The impact of stimulus modality on the EEG-correlates of associative learning and the connected memory processes

PhD Thesis

Puszta András, MD

Supervisor: Dr. habil. Nagy Attila

Department of Physiology, Faculty of Medicine, University of Szeged Szeged, 2019

Introduction

In psychology, associative memory is defined as the ability to remember the relationship between unrelated items .During associative learning novel information is first acquired, and stored first in the working memory and, if strengthened through a process called consolidation, is eventually stored in the long-term memory.

Convergent findings from neuropsychological studies in humans, together with experimental lesion studies and neuroanatomical studies in animals have shown that the prefrontal cortex together with the medial temporal lobe-hippocampal system and the basal ganglia participate in the normal performance of this task.

The acquired equivalence task is a unique associative learning task that can dissociate the contribution of the two functional system (the mediotemporal lobe-hippocampal system, and the basal-ganglia system). During the first, acquisition part, subjects learn to pair different sets of stimuli through trial-and-error. After the associations were successfully formed, subjects are asked to recall the already learnt associations (retrieval part), and to form new associations based on the rule that has been learnt during the acquisition part (generalisation part). It has been implicated, that the initial, acquisition part of the test requires intact basal-ganglia system, while patients with mediotemporal lobe-hippocampal disfunction perform poorly in the retrieval-generalization phase of the paradigm.

Although numerous studies revealed the processing of cue with different modalities, as well as the learning strategies and their neural correlates, the impact of the stimulus modality on associative learning is not well-described. Stimulus representations, and associations, are stored and (re)activated in stimulus-relevant cortical areas. Thus, associative learning requires cooperation between the learning circuit and other task-specific brain areas.

Earlier studies in primates revealed different brain areas that are primarily responsible for cross-modal and inter-modal memory. Removal of the amygdala and the subjacent cortex impair cross-modal and stimulus-reward association memory, whereas either removal of the hippocampus and the subjacent cortex or fomix transection produce impairments in spatial associative memory.

Naturally, the role of different cerebral regions not only with ablation studies, but also with modern electrophysiological recordings can be differentiated. Different regions of the brain have to communicate with each other to provide the basis for the integration of sensory information, sensory-motor coordination and many other functions that are critical for learning, memory, and perception. Hebb suggested that this is accomplished by the formation of assemblies of cells whose synaptic linkages are strengthened whenever the cells are activated synchronously. Neuronal oscillations are natural consequences of forming such cell assemblies via summation of hundreds of EPSPs and IPSPs, and the cerebral cortex generates multitudes of oscillations at different frequencies through mainly inhibiting spike-trains at a specific frequency. Each frequency band contributes in a different way to the brain's function. Although the functional role of different frequency bands is well described, but the interactions among various rhythms are not fully understood yet. A well-studied mechanism of these interactions is the analysis of cross-frequency coupling. As described first in the hippocampus, the phase of theta oscillations biases the amplitude of the gamma waves (phase-amplitude, P-A coupling or 'nested' oscillations). Cross-frequency P-A coupling can be found between different frequency bands and in different cognitive tasks (for reviews, see[111]). For example, phase modulation of gamma waves by alpha oscillations has been observed in multiple neocortical structures during working memory load.

There are number of investigations, which described the EEG-features of the different phases of the associative learning and memory. Reward-related learning key features is the positive feedback elicit beta power increment, while negative feedback causes power increment in both theta and beta power. Furthermore, studies in associative learning tasks revealed gamma coherence over parietooccipital areas. In working memory tasks frontal midline theta power increment is a well-known phenomen. Also theta/alpha-gamma cross frequency coupling was described earlier in working memory load.

Aims of the study

The studies referenced above investigated mainly visually guided equivalence learning and to our knowledge, no study addressed the cortical contribution to multisensory guided acquired equivalence learning.

Having realized, though, that we did not have normative data about the modality-dependence of the equivalence learning in humans we have developed a multisensory (audio-visual)-guided equivalence learning paradigm in order to compare the performance of healthy volunteers in visual and multisensory tasks. The primary goal of the present study is to investigate how the multisensory information changes the cortical oscillation features, i.e power-density changes, and cross-frequency coupling in different phases (acquisition, retrieval, generalization) of the acquired equivalence learning paradigm and to compare these changes to those in the visually guided learning paradigm. We asked whether the visual and multisensory tasks could share some common, modality independent changes in the cortical activation patterns or modality dependent cortical power and oscillation patterns will be found, which could be characteristic to visually and multisensory guided acquired equivalence learning, respectively.

