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OPTICAL MAPPING OF THE MOUSE CORTEX IN RESPONSE TO BLADDER STIMULATION

Hypothesis / aims of study: At present, voltage-sensitive dyes (VSDs) offer the highest spatial (0.1 μm) and temporal (0.1 ms) resolution for imaging cortical functions in animal studies [1]. Although this technique has been extensively used to investigate visual, auditory and somatic sensory systems at the cortical level, no studies have yet been published on visceral afferent system, including those from the bladder and lower urinary tract (LUT). In this study, we use the VSD, RH1691, to demonstrate the activity in the cortex in response to electrical stimulation of the bladder. This is a new dye which has an excitation wavelength that does not overlap with that of hemoglobin and therefore is unaffected by cortical blood flow.

Study design, materials and methods: C57Bl10 mice were anesthetized with 1.2 g/kg urethane. A catheter (PE-50) was then inserted through the dome of the bladder and connected to a transducer and syringe pump. Stimulation electrodes were next glued to the base of the bladder and the animal was secured in a stereotaxic frame. The crown of the skull was exposed and the boney calvarium and the dura were removed very carefully to expose a large area of right cortical hemisphere (Figure 1B, C; *permission for displaying the animal figure obtained*). A small plastic o-ring was glued to the skin overlaying the exposed cortex.

RH1691 (2 mg/ml) was dissolved in an artificial CSF solution along with the surfactant, Pluronic 127 (2%), followed by staining for 1 hour. After unabsorbed dye was removed, mineral oil was applied on top of the cortex. Action potentials were recorded using a custom-built dual-photodiode array system. Light from a 100 Watt tungsten-halogen lamp was passed through an excitation filter (630 ± 30 nm) and focused onto the cortical surface. Fluorescence emission was passed through a dichroic mirror (695 nm long pass) and directed to a 16x16 photodiode array where, at the magnification used, each diode detected light from a 560 x 560 μm area. The signal : noise ratio is ~ 200 : 1.

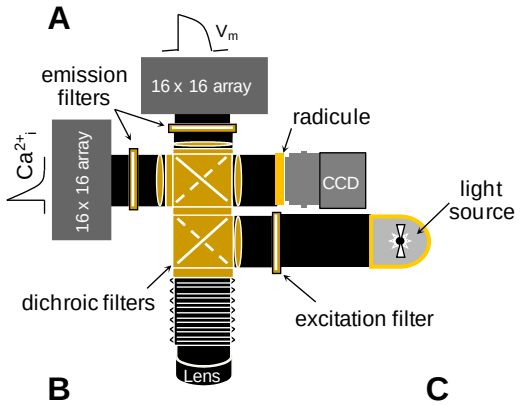
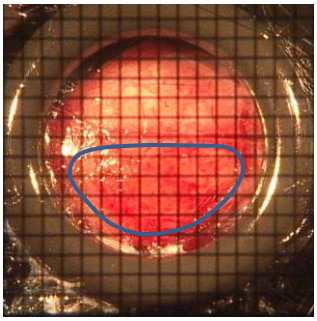
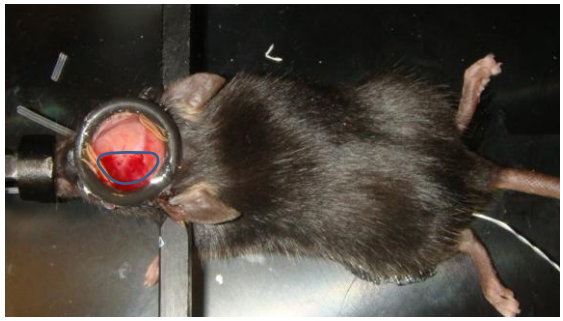


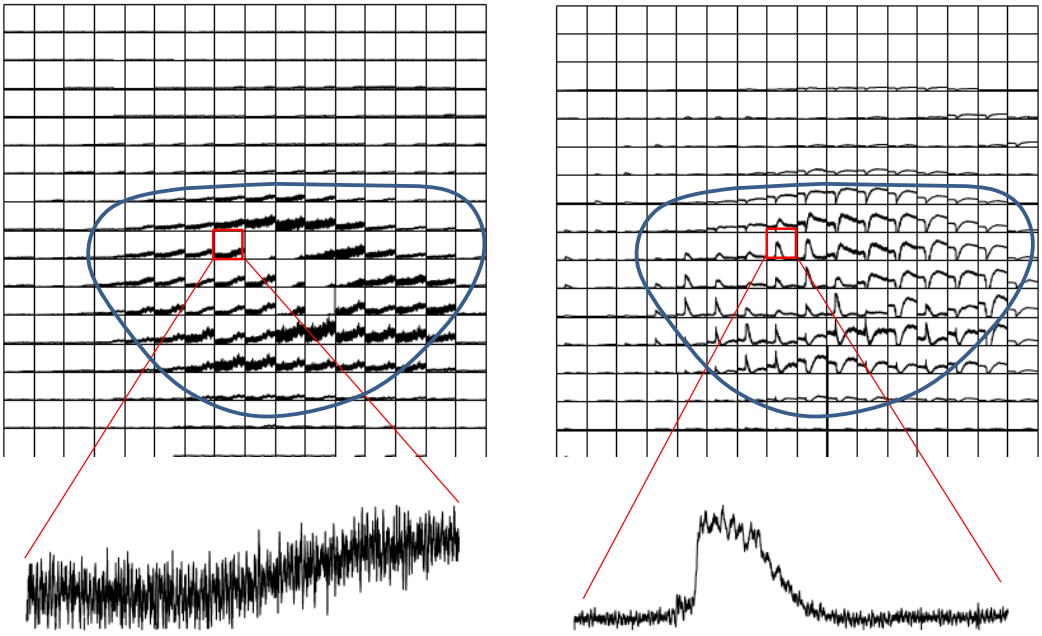
Figure 1





Results: Figure 2B shows action potentials recorded from the cortex during bladder stimulation (single train 3 sec, 0.001 sec, 100 Hz, 3.7 V) as compared to control (Figure 2A). Note: the baseline cortical activity in the control is equivalent in amplitude to the baseline in the stimulated recoding. The stimulation caused the bladder to contract and generate a pressure 30 to 40 cmH_2O (n=6). These trace maps show the spread of action potentials over the cortex, from which their conduction delays can be determined using a second derivative or cross-correlation analysis approach. In this way, isochronal maps can be generated to identify initiation sites and the spread of activity (not shown). The signal appearing at the time of bladder stimulation could be blocked by lidocaine applied to the cortex surface (not shown).

A control **Figure 2** **B** stim, 3.7 V



Interpretation of results: Our results demonstrate that VSDs and optical mapping can be used to study the sensory input to the cortex from the LUT. Using this technique we have shown the cortical response to bladder stimulation in a control mouse. Inhibition of this signal by lidocaine applied to the cortex surface demonstrated the origin of the signal to be in the cortex. Ongoing studies are characterizing these responses in mice with neurogenic detrusor overactivity.

Concluding message: Optical imaging with VSDs allows the visualization of the cortical location and distribution (spatial resolution) of neural activity, as well as the timing of the sequential activation of neurons across the cortical surface (temporal resolution). These studies have a potential to provide valuable information concerning the changes that occur in processing of LUT sensory information by the cortex in a variety of animal models of LUT dysfunction.

References

1. Grinvald A and Hildesheim R. Nat Rev Neurosc. 5: 874-85, 2004

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institutional Animal Care and Use Committee of University of Pittsburgh**