors (Porter et al., 1999; Lee et al., 2010; Bell et al., 2015). VIP cell activation in cortical regions also has significant effects on processing, including increased evoked responses in visual cortex (Fu et al., 2014) and improved behavioral performance in a memory-dependent task (Kamigaki and Dan, 2017). Our experiments expand on this previous evidence by demonstrating direct and potent VIP cell activation by low concentrations of nicotine, as well as revealing functional consequences for neural circuitry.

PV and SOM generally do not seem to express nAChRs, although there is some indication that small sub-populations within these interneuron groups may contain nAChRs (Porter et al., 1999; Gullledge et al., 2007). One study demonstrated L2/3 SOM neurons with functional nAChRs and fast-spiking (presumably PV) neurons expressing $\alpha 7$ receptors (Poorthuis et al., 2013). In hippocampus, another group discovered a population of interneurons with firing patterns similar to SOM cells that had a non-desensitizing $\alpha 2$ nAChR-mediated response to bath-application of 1 μ M nicotine, similar to our administration method (Jia et al., 2010). Yet $\alpha 7$ receptors on PV cells would rapidly desensitize with our bath-application of nicotine, and any SOM cell nicotinic depolarization might be masked by the counteracting inhibitory input we observed with nicotine application.

There is substantial evidence that VIP neurons preferentially innervate and inhibit SOM cells, consistent with the powerful sIPSC enhancement we observed in SOM cells (Lee et al., 2013; Pfeffer et al., 2013; Pi et al., 2013). Therefore, although we did not directly demonstrate that sIPSCs are enhanced in SOM neurons via VIP neurons, it appears likely that the nicotinic activation of VIP cells causes the frequency enhancement.

VIP inhibitory mechanisms

Nicotine appears to increase sIPSCs in multiple cell types, yet we found no evidence of a corresponding hyperpolarization that might be expected with enhanced inhibitory input. This could be due to the space-clamp limitations of our whole-cell recordings; i.e. the cortical cell types recorded here are known to have extensive dendritic and axonal processes, which would not be well-clamped or accurately recorded from, especially with our K^+ -based internal solution. If VIP cells primarily innervate the distal dendrites of their post-synaptic target, the inhibition and resulting hyperpolarization evoked by VIP cells might remain localized to this cellular compartment rather than spread to the soma. SOM neurons are traditionally characterized by this type of specificity, and their restricted inhibition of Pyr cell dendrites is thought to influence the integration of synaptic events. VIP cells may similarly target dendritic processes in select cell types.

In fact, in our voltage-clamp recordings (using a Cs^+ -based solution which blocks potassium channels and reduces space-clamp error) nicotine does appear to alter the baseline holding current in SOM cells (see example in Fig. 21A). This change in baseline holding current may reflect a small hyperpolarization that cannot be seen with the K^+ -based solution,

suggesting that alterations in the membrane potential of SOM neurons may occur distant from the soma. On the other hand, previous studies found that VIP cells target both the dendrites and soma of Pyr cells (Kawaguchi, 1996; Kawaguchi and Kubota, 1997). Even though direct VIP inhibition of Pyr cells is weak, it is possible that it still results in the hyperpolarization of Pyr cells. Yet it appears in our experiments and in other studies that the predominate effect of exciting VIP interneurons is that of disinhibition (Lee et al., 2013; Pfeffer et al., 2013; Pi et al., 2013; Fu et al., 2014), thus any direct hyperpolarization of Pyr cells seems to be overridden by the counteracting depolarization.

Disinhibition of Pyr cells by VIP neurons

We also observed that nicotine depolarizes Pyr cells via the activation of VIP interneurons. This finding supports a growing field of research demonstrating that VIP neurons have a strong disinhibitory effect on Pyr cells. In the auditory cortex of awake mice, optogenetic activation of VIP neurons suppresses SOM cells and increases tone-evoked responses in principal neurons (Pi et al., 2013). A similar effect occurs in visual cortex, where VIP cell activation also enhances stimulus-evoked responses (Fu et al., 2014). Given that VIP cells strongly inhibit SOM cells (Lee et al., 2013; Pfeffer et al., 2013; Pi et al., 2013), it is probable that the nicotinic disinhibition of Pyr cells involves SOM cells that are tonically inhibiting Pyr cells (Gentet et al., 2012). In our experiments nicotine is activating VIP cells which then likely inhibit SOM cells, causing a release of tonic inhibition on Pyr cells.

If this VIP to SOM to Pyr network paradigm is accurate, it seems surprising that we do not see a nicotinic hyperpolarization in SOM cells. This may be due to the space-clamp

limitations of our recordings. On the other hand, if we had recorded from more cells we might see that only a sub-population of SOM neurons are hyperpolarized by nicotine. It is also possible that the VIP-mediated disinhibition of Pyr cells does not involve SOM neurons, but instead a cell type not recorded in these experiments.

Conclusions and broader implications

Nicotine disinhibits Pyr cells by activating VIP neurons, thus modifying the cortical brain state to render Pyr cells more excitable. Pyr cells throughout auditory cortex are weakly depolarized by nicotine, but effects on Pyr cell output might only emerge when there is an auditory stimulus present. An incoming stimulus is tonotopically organized and thus exclusively activates Pyr cells assigned to process that particular stimulus. Therefore, with auditory stimulation the nicotinic depolarization that was initially generalized across all Pyr cells may transform into enhanced firing specific to the cells relevant to processing that input. Accordingly, the disinhibition of Pyr cells may serve as a preparatory mechanism for incoming input, resulting in selectively increased gain and improved cortical processing.

Though these experiments were performed in auditory cortex, other cortical regions likely consist of similar networks and nAChR functionality. Thus, these data provide insight into a probable mechanism underlying the pro-cognitive and sensory processing effects of nicotine. Moreover, our findings support the possible use of nicotine as a therapeutic by further revealing its actions at the level of neural circuitry.

