Functional Near-Infrared Spectroscopy in Non-Domesticated Grey Seals

Alexander Ruesch[a], J. Chris McKnight[b], Gordon D. Hastie[b], Barbara G. Shinn-Cunningham[a,c], Jana M. Kainerstorfer[a,c]

[a] Neuroscience Institute, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213, USA;
[b] Sea Mammal Research Unit, Scottish Oceans Institute, University of St. Andrews, St. Andrews, Fife KY16 8LB, Scotland;
[c] Department of Biomedical Engineering, Carnegie Mellon University, 5000 Forbes Avenue, Pittsburgh, PA 15213, USA.
Author e-mail: aruesch@andrew.cmu.edu

Abstract: Functional near-infrared spectroscopy is successfully used to measure brain activation to visual, auditory, and tactile stimuli in non-domesticated grey seals. The results encourage further investigation of cognition in free-ranging animals.

1. Introduction

Functional near-infrared spectroscopy (fNIRS) is commonly used in humans as well as domesticated and laboratory animals, including pigs, sheep, and non-human primates. Other devices, like biologgers, have been used for years to measure acceleration and allow GPS tracking in free ranging animals, thus uncovering foraging and migratory patterns. Yet, there are no portable sensors to measure cerebral activity, either of neuronal or vascular origin, in free-ranging, non-domesticated animals undertaking common behaviors like hunting. Adapting portable fNIRS devices for use on non-domesticated animals in their natural environment could yield insight into how animals utilize different sensory inputs to guide decision making. Here, we explore the efficacy of fNIRS in detecting cortical activation in grey seals. The hemodynamic response to neuronal activation was mapped for three stimuli, light, sound, and whisker stimulation, allowing us to identify brain regions associated with each sensory input. Though the grey seals exhibited highly variable heart rate and very strong respiratory influences on the hemodynamic signals, hemodynamic responses were identified for all stimulus types.

2. Methods

Light intensity changes caused by hemodynamic changes in the brain of grey seals were measured using the wearable continuous wave NIRS system "Brite24" (Artinis Medical Systems BV, Elst, The Netherlands). The device consists of 16 emitters shining light at 760 nm and 850 nm, as well as 8 photodiode detectors. Twenty source-detector channels with a 30 mm distance were formed, as indicated in Fig. 1A. In addition, 8 short source-detector distance channels of 10 mm length were present but not used in this analysis. The system recorded from all channels using a 10 Hz sampling rate and streamed the data to a nearby laptop via a Bluetooth connection.

To fit the probes to the head, a custom head cap for juvenile grey seals was developed. The head of an anesthetized juvenile grey seal was photographed from various angles and a 3D model was calculated using photogrammetry software (Photomodeler Scanner 2016, Photomodeler Technologies, Vancouver, Canada). The model was 3D printed ('Form 2', Formlabs Inc., Somerville, MA, USA) at a 1:1 scale. Based on the printed model, a neoprene cap was tailored such that large portions of the seals cortex would be covered by the probe.

2.1 Experimental Protocol

Procedures for capture, handling, and housing of animals conformed to the Animals Scientific Procedures Act 1986, under the Sea Mammal Research Units' Home Office license (#70/7806) and were performed by personnel deemed competent under EU directive of the protection of animals used for scientific purposes. The experiments were conducted on 5 juvenile grey seals (Halichoerus grypus). The animals were captured in Moray Firth, Scotland, and temporarily housed at the animal facility of the Sea Mammal Research Unit at the University of St. Andrews, featuring three unheated saltwater pools. All animals were released back to the wild at the sight of capture after the conclusion of the experiment.

Ten minutes prior to sensory stimulation, each seal was sedated using midazolam (Hypnovel, Roche Products Ltd., UK; 5 mg ml-1 solution, 0.03 ml kg-1) intramuscular and a combination of midazolam (0.01 ml kg-1) and ketamine (Ketaset, Zoetis, UK 100 mg ml-1 solution, 0.01 ml kg-1) intravenously. Seals were then moved to a table in an indoor lab and equipped with a respiratory band ('FLOW', Sweetzpot, Oslo, Norway) to measure chest movement. The NIRS cap was fitted to the head and good signal strength verified in each channel by checking for the presence of clear heart beats in the live data stream.

Each experiment consisted of three epochs containing 8-10 blocks of stimuli. Each block consisted of the presentation of a sensory stimulus for 15 s followed by 15 s of stimulus absence. Every epoch was preceded and followed by a 30 second rest period. The order of epochs (corresponding to different stimulus types) was randomized. During visual stimulation, the seals were presented with a 50-lumen torchlight from a 30 cm distance. Auditory stimulation consisted of the presentation of 5 different sounds that should have been familiar to the animals, presented in random order (i.e., pile driving at 500 m or 40 km distance, tidal turbines at or and high sound intensity, and a training whistle). The tactile stimulus consisted of manually brushing the seals whiskers an equal number of times, but in a randomized order, on both sides. Randomization and stimulus timings were dictated and recorded using E-Prime 3.0 (Psychology Software Tools, Sharpsburg, PA, USA). Each animal was recruited twice, with at least 6 days of rest between experiments.

