**In vivo imaging of mice brain under RF exposure**

R. Orlacchio\(^{(1)}\), Y. Percherancier\(^{(2)}\), F. Poulletier de Gannes\(^{*,(2)}\), A. Hurtier\(^{(2)}\), I. Lagroye\(^{(2,3)}\), P. Lévêque\(^{(1)}\) and D. Arnaud-Cormos\(^{(1,4)}\)

(1) University of Limoges, CNRS, XLIM, UMR 7252, 87000 Limoges, France; e-mail: rosa.orlacchio@unilim.fr; philippe.leveque@unilim.fr; delia.arnaud-cormos@unilim.fr

(2) University of Bordeaux, CNRS, Bordeaux INP, IMS, UMR 5218, 33405 Talence cedex, France; e-mail: yann.percherancier@ims-bordeaux.fr; florence.poulletier@ims-bordeaux.fr; annabelle.hurtier@ims-bordeaux.fr; isabelle.lagroye@ims-bordeaux.fr

(3) Paris Sciences et Lettres Research University, 75006 Paris, France

(4) Institut Universitaire de France (IUF), 75005 Paris, France

The central nervous system (CNS) is the primary target of the electromagnetic waves emitted by portable devices under phone call scenario. Numerous studies concerning the interaction of radiofrequency (RF) with the brain and the neuronal activity were published, with conflicting results obtained in vitro or in animal models. Electroencephalography (EEG) was widely used to non-invasively investigate the potential effects of RF exposure on human brain activity. Variations in the sleep and waking EEG power spectrum during and/or after RF exposure was consistently reported, but whether the observed physiological changes are relevant or not remains unclear and the mechanism of interaction unknown. For this reason, the possibility to image real-time brain activity in in vivo models would provide a unique contribution to assess the biological effects induced by low-level RF exposure. Functional ultrasound (fUS) is an innovative neuroimaging technique based on ultrafast Doppler. It allows to map in vivo the areas of real-time brain activation and functional connectivity by imaging the hemodynamic changes induced in the vessels that surround the activated neurons. The goal of this study is to analyze the potential modifications of brain activation in anesthetized mice during the exposure to 1.8 GHz continuous wave and GSM. To this end, a specific in vivo experimental set-up consisting of a dipole antenna for the local exposure of the brain (Fig.1) was designed and characterized through numerical and experimental dosimetry. Mice were exposed to brain-averaged specific absorption rate (SAR) levels of 2 W/kg and 6 W/kg while recording brain activation. Our results show that the fUS probe can be efficiently used during in vivo exposure with no interferences with the dipole. In addition, we demonstrated that the exposure to both tested SAR levels does not introduce significant changes in the time course of cortical activation following whisker stimulation. For the first time, to the best of our knowledge, brain activity under mobile phone exposures was evaluated in vivo with fUS imaging. The proposed technique represents a valuable instrument to provide new insights into the possible effects elicited on brain activation under RF exposure paving the way to more realistic exposure configurations including awake mice under the upcoming frequencies of the 5G telecommunications networks.

**Figure 1.** Overall view of the experimental set-up.

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