CONCLUSIONS

Nicotine enhances sensory and cognitive function via its actions at nAChRs, particularly in cortical regions (Dani and Bertrand, 2007). Although some of its benefits have been observed in a healthy population, nicotine may be especially useful as a therapeutic approach for those with impaired function such as schizophrenia, mild cognitive impairment, and sensory processing disorders (Kumari and Postma, 2005; Newhouse et al., 2012). Dysfunction of nAChRs is a common characteristic in these disorders and likely a contributing factor to their development and progression. Accordingly, nicotinic administration may help to alleviate the symptoms and partially restore normal processing function, as has been already demonstrated in individuals with schizophrenia and mild cognitive impairment (Kumari and Postma, 2005; Newhouse et al., 2012). Though some work has been done to explore the neural correlate of nicotine's pro-cognitive effects, our understanding is far from complete and it is unclear precisely how nicotine modulates brain activity at the circuit- and network-level. This dissertation aims to address this issue by exploring nicotinic effects on the mouse auditory system, especially in primary auditory cortex.

Here we found that nicotine modulates spectral processing in A1 by narrowing RFs, increasing the gain in the center of the RF, and enhancing responses to CF tones presented within notched-noise. Modulation of RFs reflected nicotine effects at several levels in the auditory pathway, including narrowed RFs in ICc and MGv, increased gain in the thalamocortical pathway, and both narrowed RFs and increased gain within A1. Upon further examination of nicotine's effects in the auditory cortex, we found that nicotine alters the membrane potential and inhibition of specific cell types. Nicotine indirectly and weakly depolarizes Pyr cells, and

directly and strongly depolarizes VIP cells, with no effect on the membrane potential of SOM or PV cells. We also observed that nicotine enhances the frequency of sIPSCs in Pyr, VIP, and SOM neurons, demonstrating increased inhibition in the cortex. Furthermore, nicotinic effects on Pyr cells are mediated by VIP neurons, thus implicating VIP cells as a main target of nicotinic actions and highlighting the role of disinhibition in the nicotinic modulation of auditory cortex.

These results integrate novel findings on the nicotinic modulation of auditory processing with cellular- and circuit-level mechanisms of nicotine's actions. By revealing both nicotine's effects on auditory RFs as well as its actions on specific cell types in auditory cortex, this dissertation provides a more complete view into how nicotine produces its well-characterized effects on cognition and sensory task performance. More specifically, the nicotinic modulation of spectral processing outlined here strongly mimics the effects of attention on RFs, suggesting that nicotine may "hijack" innate attentional processes to sharpen tuning within the auditory system. Previous studies have shown that RF widths correlate with behavioral performance, and narrowed RFs reflect better tuning and processing. Our results corroborate this idea by showing narrowed RFs and enhanced CF tone-evoked responses, implying that nicotine may improve target stimulus detection while reducing the response to distractors. Central auditory processing disorders encompass a wide array of auditory impairments, including difficulty with comprehension in noisy listening environments, and these findings suggest that nicotine may be a useful therapeutic for such impairments. Moreover, our experiments validate the use of TINN stimuli in physiological studies, rather than just their traditional use in psychoacoustic perceptual studies.

Our findings on nicotine's selective effects in cortical cell types further establish an underlying mechanism for its ability to enhance processing, which can focus future therapeutic efforts. For example, we found that nicotine disinhibits Pyr neurons via its actions at β 2-containing nAChRs in VIP cells, implying that other nAChR agonists such as those that selectively target this receptor type may have similar outcomes as nicotine. Additionally, other drugs that activate VIP cells may also produce improvements in attention and behavior. Overall, the experiments in this dissertation are likely relevant to other cortical areas and sensory modalities, as the distribution and function of nAChRs is broadly similar across cortical regions.

Future studies should examine how chronic application of nicotine influences auditory processing and circuit-level mechanisms. Although these experiments use an acute application of nicotine and reveal noteworthy effects, others have seen benefits that only emerge with a chronic administration (Newhouse 2012, Bueno-Junior 2017). It is possible that repeated doses of nicotine may modulate auditory processing or cortical function differently than what is revealed here. Moreover, chronic application is more relevant to the therapeutic use of nicotine, given that individuals will likely need multiple or long term treatments. Additional future studies could also investigate nicotine's effects on temporal auditory processing. Although spectral processing is a key component to auditory function, temporal processing is widely accepted as critical to language comprehension. Impairments in communication may arise from an inability to process auditory information at a rapid pace, and if nicotine also improves temporal auditory processing, it would provide additional evidence for its potential therapeutic use. Furthermore, studies with nicotine could extend to mouse models of disease or cognitive/sensory impairment. Since nicotine may be especially effective for individuals with

impairments, it would be useful to know how nicotine modulates neural activity in disease models. Together, these additional studies would continue to elucidate the function of nAChRs in cortical processing and clarify the potential beneficial role of nicotine in therapeutic interventions.