2.2 Hemodynamic response extraction

The raw data of light intensities was manually inspected for clear signs of motion artifacts or a lack of hemodynamic signals. Individual channels were marked for rejection on a per-block basis. The data was then loaded into the AnalyzIR toolbox [1] implemented in Matlab (The MathWorks Inc., Natick, MA, USA). The data sets were trimmed from 30 s before to 30 s after each epoch and down sampled to 5 Hz for more efficient data processing. Minor motion artifacts were removed using temporal derivative distribution repair [2]. PCA across all long-distance channels was used to remove no more than 70% of common variance, targeted towards the removal of global physiological drifts in the hemodynamic signals, such as respiration. From here, the modified Beer-Lambert law [3] was applied to extract changes in oxygenated (ΔHbO) and deoxygenated hemoglobin (ΔHbR) concentrations, as well as changes in total hemoglobin concentration (ΔHbT = ΔHbO + ΔHb). All hemoglobin signals were filtered with a pass band between 0.02 Hz and 0.3 Hz with a 4th order Butterworth filter. After applying blocks previously marked for rejection based on raw data inspection, we calculated block averages of -5 s to 35 s around the stimulation onset marker for every epoch. Tactile stimulations were sorted into left and right whisker stimulation, respectively. Group averages were calculated across the four groups of visual, auditory, left whisker, and right whisker stimulation. A two-sample t-test ('ttest2', Matlab, The MathWorks Inc., Natick, MA, USA) was used to establish the significance of an increase or decrease in hemoglobin concentrations. Baseline activity was estimated from data taken over the 3 seconds prior to stimulus presentation, put through the same processing pipeline. Measured results over a moving 3 s long window (ranging from 5 and 20 seconds after stimulus) were compared to this baseline. A change was considered to show a significant hemodynamic response if $p < 0.05$.

3. Results

The source and detector layout of the custom-built head cap relative to the seal's head shows good coverage of the cortex, presumably allowing us to measure all three stimulation types (Fig. 1A). An example of the hemodynamic changes occurring naturally in grey seals is shown in Fig. 1B. We found strong oscillations in ΔHbO and ΔHbT, characterized by an initial dip followed by a strong increase are seen approximately once a minute, accompanied by shallower opposite reactions in ΔHbR. We expected this to be related to the seals' breathing pattern, which is very abrupt and deep compared to humans, with long pauses between respiratory events.

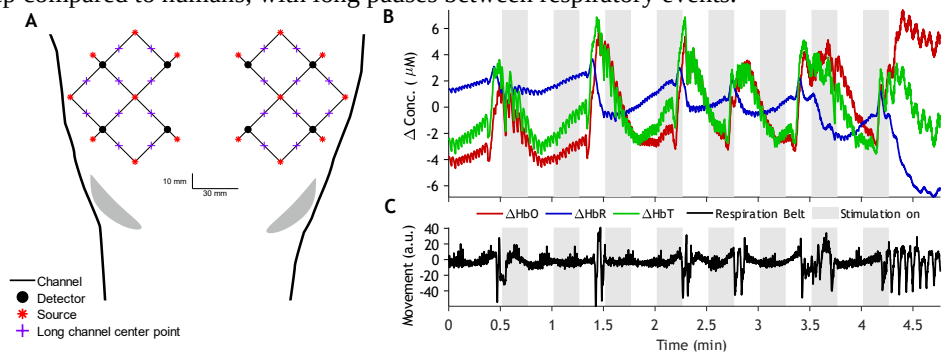

Fig. 1. (A) The multi-channel layout of the fNIRS device. Long distance channels are marked with a purple plus symbol and considered for further data processing. (B) Raw data measured from one seal. Gray shaded bars show stimulation periods. (C) The respiration belt output corresponding to the animal's chest movement. Image after [4].

This suspicion is supported by comparisons to readings from the respiration band (Fig. 1C). Grey areas show the periods of stimulation, every 15 s, and were found to not correlate with the observed respiration events. The heart

rate strongly affects the hemodynamic response, which changes rapidly during a respiration event; however, despite this, we determined that the hemodynamic responses to neuronal activity were of good quality.

