EEG based assessment of stress in horses: A Pilot Study

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As has been hypothesized more than 20 years ago, data derived from EEG (Electroencephalography) measurements can be used to distinguish between behavioral states associated with animal welfare. In our current study we found a high degree of correlation between the modulation index of phase related amplitude changes in the EEG of horses (n=6 measurements with three different horses, both sexes) and their facial expression, measured by the use of the horse grimace scale. Furthermore, the pattern of EEG derived phase amplitude coupling was significantly different between a rest condition and a stress condition in horses. This pilot study paves the way for a possible use of EEG derived phase amplitude coupling as an objective tool for the assessment of animal welfare. Beyond that, the method might be useful to assess welfare aspects in the clinical setting for human patients, as for example in the neonatal intensive care unit.

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Abstract

As has been hypothesized more than 20 years ago, data derived from EEG (Electroencephalography) measurements can be used to distinguish between behavioral states associated with animal welfare. In our current study we found a high degree of correlation between the modulation index of phase related amplitude changes in the EEG of horses (n=6 measurements with three different horses, both sexes) and their facial expression, measured by the use of the horse grimace scale. Furthermore, the pattern of phase amplitude coupling was significantly different between a rest condition and a stress condition in horses. This pilot study paves the way for a possible use of EEG derived phase amplitude coupling as an objective tool for the assessment of animal welfare. Beyond that, the method might be useful to assess welfare aspects in the clinical setting for human patients, as for example in the neonatal intensive care unit.

Introduction

Extensive research is needed in the field of animal welfare to establish animal-based indicators for well-being. Because, judging about animal welfare is most often based on or at least influenced by human assumptions and humanization (http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.7.1.htm). One reason is the lack of adequate techniques for an objective measurement of animal welfare (Barnett & Hemsworth, 1990). We addressed this question by combining a well- established technique for pain and stress assessment, the horse grimace scale (Dalla Costa et al., 2014, 2018), with telemetric EEG recordings as a promising novel tool for the measurement of objective data related to animal welfare and signs of stress (Senko et al., 2017, Hohlbaum et al., 2017, Häger et al., 2017). Facial expression scores are well-established to measure pain in human infants (Grunau & Craig, 1987) and for some species with clear facial expression, as for example horses HGS (Dalla Costa et al., 2014), mice MGS (Langford et al., 2010) and rats (Sotocinal et al., 2011). Dalla Costa et al. validated the facial expression score by promising a statistic approach to identify a classifier that can estimate the pain status of the animal based on Facial Action Units (FAUs). There exists no doubt that animals are able to experience pain, fear, stress and other moods and show these through facial expressions. The electroencephalogram (EEG), first described by Berger, is a method to measure tiny summed electrical potentials on the scalp surface which arise from pyramidal cells of the cortex (Berger, 1929). Therefore, non-invasive EEG measurements always represent network activity of cortical neurons rather than single cell activity. Cortical networks are highly dynamic and they are broadly orchestrated, which leads to electrical oscillations that can be measured on the surface of the scalp (Kida et al., 2016). These oscillations are in the range of very slow waves below 0.1 Hz, as they occur for example in preterm babies (Vanhatalo et al., 2002). Faster oscillations are categorized as theta, alpha, beta and gamma bands, some authors additionally define waves above 90 Hz as a "high gamma EEG-band" (Cavelli et al., 2017). A shift from low frequency EEG activity towards high frequency, low amplitude activity has been described during the castration procedure of calves (Coetzee, 2013). A shift of EEG band activity and lateralization have also been observed during attention-related processes in horse (Rochais et al., 2018). More than 20 years ago, it was hypothesized that new tools of EEG analysis will lead to objective measurements for the assessment of animal welfare (Klemm, 1992). One method to find qualitative changes of network activity is phase amplitude coupling (PAC) or also called cross frequency coupling (Tort et al., 2010). PAC is based on amplitude modulations between EEG bands if they occur in a certain phase relation to each other (Tort et al., 2010). This phenomenon does for example occur during certain vigilance states (Scheffzück et al., 2011) or it can be modified by the application of drugs (Scheffzück et al., 2013). Therefore we used in a proof of concept experiment the HGS as well as the PAC as a qualitative network change index and compared the intensity of amplitude modulation under resting condition and during stress condition (i.e. anticipating a medical treatment of horses in the veterinary clinic). We hypothesized that the HGS as well as the PAC are significantly different in the horses in accordance to the different conditions and second that there is an association between HGS and PAC, which means the EEG could be an objective tool for the assessment of animal welfare.

