Simultaneous Measurement of Brain Activity, Physiology & Behavior in Large Animals

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ABSTRACT
Assessing an animal's neuro-physiological and behavioral reaction to external stimuli simultaneously, continuously, and non-invasively is a major aim of applied animal welfare research to interpret a situation from the animal's perspective. The purpose of this study was to assess responses of large animals (sheep) to 3 model situations with positive, intermediate and negative emotional valence. The following reactions of up to 19 sheep were recorded: brain activity using functional near-infrared spectroscopy, facial expressions (ear postures and eye aperture), electrocardiogram, respiration rate, and electro-dermal activity. All variables were successfully recorded in the sheep and are promising to assess stress and well-being in sheep and potentially other large animals. The combination of several methods resulted in a comprehensive picture of the animals' reactions to environmental stimuli, initiating innovative animal research that combines neuroimaging, physiological monitoring and behavioral observations.

Author Keywords
animal welfare, cardiac activity, respiration, electrodermal activity, emotion, facial expression, neuroimaging, sheep

INTRODUCTION
Concurrently assessing neuro-physiological and behavioral changes in animals is highly useful for a comprehensive picture of how an animal reacts to its environment. Psycho-physiological research in humans has often focused on assessing both behavioral and physiological reactions at the same time [2]. Observations of facial expressions were combined with cardio-respiratory and electro-dermal activity. The measurement of brain activity or associated hemodynamic oxygenation changes has become increasingly applied in humans with the availability of corresponding techniques [1]. In animals, however, these methods have not been widely applied, and measuring brain activity remains a particular challenge in non-sedated animals. Here, we report concurrent assessment of hemodynamic changes in the brain, cardio-respiratory activity and facial expressions in large mammals. Electro-dermal activity was assessed in a similar setup. Our aim was to evaluate which of these methods are most promising to assess the animals' positive and negative emotional reactions. In addition, we investigated correlations between physiological and behavioral variables with regard to the possibility of substituting the latter with the former, and if any of those variables could be assessed at shorter time periods (10 seconds) in order to decrease the measurement duration and effort of signal analysis.

METHODS

Animals and Testing
Nineteen female sheep were used as experimental animals and housed at the Agroscope Reckenholz-Tänikon Research Station ART, Tänikon in Switzerland. The animals' reactions to external stimuli, which were very likely to elicit different emotional reactions, were assessed in an experimental area within their home pen. Being equipped with the measurement devices described below (see Figure 1), they were exposed to the following three exemplary situations ranging from positive to negative emotional

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valence: i) positive: animals were voluntarily groomed by a familiar human, ii) intermediate: animals were not groomed and free to move in the experimental area, iii) negative: separation from other sheep. The study was licensed by the Cantonal Veterinary Office.

Neuroimaging

Functional changes in cerebral oxygenation were assessed using a specifically designed wireless near-infrared spectroscopy sensor [3]. A halter held the small sensor (35mm x 22mm x 80mm) on the sheep's head. Light from four near-infrared sources (760nm and 870nm each) was emitted through the intact skull into the brain, and two detectors collected the transmitted light. Data were wirelessly transmitted to a nearby stationary receiver and mean changes in oxygenated and deoxygenated hemoglobin were compared for up to 9 repeated stimulations of grooming. Data were analyzed for differential reactions with respect to the localization of hemodynamic changes, i.e. right and left hemisphere, rostral and caudal as well as shallow and deep brain oxygenation. For more details see [4].

Physiology

The electrocardiogram of an animal was continuously recorded using three electrodes by means of a human Holter recorder (Modular Digital Holter Recorder, Lifecard CF, DelMar Reynolds GmbH). Using the Pathfinder software provided by the same company, mean heart rate (beats per minute) and heart rate variability (root mean square of successive difference, RMSSD) were calculated and compared across the three situations. For more details regarding all the physiological measurements, see [7].

A commercial logger (MSR145W, Modular Signal Recorder Electronics GmbH) was fixed on the animal's depilated skin with a breathable plaster in order to detect the relative body-surface humidity [%] and the body-surface temperature [°C] on the animals' skin of the rump once per second. These data were later analyzed as mean temperature, humidity and variance of both measures over a given time period.

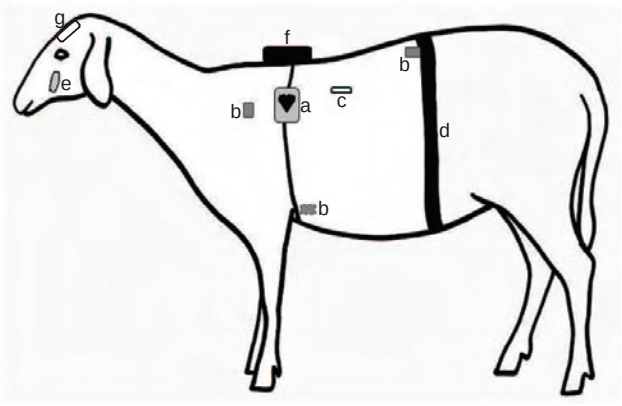

Figure 1. Sheep wearing measurement equipment (adapted from [7]: a) electrocardiogram holter and b) electrodes, c) electro-dermal activity device, d) respiration rate device, e) eye camera, f) battery pack, g) fNIRS sensor.

An extendable belt (1132 Pneumotrace II, UFI) in front of the hind legs around the abdomen of a sheep generated a continuous signal for the relative extension of the belt during inspiration and exhalation. The signal was saved at a rate of 10 Hz by the logger used for electro-dermal recordings, and the respiration rate was determined from these recordings by a smoothed spectrogram of the signal's time series in S-PLUS (Version 7.0 for Windows).

