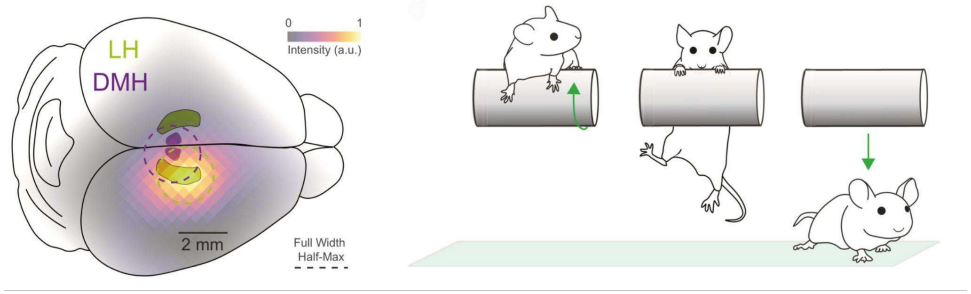
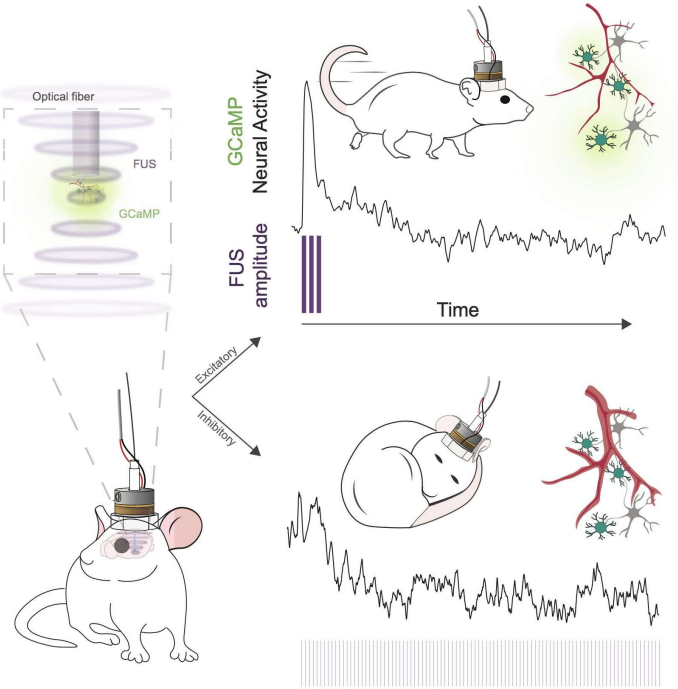
Optimized ultrasound neuromodulation for non-invasive control of behavior and physiology





Mengozzi Federico

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Context of the study

What is established and what's missing?

- Focused ultrasound stimulation (FUS) has been shown to be an effective non-invasive neuromodulation technique

- No unified practical protocol for different **brain regions**, **cell types** and **type of stimulus**

Attempt to define a protocol for targeted stimulation across

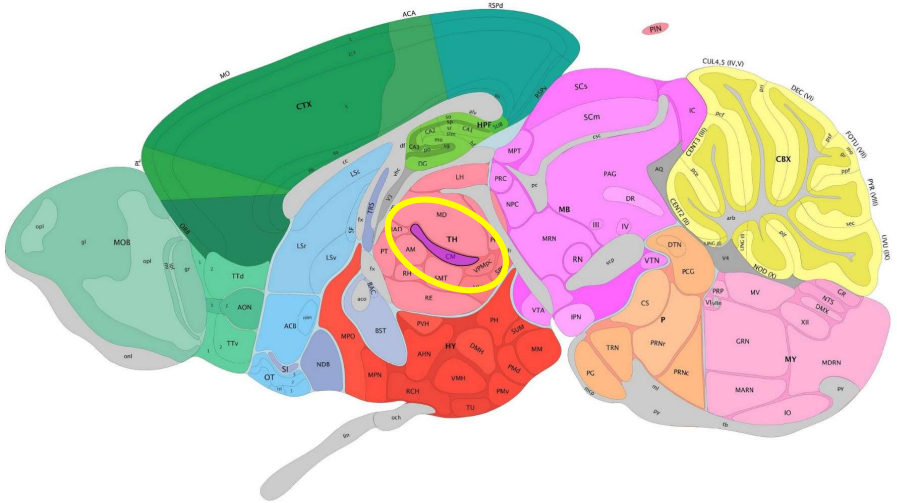
- brain region
- cell types
- stimulus paremeents

