COST EMF - MED (Action BM1309):
network for innovative uses of EMFs in biomedical applications

KTIA_NAP_13-2-2015-0011

STSM Report:

Investigation of changes in power and phase on the ongoing human EEG using novel single trial analysis methods

Researcher: Dr. Attila Trunk. email: attila.trunk@icloud.com

Home Institution: Translational Neuroscience Research Group, University of Pécs, Pécs, Hungary. Contact: Dr. István Hernádi. email: hernadi@gamma.ttk.pte.hu

Host Institution: Laboratory for Human Brain Dynamics, Nicosia Cyprus. Contact: Dr. Andreas Ioannides

STSM Reference: ECOST-STSM-BM1309-32132

STSM dates: FROM 7th March 2016 TO 18th April 2016

Abstract:

During the STSM we developed a multifunctional data analysis script programme package 1) re-reference any given electrode to its geometrically opposite neighbors 2) calculate the possible effects of a treatment (e.g., pharmacological treatments, EMF exposure from different sources, , etc.) using point-by-point non parametric permutation statistics on the amplitude accounting for single trial variability, 3) implement a novel approach to EEG analysis, namely, counting the extremes in single trial time-courses of composite channels. The approach emphasizes the importance of local activity in the EEG signal pattern. Using the developed algorithm we analyzed single trial EEG data and tested the effects of acute caffeine consumption on cognitive performance (psychomotor vigilance) in humans.

A. Purpose of the STSM

In the present STSM program I visited the Laboratory for Human Brain Dynamics (LHBD) in Nicosia, Cyprus. Our special aim was to develop new single trial analysis methods what we can use to analyze high density electroencephalography (EEG) data previously recorded in our laboratories. The host institute, LHBD is a private research company located in Nicosia, Cyprus, and this company is headed by Dr. Andreas A. Ioannides. It was established in 2004 and from 2009 provided the continuation platform for the laboratory with the same name that was started and headed by Dr. Ioannides throughout its operation (1998 – 2009) at the Japanese Research centre RIKEN, as part of the Japanese initiative to establish a pioneering Research in neuroscience with the Brain Science Institute at RIKEN. The host institute, LHBD in Cyprus, emphasizes both basic research and applications, including investigation of brain functions in both healthy humans and patients with neurological disorders. Overall the research interest of the LHBD is partly to continue the analysis of MEG data collected at BSI, RIKEN and partly to develop state-of-the-art methods for measuring, processing and interpreting EEG and neuroimaging data.

The leader of the group Dr. Ioannides has published over 100 well qualified scientific papers in peer reviewed journals such as NeuroImage, Int. J. Psychophysiol, Human Brain Mapping or Neuron, and he was invited to several international conferences where he presented his work as invited and key note speaker. He has years of experience in designing MEG and EEG experiments and analyzing high density EEG/MEG data. In cooperation with other researchers Dr. Ioannides developed new offline data referencing as well as special single

trial EEG analysis methods. For example, one of the developed methods is an epoch distribution tree (dendrogram) showing the distribution of single trial extremas around special event related potential peaks and the other one is the feature space construction of the single trials.

My previous research involved designing and supporting biological experiments using EEG and analyzing recorded EEG data. Thus visiting a laboratory with such state-of-the-art analysis methods proved extremely beneficial for both myself and my host institution at the Univ. Pécs, Hungary (UoPH).

In the next sections I will describe the work I have done during the STSM. I will describe the basics of the paradigm we used during for EEG data collection. I will differentiate between the conventional (e.g. averaged signal analysis) and the novel EEG analysis methods that we used. Then, I will present the algorithm I developed in collaboration with the team of LHBD and implemented in a concise computer program and the results we got during the STSM.

B. Work Description

Psychomotor Vigilance Test: Many test batteries have been developed so far to objectively investigate the deterioration of cognitive function e.g., through the analysis of behavioral measures such as reaction time. The Psychomotor Vigilance Test (PVT) is the most widely used test battery since the 1980s for its simplicity [(Basner & Dinges, 2011); (Basner, Mollicone, & Dinges, 2011)] and robustness. The task measures the actual arousal state, alertness and sustained attention of the participant. A huge number of studies have been using the PVT paradigm so far to investigate the effects of sleep loss as well as extended wakefulness as it is known that sleep deprivation degrades several attentional function in the human brain (Drummond et al., 2005). The subjects' task is to press a button on a reaction time measurement box at the time when the reaction time counter starts. After the button press subjects can see his or her own reaction time on the actual trial as a feedback. The test itself is based on a simple idea that the targets the subjects have to respond to occur at random intervals. In the traditional PVT the inter-stimulus intervals (ISI) before the target appears varies between 1 to 10 sec.

