Specific and nonspecific thalamocortical connectivity in the auditory and somatosensory thalamocortical slices

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Two classes of thalamic nuclei project to either middle layers or upper layers, including layer 1, of the neocortex, and are referred to as 'specific' and 'nonspecific' nuclei, respectively. The electrophysiological properties of the nonspecific nuclei have not been investigated, largely because of the paucity of *in vitro* slice preparations containing intact nonspecific pathways. In this study, we used flavoprotein autofluorescence imaging to show intact thalamocortical connectivity of nonspecific nuclei in slice preparations of the somatosensory and auditory systems. These preparations will enable the elucidation of electrophysiological properties of nonspecific pathways. *NeuroReport* **21:861–864 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Thalamocortical projections are subdivided into 'specific' and 'nonspecific' pathways, primarily targeting either middle or upper layers of cortex, respectively [1–3]. Specific pathways project topographically [4,5] and include thalamic nuclei that bring primary sensory information to somatosensory, visual and auditory cortices. Nonspecific projections, as their name implies, are more diffuse and less topographic. Although the specific pathways have been extensively investigated, the non-specific projections have received less attention, due in part to the lack of adequate experimental preparations.

Connectivity between primary thalamic and cortical regions has been shown in two commonly utilized thalamocortical slices: the somatosensory slice [6] and the primary auditory slice [7]. In the established somatosensory slice, thalamocortical connections from the ventroposterior nucleus of the thalamus (VP) and the posterior medial nucleus of the thalamus (POm) to somatosensory cortex are well characterized [6,8]. In earlier described auditory slice preparations, connections from both the MGBv and dorsal medial geniculate body (MGBd) of the thalamus to auditory cortex have been elucidated [7,8].

A recent modification of the somatosensory slice, which contains intact projections from the central lateral nucleus of the thalamus (CL) to S1, a non-specific projection, along with connections from VP to S1, has enabled the investigation of specific/nonspecific interactions in the somatosensory system [3]. Interestingly, the ventromedial nucleus of the thalamus (VM) also projects to upper layers of S1 and contain many nonspecific thalamocortical projection neurons, but it is unknown whether it is also connected to cortex in this slice. A related question in the auditory thalamocortical slice preparation is whether the medial division of the MGB (MGBm), a nucleus containing a high percentage of non-specific thalamocortical projection neurons, is connected to cortex. Such connectivity would enable the simultaneous exploration of the effects of specific and nonspecific thalamic nuclei on cortical activity in two sensory modalities and across two nonspecific relays in a single modality.

Here, using flavoprotein autofluorescence (FA) imaging, a technique recently adapted for use *in vitro* [9,10], we show that VM is connected to S1 in the modified somatosensory slice and that MGBm is connected to A1 in the standard auditory thalamocortical slice, expanding the experimental repertoire in these slice preparations.

Methods

Slice preparation

All animal procedures followed the animal care guidelines of the University of Chicago. After deeply anesthetizing the mice (*mus musculus*, BALB/C, postnatal day 13–23) with 1 mg/g ketamine (Vetaket; Phoenix Scientific, St Joseph, Missouri, USA) and 10 mg/g xylazine (AnaSed; Lloyd Laboratories, Shenandoah, Iowa, USA) and verifying that the hindlimb reflex was absent, they were perfused with cold, oxygenated sucrose-based slicing solution, composed of, in mM: 234 sucrose, 11 glucose, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4*H_2O, 10 $MgCl_2$*$6H_2O$, 0.5 $CaCl_2$*$2H_2O$. After decapitation and brain removal, tissue slices (500 μm) were cut using the techniques described earlier in a plane that maintained thalamocortical and corticothalamic connectivity in the somatosensory and auditory systems (for further details see [3,7,8,10,11]). Slices were stored in room temperature, oxygenated low calcium artificial cerebrospinal fluid, composed of (in mM): 26 $NaHCO_3$, 2.5 KCl, 10 glucose, 126 NaCl, 1.25 NaH_2PO_4*H_2O, 3 $MgCl_2$*$6H_2O$, and 1 $CaCl_2$*$2H_2O$. Experiments were

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performed on a Zeiss Axioskop FS 2 Plus (Carl Zeiss, Jena, Germany) in standard artificial cerebrospinal fluid, composed of (in mM): 26 $NaHCO_3$, 2.5 KCl, 10 glucose, 126 NaCl, 1.25 NaH_2PO_4*H_2O, 2 $MgCl_2$*$6H_2O$, and 2 $CaCl_2$*$2H_2O$.

Stimulation methods

Thalamic activation was elicited from the slice using both electrical stimulation and laser scanning photostimulation (LSPS) [10,12–14]. Although electrical stimulation yields robust FA images, LSPS only induces action potentials in cell bodies, and not axons, ensuring that remote activation results only from neurons at the stimulation site. LSPS was achieved using a computer-controlled UV Laser (355 nm; 5 laser pulses; 5 ms width at 20 Hz) to focally release 'caged' glutamate in the thalamus. Laser power at the slice was approximately 60 mW. For further details of the LSPS configuration, see [10–14].