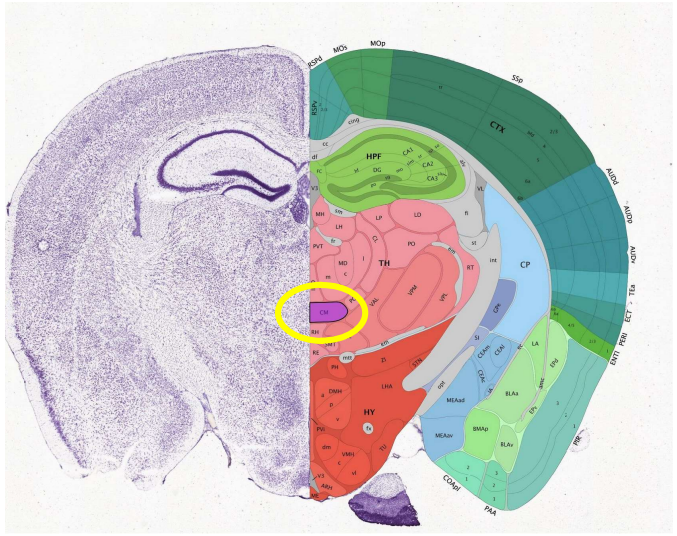
B

Methodological Approach - targets CMT

Central Medial Thalamus

- Glutamatergic neurons (excitatory)
- sleep, epilepsy

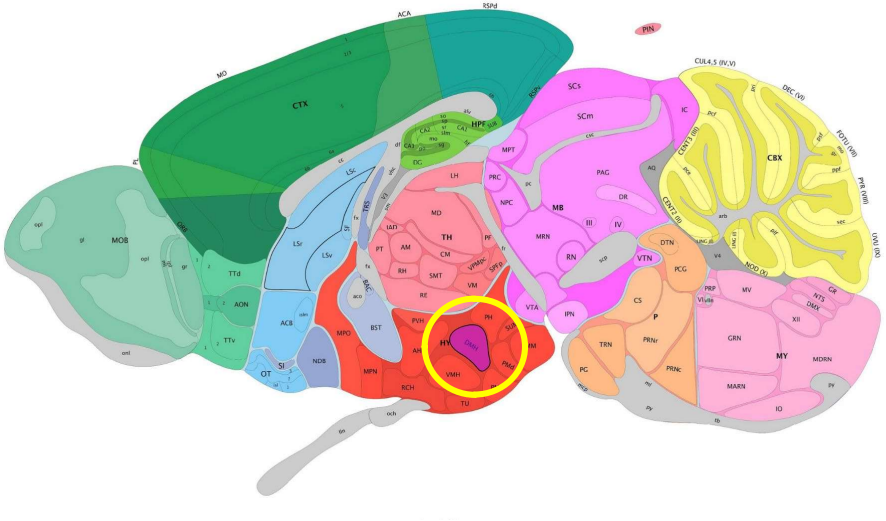


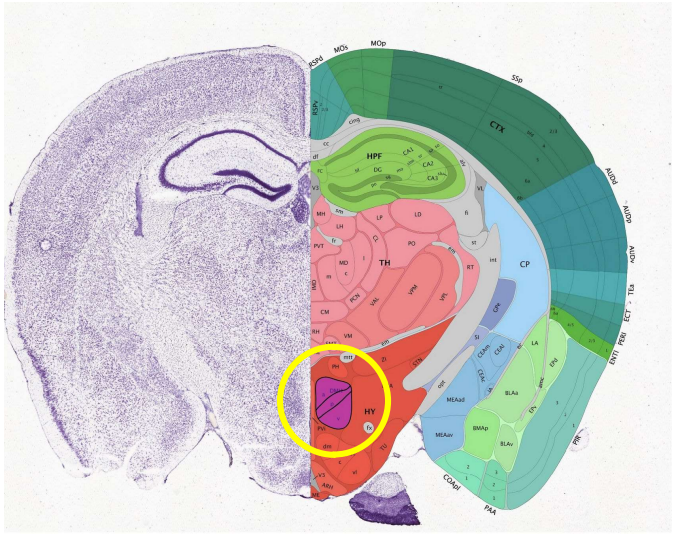


Methodological Approach - targets DMH

Dorso Medial Hypotalamus

- GABAergin neurons (inhibitory)
- anxiety, stress, fear, arousal, locomotion

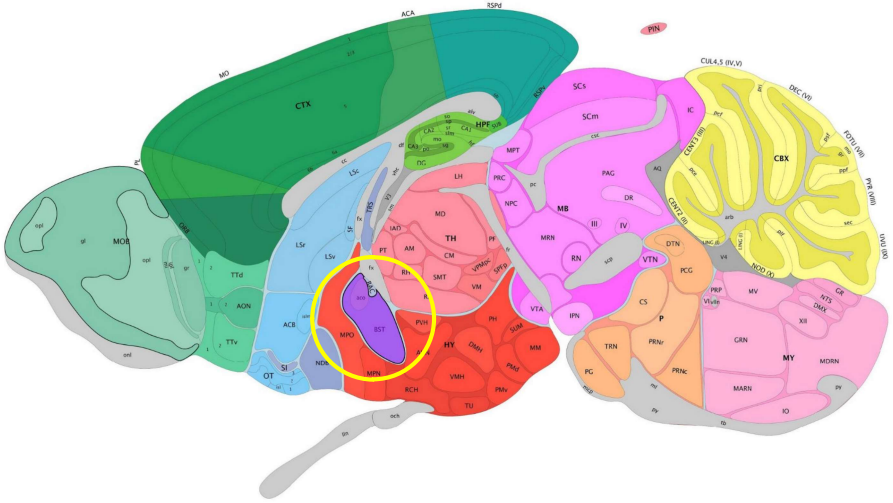


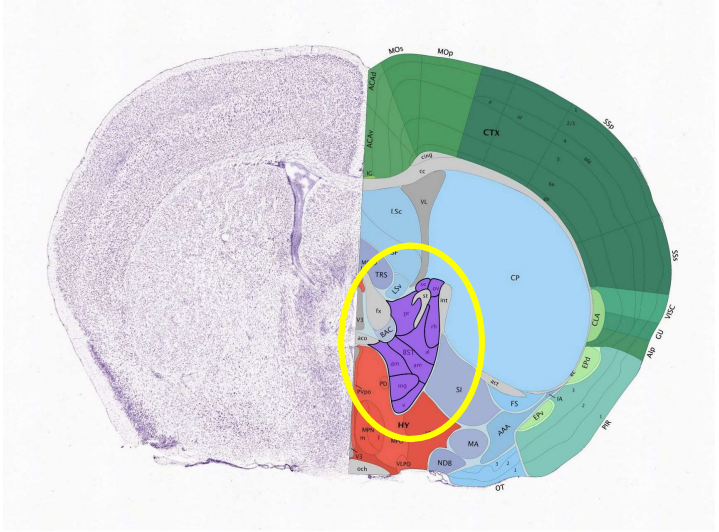


Methodological Approach - targets BNST

Bed Nucleus Stria Terminals

- GABAergin neurons (inhibitory)
- anxiety-like behavior

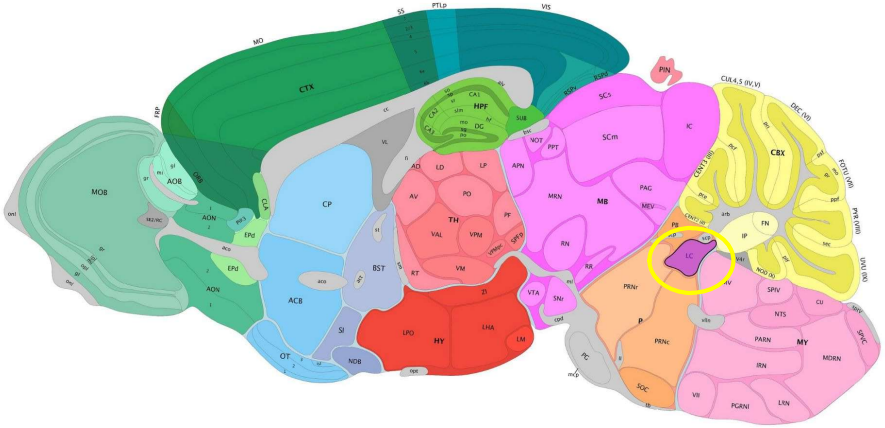


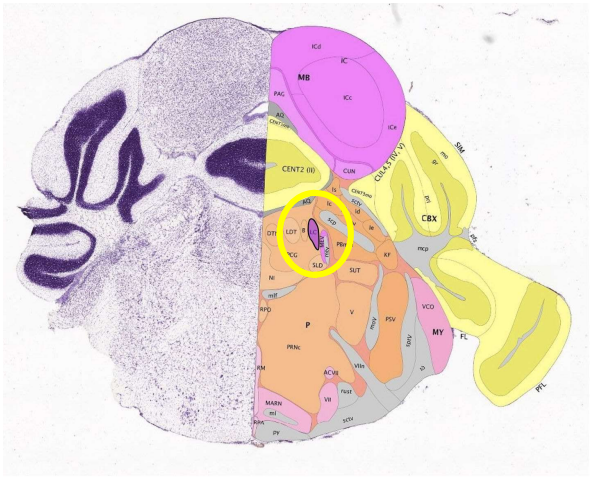


Methodological Approach - targets LC

Locus Coerules

- Noradrenergic neurons (excitatory)
- anxiety-like behavior



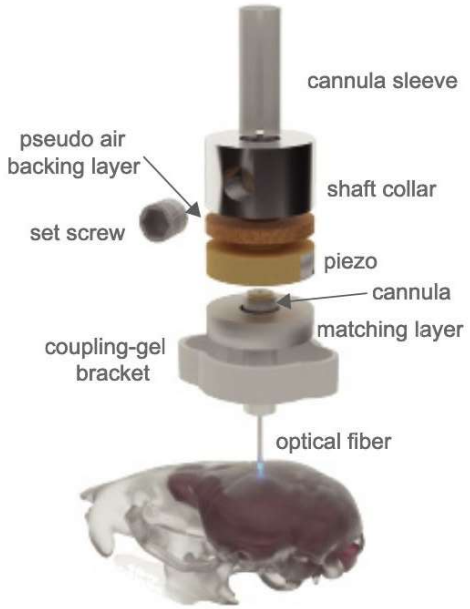


Methodological Approach - technique

What instrument to determined the protocol?

Custom tool integrating FUS and fiber photometry

- stimulation through FUS
- single-fiber photometry for recording



PhoCUS apparatus

Methodological Approach - background

Focused ultrasound (FUS) neuromodulation

- cavitation
- temperature changes (heat)*