REFERENCES

- Adesnik H, Bruns W, Taniguchi H, Huang ZJ, Scanziani M (2013) A neural circuit for spatial summation in visual cortex. *Nature* 490:226–231
- Albuquerque EX, Pereira EFR, Alkondon M, Rogers SW (2009) Mammalian Nicotinic Acetylcholine Receptors: From Structure to Function. *Physiol Rev* 89:73–120
- Alexander GM, Rogan SC, Abbas AI, Armbruster BN, Pei Y, Allen JA, Nonneman RJ, Hartmann J, Moy SS, Nicolelis MA, Mcnamara JO (2009) Remote Control of Neuronal Activity in Transgenic Mice Expressing Evolved G Protein-Coupled Receptors. *Neuron* 63:27–39
- Alitto HJ, Dan Y (2013) Cell-type-specific modulation of neocortical activity by basal forebrain input. *Front Syst Neurosci* 6:79
- Alves-Pinto A, Sollini J, Wells T, Sumner CJ (2016) Behavioural estimates of auditory filter widths in ferrets using notched-noise maskers. *J Acoust Soc Am* 139:EL19-EL24
- Amat SB, Rowan MJM, Gaffield MA, Bonnan A, Kikuchi C, Taniguchi H, Christie JM (2017) Using c-kit to genetically target cerebellar molecular layer interneurons in adult mice. *PLoS One*:1–17.
- Arroyo S, Bennett C, Aziz D, Brown SP, Hestrin S (2012) Prolonged Disynaptic Inhibition in the Cortex Mediated by Slow, Non- 7 Nicotinic Excitation of a Specific Subset of Cortical Interneurons. *J Neurosci* 32:3859–3864.
- Arroyo S, Bennett C, Hestrin S (2014) Nicotinic modulation of cortical circuits. *Front Neural Circuits* 8:30
- Askew C, Intskirveli I, Metherate R (2017) Systemic Nicotine Increases Gain and Narrows Receptive Fields in A1 via Integrated Cortical and Subcortical Actions. *eNeuro* 4.
- Aton SJ, Colwell CS, Harmar AJ, Waschek J, Herzog ED (2005) Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. *Nat Neurosci* 8:476–483.
- Barr RS, Culhane M a, Jubelt LE, Mufti RS, Dyer M a, Weiss AP, Deckersbach T, Kelly JF, Freudenreich O, Goff DC, Evins a E (2008) The effects of transdermal nicotine on cognition in nonsmokers with schizophrenia and nonpsychiatric controls. *Neuropsychopharmacology* 33:480–490.
- Behler O, Breckel TPK, Thiel CM (2015) Nicotine reduces distraction under low perceptual load. *Psychopharmacology (Berl)* 232:1269–1277.
- Bell LA, Bell KA, McQuiston AR (2015) Acetylcholine release in mouse hippocampal CA1 preferentially activates inhibitory-selective interneurons via $\alpha 4\beta 2^*$ nicotinic receptor activation. *Front Cell Neurosci* 9:115
- Bieszczad KM, Kant R, Constantinescu CC, Pandey SK, Kawai HD, Metherate R, Weinberger NM, Mukherjee J (2012) Nicotinic acetylcholine receptors in rat forebrain that bind ^{18}F -nifene: relating PET imaging, autoradiography, and behavior. *Synapse* 66:418–434
- Bringmann A (1994) Behaviour-related effects of physostigmine on the rat visual evoked potential. *Acta Neurobiol Exp* 54:355–363.
- Bryant MG, Polak JM, Bloom SR, Modlin I, Albuquerque RH, Pearse AGE (1976) Possible dual role for vasoactive intestinal peptide as gastrointestinal hormone and neurotransmitter substance. *Lancet*:991–993.

- Butt AM, Fern RF, Matute C (2014) Neurotransmitter signaling in white matter. *Glia* 62:1762–1779.
- Chen N, Sugihara H, Sur M (2015) An acetylcholine-activated microcircuit drives temporal dynamics of cortical activity. *Nat Neurosci* 18:892–902
- Christophe E, Roebuck A, Staiger JF, Lavery DJ, Charkpak S, Audinat E (2002) Two types of nicotinic receptors mediate an excitation of neocortical layer I interneurons. *J Neurophysiol* 88:1318–1327.
- Clarke PB, Schwartz RD, Paul SM, Pert CB, Pert A (1985) Nicotinic binding in rat brain: autoradiographic comparison of [3H]acetylcholine, [3H]nicotine, and [125I]-alpha-bungarotoxin. *J Neurosci* 5:1307–1315.
- Clarke PBS (2004) Nicotinic modulation of thalamocortical neurotransmission. *Prog Brain Res* 145:253–260.
- Clementz BA, Barber SK, Dzau JR (2002) Knowledge of Stimulus Repetition Affects the Magnitude and Spatial Distribution of Low-Frequency Event-Related Brain Potentials. *J Neurosci* 22:3013:303–314.
- Connors CK, Levin ED, Sparrow E, Hinton SC, Erhardt D, Meck WH, Rose JE, March J (1996) Nicotine and attention in adult attention deficit hyperactivity disorder (ADHD). *Psychopharmacol Bull* 32:67–73
- Costa S Da, Zwaag W Van Der, Miller LM, Clarke S, Saenz M (2013) Tuning In to Sound : Frequency-Selective Attentional Filter in Human Primary Auditory Cortex. *J Neurosci* 33:1858–1863.
- Couey JJ, Meredith RM, Spijker S, Poorthuis RB, Smit AB, Brussaard AB, Mansvelder HD (2007) Distributed Network Actions by Nicotine Increase the Threshold for Spike-Timing-Dependent Plasticity in Prefrontal Cortex. *Neuron*:73–87.
- Cruikshank SJ, Killackey HP, Metherate R (2001) Parvalbumin and calbindin are differentially distributed within primary and secondary subregions of the mouse auditory forebrain. *Neuroscience* 105:553–569.
- Cruikshank SJ, Rose HJ (2002) Auditory Thalamocortical Synaptic Transmission In Vitro. *J Neurophysiol*:361–384
- Cruikshank SJ, Rose HJ, Metherate R, Scott J, Rose HJ, Metherate R (2002) Auditory Thalamocortical Synaptic Transmission In Vitro. *J Neurophysiol*:361–384.
- Dani JA, Bertrand D (2007a) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu Rev Pharmacol Toxicol* 47:699–729
- DeFelipe J et al. (2013) New insights into the classification and nomenclature of cortical GABAergic interneurons. *Nat Rev Neurosci* 14:202–216
- Di Cristo G, Wu C, Chattopadhyaya B, Ango F, Knott G, Welker E, Svoboda K, Huang ZJ (2004) Subcellular domain-restricted GABAergic innervation in primary visual cortex in the absence of sensory and thalamic inputs. *Nat Neurosci* 7:1184–1186
- Ding YS, Fowler JS, Logan J, Wang GJ, Telang F, Garza V, Biegon A, Pareto D, Rooney W, Shea C, Alexoff D, Volkow ND, Vocci F (2004) 6-[18F]fluoro-A-85380, a new PET tracer for the nicotinic acetylcholine receptor: Studies in the human brain and in vivo demonstration of specific binding in white matter. *Synapse* 53:184–189.
- Disney AA, Aoki C, Hawken MJ (2007) Gain Modulation by Nicotine in Macaque V1. *Neuron* 56:701–713.