Materials and methods

Participants

EEG data of 23 adult healthy young adults were recorded (12 females, 11 males, mean age: 26 years, range=18-32). The participants were recruited on a voluntary basis. Those who decided to volunteer signed an informed consent form. The study protocol conformed to the tenets of the Declaration of Helsinki in all respects, and was approved by the Medical Ethics Committee of the University of Szeged, Hungary (Number: 50/2015-SZTE).

Visual acquired equivalence test

The test was structured as follows: in each trial of the task, the participants saw a face and a pair of fish (where each member of the pair had different color), and had to learn through trial and error learning that which fish was connected with which face. There were four faces and four possible fishes, forming eight possible pairs. In the initial, acquisition phase, the participants were expected to learn six pairs, by getting feedback in each trial. During one trial, participants were asked to indicate, which of the two possible fish matches to the given face. Each pair was introduced in a step-wise method, thus participant had to reach a certain number of good answers before a new pair was introduced. After finishing the acquisition phase, participants were asked to retrieve the pairs learned so far (retrieval phase), and the two remaining pairs (generalization phase), that based on the rule learned during the acquisition phase could easily been solved.

Audio-visual acquired equivalence test

The structure of the paradigm was the same as in case of visual associative learning test, only that the four antecedents were four sounds and the consequents were the same four faces as in the visual associative learning paradigm. The task of the participants was to determine from trial to trial which of the two given faces corresponds to the sound heard at the beginning of the trial. During the acquisition phase, six of the possible eight sound-face combinations were learned. During test phase, no feedback was given and beside the already acquired six pairs (retrieval phase); the hitherto not shown but predictable last two pairs were also presented (generalization phase).

EEG-registration

We have performed 64-channel EEG recordings from each participant while they were performed the two above-mentioned test. Additionally, 5 extra channels were placed to the mastoids and around the eyes to record ocular, and muscular movements.

Behavioural data analysis

We calculated the good answer ratio in each phase of the two paradigm, in each participant. Group-level analysis with RM-ANOVA, and Tukey-post-hoc analysis was performed.

EEG data analysis

Pre-processing

Pre-processing steps were performed using EEGLab in order to clean the EEG-data from eye-movement and muscular artefacts. The steps were used are in line with Makoto's pipeline for EEG-pre-processing, that included high-pass filter, re-reference, visual inspection of the trials, independent component analysis and Laplacian-filter.

Time-frequency (TF) analysis

Time-frequency analysis was performed using Continous Morlet wavelet convolution (CMW) via FFT algorithm. In order to avoid the edge-artefacts of the Morlet wavelet convolution, the raw data was multiplied five times before the convolution, yielding a two series-long buffer zone at the beginning and the end of the time-series, which was cut out after the time-frequency analysis. After that, we separated the EEG-data according the different phases of the paradigm (background-activity, acquisition phase, retrieval phase, generalization phase). Baseline activity was defined as the one-minute session before and after the test, and one trial was defined as 1 sec before and after the given answer.

After that, we calculated the TF-points in each phase of each participant, which was significantly differed from the baseline activity, using permutation test and cluster-mass correction for multiple comparison. To correct the mismatch between the number of trials, and the length of baseline-activity, we used bootstrap-method to randomly pick trial-length sections from the baseline-activity.

Group-level analysis of the CMW was carried out in the same way as in the individual analysis described above, with the difference that the random permutation was performed across the mean power values of the subjects and not across the power value of each individual trial.

We identified the time-windows in which we found significant difference between the visual and the audio-visual paradigm, using an interactive surface provided in one of our earlier publications. After we identified the significant time-windows and the corresponding channels in each frequency band and condition, we additionally tested if the individual normalized powers of the different frequency bands in the selected channels and time-points are significantly different in the visual and the audio-visual paradigm by using Mann-Whitney test.

Cross-frequency coupling analysis

Event related synchronisation index (SI) was calculated in order to examine whether the power of the high frequency oscillations are coupled to the phase of the low frequency oscillations on the same channel. In the first step, the power time series of the higher frequency were extracted from the concatenated trials. This was done by the combination of band-pass filtering and Hilbert transformation. First, we have narrow band pass filtered the analytic signal to each frequency of beta and gamma band (15-70 Hz). Then we obtained the power of the performed Hilbert transformation on the narrow bandpass-filtered epochs. Then we band-pass filtered the raw analytic signal to each frequency of the low frequency range (2-20 Hz, with 4 Hz-width). The phase of the band-pass filtered low and high frequency power time series were obtained from the Hilbert transform of the two time-series, respectively. The synchronization between the phase of the two power time series can be calculated using the synchronization index (SI). The SI varied between 0 and 1, with 1 indicating perfect synchronisation.

