fNIRS technology applied on animals: a study on sheep

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INTRODUCTION

The lack of data concerning the organization of the central nervous system of the animals farmed for milk and meat production is a potential handicap for the study of cognition processes, important for the assessment of animal welfare, and a gap in comparative neuroscience. We conducted several trials in order to study the functional organization of the cerebral cortex of free-moving sheep through the non-invasive application of functional near-infrared spectroscopy (fNIRS), a developing technology that has started to be applied in animals (Muehlemann et al., 2011).We intended to record the oxygen consumption of selected areas of brain involved in the performance of behavioral motor and associative tasks and to assess brain activity when animals were anticipating either a positive or negative event.

METHODS

Here we used the continuous-wave fNIRS OxyPrem device and recorded the data with Tubis software (version 4.5) (Muehlemann et al., 2008). The fNIRS data was recorded for three sheep undergoing to a series of stimuli for 20 s, each followed by a 20 s interval. The stimuli applied were: 1) auditory, consisting in a neutral sound repeated 8 times; 2) visual, involving the application of the Finnoff light intermittently on the pupil, repeated 4 times per eye and 3) physical, entailing the application of a pressure of 250 mmHg with a tourniquet on the forelimb, 4 times per limb. We repeated the protocol recording the brain activity with electroencephalography (EEG). In addition, we set up an experiment to assess the brain activity when animals were anticipating either a positive or negative event. We trained eight sheep to anticipate two events with a supposed different value via classical conditioning. Two pairs of audio-visual stimuli anticipated either the presence of feed (positive event) or the absence of it (aversive event) in a testing area hidden behind screens. On test day, each sheep performed two sessions of six trials. A session consisted in 3 positive and 3 negative trials randomly distributed (always starting with a positive). During the task sheep were wearing the fNIRS device on the head. We focused on the brain activity during the 20 s of latency between submission to stimuli and events. Furthermore, we analyzed the behavior of the sheep during the anticipation period using validated ethograms (Reefmann et al., 2009; Boissy et al., 2011).

The signal was digitized with a sampling rate of 35 Hz. Data were filtered for eight paths. Absolute [O2Hb], [HHb] and [StO2] changes were calculated by applying the modified Beer-Lambert law.

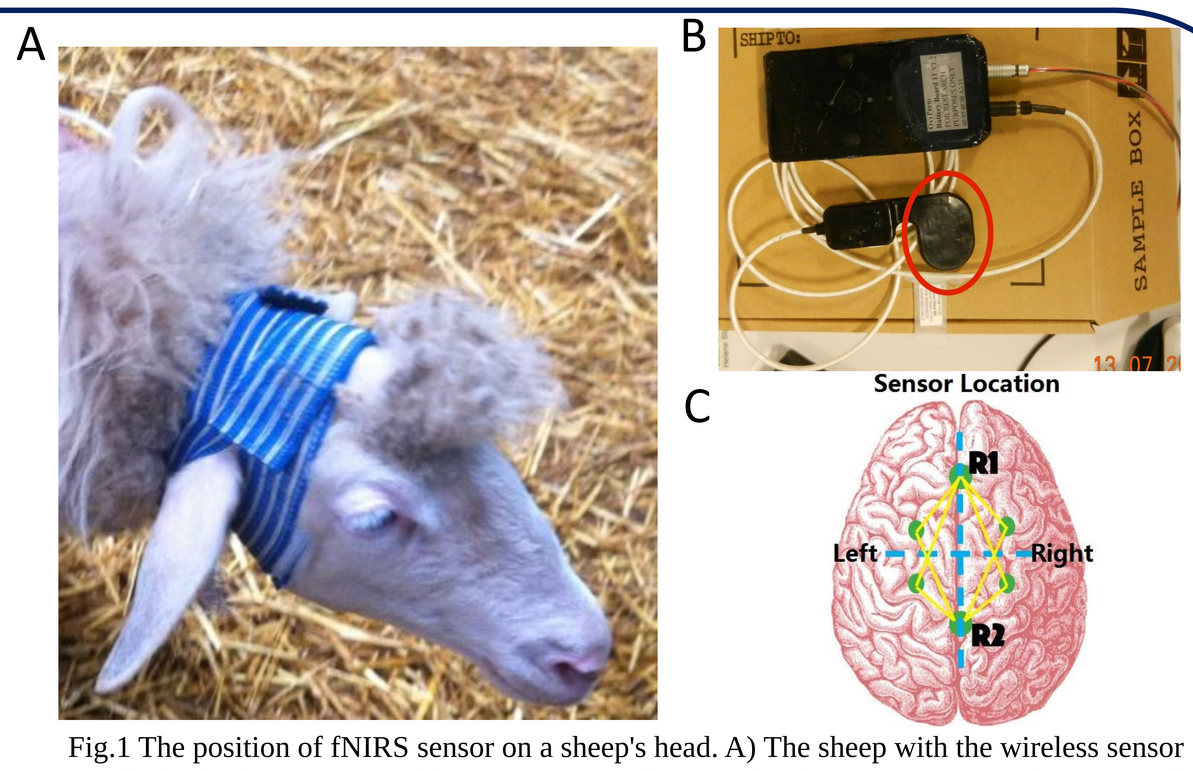

Fig.1 The position of fNIRS sensor on a sheep's head. A) The sheep with the wireless sensor attached. B) The overview of fNIRS OxyPrem device. C) Schematic top view of source and detector positions on the sheep's head. R1,R2 are two detectors,1-8 are eight light paths.

