Four FUS Regimes

High Intensity (Heat)
\[ \Downarrow \]
Ablation
Essential Tremor

300 W/cm^2
10 sec
3000 Joules
23°C Temperature Rise

Low Intensity + microbubbles
\[ \Downarrow \]
BBB opening

1-10 W/cm^2
0.1 sec
~1 Joules
< 1°C Temperature Rise

Low Intensity + drug encaged nanoparticles
\[ \Downarrow \]
Neuromodulation

Low Intensity
\[ \Downarrow \]
Neuromodulation

~ at the FDA limit for Diagnostic Ultrasound
Neuromodulation

- Evidence for at several different spatial scales
- Potential Mechanism
Precise Neural Stimulation in the Retina Using Focused Ultrasound

Michael D. Menz, Ömer Oralkan, Pierre T. Khuri-Yakub, and Stephen A. Baccus

Journal of Neuroscience 6 March 2013, 33 (10) 4550-4560; DOI: https://doi.org/10.1523/JNEUROSCI.3521-12.2013

Freq 43 MHz
Ispta 10 W/cm²
Pressure 0.55 MPa
ΔT ~0.5°C
Precise Neural Stimulation in the Retina Using Focused Ultrasound

Michael D. Menz, Ömer Oralkan, Pierre T. Khuri-Yakub, and Stephen A. Baccus

Journal of Neuroscience 6 March 2013, 33 (10) 4550-4560; DOI: https://doi.org/10.1523/JNEUROSCI.3521-12.2013

- latency shows effect after photoreceptors
- pharmacological blocking shows effect between photoreceptors and ganglion cells
Ultrasound Elicits Behavioral Responses through Mechanical Effects on Neurons and Ion Channels in a Simple Nervous System

Jan Kubanek, Poojan Shukla, Alakananda Das, Stephen A. Baccus, and Miriam B. Goodman

Department of Molecular and Cellular Physiology and Department of Neurobiology, Stanford, California 94305

The Journal of Neuroscience, March 21, 2018 • 38(12):3081–3091 • 3081

Ultrasound elicits reversal behavior
LOCALIZATION OF ULTRASOUND-INDUCED IN VIVO NEUROSTIMULATION IN THE MOUSE MODEL

Randy L. King,*† Julian R. Brown,‡§ and Kim Butts Pauly*†


Rostral

Freq 500 kHz
Ispta 3 W/cm²
Pressure 0.3 MPa
sonicated the lateral geniculate nucleus (LGN) of the cat and recorded suppression of evoked potentials in the visual cortex.
Large Animal Deep Brain Stimulation

1000 elements
500 kHz

Measured Evoked Potential, electrodes placed over visual cortex

Table:

<table>
<thead>
<tr>
<th>Animal preparation</th>
<th>EEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telazol</td>
<td>Isoflurane (0 to 1.0%)</td>
</tr>
<tr>
<td>Baseline</td>
<td>20 min tFUS</td>
</tr>
</tbody>
</table>
Large Animal Deep Brain Stimulation

- Baseline
- 20 min tFUS
- 20 min tFUS
- 20 min Control
- 20 min Control
- 20 min Control

Animal preparation

EEG

Telazol

Isoflurane (0 to 1.0%)

Light only

Ultrasound Only

Ultrasound and Light

No Stimulus

15 fUS bursts at 1 burst/s

300ms

500 Hz square wave
1ms on/off

700ms

off
Results from One Experiment

Ultrasound at LGN reduces the light-only VEP
Five Experiments

![Graph showing normalized amplitude over time for different conditions.][1]

[1]: https://example.com/graph.png
Production of Reversible Changes in the Central Nervous System by Ultrasound

Science 10 JANUARY 1958

- sonicated the lateral geniculate nucleus (LGN) of the cat and
- recorded suppression of evoked potentials in the visual cortex

Noninvasive neuromodulation and thalamic focused mapping with low-intensity focused ultrasound