Materials & Methods

Animal Experiments

All procedures were approved by the local ethics committee (L0294113), and followed the European and the German national regulations (Animal Welfare act, 2010/63/EU). All animal procedures were performed in accordance with the animal care committee's regulations [Freie Universität Berlin]. During six different days, three adult horses, belonging to the demonstration stock for veterinary students at the Freie Universität Berlin, were recorded (n=6 different veterinary interventions). All of our measurements took place during routine teaching lessons for the students, which does mean that no further experimental animals have been used solely for our study. We measured two vigilance states of each horse with the horse grimace scale and with EEG. First, the animals were recorded in a familiar situation in the stable to assess the relaxed state as a reference, called "resting condition". The second measurement took place in the examination stand in anticipation of stressful situation i.e. a veterinary treatment, called "stress condition" (Fig. 1).

Horse Grimace Scale

Panasonic Digital camera Lumix FMC-FZ200 was used to record the facial expression of the horses during the different conditions. The records were used for analyzing the HGS in accordance to Dalla Costa et al. (2014). One HGS value was calculated for each video file. Regarding the correlation with EEG coupling coefficients, corresponding files were selected, but the video files are of longer duration than EEG files.

EEG recordings

Disposable adhesive surface silver/silverchloride electrodes (Spes Medica S.r.l., 111 Genova, Italy) were placed on the nose (between the ears) as ground and reference (Fig 2 (2,3) and between eye and ear to record from the right somatosensory cortex region (parietal position, Fig 2 (1)). Before fixating the electrode, the location was shaved and the skin was cleaned with an abrasive cream (Abralyt HiCl, Easycap GmbH, Herrsching, Germany) in order to remove dead skin cells and to achieve a lower impedance. The data was recorded and sent by a telemetry unit (with an AC coupled amplifier, sampling rate 500Hz) (Lapray et al., 2009) . Only phases without artefacts were taken into account for analysis. Selected sequences lasted on an average 90s in case of the resting condition and 50 s in case of the stress condition.

EEG analysis

EEG segments without artefacts (muscle, extended line noise) were selected.

We analyzed the Data with matlab (2016, MathWorks) and with brainstorm (Tadel et al., 2011). EEG raw data were filtered with digital butterworth filters with a custom written matlab script. The filter was designed with the function butter (n=3rd order). We calculated the normalized cutoff frequency (Wn) for EEG bands delta [0-4Hz], theta [4-8Hz], alpha [8-13Hz], beta [13-30Hz], low gamma [30-80Hz] and high gamma [80-120Hz]. Wn is a number between 0 and 1, where 1 corresponds to the Nyquist frequency which is half the sampling rate (here: 500Hz).

The numerator and denominator values (IIR filter), achieved with the function butter, were used with the matlab function filtfilt to filter the EEG data. For the delta EEG band, a lowpass filter was used. We extracted all other EEG frequency bands with a bandpass filter design.

Phase amplitude coupling and Phase Locking Value (PLV) were analyzed with brainstorm software (Tadel et al., 2011).

To obtain Canolty maps (Canolty et al., 2006), following procedure was computed (neuroimage.usc.edu/brainstorm/Tutorials/Resting): The EEG was filtered at the low frequency of interest, using a narrow band pass filter. The amplitude troughs of the desired low frequency were detected in the signal. A time window is defined around the detected troughs in order to compute a time frequency decomposition using a set of narrow band-pass filters.

