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CAN THE IN VITRO GUINEA PIG BRAIN PREPARATION BE USED TO INVESTIGATE FOCUSED ULTRASOUND MEDIATED NEUROMODULATION?

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ABSTRACT

Low intensity and low frequency focused ultrasounds (LIFU) can induce neuromodulation. In the literature both excitatory and inhibitory effects of LIFU are reported. The exact mechanisms of both remain unclear, but many studies consider a key element the opening of mechanosensitive ion channels, electrophysiological mechanical coupling, and microtubule resonance. The in vitro guinea pig brain preparation has preserved vascular and neuronal structures thanks to a continuous artificial cerebrospinal fluid (aCSF) infusion. LIFU is delivered by a planar ultrasound transducer with different sonication parameters on a target hemisphere leaving the contralateral as control. To study the electrophysiological response of the brain, evoked potentials are generated in the lateral olfactory tract (LOT) and recorded in the piriform cortex (PC) pre-, during and after LIFU. Our study demonstrates that the in vitro guinea pig brain preparation is a viable method to study the effect of LIFU by analyzing evoked potential morphology and voltage amplitude.

Keywords: *in vitro guinea pig brain preparation, low intensity and low frequency focused ultrasound (LIFU), neuromodulation.*

1. INTRODUCTION

Neuromodulation refers to any intervention which modifies neuronal activity within the central or peripheral nervous system (CNS) or (PNS) to achieve a therapeutic effect via means of electrical, chemical or mechanical methodologies (Johnson et al., 2013).

Low intensity and low frequency focused ultrasound (LIFU) are mechanical energy in the form of acoustic waves exceeding audible range > 20 KHz and usually delivered at intensities below 100 mW/cm2. They are known in the literature for their transient neuromodulation properties with either enhancing or suppressing outcomes (Guo et al., 2018) (Legon et al., 2014). In most studies LIFU are delivered via planar transducers in a pulsed manner with a pulsed repetition frequency (PRF) of 1 KHz, at frequencies not exceeding 1.5 MHz, spatial average temporal average intensity (ISATA) of 30 mW/cm2 and duty cycle (DC) of 20% (Pounder & Harrison, 2008). This enables LIFU to maintain pressure levels well below cavitation thresholds, avoiding excessive tissue heating and damage, while exerting a primarily mechanical effect (Padilla et al., 2014) (Pounder & Harrison, 2008). Growing interest for the temporal blockage of neural signals by the reversible decrease in functionality of neurons (Tyler et al., 2008) poses hope for the treatment of epilepsy or chronic pain (Yoo et al., 2011).

The exact mechanisms remain unknown, but among the supported hypotheses of LIFU interactions,

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electrophysiological and mechanical coupling, opening of mechanosensitive ion channels and microtubule resonance are most popular (Dell'Italia et al., 2022). The first refers to the viscoelastic deformation capacity of neuronal membranes (Zubko et al., 2013); the second to the direct opening of mechanosensitive ion channels; and the third to microtubular oscillation induced by LIFU (Venkatachalam & Montell, 2007). In the present study, we further support these hypotheses and investigate the mechanisms in the in vitro guinea pig brain preparation (De Curtis et al., 1991) under an electrophysiological point of view.

2. MATERIALS AND METHODS

3 experiments were performed on the isolated young adult guinea pig brains maintained in vitro according to a previously described technique (De Curtis et al., 1991) (Muhlethaler' et al., 1993). After barbiturate anaesthesia (phenobarbital 30 mgkg) the brains were removed from the skull under hypothermic conditions and transferred to the incubation chamber, where arterial perfusion was established through a cannula inserted into a vertebral artery. The perfusate contained 126 mM NaCI, 26 mM NaC03,3 mM KCI, 1.2 mM K2P04, 1.3 mM MgS04, 2.4 mM CaC12, 5 mh4 HEPES, 15 mM dextrose and 0.4 mM thiourea. Dextran (3%; mol. wt 70 OOO; SIFRA, Verona, Italy) was added to the solution to balance osmolarity. The perfusate was oxygenated with a 5% C02/95% 02 gas mixture. Experiments were performed at 32°C.

