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Nicotinic alteration of functional thalamocortical topography

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Abstract

The thalamocortical pathways form highly topographic connections from the primary sensory thalamic nuclei to the primary cortical areas. The synaptic properties of these thalamocortical connections are modifiable by activation from various neuromodulators, such as acetylcholine. Cholinergic activation can alter functional properties in both the developing and mature nervous system. Moreover, environmental factors, such as nicotine, can activate these receptors, although the circuit-level alterations resulting from such nicotinic activation of sensory neural circuits remain largely unexplored. Therefore, we examined alterations to the functional topography of thalamocortical circuits in the developing sensory pathways of the mouse. Photostimulation via uncaging of glutamate was used to map these functional thalamocortical alterations in response to nicotinic receptor activation. As a result, we found that activation of forebrain nicotinic acetylcholine receptors results in an expansion and enhancement of functional thalamocortical topographies as assessed in brain slice preparations using laser-scanning photostimulation via uncaging of glutamate. These physiological changes were correlated with the neuroanatomical expression of nicotinic acetylcholine receptor subtypes ($\alpha 7$ and $\beta 2$). These circuit level alterations may provide a neural substrate underlying the plastic development and reshaping of thalamocortical circuitry in response to nicotinic receptor activation.

Keywords

nicotine; thalamus; cortex; layer 4; medial geniculate body; auditory; somatosensory; ventrobasal nucleus; thalamocortical; nicotinic acetylcholine receptor

Introduction

Nicotine exposure via smoking and secondhand smoke can adversely affect exposed juveniles [1-4]. A combination of human and animal studies suggest that offspring exposed to nicotine suffer from learning disabilities, among other developmental disorders [1,2,4-6], possibly because nicotine exposure disrupts learning that is dynamically regulated during early childhood. However, the effects of nicotine exposure on neural functions in the

developing brain are not fully understood for a number of reasons, e.g. difficulty of establishing appropriate controls in human studies, biased and underreported histories of nicotine exposure, and variation in wide-ranging epidemiological factors [2,7]. In animal models, nicotinic effects on brain function have been studied at molecular, cellular, and behavioral levels [1,2,5,7-12], but are not well understood at the circuit and system levels.

In early childhood, the brain has the largest capacity to learn and adapt to new environments [13-15]. For example, language is acquired through auditory perception during a particular span in early childhood, which often determines native language [13]. This brief time window during development is termed the critical period. If the brain is exposed to environmental toxicants, such as nicotine, during this critical period, then the occurrence of developing abnormalities increases and disruptions in learning can occur [1,2]. In particular, alterations to functional sensory circuits are likely altered as a result of nicotine exposure, particularly in higher forebrain centers [16,17]. Therefore, we sought to determine whether nicotine might affect the functional topography of thalamocortical circuitry in the developing sensory cortex using a mouse model [18,19].

The principal nuclei of the thalamus convey sensory information from the periphery to the primary sensory areas of the cerebral cortex [20,21]. These connections are organized in a highly topographic manner with limited divergence and convergence in each sensory modality [22]. This organizational plan establishes the canonical sensory maps observed in the cortex. These maps are plastic with experience and learning, which enables adaptive cortical processing to fit the sensory environment [23]. Although the roles of neuromodulatory systems, such as the cholinergic system, in this adaptive process have been well established [17,23,24], the neural substrates that establish these remapped parameters remain uncertain. Therefore, we examined the modulation of functional inputs from the thalamus to the cortex mediated by nicotine.

We utilized *in vitro* slice preparations that maintain the intact connections from the principal sensory thalamic nuclei to primary sensory cortical areas [18,19] and assessed the functional topography of projections using whole-cell patch clamp recorded responses of cortical neurons to laser-scanning photostimulation via uncaging of glutamate in the thalamus [25,26]. In this manner, we mapped the functional thalamocortical inputs in juvenile mice following activation of nicotinic acetylcholine receptors (nAChRs). We correlated these results with the neuroanatomical organization of nicotinic acetylcholine receptor subtypes.

Methods

Slice preparation

Acute slice preparations were made from juvenile mice of both sexes (ages: P10-P12). The Institutional Animal Care and Use Committee of the Louisiana State University School of Veterinary Medicine approved these procedures. To prepare acute slices containing the thalamocortical connections, animals were first deeply anesthetized using isoflurane inhalation until cessation of reflex responses. Animals were decapitated, the skull removed, and the brains quickly removed and submerged in ice cold, oxygenated, artificial cerebral spinal fluid (ACSF; in mM: 125 NaCl, 3 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 1 MgCl₂, 2

CaCl₂, 25 glucose). The brains were then blocked to preserve the thalamocortical connections in the auditory and somatosensory systems, as previously described [18,19,25]. The blocked surface was then affixed to a vibratome cutting stage using instant adhesive glue and sectioned at a thickness of 500 μ m. Slices containing intact connections between the thalamus and cortex were then transferred to a holding chamber filled with ACSF and incubated at 32°C for 1 h. Selected slices were then transferred to a recording chamber on a modified microscope stage (Siskiyou Inc., Grants Pass, OR) that was perfused constantly with ACSF at 32°C.

