Listening to Cell Membrane Potential: a new diagnostic and interventional ultrasound imaging approach

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Neuro-transmitter (NT) and electrophysiological change

- Action potential: clue of NT event
  - msec time scale
  - ~70-40 mV voltage change

1. Synthesis of the neurotransmitter: This can take place in the cell body, in the axon, or in the axon terminals.
2. Storage of the neurotransmitter in storage granules or vesicles in the axon terminals.
3. Calcium enters the axon terminal during an action potential, causing release of the neurotransmitter into the synaptic cleft.
4. After its release, the transmitter binds to and activates a receptor in the postsynaptic membrane.
5. The neurotransmitter is either destroyed enzymatically, or taken back into the terminal from which it came, where it can be reused, or degraded and removed.

Functional neuro-sensing

**Functional neuro-imaging**

Optical imaging, e.g., two-photon microscopy, shallow imaging depth due to diffraction (several mm), necessitates craniotomy to have sufficient sensitivity.

PET: low temporal resolution (> 40 ms).

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**Photoacoustic effect**

- **Equation of state**
  -Temperature $T$, density $\rho$, pressure $p$
  -In fluid with homogeneous thermodynamic property:
    - $\rho_c = \frac{1}{\rho} = \frac{1}{\frac{\partial p}{\partial T}}$
    - $\kappa = \frac{1}{\rho c_v}$: isothermal compressibility
    - $\beta_T = \frac{1}{\rho c_v}$: volume thermal expansivity

- **Heat equation**
  - $\mu \frac{\partial C}{\partial T} - p(T) + \frac{H(x,t)}{\rho}$
  - $\mu$: thermal conductivity [W/(m K)]
  - $C$: heat capacity at constant volume [J/(kg K)]
  - $H(x,t)$: Absorbed energy density [J/m$^3$]
  - $\frac{1}{\rho}$: temporal pulse shape function with unit integral [s]

Equation of state

$$\frac{\partial p}{\partial T} = -\kappa \rho_c$$

Heat equation

$$\mu \frac{\partial C}{\partial T} = p(T) + \frac{H(x,t)}{\rho}$$

Stress confinement (~ns)

$$\frac{\partial p}{\partial T} = -\kappa \rho_c$$

Isochoric condition (fixed volume)

$$\frac{\partial p}{\partial T} = -\kappa \rho_c$$

Isothermal condition (heat volume)

$$\mu \frac{\partial C}{\partial T} = p(T) + \frac{H(x,t)}{\rho}$$

Thermal confinement (~$\mu$s)

$$\frac{\partial p}{\partial T} = -\kappa \rho_c$$

Heat delivery faster than conductive heat diffusion

$$\frac{\partial p}{\partial T} = -\kappa \rho_c$$

Integral over short pulse duration

$$\mu \frac{\partial C}{\partial T} = p(T) + \frac{H(x,t)}{\rho}$$
Towards sensing electrophysiological activity in deep brain

- Objective: real-time, transcranial photoacoustic (PA) sensing of electrophysiological brain activity at deep rat brain in vivo
- Voltage-sensing mechanism using near-infrared cyanine dye

• Cyanine dye with positive polarity is attracted into cell membrane
• The aggregation of VSD leads to fluorescence (FL) quenching, which increases PA generation efficiency


Towards sensing electrophysiological activity in deep brain

- Objective: real-time, transcranial photoacoustic (PA) sensing of electrophysiological brain activity at deep rat brain in vivo
- Voltage-sensing mechanism using near-infrared cyanine dye

• Dispersion of VSD gives high FL efficiency


VSD characterization using artificial membrane potential model

- Artificial membrane diffusion potential model


Soybean-lipid membrane

IR780

K

Na

A

B

P<0.047

P<0.044

In vivo VSD characterization (6µM). (A) Absorbance and fluorescence emission spectrum of near-infrared VSD. (B) Photoacoustic spectrum and intensity change at the 760 nm of peak absorbance.


**In vivo experimental setup**

A. Male Sprague Dawley rats (275-390g) stereotaxic fixation, jugular vein catheter

B. IR780 normalized absorbance

C. SS5 SCV L-NC R-NC

**In vivo experimental protocol**

Lexiscan ▼ VSD ▼ Pentylenetetrazol (PTZ)

DAQ

The tonic-clonic movements in the fore and hind limbs of the anesthetized rat

Lexiscan ▼ VSD ▼ PTZ ▼ PTZ-

Seizure (N=2)

Lexiscan ▼ VSD ▼ PTZ +

Seizure (N=2)

**In vivo photoacoustic VSD imaging**

A. Baseline, Seizure, Seizure control, VSD control

B. Fractional PA amplitude change 0% - 2.5%
In vivo photoacoustic VSD imaging

Visual cortex stimulation and monitoring

Visual cortex stimulation and monitoring
From Brain to Prostate

Baseline (VSD+, PTZ-)

Seizure (VSD+, PTZ+)