In our present project, first we developed our own computerized version of the PVT test. Namely, a priori we binned ISIs into nine categories. Each random ISI in each bin was calculated using built-in functions in the Matlab programming environment. In order to determine the interval for randomization in each response bin we used the median values of each bin interval (e.g. for bin 1 the median time value was 1.5, for bin 2 the median time value was 2.5, etc.). Then we applied a +-0.4 sec interval variation on these median values. Hence a typical interval started from x.1 sec to x.9 sec. The 'x' corresponds to the bin categories (from 1 to 9). Hereafter these bins were named as local target probability categories (Prob1 to Prob9) as they are represent the temporal probability of the target (Trunk et al., 2015). Both fixation and target stimuli was presented on the center of the screen. Each session consisted of a total of 405 trials, with 45 trials in each local probability category. Figure 1 shows the schematic diagram of the modified version of the PVT.

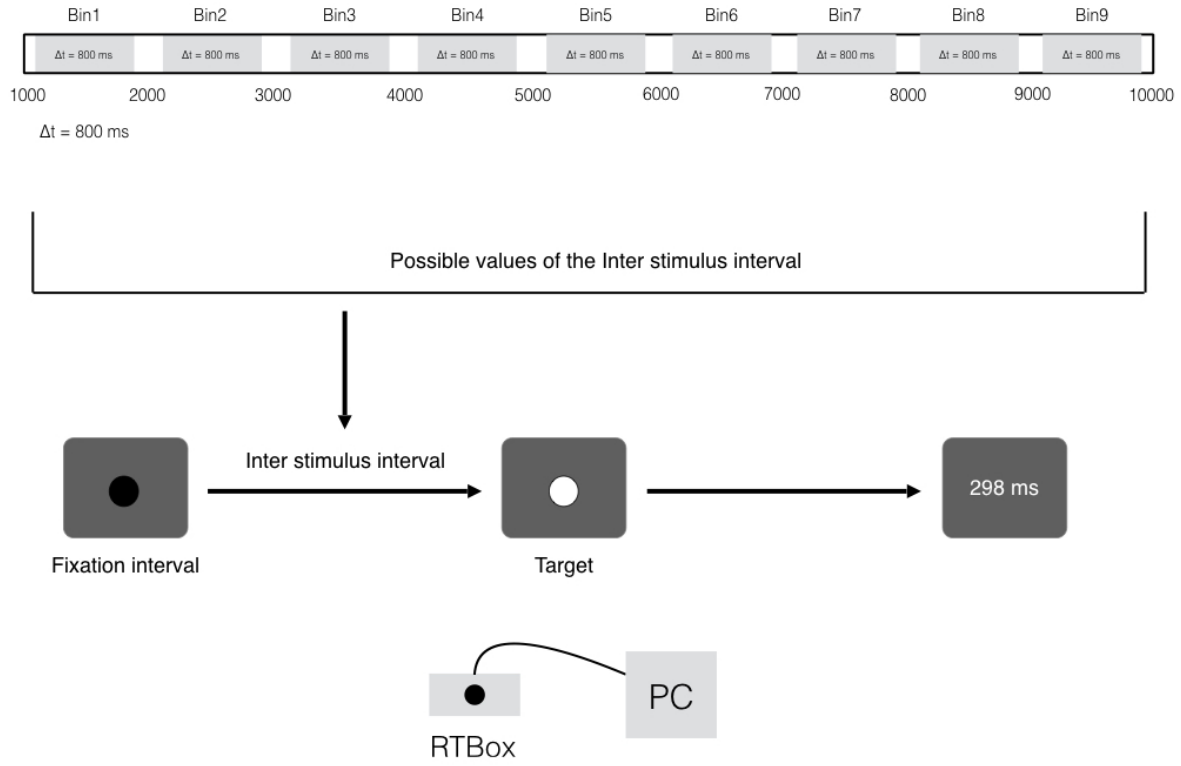

Figure 1. The schematic diagram of the Psychomotor Vigilance Task.

EEG recording design: The protocol of the study was approved by the Ethical Committee of the University of Pécs (reference number: 4174). Three mg/kg caffeine packed in identical hard gelatin capsules were administered to the participants. Control capsules contained less than 0.5 g glucose per capsule without any additional substance. Similar capsules were used for each treatment. To avoid potential influences caused by subjective bias on the number of capsules taken, participants received the same amount of capsules in the placebo sessions as in the caffeine sessions. Electroencephalography data were acquired with a 64-channel actiCap system (Easycap, Munich, Germany) and BrainAmp amplifier (Brain Products GmbH, Munich, Germany). Electrodes were placed according to the International 10–20 system. The ground and reference electrodes were placed at AFz and FPz locations, respectively. An additional EOG electrode was used to measure the eye movements and blinks. The impedance was measured at the beginning of each session and was adjusted to less than 15 kOhm at all electrodes which value meets with BrainAmp's recommendations. On-line band-pass filter were used between 0.016 Hz and 250 Hz. Raw data were digitized at 16 bit at a sampling rate of 1 kHz. Participants were asked to keep their head and eye-movements at minimum during the whole recording session.