Robert F. Dallapiazza, MD, PhD; Kelsie F. Timbie, PhD; Stephen Holmberg, CINM; Jeremy Gatesman, BA; LVT; M. Beatriz Lopes, MD, PhD; Richard J. Price, PhD; G. Wilson Miller, PhD; and W. Jeffrey Elias, MD

- pig model
- evoked potential recordings during median nerve stimulation

Immediate effect that lasts ~ minutes
adaptation
Focused ultrasound-mediated suppression of chemically-induced acute epileptic EEG activity

Byoung-Kyong Min¹, Alexander Bystritsky², Kwang-Ik Jung³, Krisztina Fischer¹, Yongzhi Zhang¹, Lee-So Maeng⁴, Sang In Park⁴, Yong-An Chung⁴, Ferenc A Jolesz¹, Seung-Schik Yoo¹

Min et al. BMC Neuroscience 2011, 12:23
http://www.biomedcentral.com/1471-2202/12/23
TRANSCRANIAL ULTRASOUND IMPACTS MONKEY CHOICE BEHAVIOR

Jan Kubanek¹, Julian Brown¹, Patrick Ye², Kim Butts Pauly², William Newsome¹

BioRxiv

Targets appear asynchronously

US stimulation of left FEF

Rightward choices (%)

Difference in target onset times (ms)

ultrasound

no ultrasound

Stimulatory Effect on FEF/behavior

US stimulation of right FEF

Rightward choices (%)

Difference in target onset times (ms)

(300 ms, 0.6 MPa, 270 kHz, 500 Hz repetition frequency)
Direct Effect from FUS?

FUS creates a difference in the early VEP peaks
Ultrasound
- has an effect at all spatial scales
- has a suppressive effect on evoked potentials
- has an “excitatory” effect in FEF/behavior
Neuromodulation

- Evidence for at several different spatial scales
- Mechanism
  - Physical Effect
  - Biophysical Mechanism

---

Physical Effect
- Radiation Force
- Cavitation
- Temperature

Biophysical Mechanism
- Ion Channel(s)
- Exocytosis

Neural Mechanisms
- Neural Pathways
- Neural Dynamics

Response
- Behavior
- Neural Response
Radiation Pressure Distorts Cells

Ultrasonic radiation pressure distorts lipid bilayer structure, causing changes in bilayer capacitance and membrane curvature.

What happens in cells?

Radiation force/
Acoustic streaming

Radiation force/
Acoustic streaming

Bending

Tension
Mechanical Effects From Radiation Force

- Radiation Force
- Retina
- Cell
- Sheep

Planning MRI
MR-ARFI Focal Spot

Displacement (µm)
Temperature

Estimates from our sheep experiments
Intramembrane cavitation as a unifying mechanism for ultrasound-induced bioeffects

Boris Krasovitski, Victor Frenkel, Shy Shoham, and Eitan Kimmel

Fig. 4. Different stages in the interaction of a BLS and an ultrasound field can induce different bioeffects on the cell membrane and the cytoskeleton. (A) As tension increases gradually in the leaflets around a pulsating BLS, from the reference stage (S₀), the slightly stretched leaflets might at first activate mechanosensitive proteins (S₁); growing tension in the leaflets might damage membrane proteins (S₂) and then might induce pore formation (S₃a, S₃b) or cause membrane rupture at high levels of stretching. (B) Pulsations of the BLSs that surround a cell initially (at C₀) might induce from reversible mild stretching of cytoskeleton fibers to irreversible rupture (C₁).
Neuromodulation

- Evidence for at several different spatial scales
- Mechanism
  - Physical Effect
  - Biophysical Mechanism

![Diagram showing the relationship between ultrasound, physical effect, biophysical mechanism, neural mechanisms, and response.]