- mechanical deformation

Ca2+ fiber photometry

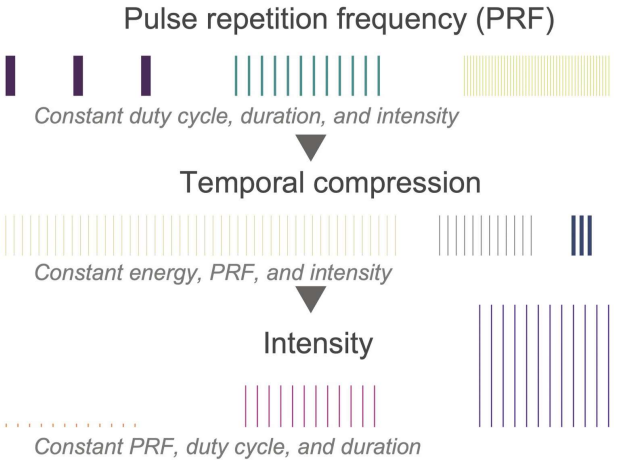
- genetic engineering in each target region to examine specific subsets of cells expressing GCaMP indicator
- GCaMP peak emission wavelength of 510 nm (necessary for measurement)
- the fiber was surgically implanted above the target area

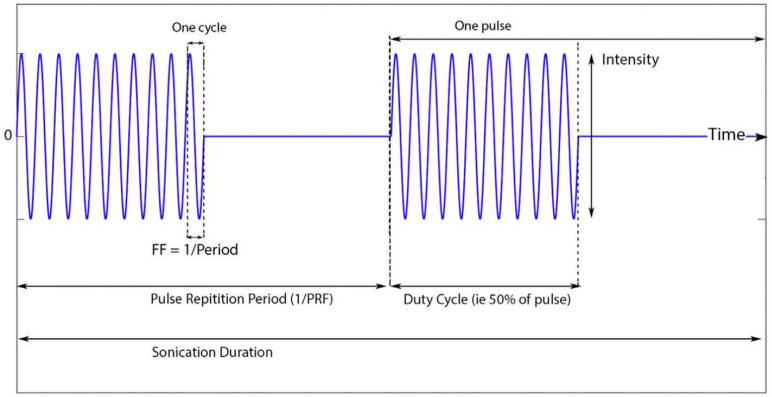
Methodological Approach - parameters

PRF: increase the frequency of each pulse
- values `2.5, 5, 10, 20, 40 Hz`
- constant energy, duration, DC, intensity
- varies #pulse/s

Temporal compression: distribute the energy over an extended time period
- values `40, 20, 10, 5 s`
- constant energy, PRF, intensity
- varies DC and duration to deliver the same energy over different time scales

Intensity: Low intensity threshold (LIUS `~3W/cm2`)
- values `0.3, 1.2, 2.3, 3.7, 5.4, 7.4 W/cm2`
- constant PRF, DC, duration
- varies intensity, energy





Protocol initialization - CMT

Starting with a 5s stimulus duration

Coordinate-descent on each dimensions (from top to bottom)

- Fix optimal PRF
- Fix optimal DC/d
- Fix optimal Intensity

For both **excitation** (left) and **inhibition** (right)