In a double blind, crossover experimental design, participants took part in two experimental sessions (caffeine and placebo) with at least one week interval between the sessions. In each session subjects performed PVT in one block without break. Each recording session lasted for 55 min (405 trials). The EEG recording started 45 minutes after caffeine or placebo capsules ingestion.

General approach: Most of the studies which investigated the possible adverse effects of the EMFs on magnetic- and electric-evoked brain potential so far used fairly limited analysis methods. Those studies have focused on comparing the averaged EEG activity or mean evoked responses over the entire scalp with time domains (e.g., across trials or through the entire recording session). The main advantages of this averaging method is that it improves the signal-noise ratio by cancelling out the ongoing background activity. However, with this traditional/conventional EEG analysis approach it is not possible to detect any biological effects of the applied

EMF energy that are not precisely time-locked to the onset of the stimulus. Therefore, the method misses that are not time-locked to the stimulus onset at the single trial level, such as treatment-time interactions modulated by the the ongoing brain activity or related to other cognitive effects. For example, effect of EMF exposure may change over time, so the observed effects may be cancelled out if we just simply average together the M/EEG activity during the whole recording session. Furthermore, averaging may eliminate those small but important treatment related relevant changes (e.g. background changes which are not precisely time-locked to the stimulus). For example, Laskaris and co-workers have demonstrated that evoked brain responses to the stimuli are extremely variable [(Laskaris & Ioannides, 2001), (Laskaris & Ioannides, 2002), (Laskaris, Liu, & Ioannides, 2003)]. The authors said that these variabilities refer to "*random ongoing background activity which is considered to be irrelevant to the processing of the stimuli and can therefore be eliminated by ensemble averaging*" (Laskaris et al., 2003). These average signals are mirrors of both local and global function-related brain dynamics and so they are a mixture of functioning of different neural networks. Therefore one has to take into account the brain related changes at the single trial level using spatial filtering of the data (e.g. neighboring re-reference of the data) to describe precisely the effects of any EMF exposures on the brain responses.

For this purpose, we developed a multifunctional script. We used built-in and self-developed functions under EEGLAB toolbox (http://sccn.ucsd.edu/eeglab/ (Delorme & Makeig, 2004) in Matlab (MathWorks, Natick, MA) programming environment.

EEG Pre-processing: The first part of the script was the EEG pre-processing routine. The pre-processing script imports the raw EEG data and converts them into EEGLAB compatible '*.set*' format, so the script applies low, high, bandpass or bandstop FIR filter, based on the user action. Then the script chops the continuous raw data into segments, using the trigger values and times that define the type of stimulus (task) and the precise timing of the target onset. A lot of emphasis was placed on making this pre-processing step generic so it could be applied (in the future) to different data recorded at each laboratory.

Neighboring re-referencing: Then, we proceeded with re-referencing the data which was the main part of the script and the work we have done. The raw data was recorded using common reference by BrainProducts (this is the EEG data acquisition system at UoPH). Usually in the EEG field most of the researchers advocate offline average re-referencing especially when the electrode montage covers nearly the whole head. This approach is based on idea that the sum of the activity of the all electrodes is zero. This is not really accurate but it is a fair approximation if the electrodes cover uniformly the entire head, something which is not true with the partial coverage of electrodes to only the top part of the head. There are two the disadvantages of this approach. First, the average reference will reflect the incomplete coverage of the head and this effect will be present in all new channel derivations. Second, any large artifact in any one channel or a group of nearby channels will be reflected in each channel derivation (but of course reduced by the facor 1/N with N the number of electrodes). Therefore, the average reference do not show spatial differentiation of activities. To do so, we adapted a method first applied for MEG data (Liu & Ioannides, 1996) for EEG. For this the first application we implemented the simplest version of the idea, assuming that any given electrode can be re-referenced to its geometrically opposite neighbors; of course with this choice we were limited to studying local activity close to electrodes which had neighbours in two nearly orthogonal directions. We called this method neighbor 4 referencing (NHB4). After applying this method the representative activity at each electrode showed more local activity rather than a mixture of signals arising from different sources. With this NHB4 re-referencing approach spatial differentiation of activities were revealed which supports the selection of the channels of interest. This new analysis allowed us to relate task and/or treatment effects with better discriminability since we were now more sensitive to neural generators near each selected electrode. The algorithm picked up each electrode after one another and then checked whether a given electrode had opposite neighbors (using a user defined configuration file). If one electrode had neighbor(s) missing, then the script skipped the processing of that electrode. Figure 2 shows the schematic diagram of the NHB4 re-referencing method.

