NON-INVASIVE MODULATION OF PAIN VIA C-LTMR SONOGENETICS

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Abstract (10 lines)*

Chronic pain affects over 30% of the global population and remains a major unmet medical challenge. Current opioid-based treatments are limited by side effects and dependency risks. This PhD project proposes a non-invasive alternative using focused ultrasound (FUS) neuromodulation. The approach targets C-low threshold mechanoreceptors (C-LTMRs) in the dorsal root ganglia (DRG), potent modulators of injury-induced pain. We hypothesize that FUS activation of C-LTMRs produces pain-relieving (antinociceptive) effects. The first part of the project will validate a transgenic mouse model for selective C-LTMR activation in vitro. The second part will map spinal circuits engaged by FUS-stimulated C-LTMRs using molecular and imaging tools. The third part will test the therapeutic potential of FUS-activated C-LTMRs in preclinical pain models. Together, these studies aim to uncover how C-LTMRs modulate pain and promote recovery. The outcomes will establish a foundation for safe, drug-free, clinically translatable chronic pain therapies.

Keywords*

Focused ultrasound (FUS); Sonogenetics; Neuromodulation; C-low threshold mechanoreceptors (C-LTMRs); Spinal circuitry; Preclinical pain models; Chronic pain; Antinociception; Non-invasive therapy.

Scientific question and Objectives (10 lines)*

The proposed project aims to **investigate focused ultrasound (FUS) neurostimulation as an alternative method to treat chronic pain from the periphery, with the advantages of being non-invasive and avoiding drug dependency**. The project is built on a solid working hypothesis in which we postulate that FUS stimulation of C-low threshold mechanoreceptors (C-LTMRs) is antinociceptive. To achieve this, a **sonogenetic approach** will be employed to enhance the neuronal sensitivity of C-LTMRs to FUS, with three main objectives: (i) validate a transgenic mouse model for selective sonogenetic activation of C-LTMRs in vitro; (ii) map the spinal circuits engaged by FUS-stimulated C-LTMRs using cFOS activity mapping and single-nucleus RNA sequencing; and (iii) assess the therapeutic efficacy of FUS-mediated C-LTMR activation in multiple preclinical pain models and explore whether pre-injury activation can prevent pain chronicity. Together, these studies will clarify the mechanisms by which C-LTMRs modulate pain and promote recovery, providing the first demonstration of selective, non-invasive FUS activation of peripheral mechanoreceptors.

Proposed approach (experimental / theoretical / computational) and research plan (20 lines) *

This project employs cutting-edge intersectional genetics and sonogenetics, introducing the mechanosensitive ion channel MscL into C-LTMRs using a novel triple transgenic mouse model (already developed at IBDM). This sonogenetic mouse model allows for precise, FUS-mediated stimulation of C-LTMRs in *in-vitro* and *in-vivo* settings. The research plan is structured into three interrelated work packages:

- **WP1** focuses on validating this newly generated mouse model *in vitro* using calcium imaging in DRG cultures to define the FUS parameters for selective sonogenetic activation of C-LTMRs, which will directly inform subsequent *in-vivo* studies (WP2 & WP3).
- **WP2** investigates the spinal neuronal circuits activated *in vivo* by sonogenetic stimulation of the peripheral nerve endings of C-LTMRs. This includes both functional mapping (via cFOS immunostaining) and molecular profiling (via single-nucleus RNA sequencing, snRNA-seq) to elucidate the molecular and cellular pathways underlying C-LTMR-mediated pain modulation.
- **WP3** aims to determine whether selective FUS activation of C-LTMRs can alleviate pain *in vivo* by using various preclinical pain models: paw incision (postoperative), Zymosan A (inflammatory), CCI (nerve injury) and Paclitaxel (chemotherapy-induced neuropathy). The main readouts will be changes in mechanical pain thresholds and conditioned place preference to evaluate both evoked and spontaneous pain.

Specific calibration ultrasonic experiments and computer simulations of acoustic fields will be conducted to ensure efficient, reproducible, and biologically safe FUS stimulation protocols (avoiding cavitation and thermal effects). By rigorously dissecting C-LTMR function, this project advances our understanding of somatosensory processing and pain regulation, while laying the scientific and technological groundwork for a non-invasive, targeted therapy for chronic pain.

Interdisciplinarity and Implication of the two labs (15 lines)*

The project is an interdisciplinary project that brings together two Centuri laboratories with complementary expertises: molecular and cellular biology for chronic pain (IBDM) and biomedical ultrasounds (LMA Marseille).

***IBDM (Institute of Developmental Biology of Marseille*).** The Moqrich team is expert in somatic sensory biology and molecular characterization of primary sensory neurons that underlie pain sensation. They will contribute to the understanding of the molecular mechanisms of FUS-induced neurostimulation (WP1 & WP2) and design and conduct *in vivo* experiments using various pain models (WP3).

LMA (Laboratory of Mechanics and Acoustics). The Franceschini team is expert in biomedical ultrasounds for the development of novel ultrasound-based techniques for imaging and therapeutic purposes, including ultrasonic neurostimulation. They will oversee the physical acoustic aspects of the project, ensuring the effective integration of FUS technologies for all WPs.

Specify with whom the person recruited will collaborate and on what aspects *

IBDM. Aziz Moqrich (DR CNRS), Ana Reynders (CR CNRS), Karine Magalon (IR CNRS): mouse genetics, somatic sensory biology, neuroimmunology, pain behavior.

LMA. Emilie Franceschini (DR CNRS), Olivier Macherey (DR CNRS), Régine Guillermin (IR CNRS), Eric Debieu (IE CNRS): Focused ultrasound-induced therapy, ultrasonic-tissue interaction, ultrasonic wave propagation.