Significant changes of the cross-frequency coupling at a population level was calculated by comparing the mean synchronisation index in a given modulating - and modulated frequency range of the baseline activity and the given phase of the paradigm. The mean SI-values in each phase of the paradigm were then compared using permutation based statistics, and the resulting Z-scores were corrected by cluster-mass correction.

Correlation between performance in the psychophysical test and the power density changes

Correlation between individual performance and power density changes in a given channel and frequency band was also calculated in each phase of the paradigm. Performance was defined as the ratio of the good trials to all trials, and the individual power changes was the individual Z-scores between the baseline activity’s power density and the given phase’s power density in a given channel in a given frequency band using Pearson-correlation.

Results

Altogether 23 healthy volunteers participated in the investigation. For the bio-mathematical analysis, the raw electrophysiological data of 18 volunteers were analysed, as in the other recordings the signal to noise ratio was low, and neither the excessive attempt to clean the data from EMG and ocular artefacts with pre-processing methods described earlier could make them acceptable.

Psychophysical results

The statistics of the correct answer ratios of the visual and audiovisual test can be found in Table 1. and Table 2.

	min	max	median	SD
Acquisition	0.83	0.98	0.92	0.02
Retieval	0.9	1	0.96	0.02
Generalization	0.92	1	0.97	0.04

ANOVA	
F=20.87	p<0.001
Tukey post-hoc p-value	
A-R	<0.001
A-G	<0.001
R-G	0.992

Table 1: *Summary of the psychophysical results (correct answer ratio) during the visual acquired equivalence task*

	min	max	median	SD
Acquisition	0.90	0.98	0.94	0.02
Retieval	0.88	1	0.96	0.03
Generalizaion	0.83	1	0.97	0.05

ANOVA	
F=7.49	p=0.002
Tukey post-hoc p-value	
A-R	0.002
A-G	0.019
R-G	0.709

Table 1: *Summary of the psychophysical results (correct answer ratio) during the audio- visual acquired equivalence task*

Time-frequency results

Acquisition phase

We found, that the power of the theta band was significantly higher during the audio-visual paradigm (mean=0.118 dB, STD=0.5 dB, Range=0 dB 2.814 dB) compared to the visual paradigm (mean=-0.042 dB, STD=0.459 dB, Range=-3.698 dB 2.012 dB) over the frontal channels, 400 ms to 170 ms before the answer. From 0 ms to 400 ms after the answer, the power of the theta band was significantly higher (p<0.001) in the audio-visual paradigm (mean=0.078 dB, STD=0.494 dB, Range=-1.943 dB 6.794 dB) compared to the visual paradigm (mean=-0.023 dB, STD=0.655 dB, Range=-6.954 dB 5.212 dB) not only over the frontal but over the parietooccpital channels, too.

In case of the alpha frequency band we found that the power was significantly lower (p<0.001) during the visual paradigm (mean=-0.278 dB, STD=1.159 dB, Range=-6.664 dB 0 dB), than in the audio-visual one (mean=0.012 dB, STD=0.140 dB, Range=-1.386 dB 1.041 dB) over the occipital channels, 350 ms to 170 ms before the answer.

We observed no significant difference in the beta power between the visual and the audio-visual paradigm.

The power of the gamma band was significantly higher (p=0.005) during the audio-visual paradigm (mean=0.093 dB, STD=0.417 dB, Range=-0.496 dB 4.472 dB) than in the visual one (mean=0.08 dB, STD=0.36 dB, Range=-0.842 dB 3.491 dB), over the parietal channels, starting from 0 ms until 500 ms after the given answer.

Retrieval phase

The Mann-Whitney test revealed that the power of the theta band was significantly higher (p<0.001) in the audio-visual paradigm (mean=0.066 dB, STD=0.383 dB, Range=-1.871 dB 3.718 dB) than in the visual paradigm (mean=-0.02 dB, STD=0.276 dB, Range=-3.549 dB 2.006 dB) over the temporal and frontal channels, 500 ms to 0 ms before the answer.