After the NHB4 re-referencing one dataset contained the single trials from each pre-processed electrode. In the present project the final maximum number of the NHB4 electrodes was 41. Some subjects had one or two bad electrodes so after the NHB4 referencing they had less than 41 electrodes.

C. Results

The next main step of the script was the statistical analysis of the data. First, we topographically plot the grand average amplitudes (across subjects) as a function of time. This visual inspection of the data showed strong

activity over the visual areas (especially over the POz electrode) starting at 130 ms. For the statistical analysis we chose the POz electrode. Point-by-point non parametric permutation statistics were used to reveal any effects of Probability or Treatment on the amplitude accounting for single trial variability. During the statistics we set the significance level to 0.05 and used 500 permutations. To control for multiple comparison we applied False Discovery Rate (FDR) correction.

Figure 3 and 4 show the results of the statistical analysis in caffeine and in placebo conditions. The point-by-point non parametric permutation statistics revealed significant differences between Prob1 and Prob9 in both placebo and caffeine treatment from 130-300 ms after the target onset.

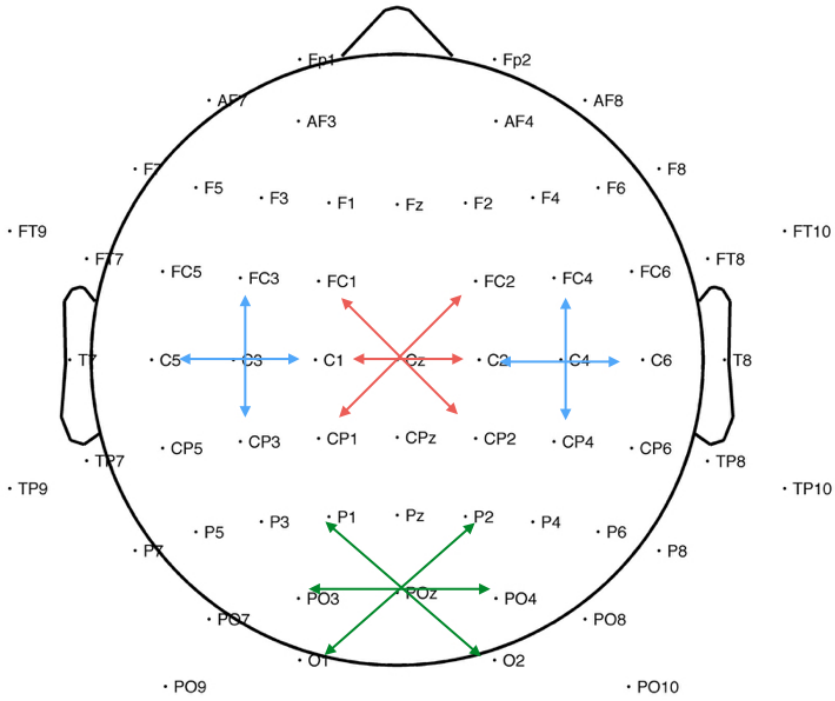

Figure 2. Illustration of the NHB4 re-referencing method. The figure shows only 4 selected channels, but the algorithm can analyze each electrode having opposite neighbors.

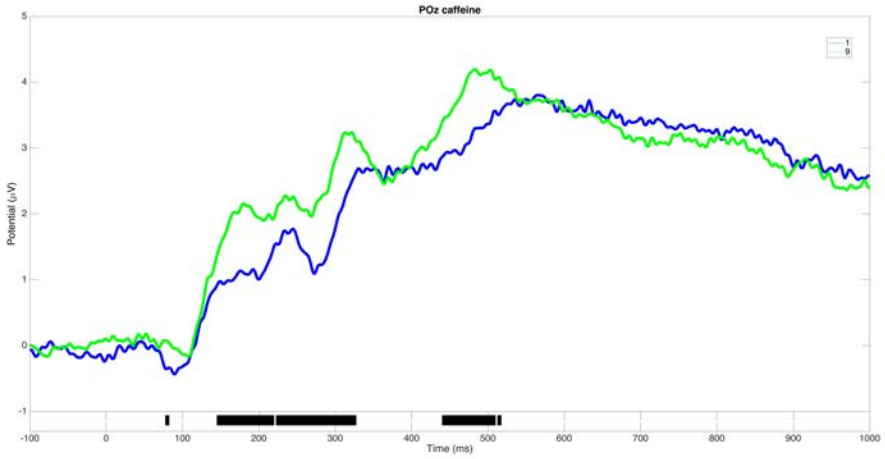

Figure 3. Results of the non-parametric permutation test between Prob1 and Prob9 in the caffeine treatment. Black bars indicate the points where the differences between the Prob1 and Prob9 are significant (after FRD correction).