- Dubno JR, Dirks DD (1989) Auditory filter characteristics and consonant recognition for hearing-impaired listeners. *J Acoust Soc Am* 85:1666–1675.
- France SJ, Rosner BS, Hansen PC, Calvin C, Talcott JB, Richardson a J, Stein JF (2002) Auditory frequency discrimination in adult developmental dyslexics. *Percept Psychophys* 64:169–179.
- Frazier CJ, Rollins YD, Breese CR, Leonard S, Freedman R, Dunwiddie T V (1998) Acetylcholine activates an alpha-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. *J Neurosci* 18:1187–1195.
- Friedman J, Horvath T, Meares R (1974) Tobacco smoking and a “stimulus barrier.” *Nature* 248:455–456.
- Fritz J, Elhilali M, Shamma S (2005) Active listening: Task-dependent plasticity of spectrotemporal receptive fields in primary auditory cortex. *Hear Res* 206:159–176.
- Fu Y, Tucciarone JM, Espinosa JS, Sheng N, Darcy DP, Nicoll RA, Huang ZJ, Stryker MP (2014) A cortical circuit for gain control by behavioral state. *Cell* 156:1139–1152.
- Fuxe K, Said SI, Instituter K (1977) Vasoactive intestinal polypeptide and the nervous system. *Neurosci Lett* 5:241–246.
- Galván V V., Chen J, Weinberger NM (2002) Differential thresholds of local field potentials and unit discharges in rat auditory cortex. *Hear Res* 167:57–60.
- Gentet LJ, Kremer Y, Taniguchi H, Huang ZJ, Staiger JF, Petersen CCH (2012) Unique functional properties of somatostatin-expressing GABAergic neurons in mouse barrel cortex. *Nat Neurosci* 15:607–612
- Gil Z, Connors BW, Amitai Y (1997) Differential regulation of neocortical synapses by neuromodulators and activity. *Neuron* 19:679–686.
- Gilbert DG, Sugai C, Zuo Y, Rabinovich NE, McClernon FJ, Froeliger B (2007) Brain indices of nicotine ’ s effects on attentional bias to smoking and emotional pictures and to task-relevant targets. *Nicotine Tob Res* 9:351–363.
- Glasberg BR, Moore BC (1986) Auditory filter shapes in subjects with unilateral and bilateral cochlear impairments. *J Acoust Soc Am* 79:1020–1033.
- Glasberg BR, Moore BCJ (1990) Derivation of auditory filter shapes from notched-noise data. *Hear Res* 47:103–138.
- Guan ZZ, Zhang X, Blenow K, Nordberg a (1999) Decreased protein level of nicotinic receptor alpha7 subunit in the frontal cortex from schizophrenic brain. *Neuroreport* 10:1779–1782.
- 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.
- Guha D, Pradhan SN (1972) Effects of nicotine on EEG and evoked potentials and their interactions with autonomic drugs. *Neuropharmacology* 15:225–232.
- Gulledge AT, Park SB, Kawaguchi Y, Stuart GJ (2007) Heterogeneity of phasic cholinergic signaling in neocortical neurons. *J Neurophysiol* 97:2215–2229
- Gupta T, Mittal VA (2014) Nicotine usage is associated with elevated processing speed , spatial working memory , and visual learning performance in youth at ultrahigh-risk for psychosis. *Psychiatry Res* 220:687–690
- Hackett TA, Barkat TR, O’Brien BMJ, Hensch TK, Polley DB (2011) Linking topography to tonotopy in the mouse auditory thalamocortical circuit. *J Neurosci* 31:2983–2995