Statistics

To extract statistically significant changes of amplitude modulation, we compared the modulation indices calculated with the open source software brainstorm [19] between the resting condition and the stress condition. First, we pooled all cycles of the phase frequency (8Hz) for each animal during a time window of 1s, corresponding to 8 phase cycles, and used the sum for each animal. We had a resolution of 125 modulation index values within a single cycle of the phase frequency, which corresponds to 360° of the 8Hz phase frequency wave. 10ms in the Canolty maps correspond to 2,9° of the phase frequency. We calculated the differences of modulation indices for every 2.9° of the phase frequency between the resting and the stress condition. The statistically significant results are shown as polar plot. Furthermore, we calculated the coefficient of correlation between the modulation index of the EEG and the results of the horse grimace scale for both behavioral states (function "corr", type “Spearman”, Matlab, 2016b).

We tested data for distribution with the Lilliefors test (Matlab 2016b). Tests were performed with the non-parametric Kruskal Wallis test and a subsequent multiple comparison test in order to achieve exact statistical relations between groups. All tests are implemented in the Matlab statistics toolbox (Matlab 2016b, MathWorks) .

Results

We graded the horses comfort behavior according to the horse grimace scale (Dalla Costa et al., 2014). EEG data and behavioral data were analyzed independently by two different persons to avoid statistical interferences. Both methods reflect changes of behavioral state.

Horse Grimace Scale

We were able to identify statistically significant changes of the facial expressions between the resting condition and the stressful condition in horses (p=0,006). The mean facial expression score for the resting condition is 4,12 (with a standard deviation of 0,57 and an upper and lower confidence interval of 5,42 and 2,83). The mean facial expression score for the condition stress is 6 (with a standard deviation of 0,61 and an upper and lower confidence interval of 7,34 and 4,66).

EEG

We were not able to detect statistically significant changes of horses EEG band power between the rest condition and the stress condition in anticipation of a veterinary treatment. Nevertheless there is a tendency towards slightly elevated EEG band power during the stress condition in comparison to the rest condition.

In contrast, we were able to identify major changes of phase amplitude coupling between the rest, and the stress condition (Fig 3). A progressive decay of phase amplitude coupling between 8Hz low frequency and the gamma and high gamma band takes place from rest towards stress. Besides the mere decay of coupling density a qualitative change regarding phase relation between the high and low frequency is clearly visible. For the rest condition, the coupling patterns extend from gamma to high gamma (up to 250 Hz) with maximal coupling coefficients during the up stroke and the down stroke of the 8 Hz low frequency (indicated as white sine wave in Fig 3a). For the condition stress (Fig 3b), the phase amplitude coupling pattern is still visible in the gamma range but overall coupling strength is lower in comparison to the resting state. Statistically significant differences of phase related amplitude modulation between the rest and the stress condition can be found during the upstroke (around 90° of the phase frequency) and during the down stroke (around 260°) (Fig 4), confirming the optical impression from the Canolty maps (supplementary table 1). Furthermore, only the down stroke of the 8Hz phase frequency reveals strong coupling coefficients with gamma and high gamma EEG bands. The coefficient of correlation (Rho) between the EEG derived data (Coupling coefficients of the Canolty map) and the horse grimace scale is -0.86 (p=0.00031) for the down stroke of the phase frequency (here at 290°) and 0,71 (p=0.01) for the up stroke of the phase frequency. The quality of raw data is good, the presence of nested activity was confirmed with a wavelet analysis (Fig 5).