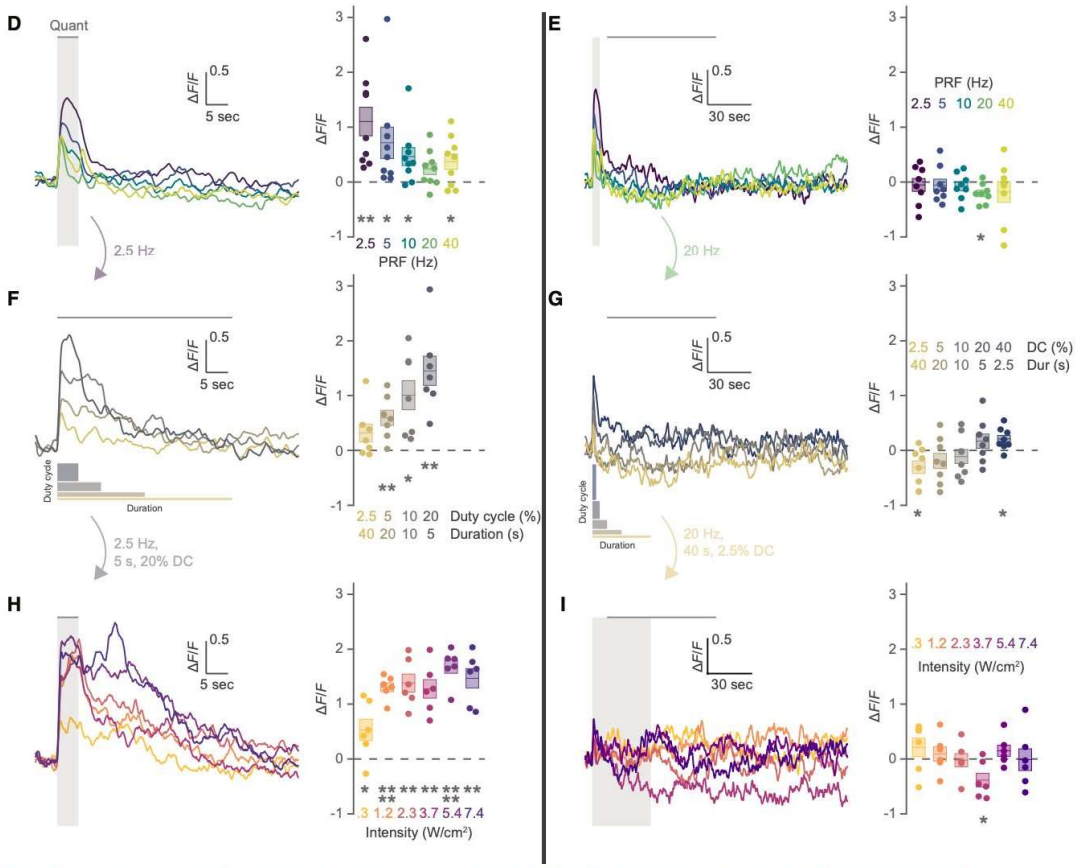
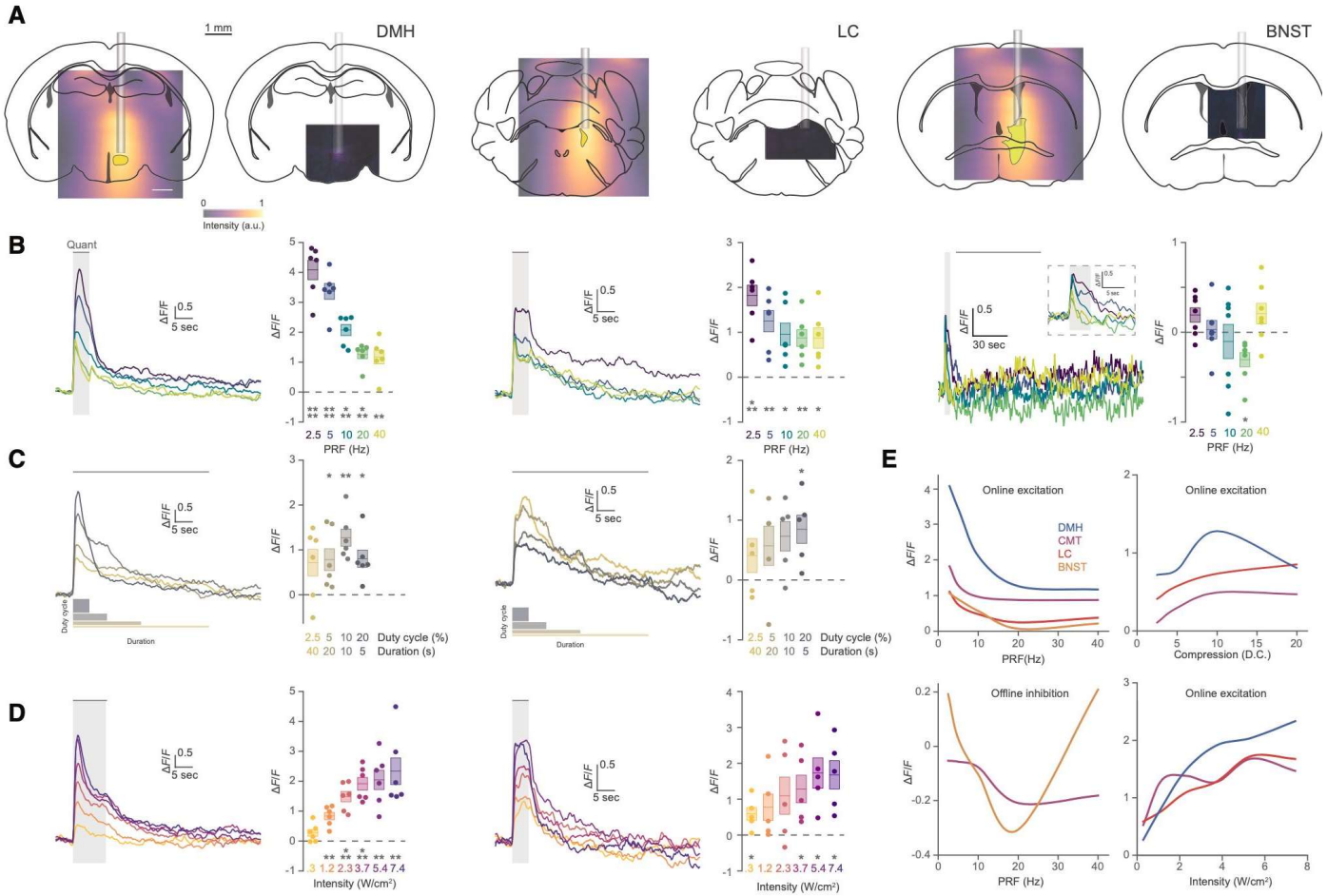


Figure 1. Stepwise parametric sweeps of ultrasound features reveal bidirectional manipulation of the midline thalamic nuclei

Protocol application - How does it generalize?