- Hamilton LS, Sohl-Dickstein J, Huth AG, Carels VM, Deisseroth K, Bao S (2013) Optogenetic Activation of an Inhibitory Network Enhances Feedforward Functional Connectivity in Auditory Cortex. *Neuron* 80:1066–1076
- Hara K, Harris RA (2002) The Anesthetic Mechanism of Urethane: The Effects on Neurotransmitter-Gated Ion Channels. *Anesth Pharmacol* 94:313–318.
- Harkrider AW, Champlin CA (2001) Acute effect of nicotine on non-smokers : III . LLRs and EEGs. *Hear Res* 160:99–110.
- Harkrider AW, Hedrick MS (2005) Acute effect of nicotine on auditory gating in smokers and non-smokers. *Hear Res* 202:114–128.
- Hasselmo ME, Sarter M (2011) Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacology* 36:52–73
- Heishman SJ (1998) What aspects of human performance are truly enhanced by nicotine? *Addiction* 93:317–320.
- Heishman SJ, Kleykamp BA, Singleton EG (2010) Meta-analysis of the acute effects of nicotine and smoking on human performance. *Psychopharmacology (Berl)* 210:453–469.
- Himmelheber AM, Sarter M, Bruno JP (2000) Increases in cortical acetylcholine release during sustained attention performance in rats. *Cogn Brain Res* 9:313–325.
- Hong LE, Schroeder M, Ross TJ, Buchholz B, Salmeron BJ, Wonodi I, Thaker GK, Stein EA (2011) Nicotine enhances but does not normalize visual sustained attention and the associated brain network in schizophrenia. *Schizophr Bull* 37:416–425.
- Hurst R, Rollema H, Bertrand D (2012) Nicotinic acetylcholine receptors: From basic science to therapeutics. *Pharmacol Ther* 137:22–54
- Inami R, Kirino E, Inoue R, Arai H (2005) Transdermal nicotine administration enhances automatic auditory processing reflected by mismatch negativity. *Pharmacol Biochem Behav* 80:453–461.
- Intskirveli I, Joshi A, Vizcarra-Chacón BJ, Metherate R (2016) Spectral breadth and laminar distribution of thalamocortical inputs to A1. *J Neurophysiol*:jn.00887.2015
- Intskirveli I, Metherate R (2012) Nicotinic neuromodulation in auditory cortex requires MAPK activation in thalamocortical and intracortical circuits. *J Neurophysiol* 107:2782–2793.
- Jackson J, Ayzenshtat I, Karnani MM, Yuste R (2016) VIP+ interneurons control neocortical activity across brain states. *J Neurophysiol* 594:jn.01124.2015
- Ji D, Dani J a (2000) Inhibition and disinhibition of pyramidal neurons by activation of nicotinic receptors on hippocampal interneurons. *J Neurophysiol* 83:2682–2690.
- Jia Y, Yamazaki Y, Nakauchi S, Ito KI, Sumikawa K (2010) Nicotine facilitates long-term potentiation induction in oriens-lacunosum moleculare cells via Ca²⁺ entry through non- $\alpha 7$ nicotinic acetylcholine receptors. *Eur J Neurosci* 31:463–476.
- Jia Y, Yamazaki Y, Nakauchi S, Sumikawa K (2009) $\alpha 2$ Nicotine receptors function as a molecular switch to continuously excite a subset of interneurons in rat hippocampal circuits. *Eur J Neurosci* 29:1588–1603.
- Kamigaki T, Dan Y (2017) Delay activity of specific prefrontal interneuron subtypes modulates memory-guided behavior. *Nat Neurosci* 20.
- Karnani XMM, Jackson J, Ayzenshtat I, Sichani XAH, Manoocheri K, Kim S, Yuste R (2016) Opening Holes in the Blanket of Inhibition : Localized Lateral Disinhibition by VIP Interneurons. *Neuron* 36:3471–3480.

- Kassam SM, Herman PM, Goodfellow NM, Alves NC, Lambe EK (2008) Developmental Excitation of Corticothalamic Neurons by Nicotinic Acetylcholine Receptors. *J Neurosci* 28:8756–8764
- Kassel ID (1997) Smoking and attention : A review and formulation of the stimulus-filter hypothesis. *Clin Psychol Rev* 17:451–478.
- Kato HK, Gillet SN, Isaacson JS, Kato HK, Gillet SN, Isaacson JS (2015) Flexible Sensory Representations in Auditory Cortex Driven by Behavioral Relevance *Neuron* 88:1027–1039
- Kaur S, Lazar R, Metherate R (2004) Intracortical pathways determine breadth of subthreshold frequency receptive fields in primary auditory cortex. *J Neurophysiol* 91:2551–2567
- Kawaguchi Y (1996) Physiological and Morphological Identification of Somatostatin- Vasoactive Intestinal Polypeptide-Containing Cells among GABAergic Cell Subtypes in Rat Frontal Cortex. *J Neurosci* 16:2701–2715.
- Kawaguchi Y, Kubota Y (1997) GABAergic Cell Subtypes and their Synaptic Connections in Rat Frontal Cortex. *Cereb Cortex*:476–486.
- Kawai H, Lazar R, Metherate R (2007) Nicotinic control of axon excitability regulates thalamocortical transmission. *Nat Neurosci* 10:1168–1175
- Kawai HD, Kang H -a. H-A, Metherate R (2011) Heightened Nicotinic Regulation of Auditory Cortex during Adolescence. *J Neurosci* 31:14367–14377
- Kepecs A, Fishell G (2014) Interneuron cell types are fit to function. *Nature* 505:318–326
- Kleeman E, Nakauchi S, Su H, Dang R, Wood MA, Sumikawa K (2016) Neurobiology of Learning and Memory Impaired function of $\alpha 2$ -containing nicotinic acetylcholine receptors on oriens-lacunosum moleculare cells causes hippocampus-dependent memory impairments. *Neurobiol Learn Mem* 136:13–20
- Klinkenberg I, Sambeth A, Blokland A (2011) Acetylcholine and attention. *Behav Brain Res* 221:430–442
- Knott VJ, Bolton K, Heenan A, Shah D, Fisher DJ, Villeneuve C (2009) Effects of acute nicotine on event-related potential and performance indices of auditory distraction in nonsmokers. *Nicotine Tob Res* 11:519–530.
- Koukoulis F, Rooy M, Tziotis D, Sailor KA, Neill HCO, Levenga J, Witte M, Nilges M, Changeux J, Hoeffler CA, Stitzel JA, Gutkin BS, Digregorio DA, Maskos U (2017) Nicotine reverses hypofrontality in animal models of addiction and schizophrenia. *Nat Publ Gr* 23:347–354
- Krashes MJ, Koda S, Ye C, Rogan SC, Adams AC, Cusher DS, Maratos-flier E, Roth BL, Lowell BB (2011) Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. *J Clin Invest* 121:2–6.
- Kumari V, Gray JA, Ffytche DH, Mitterschiffthaler MT, Das M, Zachariah E, Vythelingum GN, Williams SCR, Simmons A, Sharma T (2003) Cognitive effects of nicotine in humans: An fMRI study. *Neuroimage* 19:1002–1013.
- Kumari V, Postma P (2005) Nicotine use in schizophrenia : The self medication hypotheses. *Neurosci Biobehav Rev* 29:1021–1034.
- Lakatos P, Musacchia G, O’Connell MN, Falchier AY, Javitt DC, Schroeder CE (2013) The Spectrotemporal Filter Mechanism of Auditory Selective Attention. *Neuron* 77:750–761
- Lavine N, Reuben M, Clarke PBS (1997) A population of nicotinic receptors is associated with thalamocortical afferents in the adult rat: Laminar and areal analysis. *J Comp Neurol* 380:175–190.
- Lawrence NS, Ross TJ, Stein EA (2002) Cognitive mechanisms of nicotine on visual attention.