Discussion

Animal welfare and animal well-being is part of controversial discussions. This happens in different contexts, be it socio-political or ethical discourses, factory farming and food production, animals in private husbandry, animal-assisted therapy, zoos, wildlife or animal experiments. They all have in common that both forming and exchange of opinions are often lead rather based on emotions than on scientific findings. The assessment of welfare and well-being of animals is sometimes made by how humans feel when they find animals in certain situations. Besides the lack of ethological knowledge, in many areas related to animal husbandry or the use of animals, there are none or only imprecise legal regulations. In the field of laboratory animal science legal matters are more closely regulated, both in the context of authorization and in connection with surveillance. The commencement of Directive 63/2010/EU enforced efforts concerning animal welfare on national and European level and specified personal and institutional prerequisites. People working in this field have to prove their expertise and have to be continuously educated. Housing facilities and experiments have to be approved and procedures have to be ethically justified. Serious and extensive efforts have to be made in order to replace, reduce and refine (3Rs) experimental procedures performed on the animals or their housing conditions. There must be a close documentation of all interventions and a scientifically reasoned assessment and classification in degrees of severity according to the impairment of the animals' well-being.

For the assessment of welfare and well-being of animals in factory farming and food production, animals in private husbandry, animal-assisted therapy, zoos, wildlife or animal experiments we need adequate techniques for an objective measurement of animal welfare (Barnett & Hemsworth, 1990) and associated physiological states as for example EEG. The EEG reflects cognitive functions not only in human but also in horses. A recent paper by Rochais et al. (2018) confirmed that a higher attention state in horses is associated with a higher proportion of gamma waves. There was moreover an interaction between the attention state, the hemisphere and the EEG profile: attention towards the visual stimulus was associated with a significant increase of gamma wave proportion in the right hemisphere while "inattention" was associated with more alpha and beta waves in the left hemisphere.

As has been proposed earlier (Klemm, 1992), we were able to extract differences in EEG network activity during stress and rest in horses, mental states that are obviously characterized by significant changes in HGS. In order to validate the behavioral states "stress" and "rest" in horses, we used the horse grimace scale (Dalla Costa et al., 2018&2018). Phase amplitude coupling seems to be an extremely robust tool to extract activity patterns in the brain, associated with distinct behavioral states to access animal welfare. One reason for the robustness might be that phase amplitude coupling is extracting an information which is inherently linked to intrinsic network phenomena. The measurement does not rely on absolute values, which may be influenced by technical or intra-individual issues, but on a relational modulation index. Furthermore, phase related amplitude modulations seem to be an ubiquitous phenomenon for neural networks, as they are also involved in the generation of peristaltic movements of the intestinal tract (Huizinga et al., 2015). In fact, phase amplitude coupling in the intestinal tract is also associated with the regulation of peristaltic movements according to incoming stimuli (for example food intake). Further studies with a higher number of animals and more electrode positions may be beneficial to confirm our results and to gain further insights regarding anatomical correlations of certain behavioral representations in the EEG. Furthermore, facial expression can hardly be used to recognize welfare in species like birds, reptiles etc. with poor facial expression. If it is possible to establish reliable EEG patterns associated with stress for different species in future studies, a translational approach for species with poor facial expression might be possible. It must be kept in mind that the activity of the brain is highly dynamic, also known a neural plasticity. The context can change the activity of the brain. Every measurement of the EEG can also potentially influence the content of the EEG – some kind of uncertainty relation. Telemetric EEG recordings are extremely useful in this context, because they do not restrict the freedom of movement of the animals. Another important factor is the miniaturization of EEG amplifiers as well as comfortable electrodes which are as small as possible. For our future studies with a higher number of electrodes, we developed light curing polymer electrodes (de Camp et al., 2018). Furthermore, in a current project, the amplifier with 8 Ch will be miniaturized to 9x12mm. This EEG system will be potentially useful to assess EEG measurements in small species like birds or rodents.

Conclusions

We hypothesized that the HGS as well as the modulation index (phase amplitude coupling, PAC) derived from EEG data are significantly different in the horses in accordance to different behavioral conditions and second that there is an association between HGS and PAC, which means the EEG could be an objective tool for the assessment of animal welfare.

In conclusion, we were able to find an association between the scientifically validated horse grimace scale and an EEG pattern, associated with two distinct behavioral states, namely stress and rest in horses. Phase amplitude coupling might be a robust tool for the objective assessment of animal welfare and well-being of animals. Furthermore, it may be useful to judge about brain states as well as comfort of neonates or disabled persons, who are unable to communicate actively.