RESULTS

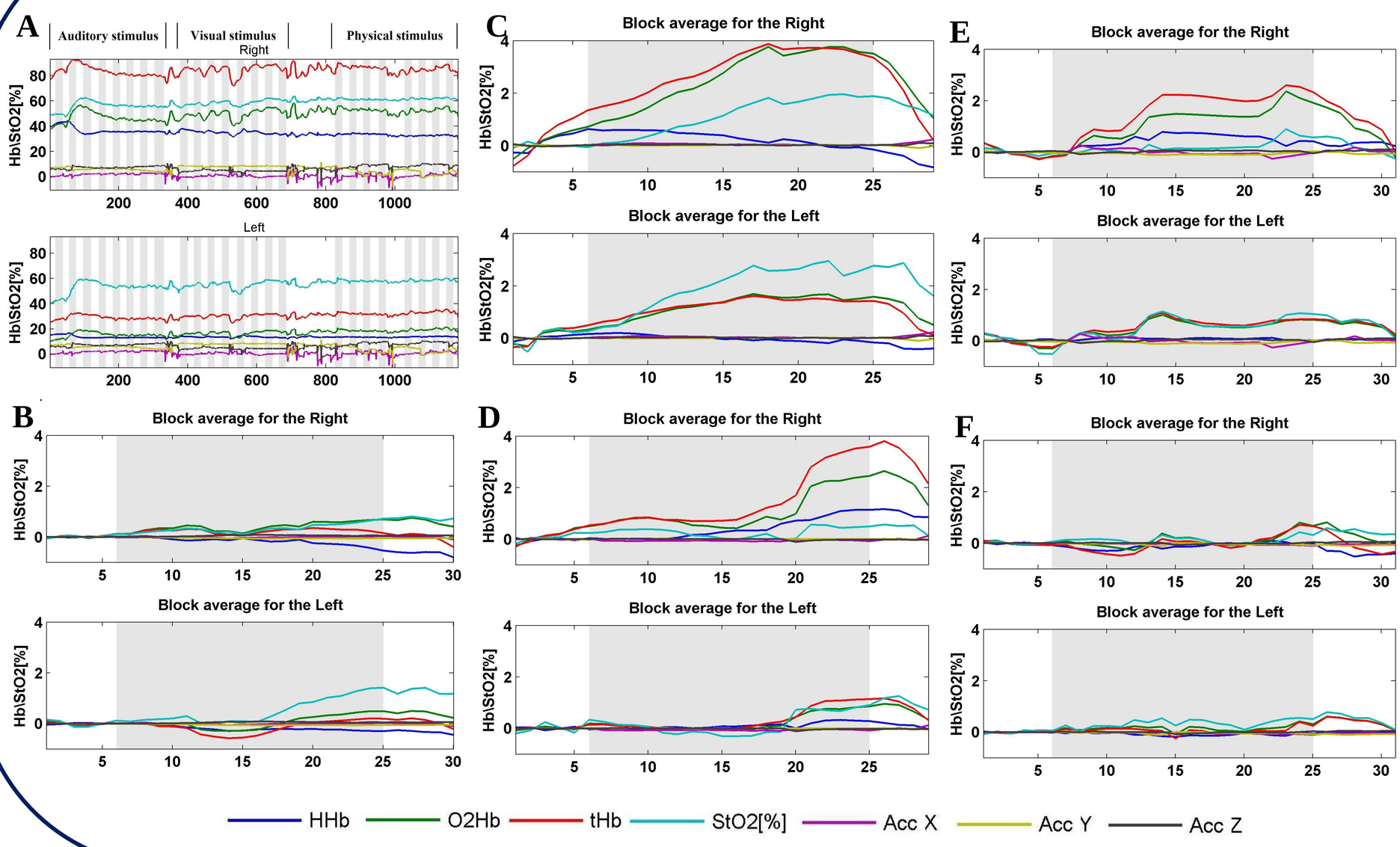

Fig.2 The absolute changes in concentration of O2Hb, HHb, tHb and StO2%, as well as the position change of the sensor for two regions of one sheep's head during the auditory-visual-physical stimuli. The grey areas are the stimuli. A)The whole stimuli. B) The block average for auditory stimulus; C) The block average for visual stimulus to the right eye. D) The block average for visual stimulus to the left eye. E) The block average for physical stimulus to the right forelimb. F) The block average for physical stimulus to the left forelimb.

Here we present the preliminary fNIRS results from one sheep during the sensory stimulation. When the sheep was exposed to the stimuli, cortical response could be observed in response to each stimulus. Specifically, the response on right hemisphere and left hemisphere were different for all the events, with the right hemisphere showing stronger response compared the left hemisphere. When comparing the different stimuli, the results show that the sheep had the strongest response during visual stimulus to the right eye (see Fig.2(C)), the weakest response during the physical stimulus to the left forelimb (see Fig.2(F)). However, due to head motion and limited sample, further study need to be done.

CONCLUSION

This was a pilot phase of a larger study and sample size was very limited. Further investigation is necessary in order to understand the cortex activity during sensorial stimulation in sheep. In the future we'll consider also process of lateralization in order to highlight a possible influence on one hemisphere.

fNIRS represents an innovative non-invasive method to conduct more objective assessments of animal behavior and helps to improve the evaluation of animal welfare. The data will allow the identification of the cerebral areas involved in the physiological regulation of complex and integrated behaviors with different emotional valences. Combining the exploration of the neural substrates underlying cognitive functions with existing behavioral and physiological measures will strengthen knowledge of how animals perceive different environmental situations. Validation of the data will also promote the use of large herbivores' brains as suitable scientific models in neuroscience.

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