- Neuron 36:539–548.
- Leão RN, Mikulovic S, Leão KE, Munguba H, Gezelius H, Enjin A, Patra K, Eriksson A, Loew LM, Tort ABL, Kullander K (2012) OLM interneurons differentially modulate CA3 and entorhinal inputs to hippocampal CA1 neurons. *Nat Neurosci* 15:1524–1530
- Lee S, Hjerling-Leffler J, Zagha E, Fishell G, Rudy B (2010) The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. *J Neurosci* 30:16796–16808
- Lee S, Kruglikov I, Huang ZJ, Fishell G, Rudy B (2013) A disinhibitory circuit mediates motor integration in the somatosensory cortex. *Nat Neurosci* 16:1662–1670
- Lendvai B, Vizi ES (2008) Nonsynaptic chemical transmission through nicotinic acetylcholine receptors. *Physiol Rev* 88:333–349.
- Levin ED, Mcclernon FJ (2006) Nicotinic effects on cognitive function : behavioral characterization , pharmacological specification , and anatomic localization. *Psychopharmacology (Berl)* 184:523–539.
- Li L-y., Ji X -y., Liang F, Li Y -t., Xiao Z, Tao HW, Zhang LI (2014) A Feedforward Inhibitory Circuit Mediates Lateral Refinement of Sensory Representation in Upper Layer 2/3 of Mouse Primary Auditory Cortex. *J Neurosci* 34:13670–13683
- Liang K, Poytress BS, Chen Y, Leslie FM, Weinberger NM, Metherate R (2006) Neonatal nicotine exposure impairs nicotinic enhancement of central auditory processing and auditory learning in adult rats. *Eur J Neurosci* 24:857–866.
- Liang K, Poytress BS, Weinberger NM, Metherate R (2008) Nicotinic modulation of tone-evoked responses in auditory cortex reflects the strength of prior auditory learning. *Neurobiol Learn Mem* 90:138–146.
- Lichtenberg NT, Pennington ZT, Holley SM, Greenfield VY, Cepeda X, Levine MS, Wassum KM (2017) Basolateral Amygdala to Orbitofrontal Cortex Projections Enable Cue-Triggered Reward Expectations. *J Neurosci* 37:8374–8384.
- Logemann HNA, Bocker KBE, Deschamps PKH, Kemner C, Kenemans JL (2014) The effect of the augmentation of cholinergic neurotransmission by nicotine on EEG indices of visuospatial attention. *Behav Brain Res*:67–73
- Martin-Ruiz CM, Lee M, Perry RH, Baumann M, Court JA, Perry EK (2004) Molecular analysis of nicotinic receptor expression in autism. *Mol Brain Res* 123:81–90.
- McArthur GM, Bishop DVM (2004) Frequency discrimination deficits in people with specific language impairment: reliability, validity, and linguistic correlates. *J Speech Lang Hear Res* 47:527–541
- McDonald JK, Parnevelas JG, Karamanlidis AN, Brecha N (1982) The morphology and distribution of peptide-containing neurons in the adult and developing visual cortex of the rat. II. Vasoactive intestinal polypeptide. *J Neurocytol* 11:825–837.
- Mengler ED, Hogben JH, Michie P, Bishop DVM (2005) Poor frequency discrimination is related to oral language disorder in children: A psychoacoustic study. *Dyslexia* 11:155–173.
- Mesik L, Ma W-P, Li L-Y, Ibrahim LA, Huang ZJ, Zhang LI, Tao HW (2015) Functional response properties of VIP-expressing inhibitory neurons in mouse visual and auditory cortex. *Front Neural Circuits* 9:22
- Metherate R, Ashe JH (1994) Facilitation of an NMDA receptor-mediated EPSP by paired-pulse stimulation in rat neocortex via depression of GABAergic IPSPs. *J Physiol* 481 (Pt 2:331–