Electrophysiology and photostimulation

Whole-cell recordings were made using a Multiclamp 700B amplifier and digitized on a custom PC (ADEK Industrial Computers, Raymond, NH) using a Digidata 1440A acquisition system, pCLAMP10 software (Molecular Devices, Sunnyvale, CA) and the Ephys software package (Janelia Farms, Ashburn, VA).

The transferred thalamocortical slices were submerged in the recording chamber under constant bath perfusion of ACSF at 32°C and neurons were visually targeted for recording using an Olympus BX-51 microscope (Olympus America, Center Valley, PA). Recording pipettes with tip resistances of 4-8 M Ω were filled with either a normal intracellular solution (in mM: 135 KGluconate, 7 NaCl, 10 HEPES, 1-2 Na₂ATP, 0.3 GTP, 2 MgCl₂ at a pH of 7.3 obtained with KOH and osmolality of 290 mosm obtained with distilled water). Recordings were made in current or voltage clamp modes, which were uncorrected for liquid junction potentials of ~10 mV. Intrinsic membrane properties were measured in current-clamp using hyperpolarizing and depolarizing current steps. Recorded layer 4 neurons were classified as regular-spiking or fast-spiking, as described previously [25].

Laser-scanning photostimulation (LSPS) with caged glutamate was used to map the thalamocortical regions eliciting EPSCs in recorded layer 4 cortical neurons. Nitroindoliny1 (NI)-caged glutamate (0.37 mM; Sigma-Aldrich, St. Louis, MO) was added to the recirculating ACSF. Photolysis of the caged glutamate was done focally with a pulsed ultraviolet (UV) laser (DPSS Lasers, Inc., Santa Clara, CA) [27]. Custom software (Ephys) written in MATLAB (MathWorks, Inc., Natick, MA) was used to control the photostimulation parameters and analyze the data [28]. Interpolated plots were superimposed on differential interference contrast (DIC) photomicrographs corresponding to the stimulation sites.

Immunohistochemistry

Mice were anesthetized with isoflurane anesthesia followed by a lethal dose of sodium pentobarbital. For these anatomical experiments, in order to examine expression of nicotinic receptors relative to inhibitory interneurons, we utilized a transgenic mouse line with vesicular gamma-aminobutyric acid transporter (VGAT) positive interneurons expressing the Venus fluorescent protein (developed by Atsushi Miyawaki at RIKEN, Wako, Japan). Immediately following cessation of reflex responses, the mice were transcardially perfused with a fixative solution containing 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) in 0.01M phosphate buffered saline (PBS). The perfused animals were left for

1 h prior to dissection of brains, which were then post-fixed overnight in the 4% paraformaldehyde. The following day, the brains were transferred to a 4% paraformaldehyde / 30% sucrose solution for 2-3 days for cryoprotection. The brains were blocked using a clean razor blade in the same manner as for slice physiology and were then sectioned at 50 μ m using a cryostat (Leica Microsystems, Buffalo Grove, IL).

The sections were then rinsed 4 times in 0.01M PBS, then placed in a solution containing 0.3% Triton X-100 in 0.01M PBS for 1 h. The sections were transferred to 10% normal goat serum (Vector Labs, Burlingame, CA) and 0.5% Triton X-100 in 0.01 M PBS for 1 h in order to block non-specific binding of the primary antibody. Sections were then incubated at 4°C for 24 h in a primary antibody solution for the nicotinic acetylcholine receptor subunit (α 7 or β 2) (α 7(1:200): Abcam #AB23832; β 2(1:1000): Millipore #AB15325). The next day, sections were rinsed in 0.01M PBS and incubated for 2 h with an Alexa 568 fluorescently labeled secondary antibody. These sections were then rinsed in 0.01M PBS, mounted on gelatinized slides and coverslipped with Vectashield anti-fade medium with DAPI to non-specifically stain cell bodies (Vector Labs).

Results

In order to assess whether nicotine affected the functional topography of thalamocortical projections, we prepared *in vitro* slice preparations containing the intact thalamocortical connections in the auditory and somatosensory systems [18,19,25]. Neurons were recorded in layer 4 of either the primary somatosensory barrel cortex (S1) or the primary auditory cortex (A1) respectively using whole-cell patch clamp to measure evoked postsynaptic currents in voltage-clamp mode [25,26]. We characterized the functional thalamocortical topography of regular-spiking neurons in layer 4 of each sensory cortical area (A1: n=9; S1: n=7) (average resting potentials: -64.9 ± 5.2 mV; average input resistance: 258.3 ± 102.0 M Ω) that were connected to the thalamus in the relevant thalamocortical slice preparations.