Our pilot study gives strong evidence that EEG measurements can be an objective tool for the assessment of animal welfare and stress.

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References

Barnett J L, Hemsworth, P H. 1990. The Validity of Physiological and Behavioural Measures of Animal Welfare. *Applied Animal Behaviour Science* 25: 177-187.

Berger H. 1929. Ueber das Elektrenkephalogramm des Menschen. *Archiv für Psychiatrie* 87: 527-570.

de Camp N V, Kalinka G, Bergeler J. 2018. Light-cured polymer electrodes for non-invasive EEG recordings. *Sci Rep* 8:14041.

Canolty R T. et al. 2006. High gamma power is phase-locked to theta oscillations in human neocortex. *Science* 313.5793 : 1626-28.

Cavelli M, Rojas-Líbano D, Schwarzkopf N, Castro-Zaballa S, Gonzalez J, Mondino A, et al. 2017. Power and coherence of cortical High Frequency Oscillations during wakefulness and sleep. *European Journal of Neuroscience* 18: ejn 13718.

Coetzee J F. 2013 Assessment and management of pain associated with castration in cattle. *The Veterinary Clinics of North America Food animal practice* 29(1): 75-101.

Dalla Costa E, Minero M, Lebelt D, Stucke D, Canali E, Leach M C. 2014. Development of the Horse Grimace Scale (HGS) as a Pain Assessment Tool in Horses Undergoing Routine Castration. *PLoS ONE* 9(3): e92281.

Dalla Costa E, Pascuzzo R, Leach MC, Dai F, Lebelt D, Vantini S, et al. 2018. Can grimace scales estimate the pain status in horses and mice? A statistical approach to identify a classifier. *PLoS ONE*. 13(8): e0200339 DOI: 10.1371/journal.pone.0200339

Grunau R V E., Craig K D. 1987. Pain expression in neonates: facial action and cry. *Pain* 28: 395-410.

Häger C et al. 2017. The Sheep Grimace Scale as an indicator of post-operative distress and pain in laboratory sheep. *PLoS One* 12(4):e0175839. DOI: 10.1371/journal.pone.0175839

Hohlbaum K, Bert B, Dietze S, Palme R, Fink H, Thöne-Reineke C.2017. Severity classification of repeated isoflurane anesthesia in C57BL/6JRj mice-Assessing the degree of distress. *PLoS One* 12(6):e0179588 DOI: 10.1371/journal.pone.0179588

Huizinga J D, Parsons S P, Chen J-H, Pawelka A, Pistilli M, Li C, et al. 2015 Motor patterns of the small intestine explained by phase-amplitude coupling of two pacemaker activities: the critical importance of propagation velocity. *Am J Physiol Cell Physiol* 309: C403-C414.

Kida T, Tanaka E, Kakigi R. 2016. Multi-Dimensional Dynamics of Human Electromagnetic Brain Activity. *Frontiers in Human Neuroscience* 9:713.

Klemm W R. 1992. Are there EEG correlates of mental states in animals? *Neuropsychobiology* 26(3): 151-65.

Langford D J, Bailey A L, Chanda M L, Clarke S E, Drummond T E, Echols S et al. 2010. Coding of facial expressions of pain in the laboratory mouse. *Nature Methods* 7(6): 447-9.

Lapray D, Bergeler J, Dupont E, Thews O, and Luhmann H J. 2009. A novel telemetric system for recording brain activity in small animals. In Telemetry: Research, Technology and Applications, D. Barculo and J. Daniels, eds. Nova Science Publishers), pp. 195-203.

OIE (World Organization for Animal Health). Chapter 7.1. Introduction to the recommendations for animal welfare. Terrestrial Animal Health Code 2010. Available at http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.7.1.htm.

Rochais C, Sébilleau M, Menoret M, Oger M, Henry S, Hausberger M & Cousillas H. 2018. Attentional state and brain processes: state-dependent lateralization of EEG profiles in horses. *Scientific Reports* 8:10153 | DOI:10.1038/s41598-018-28334-9.