- Miwa JM, Freedman R, Lester HA (2011) Neural Systems Governed by Nicotinic Acetylcholine Receptors : Emerging Hypotheses. *Neuron* 70:20–33
- Miyoshi G, Hjerling-Leffler J, Karayannis T, Sousa VH, Butt SJB, Battiste J, Johnson JE, Machold RP, Fishell G (2010) Genetic Fate Mapping Reveals That the Caudal Ganglionic Eminence Produces a Large and Diverse Population of Superficial Cortical Interneurons. *J Neurosci* 30:1582–1594
- Moore DR (2015) Sources of pathology underlying listening disorders in children. *Int J Psychophysiol* 95:125–134
- Moore DR, Ferguson M a, Edmondson-Jones a M, Ratib S, Riley A (2010) Nature of auditory processing disorder in children. *Pediatrics* 126:e382–e390.
- Morley BJ, Happe HK (2000) Cholinergic receptors: Dual roles in transduction and plasticity. *Hear Res* 147:104–112.
- Morrison JH, Magistretti PJ, Benoit R, Bloom FE (1984) The distribution and morphological characteristics of the intracortical VIP-positive cell: An immunohistochemical analysis. *Brain Res* 292:269–282.
- Muller-Preuss P, Mitzdorf U (1984) Functional anatomy of the inferior colliculus and the auditory cortex : current source density analyses of click-evoked potentials. *Hear Res* 16.
- Newhouse P, Kellar K, Aisen P, White H (2012) Nicotine treatment of mild cognitive impairment. *Neurology*
- Norena A, Eggermont JJ (2002) Comparison between local field potentials and unit cluster activity in primary auditory cortex and anterior auditory field in the cat. *Hear Res* 166:202–213.
- O’Connell MN, Barczak A, Schroeder CE, Lakatos P (2014) Layer Specific Sharpening of Frequency Tuning by Selective Attention in Primary Auditory Cortex. *J Neurosci* 34:16496–16508
- Obata-Tsuto HL, Okamura H, Tsuto T, Terubayashi H, Fukui K, Yanaihara N, Iyata Y (1983) Distribution of the VIP-like immunoreactive neurons in the cat central nervous system. *Brain Res Bull* 10:653–660.
- Okamoto H, Stracke H, Wolters CH, Schmael F, Pantev C (2007) Attention improves population-level frequency tuning in human auditory cortex. *J Neurosci* 27:10383–10390
- Patterson RD (1976) Auditory filter shapes derived with noise stimuli. *J Acoust Soc Am* 59:640–654.
- Pfeffer CK, Xue M, He M, Huang ZJ, Scanziani M (2013) Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. *Nat Neurosci* 16:1068–1076
- Pi H-J, Hangya B, Kvitsiani D, Sanders JI, Huang ZJ, Kepecs A (2013) Cortical interneurons that specialize in disinhibitory control. *Nature* 503:521–524
- Pinto L, Goard MJ, Estandian D, Xu M, Kwan AC, Lee S-H, Harrison TC, Feng G, Dan Y (2013) Fast modulation of visual perception by basal forebrain cholinergic neurons. *Nat Neurosci* 16:1857–1863
- Poorthuis RB, Bloem B, Schak B, Wester J, De Kock CPJ, Mansvelder HD (2013a) Layer-specific modulation of the prefrontal cortex by nicotinic acetylcholine receptors. *Cereb Cortex* 23:148–161.

- Porter JT, Cauli B, Staiger JF, Lambolez B, Rossier J, Audinat E (1998) Properties of bipolar VIPergic interneurons and their excitation by pyramidal neurons in the rat neocortex. *Eur J Neurosci* 10:3617–3628.
- Porter JT, Cauli B, Tsuzuki K, Lambolez B, Rossier J, Audinat E (1999) Selective excitation of subtypes of neocortical interneurons by nicotinic receptors. *J Neurosci* 19:5228–5235.
- Pouille F, Scanziani M (2001) Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. *Science* 293:1159–1163.
- Prönneke A, Scheuer B, Wagener RJ, Möck M, Witte M, Staiger JF (2015) Characterizing VIP Neurons in the Barrel Cortex of VIPcre/tdTomato Mice Reveals Layer-Specific Differences. *Cereb Cortex* 25:4854–4868
- Provost SC, Woodward R (1991) Effects of nicotine gum on repeated administration of the stroop test. *Psychopharmacology (Berl)* 104:536–540.
- Reinhold K, Lien AD, Scanziani M (2015) Distinct recurrent versus afferent dynamics in cortical visual processing. *Nat Neurosci* 18:1789–1797.
- Rezvani a H, Levin ED (2001) Cognitive effects of nicotine. *Biol Psychiatry* 49:258–267.
- Rezvani AH, Bushnell PJ, Levin ED (2002) Effects of nicotine and mecamylamine on choice accuracy in an operant visual signal detection task in female rats. *Psychopharmacology (Berl)*:369–375.
- Rudy B, Fishell G, Lee S, Hjerling-Leffler J (2011) Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Dev Neurobiol* 71:45–61.
- Sahin M, Bowen WD, Donoghue JP (1992) Location of nicotinic and muscarinic cholinergic and mu-opiate receptors in rat cerebral neocortex: evidence from thalamic and cortical lesions. *Brain Res* 579:135–147.
- Sarter M, Parikh V, Howe WM (2009) Phasic acetylcholine release and the volume transmission hypothesis: time to move on. *Nat Rev Neurosci* 10:383–390.
- Simple MN (1995) Monaural Inhibition in Cat Auditory Cortex. *J Neurophysiol* 73.
- Sims KB, Hoffman DL, Said S, Zimmerman EA (1980) Vasoactive intestinal polypeptide (VIP) in mouse and rat brain. *Brain Res* 186:165–183.
- Slotkin TA (2002) Nicotine and the adolescent brain : Insights from an animal model. *Neurotoxicol Teratol* 24:369–384.
- Smucny J, Olincy A, Eichman LS, Tregellas JR (2015) Neuronal effects of nicotine during auditory selective attention. *Psychopharmacology (Berl)*:2017–2028.
- Soma S, Shimegi S, Suematsu N, Sato H (2013) Cholinergic modulation of response gain in the rat primary visual cortex. *Sci Rep* 3:1138
- Stachniak TJ, Ghosh A, Sternson SM (2014) Chemogenetic Synaptic Silencing of Neural Circuits Localizes a Hypothalamus to Midbrain Pathway for Feeding Behavior. *Neuron* 82:797–808
- Stiebler I, Ehret G (1985) Inferior colliculus of the house mouse. I. A quantitative study of tonotopic organization, frequency representation, and tone-threshold distribution. *J Comp Neurol* 238:65–76
- Summers V, Leek MR (1994) The internal representation of spectral contrast in hearing-impaired listeners. *J Acoust Soc Am* 95:3518–3528.
- Sun XY, Yang XY, Galvin XVC, Yang S, Arnsten AF, Wang XM (2017) Nicotinic $\alpha 4\beta 2$ Cholinergic Receptor Influences on Dorsolateral Prefrontal Cortical Neuronal Firing during a Working Memory Task. *J Neurosci* 37:5366–5377.

