**Talk**

## CAN FUNCTIONAL NEAR-INFRARED SPECTROSCOPY (fNIRS) GIVE US RELIABLE INSIGHTS ON SHEEP BRAIN ACTIVITY?

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Functional near-infrared spectroscopy (fNIRS) is a noninvasive optical technique for monitoring the concentration of oxygenated and deoxygenated haemoglobin in human cerebral cortex. Recently, fNIRS has been increasingly used in animal studies, but research is needed to improve measurement accuracy and result reliability (e.g. rejecting possible extracerebral contributions). A wearable continuous wave (CW) fNIRS system (Octamon, Artinis Medical Systems, The Netherlands) was used to measure the cerebral activity of ten freely moving ewes (Sarda breed, 8-month-old) undergoing a motor task (30s baseline, 30s walking; ten repetitions) and a startling test (after 30s baseline an umbrella was opened in front of the sheep for 3s, followed by 60s recovery; five repetitions). fNIRS sensors were applied on the depilated sheep forehead and held in place with a customized head cap. Two pairs of transmitter-receiver at short (10mm) and long (30mm) distance were used to record light intensity at two wavelengths (760nm, 850nm) from the left and right hemisphere. Data were fitted to a CW model for photon diffusion in a two-layer geometry to estimate absorption changes with respect to the baseline in the bottom layer (representing cerebral cortex) and in the upper layer (representing tissues above brain: scalp, skull, CSF). Upper layer thickness was derived from anatomical measurements, while baseline optical properties (absorption and reduced scattering coefficients) were derived from time-resolved reflectance measurements on the same sheep. Beer's law was then used to calculate oxygenated haemoglobin changes ($\Delta O_2Hb$) and deoxygenated haemoglobin changes ($\Delta HHb$) in both layers. Results of the motor task showed no changes in the upper layer and a canonical response in the bottom layer ($\Delta O_2Hb$ increase and $\Delta HHb$ decrease) in both hemispheres when sheep were walking (Fig 1a). For the startling test, we found no changes in the upper layer and, after the stimulus, a canonical response only in right bottom layer ($\Delta O_2Hb$ increase and $\Delta HHb$ decrease) (Fig 1b). These results confirm that CW fNIRS allowed to non-invasively measure cerebral cortex activity in freely moving sheep and that the use of short and long distance pairs of transmitter-receiver, coupled to a two-layer model for photon diffusion, was useful to reject extracerebral contributions. Measuring cerebral areas activation has the potential to give us new insights in the study of animal emotion and welfare.

This study was approved by the Italian National Ethical Commission (authorization n°457/2016-PR) and supported by MIUR-PRIN2015 (Grant 2015Y5W9YP).

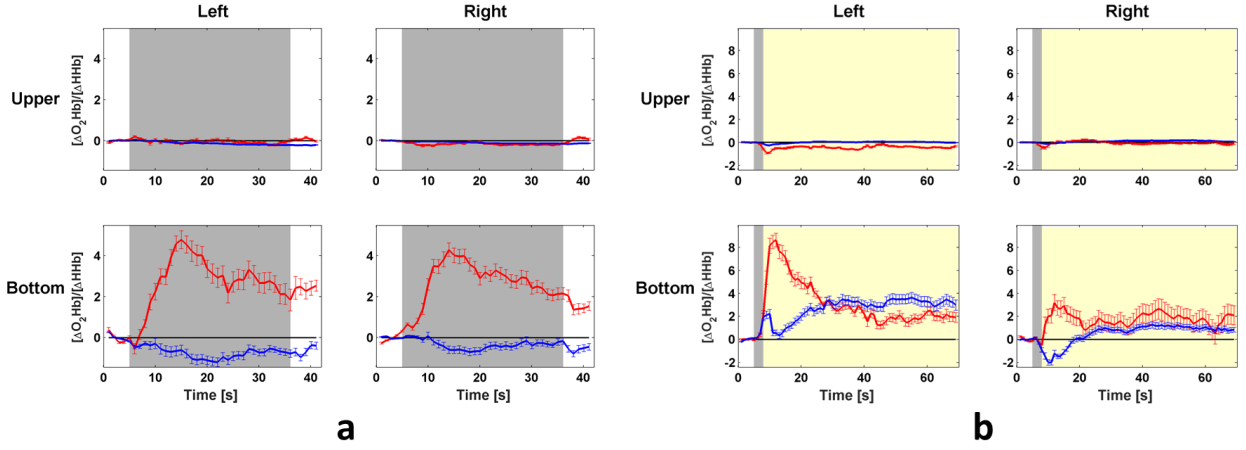


**Fig 1** - $\Delta O_2Hb$ (in red) and $\Delta HHb$ (in blue), average value ± SD calculated over repetitions and sheep, for the upper and bottom layer for both hemispheres (left and right). (a) Motor task: white part refers to baseline (sheep stand still), while grey part refers to sheep walking. (b) Startling test: white part refers to baseline (sheep stand still), grey part refers to umbrella opening and yellow part refers to fear reaction.