Scheffzück C, Kukushka V I, Vyssotski A L, Draguhn A, Tort A B L, Brankack, J. 2011. Selective Coupling between Theta Phase and Neocortical Fast Gamma Oscillations during REM-Sleep in Mice. *PLoS ONE* 6(12): e28489.

Senko T, Olexova L, Mokosakova M, Krsková L. 2017. Angiotensin II enhancement during pregnancy influences the emotionality of rat offspring (Rattus norvegicus) in adulthood. Potential use of the Rat Grimace Scale. *Neuro Endocrinol Lett* 38(2):117-123

Scheffzück C, Kukushka V I, Vyssotski A L, Draguhn A, Tort A B L, Brankack J. 2013. Global slowing of network oscillations in mouse neocortex by diazepam. *Neuropharmacology* 65: 123-133.

Sotocinal S G, Sorge R. E, Zaloum A, Tuttle A H, Martin L J, Wieskopf J S et al. 2011. The Rat Grimace Scale: A partially automated method for quantifying pain in the laboratory rat via facial expression. *Molecular Pain* 7:55.

Tadel F, Baillet S, Mosher J C, Pantazis D, Leahy R M. 2011. Brainstorm: a user-friendly application for MEG/EEG analysis. *Comput. Intell. Neurosci*. 879716.

Tort A B, Komorowski R, Eichenbaum H, Kopell N. 2010. Measuring Phase-Amplitude Coupling Between Neuronal Oscillations of Different Frequencies. *J Neurophysiol* 104: 1195–1210.

Vanhatalo S, Tallgren P, Andersson S, Sainio K, Voipio J, and Kaila K. 2002. DC-EEG discloses prominent, very slow activity patterns during sleep in preterm infants. *Clin.Neurophysiol*. 113: 1822-1825.

Figure legends

Fig 1. Horse in examination stand. The telemetry unit is fixed at the shoulder with a piece of tape. As an example, typical facial expressions in this picture are: Ears oriented backwards (score 2), closed eyes (here 0), tension above eyes (score 1), tension in the region around jaw muscles (score 1), tension around muzzle and prominent chin (score 1), tension around nozzles, flattened profile (score 2). The sum of all facial expression scores is 7 for this image.

Fig 2. Electrode positions. The ground and reference electrode were placed above the nose (2,3), the recording electrode was placed in a parietal position between the right eye and ear (1).

Fig 3. Canolty maps for rest EEG and stress EEG. Phase related amplitude modulation is shown as heat map. The low frequency for both behavioral conditions is 8 Hz (indicated as white sine wave in subplot a). Maximal amplitude modulation is visible during the up- an down stroke of the low frequency in the gamma and high gamma band with a peak at 130 Hz during rest (subplot a). In the stress condition (subplot b) the coupling pattern is quantitatively weaker, additionally the phase relation is changed. Amplitude modulation is maximal during the down stroke, the upstroke is only weakly associated with amplitude modulation in contrast to the resting condition (subplot a). Maximal modulation index values are again visible around 130 ° in the high gamma range.

Fig 4. Number of statistically significant differences of phase related amplitude modulation between rest and stress. Most differences of the EEG derived modulation index between behavioral states can be found for the upstroke of the phase frequency around 60°. A minor fraction of significant differences is visible during the down stroke around 210°.

Fig 5. EEG raw data trace with nested activity of multiple bands. One second of original EEG recording is shown as raw data trace (upper panel) and wavelet analysis (lower panel). High power is coded as warm color (red). The wavelet analysis reveals nested activity in the alpha, beta and gamma band of the EEG.

Figure 1

Figure 1 Horse in examination stand.

The telemetry unit is fixed at the shoulder with a piece of tape. As an example, typical facial expressions in this picture are: Ears oriented backwards (score 2), closed eyes (here 0), tension above eyes (score 1), tension in the region around jaw muscles (score 1), tension around muzzle and prominent chin (score 1), tension around nozzles, flattened profile (score 2). The sum of all facial expression scores is 7 for this image.