- Sutter ML, Schreiner CE, McLean M, O'Connor KN, Loftus WC (1999) Organization of inhibitory frequency receptive fields in cat primary auditory cortex. *J Neurophysiol* 82:2358–2371.
- Swan GE, Lessov-schlaggar CN (2007) The Effects of Tobacco Smoke and Nicotine on Cognition and the Brain. *Neuropsychol Rev*:259–273.
- Taly A, Corringer P, Guedin D, Lestage P (2009) Nicotinic receptors : allosteric transitions and therapeutic targets in the nervous system. *Nat Rev Drug Discov* 8:733–750
- Taniguchi H, He M, Wu P, Kim S, Paik R, Sugino K, Kvitsani D, Fu Y, Lu J, Lin Y, Miyoshi G, Shima Y, Fishell G, Nelson SB, Huang ZJ (2011) A Resource of Cre Driver Lines for Genetic Targeting of GABAergic Neurons in Cerebral Cortex. *Neuron* 71:995–1013.
- Tassonyi E, Charpantier E, Muller D, Dumont L, Bertrand D (2002) The role of nicotinic acetylcholine receptors in the mechanisms of anesthesia. *Brain Res Bull* 57:133–150.
- Timmermann DB, Sandager-Nielsen K, Dyhring T, Smith M, Jacobsen AM, Nielsen E, Grunnet M, Christensen JK, Peters D, Kohlhaas K, Olsen GM, Ahring PK (2012) Augmentation of cognitive function by NS9283, a stoichiometry-dependent positive allosteric modulator of $\alpha 2$ - and $\alpha 4$ -containing nicotinic acetylcholine receptors. *Br J Pharmacol* 167:164–182.
- Turrini P, Casu MA, Wong TP, De Koninck Y, Ribeiro-da-Silva A, Cuellar AC (2001) Cholinergic nerve terminals establish classical synapses in the rat cerebral cortex: Synaptic pattern and age-related atrophy. *Neuroscience* 105:277–285.
- Umbriaco D, Watkins KC, Descarries L, Cozzari C, Hartman BK (1994) Ultrastructural and morphometric features of the acetylcholine innervation in adult rat parietal cortex: An electron microscopic study in serial sections. *J Comp Neurol* 348:351–373.
- Urban DJ, Zhu H, Marcinkiewicz CA, Michaelides M, Oshibuchi H, Rhea D, Aryal DK, Farrell MS, Lowery-gionta E, Olsen RHJ, Wetsel WC, Kash TL, Hurd YL, Tecott LH, Roth BL (2016) Elucidation of The Behavioral Program and Neuronal Network Encoded by Dorsal Raphe Serotonergic Neurons. *Neuropsychopharmacology* 41:1404–1415
- Wada E, Wada K, Boulter JIM, Deneris E, Heinemann S, Patrick JIM, Swanson LW (1989) Distribution of Alpha2, Alpha 3, Alpha 4, and Beta2 Neuronal Nicotinic Receptor n the Central Nervous Subunit mRNAs in the Central Nervous System : A Hybridization Histochemical Study in the Rat. *J Comp Neurol* 336:314–345.
- Wallace MN, He J, Winer JA, Schreiner CE (2011) Spectral Processing in Auditory Cortex. In: *The Auditory Cortex*, pp 133–145
- Warbrick T, Mobascher A, Brinkmeyer J, Musso F, Stoecker T, Shah NJ, Fink GR, Winterer G (2012) Nicotine Effects on Brain Function during a Visual Oddball Task : A Comparison between Conventional and EEG-informed fMRI Analysis. *J Cogn Neurosci*:1682–1694.
- Wehr M, Zador AM (2005) Synaptic mechanisms of forward suppression in rat auditory cortex. *Neuron* 47:437–445.
- Wehr MS, Zador AM (2003) Balanced inhibition underlies tuning and sharpens spike timing in auditory cortex. *Nature* 426:442–446
- Wesnes K, Warburton DM (1984) Effects of scopolamine and nicotine on human rapid information processing performance. *Psychopharmacology (Berl)* 82:147–150.
- Whitehouse PJ, Martino AM, Antuono PG, Lowenstein PR, Coyle JT, Price DL, Kellar KJ (1986) Nicotinic acetylcholine binding sites in Alzheimer's disease. *Brain Res* 371:146–151.
- Wonnacott S (1997) Presynaptic nicotinic ACh receptors. *Trends Neurosci* 20:92–98.
- Wu GK, Arbuckle R, Liu B, Tao HW, Zhang LI (2008) Article Lateral Sharpening of Cortical

- Frequency Tuning by Approximately Balanced Inhibition. *Neuron* 58:132–143.
- Xiao Y, Kellar KJ (2004) The Comparative Pharmacology and Up-Regulation of Rat Neuronal Nicotinic Receptor Subtype Binding Sites Stably Expressed in Transfected Mammalian Cells. *J Pharmacol Exp Ther* 310:98–107.
- Xu X, Roby KD, Callaway EM (2010) Immunochemical characterization of inhibitory mouse cortical neurons: Three chemically distinct classes of inhibitory cells. *J Comp Neurol* 518:389–404.
- Yuan K, Fink KL, Winer JA, Schreiner CE (2011) Local connection patterns of parvalbumin-positive inhibitory interneurons in rat primary auditory cortex. *Hear Res* 274:121–128
- Zolles G, Wagner E, Lampert A, Sutor B (2009a) Functional expression of nicotinic acetylcholine receptors in rat neocortical layer 5 pyramidal cells. *Cereb Cortex* 19:1079–1091.