Protocol : specific behavioral effects

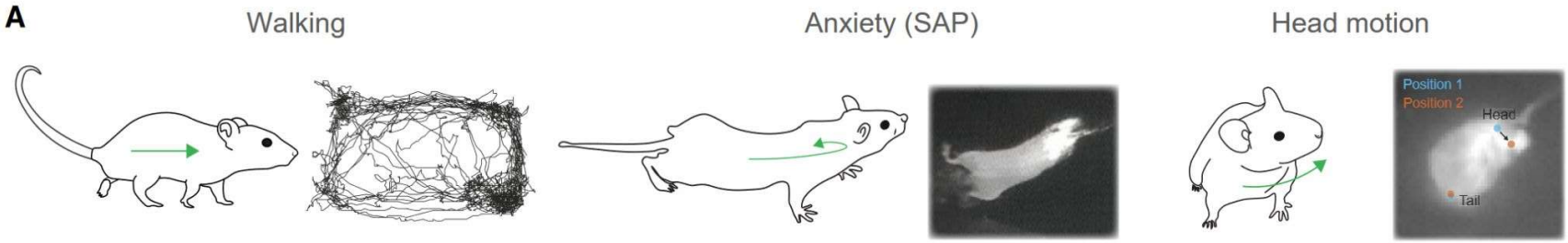
The *thalamus*, *hypothalamus*, and *lateral cortex* :

linked to varying levels of consciousness, anxiety and locomotion that manifest in visible changes in behavior !

Protocol : specific behavioral effects

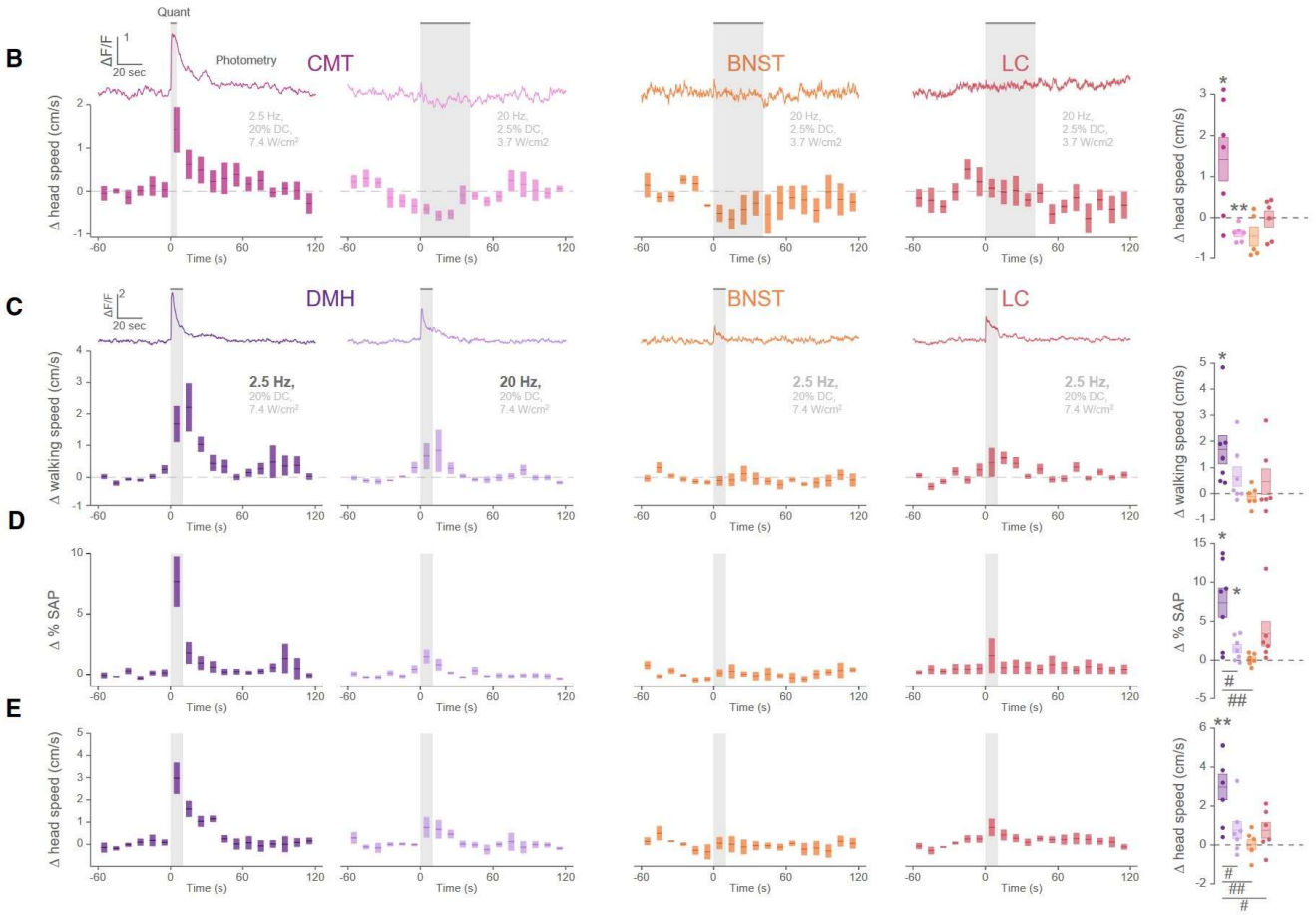
Automated video tracking to quantify changes in :

- head motion
- walking
- body shape



Goal : Stimulate various brain targets at optimal, suboptimal excitation and inhibition parameters

Protocol : specific behavioral effects

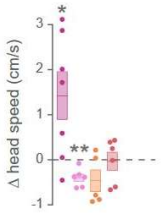


shaded gray area represents stimuli period

Protocol : specific behavioral effects

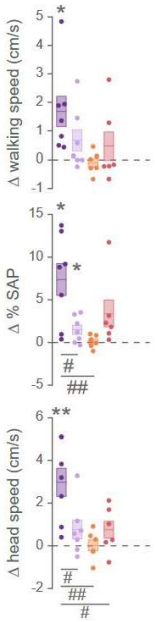
CMT :

- **Optimal excitatory stimulation** *increased* head motion
- **Optimal inhibitory stimulation** significantly *reduced* head motion.
  - demonstrates that optimized parameters allow for **bidirectional control** of behavior