In case of the power of the alpha frequency band we found that it was significantly lower (p<0.001) during the visual paradigm (mean=-0.253 dB, STD=1.058 dB, Range=-8.308 dB 0 dB) than in the audio-visual paradigm (mean=-0.036 dB, STD=0.222 dB, Range=-2.324 dB 0.971 dB) over the parietooccipital channels, from 500 ms before the answer.

The power of the beta frequency band was significantly higher ($p<0.001$) during the audio-visual paradigm (mean=0.027 dB, STD=0.276 dB, Range=-3.61 dB 2.994 dB) than in the visual paradigm (mean=-0.093 dB, STD=0.47 dB, Range=-5.524 dB 1.11 dB), over the occipital and parietooccipital channels, from 500 ms before the answer.

The power of the gamma band was significantly higher ($p<0.001$) during the audio-visual paradigm (mean=0.026 dB, STD=0.27 dB, Range=-3.205 dB 4.133 dB) compared to the visual paradigm (mean=-0.041 dB, STD=0.302 dB, Range=-4.662 dB 2.088 dB), over the frontal and parietooccipital channels, starting from 500 ms before the answer.

Generalization phase

The Mann-Whitney test revealed that the power of the theta band was significantly higher ($p<0.001$) during the audio-visual paradigm (mean=0.009 dB, STD=0.356 dB, Range=-3.022 dB 3.157 dB) than in the visual one (mean=-0.127 dB, STD=0.677 dB, Range=-8.810 dB 2.529 dB) over the frontal and parietooccipital channels, from 500 ms before the answer.

The power of the alpha frequency band was significantly lower ($p<0.001$) during the visual paradigm (mean=-0.245 dB, STD=1.065 dB, Range=-8.11 dB 0.381 dB), compared to the audio-visual paradigm (mean=-0.033 dB, STD=0.409 dB, Range=-4.785 dB 3.102 dB) over the occipital and parietooccipital channels, from 500 ms before the answer.

The power of the beta frequency band was significantly higher ($p<0.001$) during the audio-visual paradigm (mean=0.017 dB, STD=0.347 dB, Range=-5.557 dB 3.102 dB) compared to the visual paradigm (mean=-0.066 dB, STD=0.438 dB, Range=-6.089 dB 3.068 dB), over the parietooccipital channels, starting from 500 ms before the answer.

The power of the gamma band was significantly higher in the audio-visual paradigm (mean=0.026 dB, STD=0.346 dB, Range=-4.803 dB 4.537 dB) than in the visual paradigm (mean=-0.038 dB, STD=0.376 dB, Range=-5.521 dB 3.49 dB), over the frontal and parietooccipital channels, starting from 500 ms before the answer.

Cross-frequency coupling

We found significantly higher synchronization index (SI) in each phase of the paradigm compared to baseline activity both in the visual and audio-visual acquired equivalence tasks. Comparing the visual and the audio-visual task, we found that the theta-beta and alpha-beta SI was significantly higher during the acquisition phase of the audio-visual task compared to the visual task over almost every channels except the occipital visual areas.

Correlation between performance in the psychophysical test and the power density changes of the EEG signals

In general, there was strong correlation between the performance and the changes of power densities in the acquisition phase of both the visual and audio-visual paradigms but such correlation was not remarkable during retrieval and generalization phases of both visually guided and multisensory guided learning paradigms. we also found, that the performance was negatively correlated with the power of the >8 Hz oscillations during the audio-visual task, mainly over the parietooccipital-occipital channels, while we observed positive correlation between the performance and the power of the theta-beta- gamma band during the visual task over central-parietooccipital channels.

Discussion

In the present study we have analyzed the EEG correlates in a visually-guided and an audio-visually (bimodal or multisensory) guided acquired -equivalence learning tasks. However, this learning paradigm requires critically the normal function of subcortical structures, i.e. hippocampi and basal ganglia, the cortical contribution seems to be necessary in the visually and audio-visually guided learning paradigm, too. To our knowledge, this is the first study, which addresses the comparison of the cortical power spectra and their changes in a unimodal visual and a multisensory associative learning task. The major findings of the study are that the cortical activity depends critically on the phase of the paradigm, and some changes in cortical powers are characteristic to unimodal visual and multisensory audio-visual learning tasks. In general, during the audio-visual paradigm, the power changes of the event-related low and high-oscillations were higher compared to the visual paradigm, but the psychophysical performance of the acquisition phase only correlated with the power of different frequency bands in the visual paradigm.