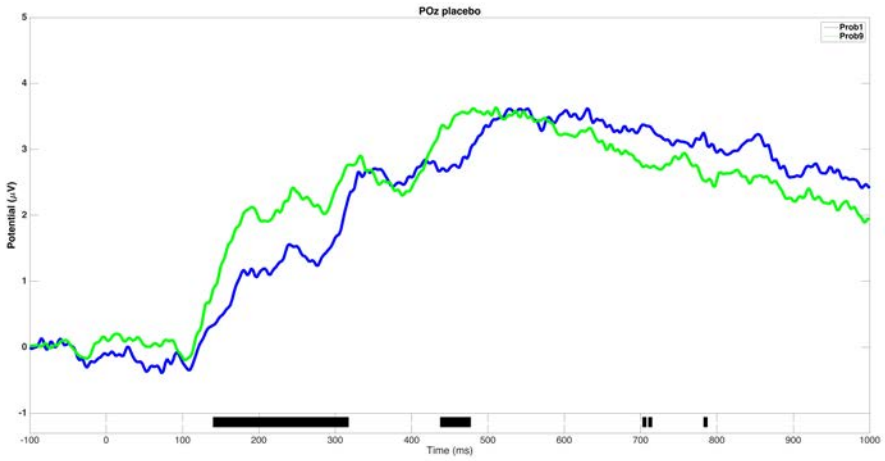

Figure 4. Results of the non-parametric permutation test between Prob1 and Prob9 in the placebo treatment. Black bars indicate the points where the differences between the Prob1 and Prob9 are significant at the $p < 0.05$ level (after FRD correction).

In another statistical model we tested the interaction effects of Prob1 vs. Prob9 X Caffeine vs. Placebo. Here we focused on the onset of the processing from 130 ms to 160 ms after the onset of the target. Figure 5 shows the results of the statistical test. The applied permutation statistic revealed significant Probability effect in both treatment (caffeine, placebo). The analysis of the Treatment in both Prob1 and Prob9 categories indicate significant differences between the treatments only in the Prob1. These results indicate strong probability effect in both conditions and further indicated the facilitating effects of caffeine on information processing in the lowest probability category (Prob1) where the subjects' RT were the slowest. These results is in line with the difference scores of the RT between caffeine and placebo in the Prob1 and Prob9 (Figure 6.) while the difference of the reaction time between caffeine and placebo in the Prob1 was much higher than in the Prob9. Namely the difference between the Prob1 and Prob9 in the caffeine treatment was smaller than that in the placebo treatment. These results suggest differential effects of caffeine on visual information processing: caffeine has the largest effects on demanding task where there a higher attentional load.