In order to first map the functional thalamocortical topography of projections to the recorded layer 4 neurons, a biologically inert form of glutamate, NI-caged glutamate, was added to the recirculating ACSF bath [27,29]. A pulsed-UV laser was then used to focally ‘uncage’ glutamate and stimulate thalamic neurons within local (<50 μ m) regions of the slice. In these experiments, we used a 16×16 stimulation grid with 80 μ m spacing between stimulation sites (~ 1.6 mm²) to survey the potential thalamic areas innervating the layer 4 cortical neuron.

Consistent with our prior studies, we found robust functional connectivity between the thalamus and cortex in both the somatosensory (not shown) and auditory (Fig. 1A) thalamocortical slice preparations [25,26]. In the case of the somatosensory thalamocortical slice, EPSCs were elicited from focal regions in the ventroposterior medial nucleus (not shown). In the auditory thalamocortical slice, EPSCs were elicited from the medial geniculate body (Fig. 1A-C). The peak amplitudes (Figs. 1B) and latencies (Figs. 1C) were also consistent with our prior examinations of these pathways [25,26].

We then tested whether nicotine could modulate the organization of these functional thalamocortical topographies. As a first test, following the initial mapping described above, we then added nicotine (1 μ M) to the recirculating caged glutamate bath and then subsequently remapped the photostimulation-evoked thalamocortical responses following a 5 min equilibration interval. Interestingly, we observed that the thalamic regions capable of eliciting EPSCs increased following bath application of nicotine (Figs. 1D), which was not observed when the non-specific nicotinic antagonist, mecamylamine (10 μ M), was added prior to the nicotine. Moreover, these alterations to functional thalamocortical topography were also found when nicotine was locally applied near the recording electrode in the cortex using a pipette containing nicotine (1 μ M) in ACSF, however these changes were not found with local nicotine application in the thalamus. On average, nicotine-mediated changes to these functional thalamic areas was similar in both the auditory and somatosensory TC systems, increasing from $39,680 \pm 11,448 \mu\text{m}^2$ to $80,640 \pm 16,063 \mu\text{m}^2$ ($p < 0.01$, paired t-test) (Figs. 1D). In addition, average peak amplitudes increased from $172 \pm 150 \text{ pA}$ to $270 \pm 204 \text{ pA}$ ($p < 0.05$, paired t-test) (Figs. 1E) and average latencies increased from $28 \pm 12 \text{ ms}$ to $40 \pm 15 \text{ ms}$ ($p < 0.05$, paired t-test) (Figs. 1F).

In addition, we assessed whether distributions of nicotinic acetylcholine receptors (nAChRs) corresponded to these physiological results. Therefore, we immunohistochemically examined thalamic and cortical regions for nicotinic acetylcholine receptor subtypes in the same auditory and somatosensory thalamocortical slice planes used for our physiological studies. We found a unique distribution of the nicotinic acetylcholine receptor subtype $\beta 2$ in discrete puncta that were localized to the upper cortical layers of S1 (not shown) and A1 (Fig. 2A, B). These puncta were found primarily juxtaposed next to non-inhibitory neurons (Fig. 2B: red puncta and blue cells). Interestingly, this receptor subtype was not expressed in the corresponding somatosensory and auditory thalamic nuclei (Fig. 2C). In contrast, we found that the nicotinic acetylcholine receptor subtype $\alpha 7$ was distributed across cortical lamina (not shown) [30,31].

Discussion

We found that the thalamocortical projections form highly topographic functional connections in our slice preparation revealed by laser-scanning photostimulation via uncaging of glutamate (LSPS). These results are in agreement with previous studies indicating robust connectivity between the thalamus and cortex in the auditory and somatosensory thalamocortical slice [18,19,25,26]. However, our current results demonstrate that the thalamic regions eliciting photostimulation-evoked EPSCs from layer 4 neurons expanded in the presence of bath- and locally-applied nicotine in the cortex. Moreover, the average thalamic photostimulation-evoked EPSCs also exhibited increases in average response amplitudes and slight increases in average response latencies. These alterations persisted when nicotine was applied locally near the recording electrode in the cortex, but not when applied in the thalamus. However, we did not assess whether these changes persisted following nicotine removal, due to technical constraints with maintaining the caged glutamate in the recirculating bath. These physiological findings correlate with the neuroanatomical distribution of the nAChR $\beta 2$ subtypes in the cortex, which were not observed in the thalamic nuclei, similar to prior reports [30,31].