PhD student's expected profile*

The PhD candidate should hold a MSc (or equivalent degree) in molecular or cellular biology, neurosciences, or biophysics. We aim to find a highly-motivated student with creative skills and appeal for experimental work. Ability to work in an interdisciplinary environment involving several research teams will be required.

Is this project the continuation of an existing project or an entirely new one?
In the case of an existing project, please explain the links between the two projects (5 lines)*

The project is in continuation of a 4-years PhD project of Elena Brunet (IBDM-LMA 2020-2024). By combining primary neuron culture, calcium imaging and single-cell RNA sequencing, focused ultrasound has been shown to stimulate DRG neurons with preferential activation in the neurons expressing Gαi-interacting protein, encompassing the C-LTMRs (potent modulators of injury-induced pain) and the C high-threshold mechanoreceptors C-HTMRs (potent contributors to nociception) (Brunet *et al.*, 2025). The present PhD project aims to pursue this research to stimulate *in vivo* the C-LTMRs for chronic pain treatment.

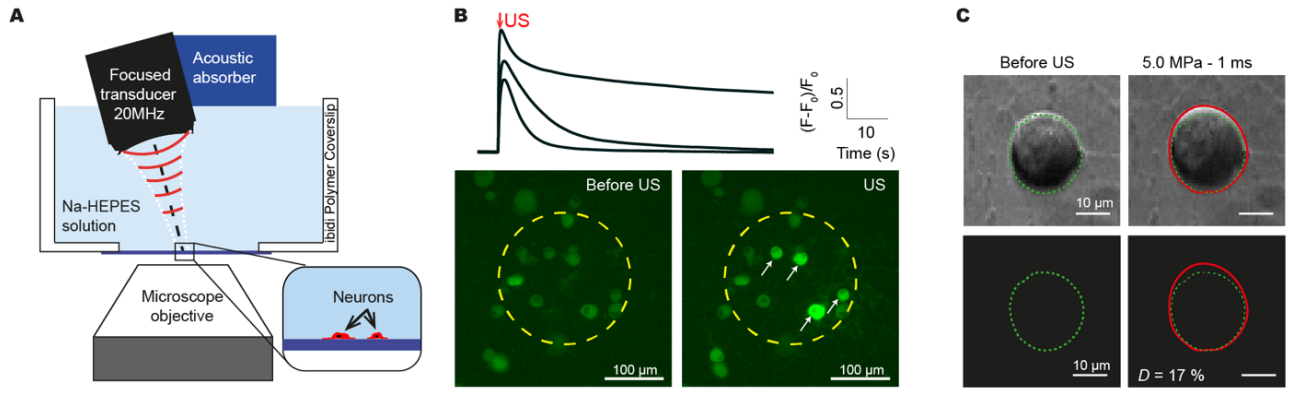
Two to five references related to the project*

- Hoffmann et al., "*Focused ultrasound excites action potentials in mammalian peripheral neurons in part through the mechanically gated ion channel PIEZO2*". ***PNAS*** 119(21), 2022
- Cadoni S et al., "*Ectopic expression of a mechanosensitive channel confers spatiotemporal resolution to ultrasound stimulations of neurons for visual restoration*". ***Nature Nanotechnology*** 18, 667-676, 2023

Two main publications from each PI over the last 5 years*

- Yoo S., Santos C., Reynders A., Marics I., Malapert P., Gaillard S., Charron A., Ugolini S., Rossignol R., El Khallouqi A., Springael J.-Y., Parmentier M., Saurin A. J., Goaillard J.-M., Castets F., Clerc N., Moqrich A., "*TAFA4 relieves injury-induced mechanical hypersensitivity through LDL receptors and modulation of spinal A-type K(+) current*". ***Cell Reports*** *37*, 109884, 2021
- Hoeffel G., Debroas G., Roger A., Rossignol R., Gouilly J., Laprie C., Chasson L., Barbon P.V., Balsamo A., Reynders A., Moqrich A., Ugolini S., "*Sensory neuron-derived TAFA4 promotes macrophage tissue repair functions*". ***Nature*** *594*, 94-99, 2021
- Merlo A., Losserand S., Yaya F., Connes C., Faivre M., Lorthois S., Minetti C., Nader E., Podgorski T., Renoux C., Coupier G., Franceschini E., "*Influence of storage conditions and buffer composition on the mechanical behaviour of flowing red blood cells*", ***Biophysical journal***, 122 360-373, 2023
- Brunet E., Parpaite T., Yoo S., Debieu E., Metwally K., Mensah S., Malapert P., Saurin A., Macherey O., Franceschini E., Moqrich A., *"Focused ultrasound activation of cultured primary sensory neurons: molecular and biophysical characterization", under press* ***Scientific Reports***, 2025 (doi: 10.21203/rs.3.rs-6049101/v1)

Project's illustrating image



Focused ultrasound triggers calcium responses preferentially in GINIP-expressing neurons (Brunet *et al.*, 2025)

(**A**) Schematic view of the US transducer placed in order to have the US focal zone at the center of the optical microscope's visual field. The transducer was tilted with an angle of about 20° to reduce standing wave formation. FUS are delivered to GCaMP6f DRG neurons cultured on a polymer dish, while neural responses are recorded by calcium imaging. (**B**) Examples of images of GCaMP6s fluorescence before and during FUS stimulation for the stimulus [20 MHz, p^+=5 MPa, Δt=1 ms], and corresponding calcium responses of DRG neurons within the -6 dB beam area (yellow circles). (**C**) Deformations of the DRG neurons during FUS stimuli monitored by high-speed camera showing that cell deformations are necessary to activate DRG neurons.

*: Mandatory