Neuronal Stimulation by Acoustic Fields

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Introduction
Hydrogen Bonds Form Protein Structures

α Helix secondary structure

hydrogen (electrostatic) bond

courtesy U Penn
Heat Denatures Protein

H bonds break at $\frac{1}{2}$ eV, destroying 4th, 3rd, 2nd order structures. Example:

ovalbumin in egg white

turns from optically clear to opaque

Kjaergaard 2007

What’s Cooking America
Lossy Acoustic Beam Heats Tissue

$Q \sim I$

$I(x) = I_0 e^{-2\alpha f x}$

- $Q$: heat
- $I$: local beam intensity
- $I_0$: incident intensity
- $f$: frequency
- $\alpha$: attenuation coefficient
- $x$: depth
Heating > 43 °C Denatures Proteins

normal myocardium

ablated

TEM 1 μm
Ablation Invisible to Ultrasound B-mode

1 cm

9 MHz f/3.5 transducer mechanical scan
ex vivo chicken breast

conventional B-mode image

enhanced image reveals HIFU lesion
Acoustic Radiation Force for Lesion Palpation

\[ \frac{F}{V} \sim \alpha f I / c \]

- \( F \): acoustic radiation force
- \( V \): volume
- \( \alpha \): attenuation coefficient
- \( f \): frequency
- \( I \): local beam intensity
- \( c \): speed of sound
Acoustic Field Conserves Linear Momentum

Incident ultrasound beam momentum $q_i$

Reflected momentum $q_r$

Transmitted momentum $q_t$

Net momentum imparted to $V$:

$$q = (q_i - q_t) + q_r$$

Absorption and reflection

Attenuation
Flat Absorbing Target Wattmeter (FDA)

RF source
transducer
beam
water
sponge
balance

0.40 g
Ballistic Pendulum (NPL, UK)

\[ mgh = \frac{1}{2} mv^2 \]
A-mode Imaging of Displacement

idealized backscattered RF signal

acoustic force field can be steady state (oscillatory) or transient (impulse)
Displacement < Cell Diameter

Lizzi, Muratore, Deng, et al. UMB 2003
Induced Cell Transformations

Acoustic field

\[ \downarrow \]

Stress \rightarrow Structural response \rightarrow Functional response

- translation
- rotation
- deformation

displacement \rightarrow cell-cell adhesive triggers
membrane-bound & internal triggers

e.g.,
integrin-mediated response
cytoskeletal response

Giancotti 1999 Science
Ingber 2003 J Cell Sci

continuum mechanics view
Hypothesis

Acoustic field

↓

Stress  ➔  Structural response  ➔  Functional response

brightfield & fluorescence microscopy, ultrasound

e.g., electrical activity (neuronal tissue), enhanced / disrupted activity (many tissues)
Application: Non-invasive Brain Stimulus
	ransducer
ultrasound beam
neuronal stimulus
Methods – Structural Studies
System

Sonocare CST-100 therapy transducer 4.67 MHz

x-y stage

cell culture

μscope

camera

Exposé Out

acquisition PC

Panametrics 5601A/ST T/R pulser/receiver

ENI 2100L RF amplifier

Agilent 33250A waveform generator

PC
Therapy Transducer

Sonocare CST-100
PZT-4 spherical cap
80 mm diameter, 90 mm focal length
f/1.1, 19 mm diameter central hole
4.67 MHz

Panametrics MD3657
A-mode diagnostic transducer, 7.5 MHz

water-filled coupling cone

-3 dB focal region ≈ 0.4 mm x 1.2 mm

Muratore 2005 ISTU
Results – Structural Studies
Translation

PC12 cells at 32 x

30 ms pulse

~100 kPa

100 µm
Deformation
PC12 cells adhered to polystyrene with poly-L-lysine
PC12 cells adhered to polystyrene with poly-L-lysine
PC12 cells adhered to polystyrene with poly-L-lysine
PC12 cells adhered to polystyrene with poly-L-lysine
Methods – Functional Studies
Ultrasonic Subsystem

- multi-axis support armature
- matching network
- PZT spherical cap transducer f/2 4MHz
- water-filled coupling cone
- cell culture dish
Ultrasound Stimulus

peak pressure $\sim 0.1$ MPa per Onda HNA-040 hydrophone

calculated beam profiles
60 channels
For recording:
one is selected as ground
Rat Hippocampal Slice Culture

- O₂-saturated artificial cerebro-spinal fluid control
- coupling cone
- hippocampal slice
- 60 electrode array
- multi-electrode array cassette
Hippocampal Culture in situ

CA$_1$: Cornu Ammonis pyramidal cells

DG: Dentate Gyrus granular cells

1 mm
Results – Functional Studies
Functional Response to Electrical Stimulus

100 μs 100 μA stim

avg 3 sweeps
Functional Response to Ultrasonic Stimulus

- 4.04 MHz
- 0.1 MPa
- 100 μs (to match e-stim)
- (400 cycles/burst)

Amplitude (mV)

Time (ms)

avg 8 sweeps
Post-insonification Response to Electrical Stimulus

100 μs 100 μA stim

still viable!

avg 3 sweeps
Detail of Aggregate Response

- Ultrasonic stimulus at 4.04 MHz ranging from 77 kPa.
- 20-50 μV electrical potential ranging from 0 to 60 μV.
- Time in milliseconds ranging from 0 to 150 ms.
Dentate Gyrus Field Potential (μV pp)

Acoustic Power (W)

Channel 78 sweep 3

138 μV

300 ms
Dose-Response Quartiles:

- DG: similar behavior
- CA1: apparent threshold

Low dose “quiets” system

Possible fatigue
Discussion
Functional Response Mechanism

- Ultrasonic device stimulus
  - Electrical leakage: $R > 50 \, M\Omega$; no measured pulse with agarose; no response with air block
  - Acoustic effect: possible; less likely at high $f$

- Radiation force
  - Heat: hypothesis; observe mechanical deformation under similar conditions
  - Direct: streaming blocked

- Cavitation: possible; less likely at high $f$
Conclusions

Ultrasonic stimulus acting on rat hippocampal culture:

- elicits response similar to electrical stimulus
- does not apparently harm culture
- radiation force is possible mechanism
- exhibits threshold behavior