- **Physical Effect**
  - Radiation Force
  - Cavitation
  - Temperature

- **Biophysical Mechanism**
  - Ion Channel(s)
  - Exocytosis

- **Neural Mechanisms**
  - Neural Pathways
  - Neural Dynamics

- **Response**
  - Behavior
  - Neural Response
Activation of Mechanically Gated Channels

HEK cell expressing Piezo1 channels

25-micron polystyrene film

Prieto et al., 2018
Activation of Mechanically Gated Channels

Piezo1

Effect on cells with strong mechanical sensitive ion channels

Prieto et al., 2018
Ultrasound Elicits Behavioral Responses through Mechanical Effects on Neurons and Ion Channels in a Simple Nervous System

Jan Kubanek,1 Poojan Shukla,1 Alakananda Das,1 Stephen A. Baccus,2 and Miriam B. Goodman1

1Department of Molecular and Cellular Physiology and 2Department of Neurobiology, Stanford, California 94305

The Journal of Neuroscience, March 21, 2018 • 38(12):3081–3091 • 3081

Ultrasound elicits reversal behavior
Ultrasound Elicits Behavioral Responses through Mechanical Effects on Neurons and Ion Channels in a Simple Nervous System

Experiments with Knockouts

Ultrasound elicits reversal behavior

Loss of mechanosensation, but not thermosensation, disrupts ultrasound-evoked reversals.
Hippocampal Neurons in Rat Brain Slices Exhibit a Robust Response to Ultrasound

Prieto et al., unpublished

P = 5*10^{-6}

N=24
What are the Mechanically Sensitive Ion Channels in Hippocampal Pyramidal Neurons?

KCNQ unaffected by blocking
Nav1.6 Channels Mediate the Inhibitory Response in Hippocampal Neurons in Brain Slice Recording

**Control**

- **10 µM riluzole (Nav1.6 inhibitor)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>10 µM riluzole</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>0.009</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Prieto et al., unpublished
Summary

Mechanical Force

For:
- hippocampal slice: Nav1.6
- cultured neurons: TRPP1, TRPP2, TRPC1 (M. Shapiro @ BI)
- C. elegans knockouts retina
- freq response
- intensity response

Against:

Physical Effect
- Radiation Force
- Cavitation
- Temperature

Biophysical Mechanism
- Ion Channel(s)
- Exocytosis

Neural Mechanisms
- Neural Pathways
- Neural Dynamics

Response
- Behavior
- Neural Response

Baccus Lab (BioRxiv)
Retina

43 MHz 15 MHz 1.9 MHz

Normalized response vs. Intensity (W/cm²) SPPA

n>2
Temperature

For:
- retina freq response
- intensity response

Against:
- c-Elegans knockouts
- Doesn’t get that hot
Cavitation

For:
- my mouse data (mouse complicated)

Against:
- These are low intensities MI<1.9 (FDA limit)

For:
- Radiation Force
- Cavitation
- Temperature

Biophysical Mechanism
- Ion Channel(s)
- Exocytosis

Neural Mechanisms
- Neural Pathways
- Neural Dynamics

Response
- Behavior
- Neural Response

\[
MI = \frac{P_{PNP} (MPa)}{\sqrt{f (MHz)}}
\]

I=3 W/cm²
P=.3 MPa

\[
P_{PNP} = P_{PPP}
\]

\[
MI = \frac{.3}{\sqrt{.5}} = .4
\]
Outline

- How is FUS different for ablation, drug delivery, and neuromodulation?
- FUS neuromodulation at several scales (Stanford-slant)
- What is the mechanism?
- What about the mouse model?
- What will it take to do this in humans?
Unique Tools Available in the Mouse

- double stain for cell type, ion channels
Mice are hearing something…
Experimental Setup

Auditory Evoked Potential

Auditory Brainstem Response (ABR)
Genetically Deaf Mice Respond to Ultrasound

Representative ABR Responses to 16 kHz Sound

Representative ABR Thresholds to Sound

Mean success rate (%)

TRIOBP Deaf Mice, N=7
Hearing WT Mice, N=7

Samba LOXHD1 Deaf Mice, N = 4
Hearing WT Mice, N = 8
Waveform Envelope Affects What They Hear
They hear < 40dB Sound Click
And They Still Respond

**ABR Signal Power**

- Sound Click 40 dB SPL
- CW (2.9 w/cm²)
- PW-8kHz (2.9 w/cm²)
- Smoothed CW (2.9 w/cm²)
- PW-1.5kHz (2.9 w/cm²)
- Sham