Need for nerve guidance during peeling out procedure of fascia

Proposed nerve-guided robot-assisted laparoscopic prostatectomy

Step 1: Robotic tool approach through the ports on the abdominal incisions.
Step 2: Direct VSD staining of a prostate tissue through the abdominal incision port.
Step 3: Flushing out of the VSD on the prostate surface which is not bound at tissue membrane.
Step 4: Stimulation on nerves in the surgical region of interest, and
Step 5: Nerve-sparing prostatectomy with the augmented nerve map
Available time for VSD staining

<table>
<thead>
<tr>
<th>Procedure Description</th>
<th>Time</th>
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<tbody>
<tr>
<td>Dissection of colon adhesions</td>
<td>~0:31</td>
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<tr>
<td>Posterior approach with dissection of the seminal vesicles</td>
<td>~2:47</td>
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<tr>
<td>Dissection of the anterior abdominal wall</td>
<td>~10:50</td>
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<tr>
<td>Opening of the endopelvic fascia and dissection of the periprostatic fat</td>
<td>~8-10 min</td>
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<tr>
<td>Suture of the dorsal venous complex</td>
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<td>Preservation of neurovascular bundles during left sided dissection</td>
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<tr>
<td>Dissection of the left posterior pedicle</td>
<td>~33:20</td>
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<tr>
<td>Apical and urethral dissection</td>
<td>~49:01</td>
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<tr>
<td>Evaluation of nerve sparing with the ProPep electrodes</td>
<td>~54:14</td>
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<tr>
<td>Vesicourethral anastomosis</td>
<td>~55:12</td>
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<tr>
<td>Surgery ended</td>
<td>~1:07</td>
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</tbody>
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In vivo experimental protocol

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Procedure</th>
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<tbody>
<tr>
<td>0 min</td>
<td>Direct VSD administration</td>
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<tr>
<td>1 min</td>
<td>Flushing with saline solution</td>
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<tr>
<td>1 min</td>
<td>Electrode stimulation</td>
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<td>3 min</td>
<td>Post-stimulation</td>
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<tr>
<td>10 min</td>
<td>Time point completing exposure of prostate capsule with periprostatic fascia</td>
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Fluorescence recording (500 ms exposure, ~2 fps)

1-10 min 1 mM IR780 solution with DMSO and Cremaphor

10 min 5 min Saline solution

In vivo experimental setup

Pr: prostate; Pn: penis; RCN: right cavernous nerve; RCC: right corpus cavernosum

ICP: intracavernos pressure
Validation of erectile stimulation

Electrical stimulating electrodes
Cannula for ICP measurement

Real-time prostate nerve mapping
in vivo

Time-averaged F/F₀ trace

Right cavernous nerve
MPG: major pelvic ganglion
Bipolar electrodes

Fractional change in fluorescence intensity [%]

RCN: right cavernous nerve;
MPG: major pelvic ganglion

Real-time prostate nerve mapping
in vivo (2nd attempt)

Preliminary in vivo results on nerve localization on rat prostate:

(A) White light and FL images;
(B) Evolution of FL intensity during stimulation. The gradual decrease is due to photo-bleaching;
(C) Subtracted images between indicators, and its fusion on FL images.
Histological validation of direct VSD administration

- Confirmed direct staining procedures can deliver VSD > 2-mm deep in prostate

Discussion

- We presented the preliminary results of real-time nerve guidance using dual-modal VSD and intra-operative FL imaging

- Our further works will be focused on
  - Developing pulsed laser-based dual-modal intra-operative guidance
  - Integrating non-invasive ultrasound neuromodulation
Discussion

- We presented preliminary results of real-time nerve guidance using dual-modal VSD and intra-operative FL imaging

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  - Constructing control group with CN block

Thank you
Quantitative comparison

- Normalized neuro-activity index (projected during 2-10 min)

### Table

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<thead>
<tr>
<th>Seizure (Seizure)</th>
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<th>VSD control (VSD, Post)</th>
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<td>VSD absent, PTSD</td>
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Photoacoustic effect

- Equations and diagrams related to stress confinement and heat delivery.
**Clinical applications**

- Brain imaging
- Ocular imaging
- Gastrointestinal tract
- Cardiovascular system
- Ovarian / Prostate cancers
- Breast cancer
- Skin disease imaging
- Bone/joint disease


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**EEG confirmation of in vivo protocol**

- EEG confirmation of seizure induction using PTZ administration

Evolution of EEG signal in the in vivo protocol identical to transcranial PA imaging:

(A) Representative EEG traces recorded from rat motor cortex before and during induction of status epilepticus using chemoconvulsant PTZ. (B) EEG spectral quantitation of the EEG recording done every 10 sec epoch during the EEG showed the expected progressive rise in EEG power associated with evolution of the PTZ induced status epilepticus.

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**Validation of important assumptions**

- Pharmacological treatment for BBB opening
  - Blood-brain barrier (BBB): semipermeable membrane barrier separating the circulating blood from the brain in the central nervous system (CNS).
  - Regadenoson (i.e., Lexiscan) can modulate adenosine receptor signaling to enhance the permeability of VSD through BBB.

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Validation of Important assumptions

- Robustness on VSD interference on neuro-activity