For electrical stimulation experiments, standard concentric bipolar stimulating electrodes were used to deliver trains of stimuli (pulse width 10 ms, 20 Hz, 0.5–1 s duration).

Imaging

Metabolic activity was measured by capturing green light (~510–540 nm) generated by mitochondrial flavoproteins, most prominently flavin mononucleotide and flavin adenine dinucleotide, in the presence of blue light (~450–490 nm; see [9,15] for further details) with a high-sensitivity camera (Retiga-SRV, Qimaging). We earlier showed that deviation in the signal correlates to the degree of postsynaptic activity (see [10] for more details and a demonstration of some capabilities of FA imaging). Optical recordings were taken during 14 s runs, with stimuli lasting for 1000 ms. Image exposure time ranged from 80 to 150 ms. All images were taken at 2.5× magnification and processed using programs generated in-house made to run on Matlab.

Results

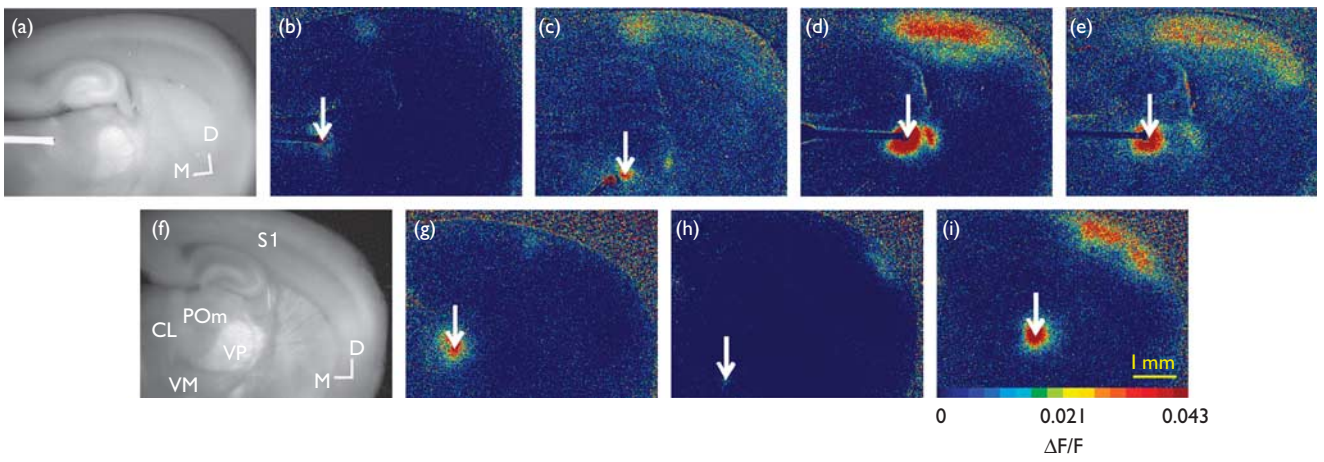
Somatosensory thalamocortical slice

We assessed connectivity in the modified somatosensory thalamocortical slice using both electrical stimulation and photostimulation. Results, shown in Fig. 1, show thalamocortical connectivity from VM, CL, POm, and VP to cortex. Trains of stimuli were used to activate each of the nuclei, which led to robust stimulus sites in both VP and VM (Figs. 1c and d), but a very weak stimulation site in CL (Fig. 1b). Cortical activation was both focal and subtle, and ΔF/F values rose above 0.75% in response to the stimulus train. This is the first demonstration that VM, a thalamic nucleus containing many nonspecific thalamic relay cells, is connected to cortex in this slice. We saw this connectivity in slices from six animals, out of seven tested. We used electrical stimulation in four animals, and photostimulation in three animals, one of which overlapped with an electrical stimulation experiment.

Auditory thalamocortical slice

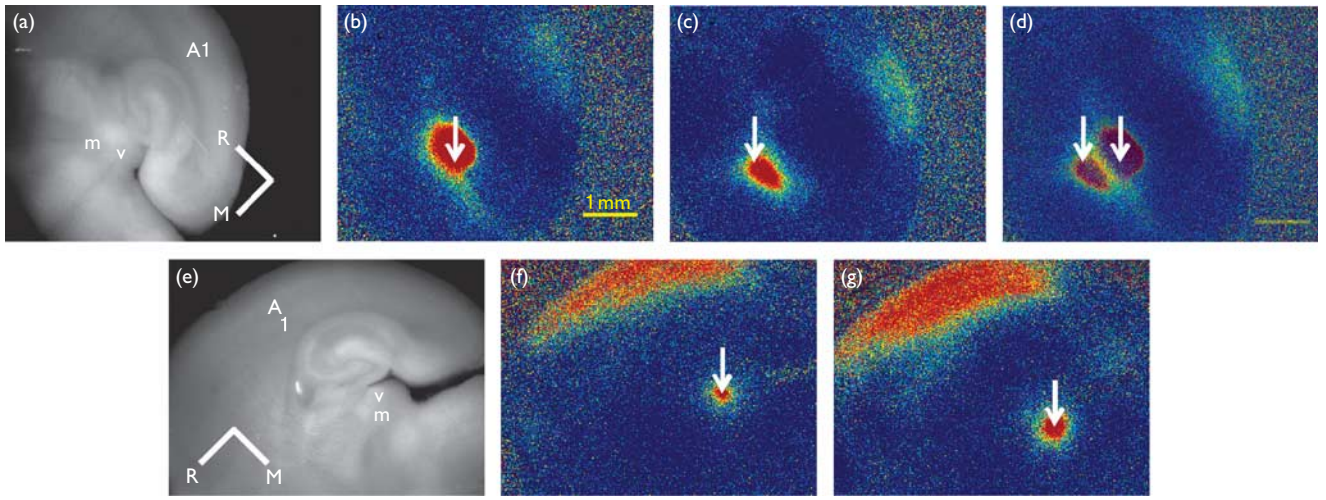
We also assessed connectivity in the auditory thalamocortical slice using both electrical stimulation and photostimulation. Results, shown in Fig. 2, show thalamocortical