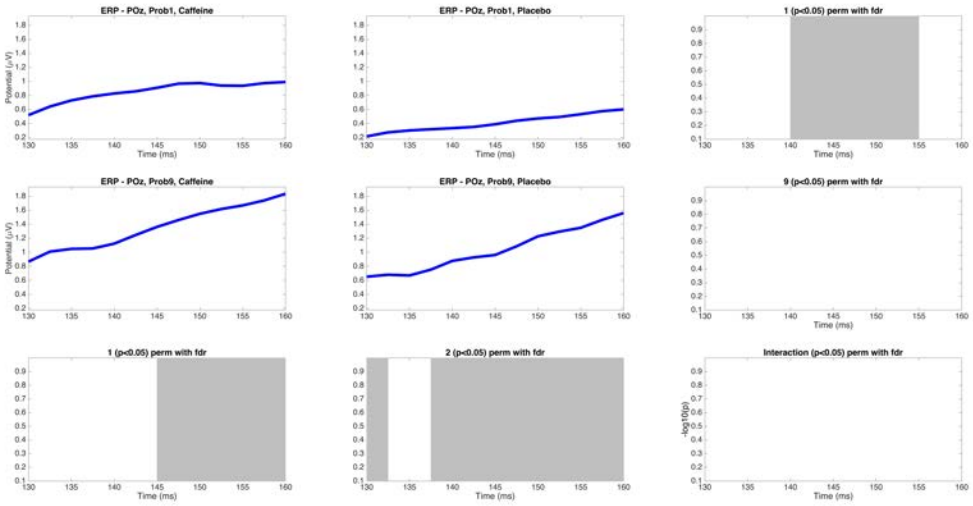

Figure 5. Results for the interaction test of the Treatment (Caffeine, Placebo) and Probability (Prob1, Prob9). The non-parametric permutation test showed significant Probability effect in both treatment similarly as it was shown on the Figure 3 and 4. We also found caffeine effect but only on the Prob1. The first and the second column correspond to the Probability tests in Caffeine and Placebo, respectively. Grey bars in the last two figures of the first two column indicate the time points where the permutation statistic showed significant differences between the probabilities. First and the second rows correspond to the Treatment tests in the Prob1 and Prob9, respectively.

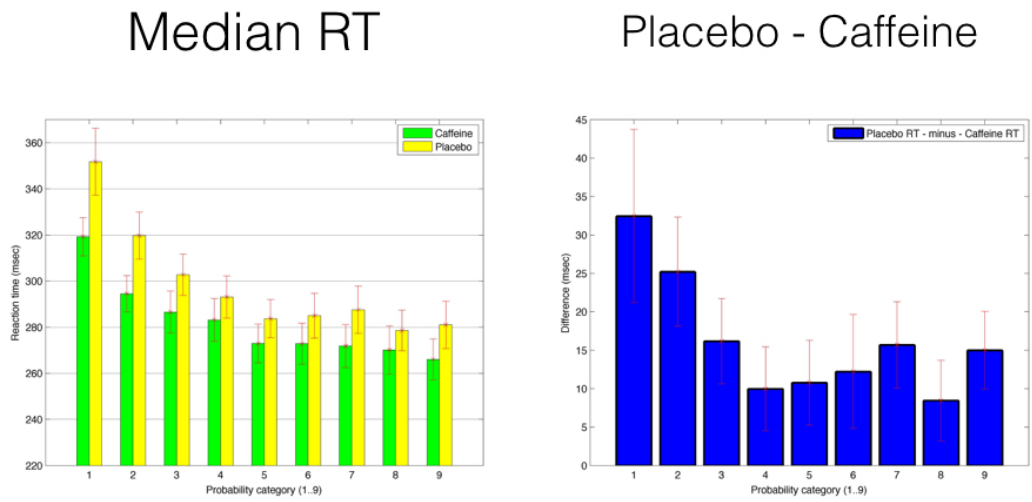

Figure 6. Results for the reaction time in each probability category of the treatments. The left figure shows median reaction times in the caffeine and placebo treatments. The right figure shows the difference between caffeine and placebo reaction times in each probability category.

Quantification of the single trials: Another novel and robust quantitative single trial analysis method is to count the single trial peaks passing a predefined threshold within a predefined time interval. During the STSM we finally developed a script which uses the idea of counting extrema in single trial time-courses of composite channels emphasizing local activity, published in (Liu, Ioannides, & Müller-Gärtner, 1998). The algorithm I designed was based on a parametric description of each time-course and the implementation script was written in Matlab programming environment. The strength of this method is its simplicity. The main idea is that the single trial ERPs are quantified as a function of time by searching through the single trial itself and identifying its peaks. The method uses a predefined moving window to go through the signal. The peaks are categorized into three classes. This means that the peaks can be positive or negative, or there is no peak. Only those peaks can be categorized into positive (negative) classes which amplitude is at least 80% of the amplitude of the strongest positive (negative) in the predefined time interval of the average signal (per subject, treatment and probability). During the analysis we used the time interval from 30 ms to 300 ms after the onset of the stimulus using a 24 ms moving window. We investigated the effects of the independent variables Probability and Treatment on the number of trials passing the positive (negative) threshold. As described above we applied this analysis on the POz electrode. Figure 7 and Figure 8 show the number and the percentage of trials passed the threshold in the different treatments (caffeine and placebo) and probabilities [Prob1 (as low probability), Prob4 (as mid probability) and Prob9 (as high probability)], respectively.