DMH :

- **Optimal excitatory stimulation** caused a clear *increase* in walking, head motion and SAP.
- **Suboptimal stimulation** *increase less* h.m compared to optimal excitatory stimulation



BNST and LC :

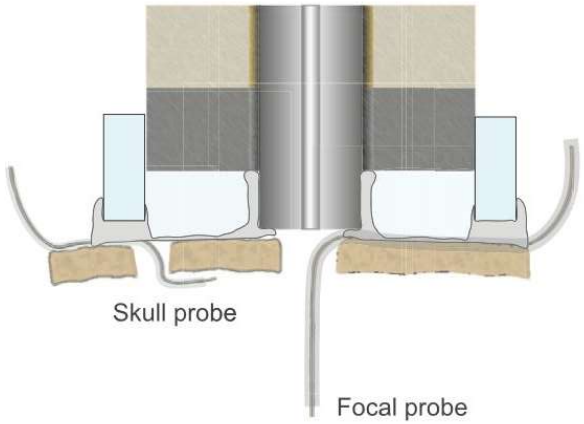
- The **optimized excitatory stimulation** for the DMH or CMT did not produce any significant changes in behavior when applied to the **BNST** or **LC**, showing that the effects are **target-specific** and *don't generalize* across regions.

R

Brain cooling and vasoconstriction

Method: Replaced optical fiber with a thermocouple to monitor local brain temperature at the ultrasound focus.

Goal: Investigate whether FUS-induced temperature changes explain differences in neuromodulation effects..



Brain cooling and vasoconstriction

Neural Activity and Blood Flow:

- Normally, **neural activity increases vasodilation**, but substantial increases can cause **delayed vasoconstriction**

Ibuprofen:

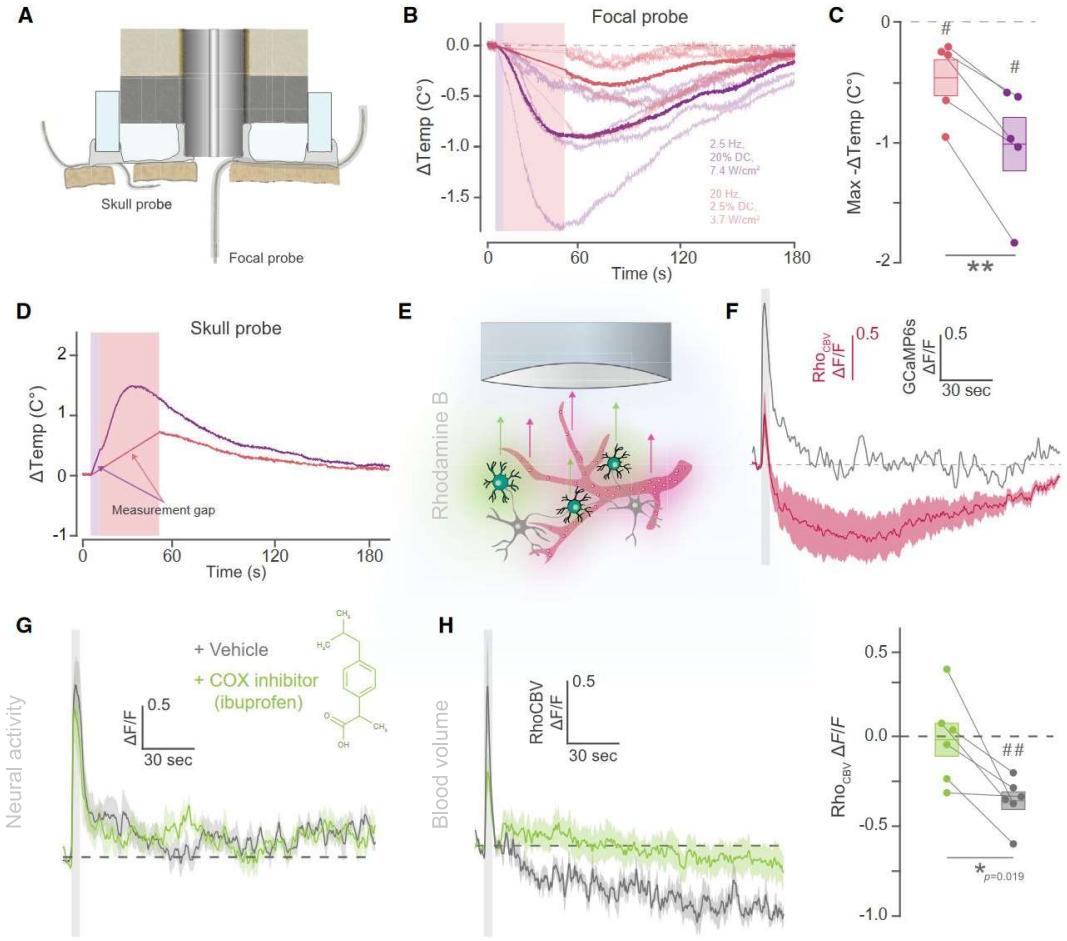
- Acts as a **COX1/2 inhibitor**.
- Injected to test whether **blocking COX1/2** prevents the sustained decrease in **blood volume** after FUS-induced neural activity.

Saline: Used as a **control injection** to compare with ibuprofen's effects on **blood volume** and neural activity.

Goal: Examine if **COX1/2 signaling** is responsible for the **delayed vasoconstriction** observed after substantial neural activity in **CMT.**

M

Brain cooling and vasoconstriction



Brain cooling and vasoconstriction

- **Temperature Changes**:
  - **Increased temperature** just above the cortex due to skull absorption of ultrasound waves (D)
  - Contrary to expectations, **temperature decreased at the focus** by nearly 1°C (B)

- **Neuronal Activity and Blood Flow**:
  - FUS stimulation caused a **rapid rise in neuronal activity and blood volume**, followed by **prolonged vasoconstriction**.