On the other hand, while the power changes of the event-related oscillations were higher during the audio-visual paradigm, the performance did not depend on the power of different oscillations, and the strength of the cross-frequency coupling was higher. Furthermore, the performance of the acquisition phase seems to be connected primarily to the strength of the alpha-beta coupling during the audio-visual paradigm.

We are convinced that the cortical power differences in the two paradigms cannot be the result of having previously completed the first task (precondition), hence the order of the two paradigms (visual and audio-visual) varied randomly across subjects. The performance of the investigated population in the psychophysical test was in the same range as that of the earlier investigated healthy controls of neurological and psychiatric patients. Based on this we are strongly positive that the electrophysiological results showed here are representative.

While former studies revealed that mainly cortical areas are involved in associative learning, only few electrophysiological studies showed the functional basis of the multisensory integration[138], and to our knowledge, our study is the first that describes the role of different oscillations in multisensory guided learning and the role of multisensory integration in assocaitive learning.

Conclusion

We can conclude that the changes in the power of the different frequency-band oscillations were more enhanced during the audio-visual paradigm than in the visual one. On the other hand, we found strong correlation during the acquisition phase between the power of different frequency bands and the psychophysical performance both in the visual and the audio-visual task. In addition, the acquisition the retrieval and the generalization phases of the bimodal, audio-visual task required showed more synchronized cortical activity than the visual one. Our results suggest that the multisensory associative learning and the connected memory processes (retrieval, and generalization) require a prominent, and more synchronized cortical activation, while the unimodal visual associative learning and the connected memory processes require prominent and less synchronized cortical activity. These findings further emphasize the effect of multimodal integration during associative learning and memory processes.

Acknowledgements

First of all, I would like to express my deepest gratitude to my supervisor Attila Nagy, who guided my path from 2nd year physiology (as my seminar teacher), through three years of scientific students' research, thesis and PhD work to writing this dissertation, helping me through in difficult situations. I would also like to express my gratitude to Professor Gábor Jancsó for giving me the chance to participate in the Neuroscience PhD program. and to Professor Gyula Sáry for allowing me to do my research in the Department of Physiology, as well as the teaching possibility, and the kindness they showed towards me every time I had something to discuss with them. I would also like to thank Professor Ute Habel, and my colleagues in RWTH Uniklinik Aachen, Han-Gue Jo, Thilo Kellermann and Mikhail Votinov to hosted me in Aachen. I would also like to thank Professor Rufin Vogels for hosting me in Leuven, and Francesco Fabbrini, and all the colleagues in Leauven for teaching me the basics of the optogenetical manipulation. I express my gratitude to Gábor Braunitzer, who helped during my earliest research carrier. I would also like to thank my fellow PhD students (Attila Őze, Diána Nyujtó, Ákos Pertich, Zsófia Giricz) and postdocs (Balázs Barkóczi, Balázs Bodosi, and Gabriella Eördegh) and my former master students, Xénia Katona, and Blanka Bindics as well as other master students in the Laboratory, including but not limited to Nándor Görög, Viktória Balikó, Nóra Cserháti, Anna Pihokker, Petra Rózsa, Kálmán Tót. A deep thank goes to Péter Liszli, who did a tremendous amount of work helping me with the technical part of the research. I would also like to thank all the collegues of the Department of Physiology, for the friendly atmosphere, especially to Anna Bognár, Péter Kaposvári, Alexandra Büki. I couldn't emphasize the gratitude towards my family, Lajos Puszta, Ágnes Sági, Dániel Puszta, Katalin Szabó, and the youngest member of the family, Ferenc Puszta who did much more inspiration during my PhD that they can probably imagine. I am deeply grateful to my friends, who were always there for me: Alexis Echeff, Rita Wiesner-Deák, Péter Kátai, Dániel Boglári, Heni Tarpai, and Péter Wiesner, who are the greatest friends one can ever ask for. This work was supported by Hungarian Brain Research Program Grant KTIA_13_NAP-A-I/15, SZTE ÁOK-KKA Grant No: 2019/270-62-2. Campus Mundi Scholarship (Grant No.:CA-SMP-KA103/596/2018), IBRO PERC inEurope Short Stay Grant (Grant No.: PERCInEUROPE-2019-21)