Behavior

In analogy to human facial expressions, the animals' ears and eyes were observed. A number of different ear postures and movements were recorded in continuous direct observations (see [5] for more details). Two finger cameras (DV-2000B Weatherproof, CCD B&W finger camera, Conrad Electronics) placed on the sheep's head recorded the pictures for analyzing the eye aperture and amount of visible eye white of the sheep. The size of the eyes were electronically measured on the digitized images with on-screen mouse clicks in R, and compared across the situations.

RESULTS & DISCUSSION

All measures were simultaneously assessable in sheep using the devices as described. Cerebral changes in relative tissue oxygenation could be assessed during the positive compared to the intermediate situation (please see [4] for details). Motion artifacts in the negative situation were too strong for reliable data analysis. Nevertheless, the fNIRS sensor is the only device that can be used to measure hemodynamic oxygenation changes non-invasively in non-sedated and moving animals.

The device to assess cardiac activity was well suited to record the animals' electrocardiogram. The analysis tool is programmed for humans and special adaptation of the software to detect R-peaks specifically for other species would be desirable in the future to analyze the data in less time. The respiration rate was measurable in sheep using the Pneumotrace belt. Raw data should be pre-processed to remove movement artifacts before automatically calculating mean respiration rate based on the signal's time series during a given time period. Assessing the relative electro-dermal activity posed an easy way to monitor body surface humidity and temperature of the animals, which are related to sympathetic nervous system activity.

Observations of ear postures presented the most non-invasive way to externally monitor an animal's reaction to a situation. The number of ear-posture changes or, for example, forward ear postures (Figure 2), were higher in the negative than the positive emotional situation (for more results see [5]). The ear postures can be recorded in direct observations or video-recorded and later analyzed using commercially available software for behavioral analysis.

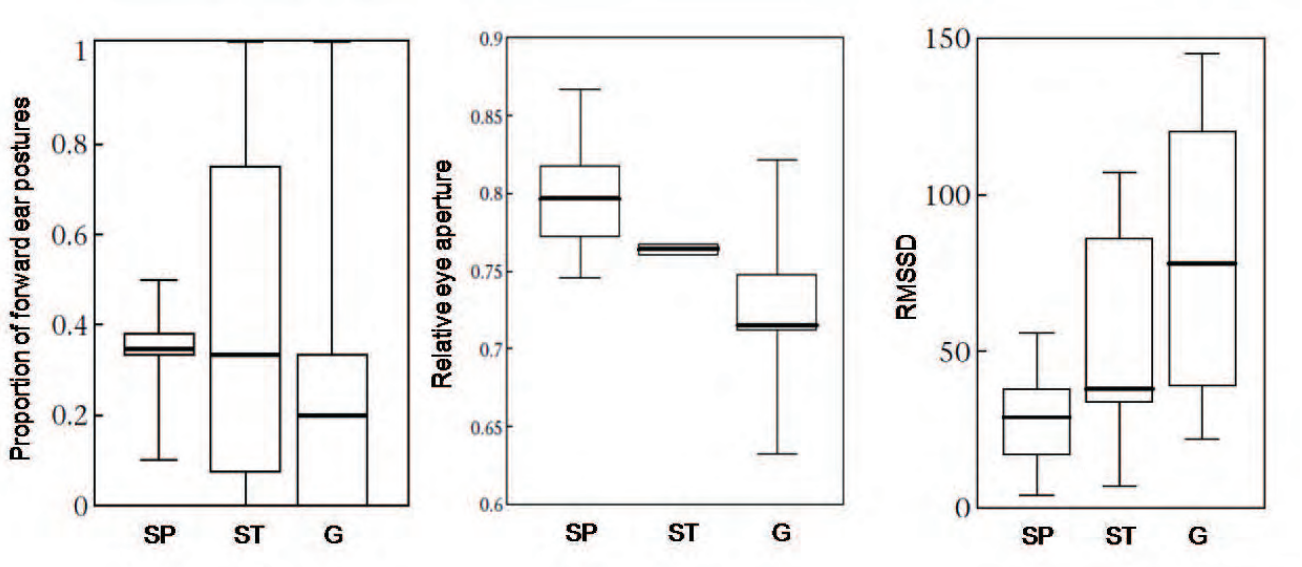

Figure 2. Sheep's behavioural and physiological responses to separation from group members (SP, negative valence), standing in the feeding area (ST, intermediate valence), and grooming (G, positive valence); adapted from [7].

Observations of the eyes of animals using the robust and water-proof finger cameras were also possible. Nevertheless, the development of eye trackers for animals would be highly appreciated by researchers since these observations are not possible in direct observations and would contribute greatly to assess sympathetic physiological responses in animals.

The number of ear-posture changes and the relative eye aperture both correlated strongly negative with heart rate variability, indicating that behavioral observations could replace the monitoring of cardiac activity (see [7] for more details). Given that some physiological reactions were reflected in the behavioral responses, future experiments do not have to assess all measures so that the required time and effort can be reduced but the animal's reaction to environmental stimuli is still fully assessed.

With respect to the measurement duration, cardiac activity is regulated very rapidly and can be assessed within ten-second time windows. This short interval also seemed to be sufficient for assessing eye aperture (Figure 2). All other physiological and behavioral measures should be assessed continuously within longer time windows to reliably assess the animals' reactions. Ear postures [5] and respiration rate, for example, are best monitored within at least 30-second time windows.

CONCLUSION

This study demonstrates that cerebral, physiological and behavioral responses can be concurrently, continuously and non-invasively assessed in sheep. Innovative behavioral measures like ear postures may even substitute physiological reactions, and measuring hemodynamic changes is possible in unsedated and freely moving animals, so that a whole new range of possibilities opens up to assess subjective states in animals in response to their environment.

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