**Motor Response Mean Success Rate (%)**

- PW-8kHz PRF
- PW-1.5kHz PRF
- CW (Rect)
- CW (Smoothed)
- Sham

**Statistics**

- PW-8kHz: P = 0.96
- PW-1.5kHz: P = 0.46
- CW (Rect): P = 0.48
- CW (Smoothed): P = 0.78
- Sham: P = 0.78

**Note:** The images show graphs and bars representing data, with statistical significance marked by asterisks (**) and p-values.
Further Evidence Against Startle

- **Sham**
- **80 ms**
- **160 ms**
- **320 ms**
- **640 ms**

**EMG activation duration (N = 6)**

\[ R^2 = 0.98 \]
\[ p < 0.01 \]
Outline

- How is FUS different for ablation, drug delivery, and neuromodulation?
- FUS neuromodulation at several scales (Stanford-slant)
- What is the mechanism?
- What about the mouse model?
- What else will it take to do this in humans?
evoked potential recordings during medial nerve stimulation before and after sonication of thalamus

no evidence of damage
Stimulated V1

- seen in those with repetitive sonications (~600)
Safety Study 1

- 2 NHP that were to be euthanized (disease)
- Various pressures, 500 repetitions
- Nothing to be seen on histology

Jan Kubanek
Donna Bouley
Kim Butts Pauly
Bill Newsome

Freq  
270 kHz

Ispta  
91 W/cm²

Pressure  
2.4 MPa

2.4 MPa

Normal
Safety Study 2

MI=1.7

FUS bursts per location vs estimated \textit{in situ} I_{SPTP}

FUS bursts per location vs estimated \textit{in situ} I_{SPTA}

MR-ARFI

Neuromodulation

# locations

1 2 3 5 6
Safety Study

- Didn’t see anything that wasn’t also seen in controls
- No sign of inflammatory response
Conclusions

- Focused Ultrasound can act on tissue via
  - thermal ablation (high intensity)
  - BBB opening (low intensity with microbubbles)
  - drug uncaging (low intensity with nanoparticles)
  - direct neuromodulation (low intensity)

Ultrasound neuromodulation

- has effect at all levels from cells, to slice, to whole animals, incl. humans
- has a suppressive effect on evoked potentials
- has an excitatory effect in FEF/behavior
- Leaning strongly toward radiation force effect on stretch sensitive ion channels

More work to be done

- Sort out effect on the histological level
- Put together the tools for human use/control
Questions for our discussion

1) Auditory Stimulus Conditions
   A) Auditory stimulus dB reference is not given (SPL?)
   B) T=period= 2µs for a 500 kHz US signal, 100 cycles/pulse=200 µs/pulse
      120 pulses = 24 msec, not 80 msec
   I cannot determine relation between 1500 Hz repetition and duty cycle
   Envelope of stimulus not stated (rectangular?)
   C) We should discuss differences between sound power and intensity
   D) They varied pulse duration without considering temporal summation

2) Stimulus Pathway
   They reasoned that "If the effects are mediated by the inner ear, one would expect the ear closest to the ultrasound focus to receive more of the stimulus, resulting in stronger activation of the contralateral auditory cortex due to auditory pathway decussation in the brainstem". This may not be true if the mechanism is by bone conduction because there is 0 dB between-ear attenuation in the skull. There likely is 0 dB between-ear attenuation for CSF mechanism too.
   On the other hand, in separate trials at both right and left visual-cortical targets, the results showed a clear contralateral bias, present in all animals tested

3) Auditory Frequency Range of Mouse
   The value of 1500 Hz selected as being audible to the mouse is questionable based on some measures of the frequency range of mouse hearing.

4) Auditory Startle Reflex
   The auditory startle reflex adapts

---

**Apples to Oranges Comparison**

Stanford: 2.9 W/cm\(^2\) (Broadband) $$\rightarrow$$ 40dB (Broadband) Sound Click
Caltech: 4.2 W/cm\(^2\) (Broadband) $$\rightarrow$$ 108dB (Narrowband) 1.5 kHz Sound