Visual stimulation causes activity bilaterally, predominantly in the posterior region, as in other mammals. Cortical responses to auditory stimuli were found ventral to the supra-Sylvian sulcus, as previously described [5]. Auditory activation reached into the presumed visual cortex. Finally, tactile activation appeared in the hemisphere contralateral to the side of stimulation, but with a poorly defined location. The three sensory stimuli each elicited distinct activation patterns involving different brain regions. Fig. 2 shows one example of left whisker stimulation, which produced a response in ΔHbO in the right hemisphere.

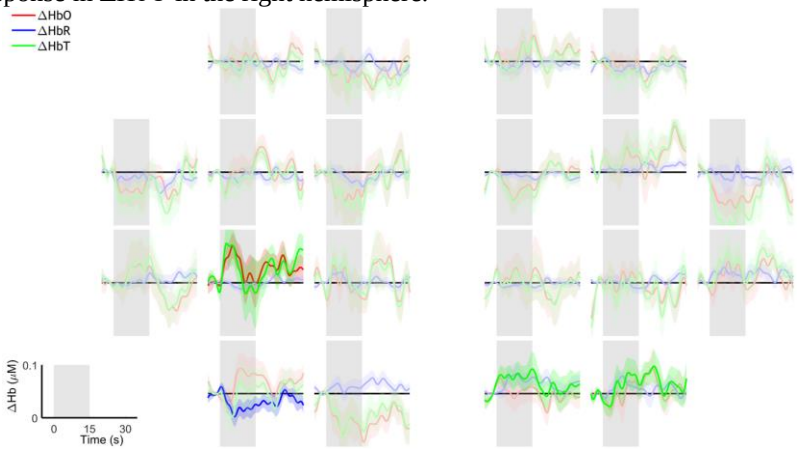

Fig. 2. Group averaged responses to **left** vibrissal stimulation in ΔHbO (red), ΔHbR (blue), and ΔHbT (green). Shaded ribbons represent the standard deviation. Gray bars show the time of stimulation. Strong colors represent measurements that were found to have a significant (Student's t-test, $p<0.05$) change as compared to the pre-stimulus time. Significant activation was observed on the right hemisphere. Image from [4].

4. Discussion

Here we have shown that fNIRS can be successfully applied to marine mammals, specifically grey seals. We measured differences in brain activity in response to visual, auditory, and somatosensory stimulation similar to what free-ranging animals likely encounter naturally. The hemodynamics in grey seals offer unique challenges due to the strong and fast respiratory events that are accompanied by extreme heartrate changes (from about 25 bpm to 45 bpm in Fig. 1B) that far exceed hemodynamic changes observed in terrestrial mammals. What on the one hand presents a challenge in the processing of hemodynamic responses to neuronal activation, can on the other hand yield valuable additional information about physiological processes in these animals related to tissue oxygenation, arterial saturation with oxygen, heart and respiration rates, and blood volume and flow changes. Such data can inform interpretation of future resting state measurements and recordings of free-ranging animals that performing everyday behaviors in natural environments. While the current results are a proof of concept, a larger cohort of animal should be measured to confirm the correspondence between sensory stimulation and brain activation patterns in future fNIRS studies. Our results suggest that fNIRS could be usefully incorporated into biologging systems to support investigations into how these animals utilize and process different sensory inputs in daily activities like hunting or avoiding predators.

5. Acknowledgement

This project was funded as part of the Department for Business, Energy and Industrial Strategy Offshore Energy Strategic Environmental Assessment programme. Supplementary funding was provided by the Office of Naval Research Ocean Acoustics Program (N00014-19-1-1223). Assistance in funding for acquisition of the fNIRS system was provided by SMRU Consulting's 10th Anniversary award.

6. References

[1] H. Santosa, X. Zhai, F. Fishburn, and T. Huppert, "The NIRS Brain AnalyzIR toolbox," *Algorithms*, vol. 11, no. 5, 2018, doi: 10.3390/A11050073.

[2] F. A. Fishburn, R. S. Ludlum, C. J. Vaidya, and A. V. Medvedev, "Temporal Derivative Distribution Repair (TDDR): A motion correction method for fNIRS," *Neuroimage*, vol. 184, no. June 2018, pp. 171–179, 2019, doi: 10.1016/j.neuroimage.2018.09.025.

[3] A. Sassaroli and S. Fantini, "Comment on the modified Beer–Lambert law for scattering media," *Phys. Med. Biol.*, vol. 49, no. 14, pp. N255–N257, 2004, doi: 10.1088/0031-9155/49/14/N07.

[4] J. C. McKnight *et al.*, "Shining new light on sensory brain activation and physiological measurement in seals using wearable optical technology," *Philos. Trans. R. Soc. B Biol. Sci.*, Nov. 2021.

[5] A. M. Alderson, E. Diamantopoulos S, and C. B. Downman, "Auditory cortex of the seal (Phoca vitulina).," *J. Anat.*, vol. 94, pp. 506–11, 1960.