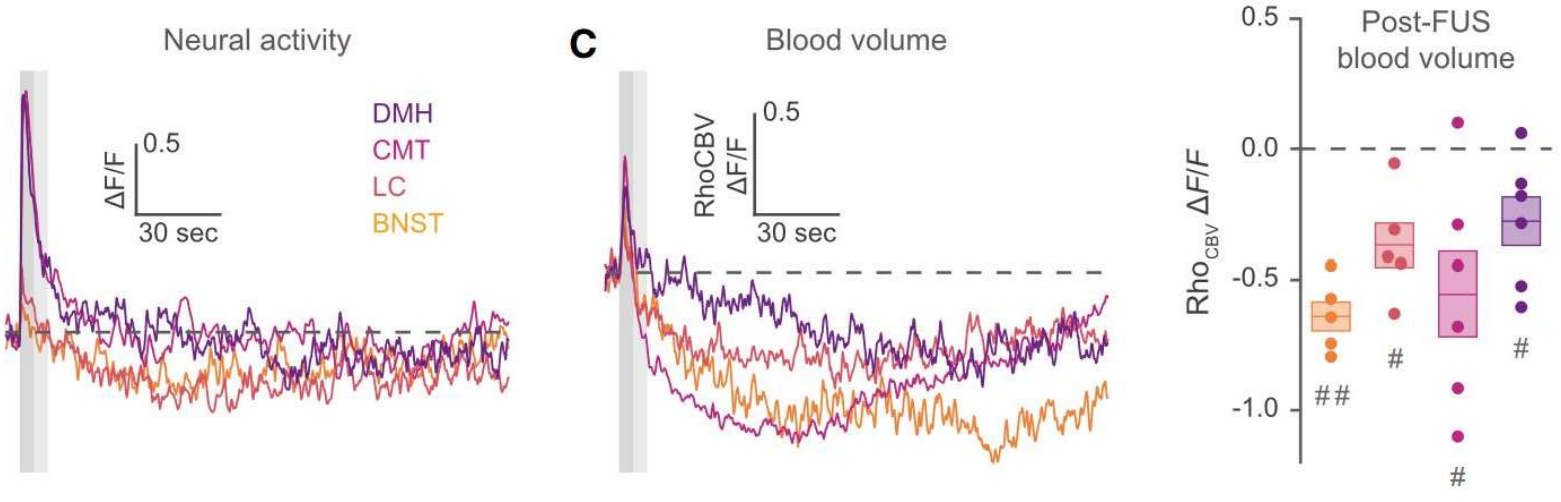
Important result : With **ibuprofen**, the **decrease in blood volume** was **eliminated**, confirming that COX1/2 signaling drives the **long-lasting vasoconstriction** !

Brain cooling and vasoconstriction

Question :

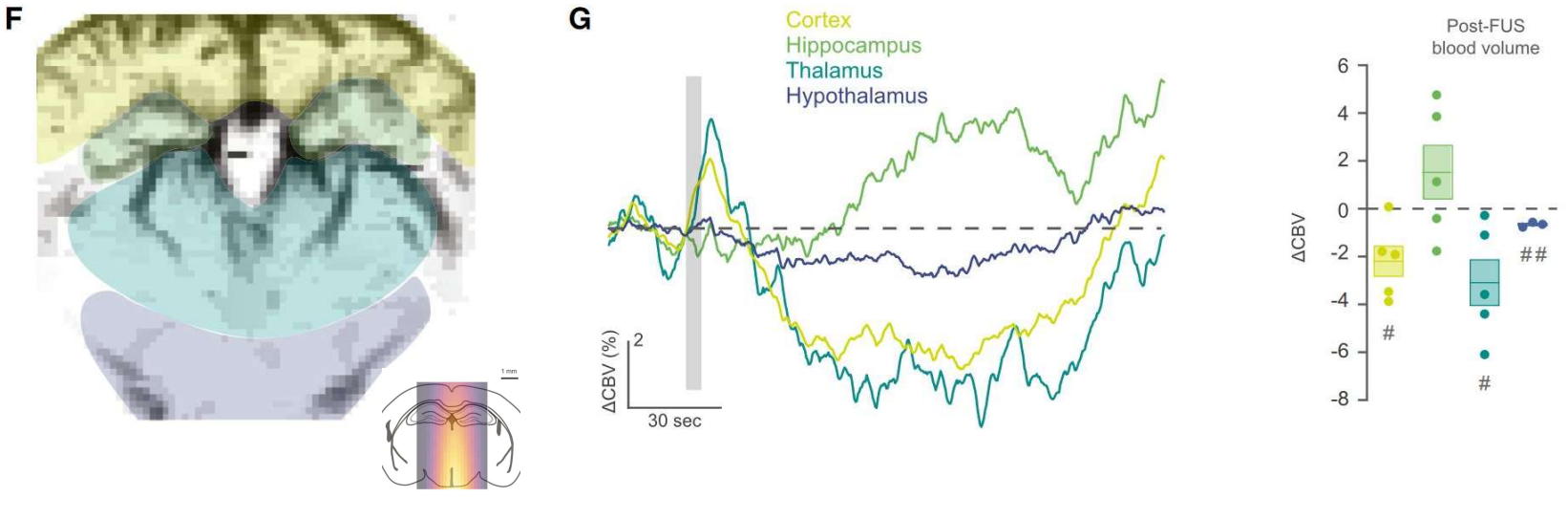
- Is strong FUS activation which drive long-lasting neurovascular, related to the difference observed in neural activity of different regions (DMH, LC and BNST) ?

Method : Measured blood volume following optimized stimulation



Brain cooling and vasoconstriction

Significant vasoconstriction occurred broadly across the thalamus, hypothalamus, and cortex. Hippocampus was resilient against vasoconstriction, suggesting that vasodynamic response propagates *beyond* the ultrasound focus in a brain-region-dependent manner



Limitations

- **Animal Model Constraints**
  - **Mice models** may not fully represent human brain structure and function.
  - Differences in **brain size and neuroanatomy**
- **Short-Term Stimulation and Effects**
  - The study focused on **immediate effects** of FUS.
  - **Long-term impacts** of repeated stimulation remain unknown.
  - Chronic exposure could lead to **neural adaptation**, **tissue damage**
- **Potential for Auditory Confounds**
  - FUS may generate **audible sounds**, influencing behavior, especially in mice.

Challenges

- **Limited Behavioral Metrics and Interpretations**
    - Behavioral assessments focused on **head motion, walking, and anxiety** but lacked evaluation of **complex behaviors** like learning and memory.
    - Differentiating **FUS-induced behaviors** from natural behaviors in awake animals

- Challenges in **optimizing FUS parameters**, **managing auditory confounds**, dealing with **ethical concerns**.
- **Interpreting unexpected effects like cooling**.