The general finding of nicotinic alterations to thalamocortical responsiveness also matches prior reports of such synaptic changes to cholinergic agonists [17,32-34]. In their study, Gil et al. [17] demonstrated a nicotinic enhancement of evoked thalamic EPSPs in the somatosensory thalamocortical slice, similar to the enhancement of EPSCs that we found. Our results extend those findings to include the auditory thalamocortical system and further demonstrate a functional topographic expansion of enhanced thalamocortical connections in these two sensory systems. Moreover, the results of Gil et al. [17] suggest that these nicotinic synaptic enhancements were restricted to thalamocortical synapses and did not affect intracortical synapses. Again, our findings are consistent with those results, although we did not directly examine alterations to intracortical functional topographies, which remain for future studies.

Our results suggest that activation of cortical nAChR strengthens thalamocortical synaptic responses, potentially by enhancing previously weak or silent synapses from heterotopic thalamic territories [17,35,36]. Such cholinergic modifications of functional thalamocortical circuitry may account for the developmental plasticity of *in vivo* physiological properties and provide possible neural substrates for learning and strengthening of remapped sensory parameters [23,24,37-39]. Alterations in this system during development have been linked with behavioral changes, such as hyperactivity and impaired spatial learning [40-42]. Moreover, these results suggest a possible mechanism for the plastic reorganization of thalamocortical synaptic responses via nAChR mediated enhancement of weak synapses, coupled with correlated strengthening of unmasked inputs, which may also include those arising from other cortical and subcortical sources [17,35,36,43].

The current study focused particularly on the role of nicotinic modulation of functional thalamocortical topography in the developing cortex. However, the potential role of other cholinergic receptors, i.e. muscarinic receptors, in the forebrain has been appreciated [44,45]. For instance, Zhang et al. [44] have demonstrated an important role for M1 muscarinic receptors in the developmental establishment of orderly tonotopic maps in the mouse. It is therefore plausible that muscarinic activation also may affect functional thalamocortical topography, but likely in a different manner consistent with its differential synaptic effects [17]. Thus, it remains for future studies to assess the differential role of this and other neuromodulators on shaping functional forebrain topographies.

Conclusion

Activation of nicotine acetylcholine receptors is important for regulating the plasticity of synapses in the forebrain. Prior studies have demonstrated aspects of the synaptic physiological changes that arise in forebrain connections in response to nicotinic activation. Here, we have demonstrated a substantial expansion of the functional topography of thalamocortical connections in response to nicotinic receptor activation in the developing cerebral cortex. These results suggest a potential plastic mechanism for nicotinic sculpting of thalamocortical circuitry.

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Figure 1.

Cholinergic alterations to functional topography in the auditory thalamocortical slice. **A.** Whole-cell patch clamp recorded responses from a layer 4 neuron in the primary auditory cortex (A1) following laser-scanning photostimulation (LSPS) of the auditory thalamus, medial geniculate body (MGB). Panels A and D depict photomicrographs of the MGB with recorded traces from the layer 4 neuron superimposed on the spatial location in the thalamus that elicited the response following LSPS. Recordings in voltage clamp mode. Downward deflections in the traces indicate excitatory postsynaptic current (EPSCs) responses. **B.** The peak amplitude of the EPSCs in A are plotted, with hot colors indicating bigger amplitudes, as indicated by the scale bar. **C.** The onset response latencies of the EPSCs in A are plotted, with cooler colors indicating longer latencies, as indicated by the scale bar. **D.** Bath application of nicotine results in alterations to evoked responses. The same thalamic region is photostimulated again following application of nicotine to the bath and changes to the peak amplitudes and latencies were observed. Compare traces at similar grid locations with those in panel A. **E.** Plot of peak responses in D, in the same manner as described for panel B. **F.** Plot of onset latencies in D, in the same manner as described for panel C. Figure abbreviations: Hip, hippocampus; L, lateral; MGB, medial geniculate body; R, rostral.

Figure 2. Cortical and thalamic expression of nicotinic acetylcholine receptor (nAChR) subtype $\beta 2$. **A.** Laminar expression of nAChR $\beta 2$ (red puncta) in the primary auditory cortex (A1), relative to VGAT-positive inhibitory neurons (green cells). Photomontage of A1 labeling following immunohistochemistry for nAChR $\beta 2$ (red puncta) and DAPI staining (blue cells) in a VGAT-Venus transgenic mouse that expresses Venus fluorescent protein in inhibitory neurons (green cells). **B.** Terminals positive for nAChR $\beta 2$ (red puncta) terminate on cell bodies that are primarily non-inhibitory (blue cells), while inhibitory neurons (green cells) have completely no puncta juxtaposed. Higher magnification view of upper cortical layers in panel A. **C.** Thalamic expression of nAChR $\beta 2$ is completely absent. Figure abbreviations: C, caudal; Hip, hippocampus; L, lateral; MGB medial geniculate body.