Glass electrodes filled with 0.5 M NaCl (2-8 MSZ resistance) were used to record field potentials. Bipolar electrodes (twisted silver wires) were positioned on the LOT for stimulation bilaterally and recording electrodes in the APC bilaterally (see figure 1). A multi-channel amplifier (Biomedical Engineering Co., Thomwood, NY) was used for extracellular recordings. Field potential laminar profiles were obtained by averaging 5-7 evoked responses at each 50 pm step along a penetration perpendicular to the piriform cortex (rAPC), perpendicular to cortical lamination (Boido et al., 2014) (Uva & De Curtis, 2005) (Uva L, 2005).

Single pulses and paired pulses were alternated at fixed rate of 5 seconds. The paired pulses were given at an intersynaptic delay of 30 ms (Haberly et al., 1986) (Muhlethaler et al., 1993). Inputs were given in both hemispheres in an alternated fashion and recordings were performed throughout the whole experiment with Reader (lozzino e. Condarelli, 1998). The sonication was performed only in one hemisphere. Before the sonication with LIFU, 10 minutes of EPs recordings was performed. Then EPs were registered during sonication and for a total of 50 minutes after sonication to detect long term modifications. A planar ultrasound transducer was used with the following parameters: frequency 0.485 MHz, duty cycle (DC) 30%, amplitude 400 mVpp, pulse repetition frequency (PRF) 1 kHz, sonication duration (SD) 2 minutes. EPs were analysed on a software called CLAMPFIT 11.2 (Axon™ pCLAMP™ 11 Electrophysiology Data Acquisition & Analysis Software).

3. RESULTS

Target: left piriform cortex. Single and paired pulse during and after LIFU (see graph 1).

4. DISCUSSION

Both single and paired pulses change morphology and voltage amplitude of the evoked potentials pre-, during and after LIFU.

5. CONCLUSIONS

FUS has an impact on neuronal excitability. The experimental set-up of the in vitro guinea pig brain preparation with the use of evoked potentials as a representation of neuronal excitability is a viable method to investigate FUS neuromodulatory effects. Further experiments need to be done to confirm these preliminary results and can be implemented with immunohistochemistry analysis.

6. FIGURES

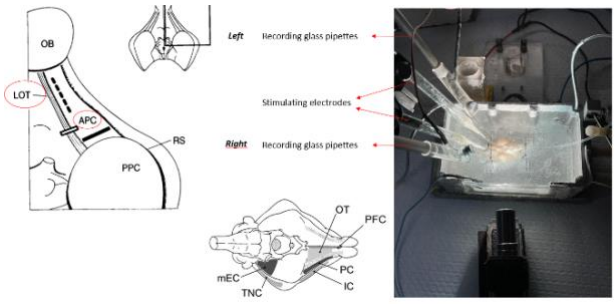
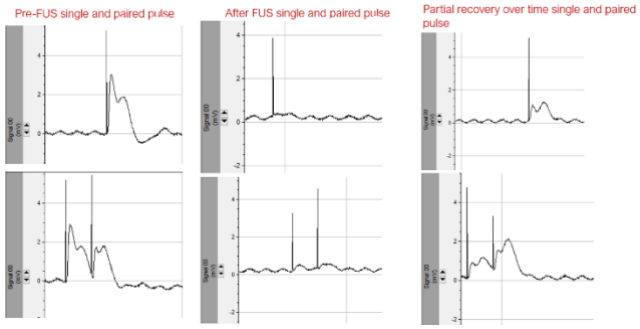


Figure 1: Experimental setup: recording and stimulating electrodes.

Figure 2: FUS probe embedded with parafilm.



Graph 1: Left piriform cortex (target) single and paired pulses pre-FUS, 5 minutes after FUS and 40 minutes after FUS.

7. ACKNOWLEDGMENTS

The experimental protocol was reviewed and approved by Committee on Animal Care and Use and by the Ethics Committee of the Fondazione IRCCS Istituto Neurologico Carlo Besta (DO-01-2020) and by the Italian Ministry of Health (Aut. Min. No. 271-2012/A).

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