Fig. 1



Connectivity in the modified somatosensory thalamocortical slice preparation. (a) Raw flavoprotein autofluorescence (FA) image for anatomical reference in electrical experiments (b–e). (b) Electrical stimulation of central lateral nucleus of the thalamus (CL) and resulting cortical activation. (c) Electrical stimulation in ventromedial nucleus of the thalamus (VM) and resulting cortical activation. (d) Electrical stimulation in ventroposterior nucleus of the thalamus (VP) and cortical activation. (e) Posterior medial nucleus of the thalamus (POm) electrical stimulation and cortical activation. (f) Raw FA image for anatomical reference in photostimulation experiments (g–i). (g) Cortical response to photostimulation of CL. (h) Photostimulation of VM and resultant activity in S1. Arrow indicates stimulation site here and in all other imaging figures. (i) Cortical response to photostimulation in VP. Arrows in the lower right inset of (a and f) are for orientation: D, Dorsal; M, Medial. Color bar at bottom of (i) represents values of ΔF/F corresponding to colors in panels (b–e and g–i). Color bar also applies to all FA color map images in Fig. 2.

Fig. 2



Connectivity in the auditory thalamocortical slice preparation. (a) Electrical stimulation of MGBm and flavoprotein autofluorescence (FA) response in auditory cortex. (b) Electrical stimulation in MGBv and cortical response. (c) Raw FA image for anatomical reference. (d) Overlay of (a and b). Note the lack of overlap between stimulation sites and limitation of activation to the borders of the subnuclei. (e) Raw FA image for anatomical references for panels (f and g). (f) Cortical response to MGBv photostimulation. (g) Cortical response to photostimulation in MGBm. See Fig. 1 for colormap. In (a and e): m, MGBm; M, Medial; R, Rostral; v, MGBv. See Fig. 1 for FA colorbar and corresponding $\Delta F/F$ values. Single arrows in (b, c, f and g) correspond to stimulation sites.

connectivity from MGBv to cortex, which is the 'specific' thalamic nucleus in the auditory system, as well as from MGBm, the 'nonspecific' thalamic nucleus in the auditory system, to cortex. $\Delta F/F$ values reached $> 4\%$ after both MGBv and MGBm photostimulation (Figs. 2f and g, suggesting a robust slice for both projections. Electrical stimulation trains (see Methods section) in another slice from a different animal elicited less robust activation of cortex, though levels reached $> 2\%$ $\Delta F/F$ in maximally activated areas (Figs. 2b and c). Note that the stimulation sites during electrical stimulation remained confined to MGBm and MGBv. MGBm stimulation elicited cortical activation in four out of four auditory thalamocortical slices. We used electrical stimulation in slices from four animals, as well as photostimulation in two of these slices.

Discussion

Here, we showed that two thalamocortical slice preparations from different sensory systems contain both specific and nonspecific thalamocortical pathways. Nonspecific projections, from VM and CL, to cortex are maintained in a modified version of the somatosensory slice [3] along with projections from first-order and higher-order specific thalamic nuclei, VP and POm, to cortex. We have also showed that MGBm projections to cortex are intact in the standard auditory thalamocortical slice preparation [7]. Interactions between specific and nonspecific thalamocortical circuits are thus viable in these slice preparations, enabling the comparative study of interactions among VM, POm, CL and VP thalamocortical circuitry in the somatosensory slice and MGBv and MGBm projections to cortex in the auditory slice. This provides a powerful tool for future elucidation of the physiological interactions of these pathways at their cortical targets.

Conclusion

The potential interactions between specific and nonspecific pathways are particularly intriguing, since the role of layer 1-projecting thalamocortical nuclei is unknown. The apical dendrites of corticothalamic pyramidal cells in layer 5 extend into layer 1, facilitating possible interactions with the robust corticothalamocortical circuit. Furthermore, layer 5 pyramidal cells exhibit a strong form of coincidence detection involving the layer 1 projecting thalamocortical neurons and cortical circuitry that is closely related to specific thalamocortical input [3,16]. The utility of these slices containing both systems can qualitatively elucidate whether coincidence detection is used for these two projections and quantitatively determine the relationship between the timing of inputs and the degree of coincidence detection.

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The authors report no conflicts of interest.

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