Figure 2

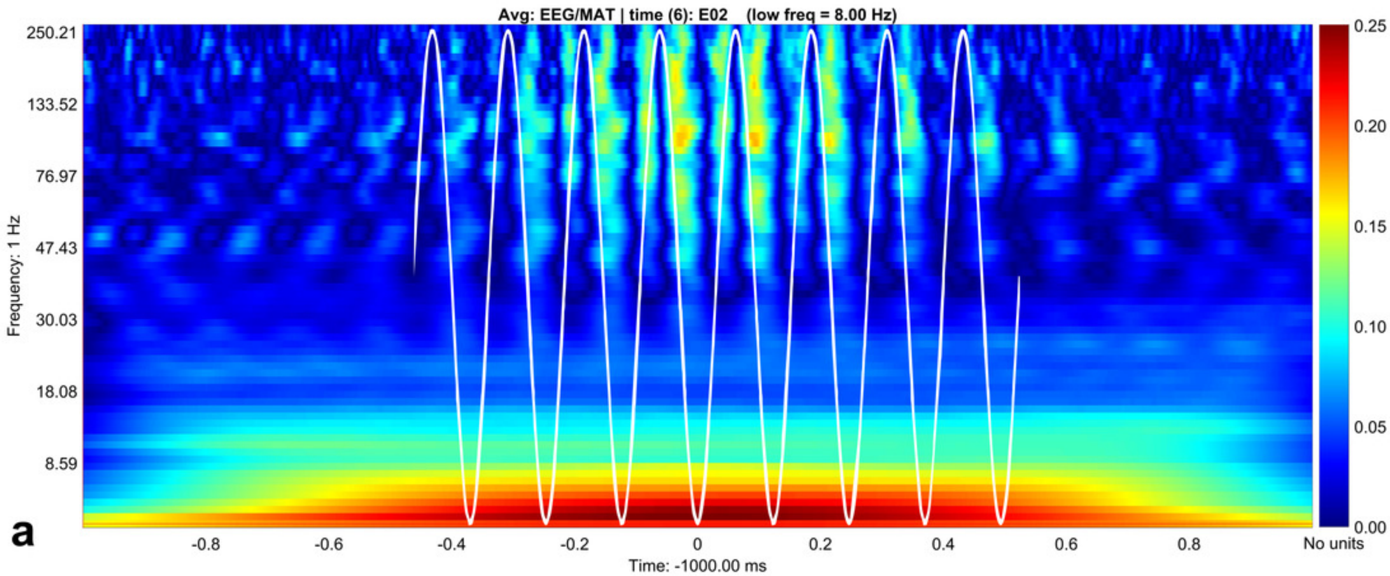
Figure 2 Electrode positions.

The ground and reference electrode were placed above the nose (2,3), the recording electrode was placed in a parietal position between the right eye and ear (1).

Figure 3

Figure 3 Canolty maps for rest EEG and stress EEG.