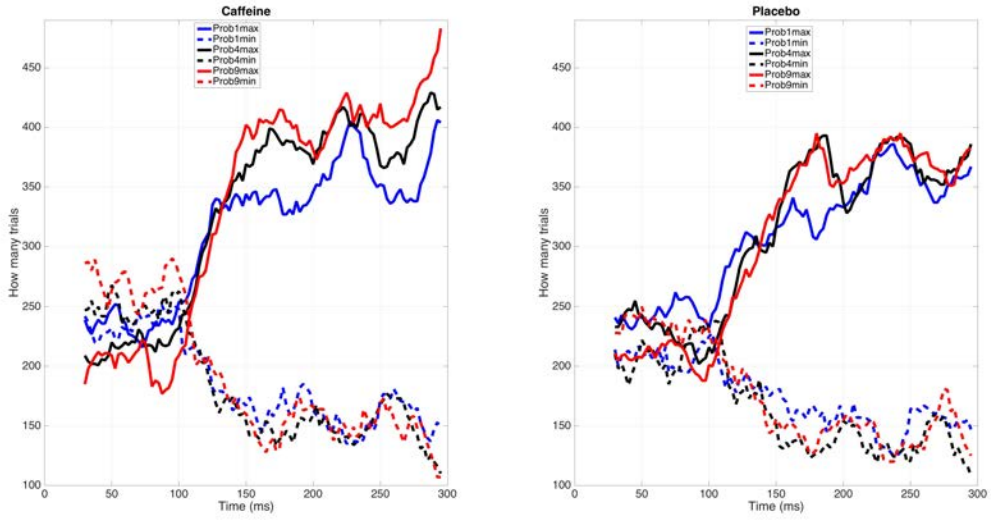

Figure 7. The figure shows the number of trials which passed the 80% amplitude threshold as a function of time. The left and right column shows the Prob1, Prob4 and Prob9 in the caffeine and placebo treatment, respectively. Clear difference can be seen from 100 to 130 ms between caffeine and placebo in the Prob1. Note that the time axis marks the beginning of the 24 ms moving window.

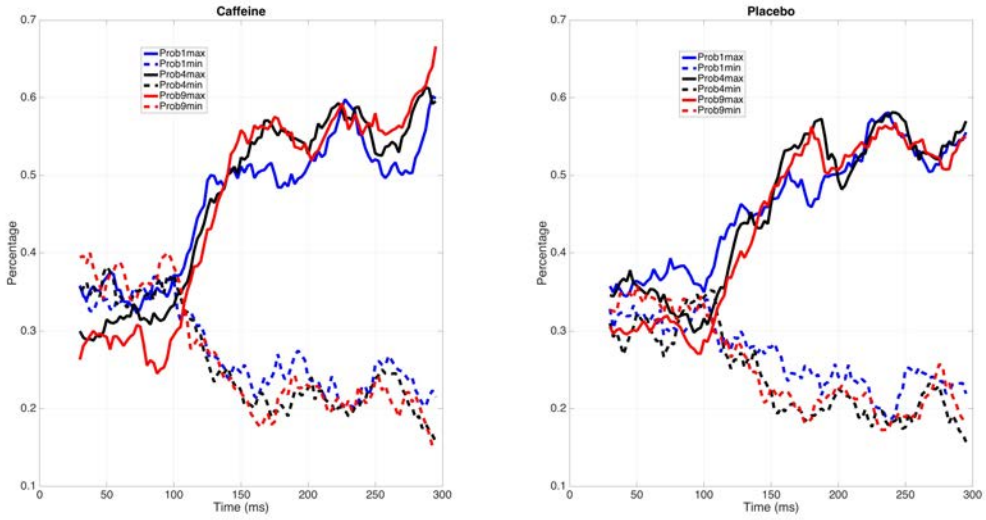

Figure 8. he figure shows that how many percentage of the trials passed the 80% amplitude threshold as a function of time. In accordance with (Liu et al., 1998) our data supports the notion that 50-70% of the trials describe the peaks in the averaged ERP. The left and right column shows the Prob1, Prob4 and Prob9 in the caffeine and placebo treatment, respectively. Similarly, to the Figure 7 there is clear difference from 100 to 130 ms between caffeine and placebo in the Prob1. Note that the time axis marks the beginning of the 24 ms moving window.

D. Future collaboration with host institution

The STSM had a twofold motivation with a short and a long term objective. The short term motivation was to make sufficient progress so that the output of the novel analysis approach would include results which will constitute at least one significant advance for each one of the participating laboratories and teams. It was hoped that the novel analysis results would also be sufficient for one or two publications to be submitted soon after the completion of the STSM. The long term objective was to establish a common framework for analysis that will be the foundation for a longer term collaboration between the two teams. To satisfy the above goals the actual STSM was preceded by excessive discussion and prior work to decide what methodology was to be adopted during the STSM and initial and time consuming preparatory work was also done such as agreeing in format definitions and conversion of data recorded by each team. It was decided to use the EEGLAB for the STSM and associated formats starting with scripts which were already in use at UoPH and extending them to new single trial applications along the items which were already successfully applied to the analysis of MEG data at LHBD both in Japan and Cyprus. With these preliminary results and data handlind in place we were able to start working on the main goal of the STSM almost immediately and hence we successfully completed the program of work as planned.