Novelty

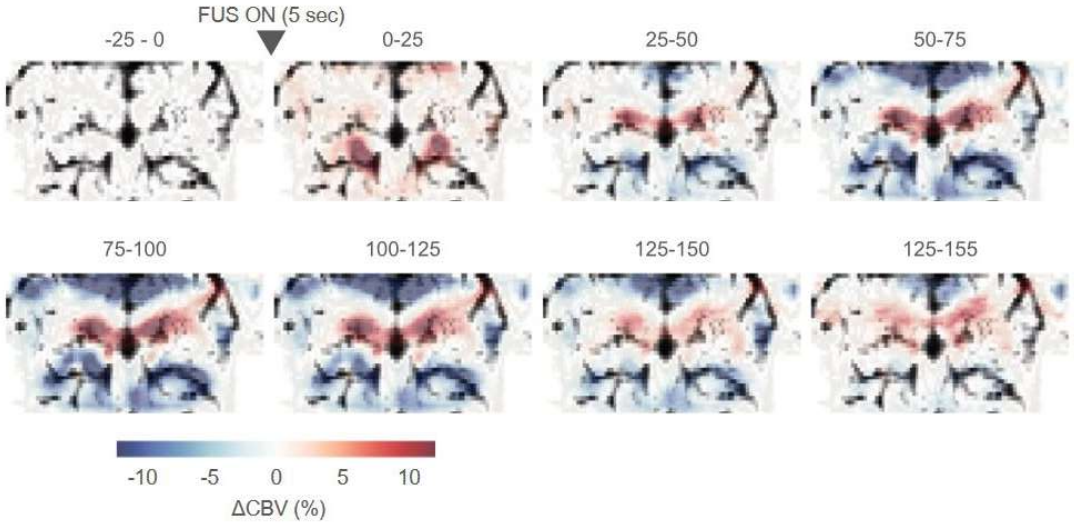
- Bidirectional stimulation (excitatory and inhibitory)
- First attempt at creating a standard protocol
- Importance of parameter optimization
- Behavioral effects observed without anesthetic confounds
- Level of control comparable to optogenetics methods

Open Qs

- Translational potential?
- Long term effect of FUS?
- Cell-response specificity recognized but not completely addressed?
- Effect on larger networks?
- Can a similar protocol be established for other NM techniques?
- Complex temporal dynamics between blood-flow and brain-region activity is not clear?

Questions?

Brain cooling and vasoconstriction



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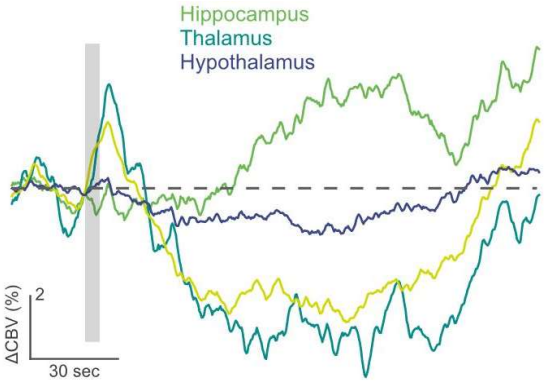
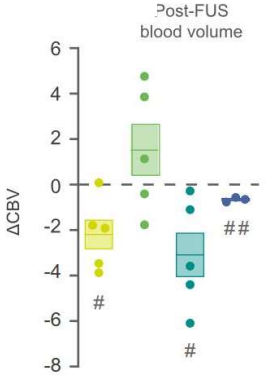
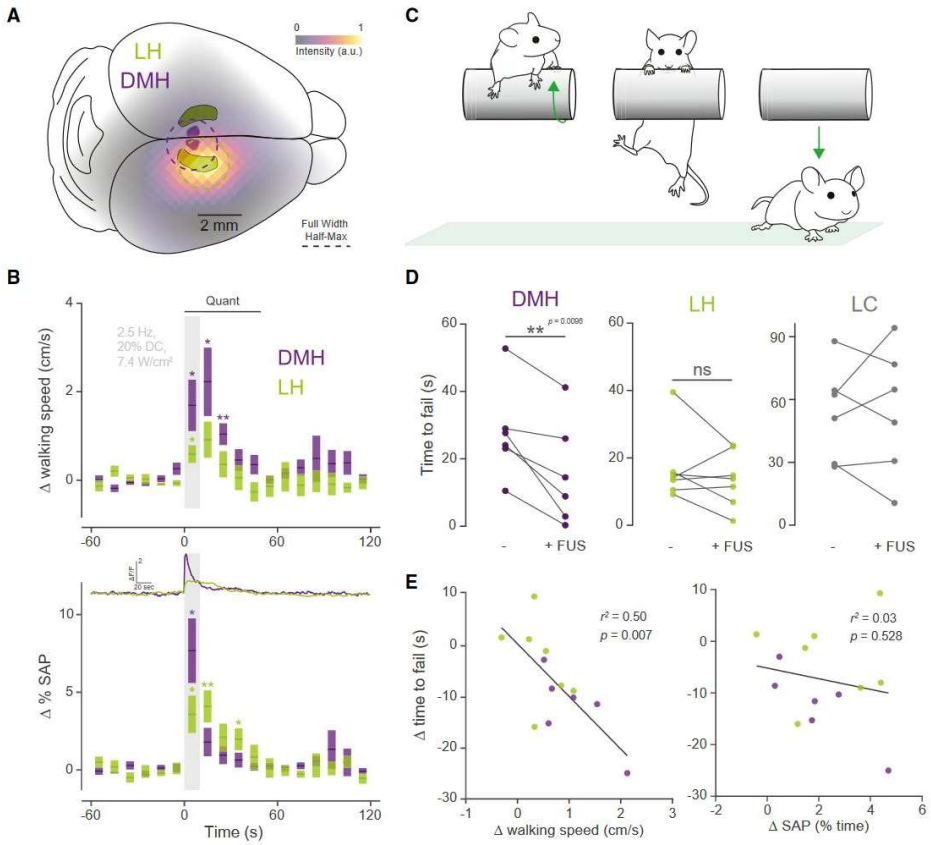




Protocol and specific behavioral effects

Interruption of walking behavior :
not observed during LC stimulation

highlight the specificity of this response to the hypothalamic locomotor region



- **Hypothalamic subregions produce differential behavioral response to FUS stimulation**