Phase related amplitude modulation is shown as heat map. The low frequency for both behavioral conditions is 8 Hz (indicated as white sine wave in subplot a). Maximal amplitude modulation is visible during the up- an down stroke of the low frequency in the gamma and high gamma band with a peak at 130 Hz during rest (subplot a). In the stress condition (subplot b) the coupling pattern is quantitatively weaker, additionally the phase relation is changed. Amplitude modulation is maximal during the down stroke, the upstroke is only weakly associated with amplitude modulation in contrast to the resting condition (subplot a). Maximal modulation index values are again visible around 130 ° in the high gamma range.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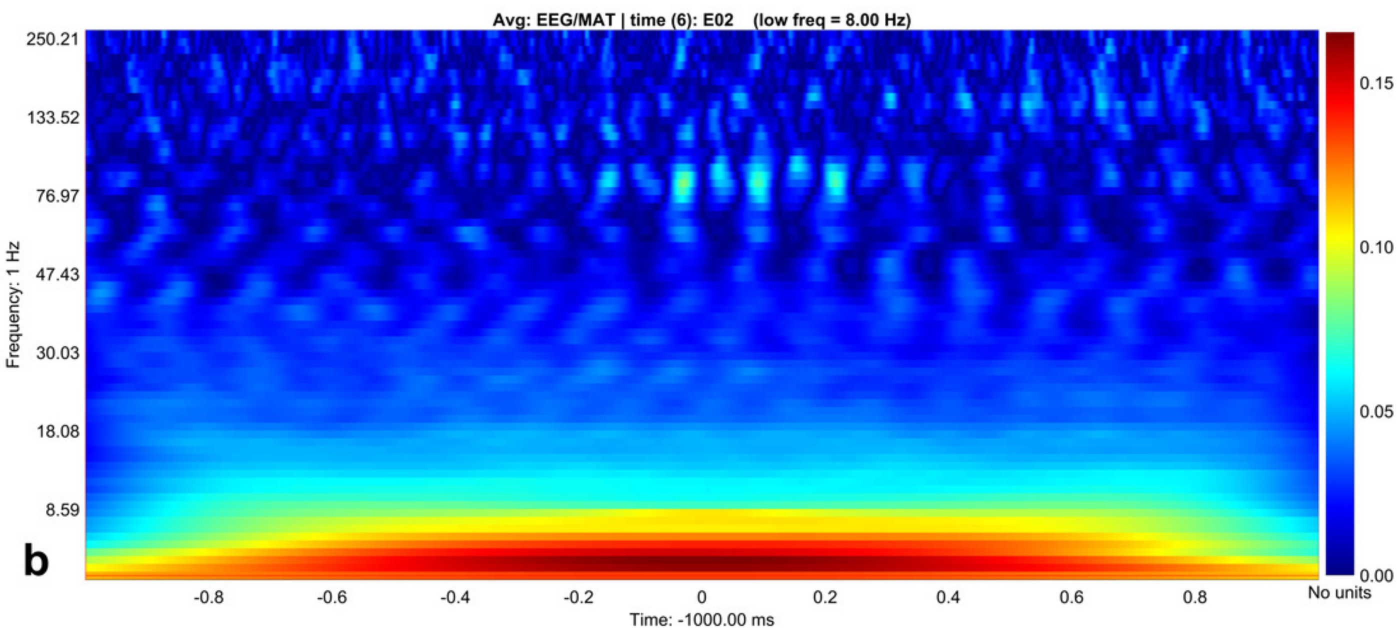
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Figure 4

Fig 4. Number of statistically significant differences of phase related amplitude modulation between rest and stress.

Most differences of the EEG derived modulation index between behavioral states can be found for the upstroke of the phase frequency around 60°. A minor fraction of significant differences is visible during the down stroke around 210°.

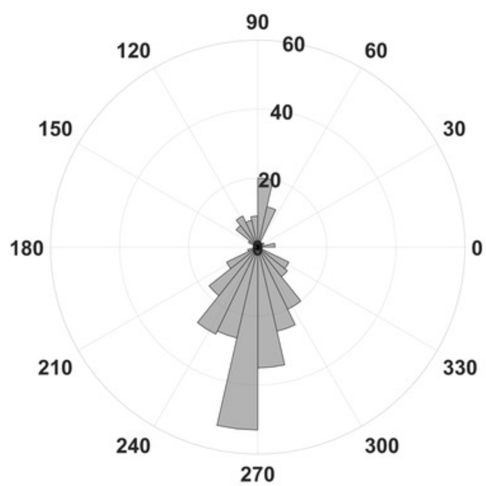

Figure 5

Fig 5. EEG raw data trace with nested activity of multiple bands.

One second of original EEG recording is shown as raw data trace (upper panel) and wavelet analysis (lower panel). High power is coded as warm color (red). The wavelet analysis reveals nested activity in the alpha, beta and gamma band of the EEG.