We previously described the main components of the completed work that are now a new addition to the analysis repertoire of both teams.

E. Expected Publications

We believe the data reported here, after some additional statistical treatment of the results of single trial counting method are enough for a good publication and this is the immediate follow up step of this work. In addition, the scripts developed during the STSM are now adapted for use with the EEG measurements of LHBD and we hope a second publication will follow soon.

F. Other Comments

In my report I described what I have worked on and what we achieved during the STSM at LHBD in Nicosia. To sum up, during the short term mission I have acquired a set of single trial analysis methods and applied them to raw EEG data recorded in a Psychomotor Vigilance Test (PVT). Before the visit our primary aim was to identify and document whether changes in power and/or phase are correlated with performance in the available EEG data. We anticipated that the new single trial analysis approaches would provide us better new perspectives to detect any possible beneficial (or adverse) effects of caffeine on the PVT measures compared to conventional average based analysis approaches. Thus with these new analysis methods our pharmacologically validated behavioral test paradigm may be introduced to be used in connection with novel innovative biomedical technologies (e.g. rTMS, tDCS and neurofeedback). Our results showed that the applied single trial analysis approach is far more sensitive than the conventional measures. Thus we suggest that the brain signal should be analyzed at the single trial level together with the behavioral (e.g. reaction time) changes.

Overall, from my personal perspective, the mission was very successful, provided mutual benefits for both participating laboratories, and I had a great chance to become up-to-date on the latest analysis methods in the EEG field. Certainly, I will be able to use the knowledge acquired during my visit in my future work.

I would like to thank my supervisor Dr. István Hernádi, the head of the host institute, Dr. Andreas Ioannides and the COST BM1309 for giving me the opportunity to learn at the LHBD. I also thank the technical help of Vilmos Oláh, Dr. Lichan Liu, Tina Cleanthous and Constantinos Kourouyiannis. I really appreciated the time I spent there and enjoyed the work with all the colleagues at the host institute.

References

Basner, M., & Dinges, D. F. (2011). Maximizing sensitivity of the psychomotor vigilance test (PVT) to sleep loss. *Sleep*, *34*(5), 581–591.

Basner, M., Mollicone, D., & Dinges, D. F. (2011). Validity and sensitivity of a brief psychomotor vigilance test (PVT-B) to total and partial sleep deprivation. *Acta Astronautica*, *69*(11-12), 949–959. http://doi.org/10.1016/j.actaastro.2011.07.015

Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods*, *134*(1), 9–21. http://doi.org/10.1016/j.jneumeth.2003.10.009

Drummond, S. P. A., Bischoff-Grethe, A., Dinges, D. F., Ayalon, L., Mednick, S. C., & Meloy, M. J. (2005). The neural basis of the psychomotor vigilance task. *Sleep*, *28*(9), 1059–1068.

Laskaris, N. A., & Ioannides, A. A. (2001). Exploratory data analysis of evoked response single trials based on minimal spanning tree. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *112*(4), 698–712.

Laskaris, N. A., & Ioannides, A. A. (2002). Semantic geodesic maps: a unifying geometrical approach for studying the structure and dynamics of single trial evoked responses. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *113*(8), 1209–1226.

Laskaris, N. A., Liu, L. C., & Ioannides, A. A. (2003). Single-trial variability in early visual neuromagnetic responses: an explorative study based on the regional activation contributing to the N70m peak. *NeuroImage*, *20*(2), 765–783. http://doi.org/10.1016/S1053-8119(03)00367-7

Liu, L. C., Ioannides, A. A., & Müller-Gärtner, H. W. (1998). Bi-hemispheric study of single trial MEG signals of the human auditory cortex. *Electroencephalography and Clinical Neurophysiology*, *106*(1), 64–78.

Liu, L., & Ioannides, A. A. (1996). A correlation study of averaged and single trial MEG signals: the average describes multiple histories each in a different set of single trials. *Brain Topography*, *8*(4), 385–396.

Trunk, A., Stefanics, G., Zentai, N., Bacskay, I., Felinger, A., Thuroczy, G., & Hernádi, I. (2015). Effects of concurrent caffeine and mobile phone exposure on local target probability processing in the human brain. *Scientific Reports*, *5*, 14434. http://doi.org/10.1038/srep14434

Confirmation by the host institution of the successful execution of the STSM:

We confirm that Attila Trunk has performed the research work as described above.

Dr. Andreas Ioannides

Contact Person of Host Institution

Dr. Attila Trunk

Name of the researcher