Sensory guided associative learning in paediatric migraine without aura

Summary of Ph.D. Thesis

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1. Introduction

Associative learning, a form of learning based on the formation of relationships between unrelated items. This relationship can usually develop because of learning based on similar or opposite characteristics, which coincide in time and space. The **Rutgers Acquired Equivalence Test** developed by Myers et al. (2003) is to investigate a specific kind of associative learning, the visually guided equivalence learning. In this also called face-fish paradigm, associations need to be acquired between antecedent stimuli (cartoon characters) and consequent responses (drawings of fish) through trial and error learning.

The test is structured into sections including the acquisition phase (a feedback-guided learning of face-fish associations), the retrieval part of the testing phase (recalling already acquired associations without feedback), and the generalization part of the testing phase (application of associative knowledge in new situations). In the acquisition phase participants had to learn which fish belongs to which character through trial and error, furthermore they also get the point of that some stimuli are equivalent concerning the associated consequents, as some cartoon characters have the same fish. In the test phase, the subjects had to recall the associations without feedback and they had to apply their knowledge in new situations.

Feedback guided associative learning of the stimulus-consequent pairs and implicit learning of the stimulus categories based on the hidden acquired equivalence of stimuli are both involved in the acquisition phase. With this paradigm, we could examine the medial temporal lobe (including the hippocampus) and the basal ganglia-related learning and memory separately.

The **medial temporal lobe** plays an important role in the declarative (explicit) and the long-term memory. The central part of MTL memory system is the **hippocampal region**: the Cornu Ammonis fields 1-4, the dentate gyrus and the subiculum. The hippocampus is responsible not only for explicit encoding, but also for recall, spatial orientation, and some implicit processes. The hippocampus also has a role in procedural learning as well as in recognizing and recalling complex correlations. Patients with histological evidence of hippocampal damage have moderate memory impairment: deficiency in episodic memory, intact working memory and remote autobiographical memory.

The **basal ganglia system** is responsible for the regulating muscle tone and voluntary movements, and also plays an important role in the regulation of emotionally motivated movements, and the cognitive (the procedural and habit learning) and affective functions. In

Parkinson's disease the basal ganglia system is damaged, and consequently, the stimulus-response-based habitat learning as well. In the previous acquired equivalence test, patients suffering from Parkinson's disease performed significantly slower in the acquisition phase, but transferred the previously learned information as well as the control group.

Migraine is a primary headache, one of the most prevalent chronic neurological disorder with extremely incapacitating symptoms affecting about 1.04 billion people worldwide. Migraine is most common between the ages of 20 and 45, but affects children as well. **Migraine without aura** is characterized by recurrent headaches manifesting in attacks lasting 4-72 hours typically accompanied by at least two of the following symptoms: unilateral location, pulsating quality, aggravation by routine physical activity. Further associated migraine symptoms include nausea, vomiting, phonophobia, and photophobia. Diagnosis of **childhood migraine** is challenging because of its varying symptoms, triggers and aetiology. Pediatric migraine headache is shorter in duration, more often bilateral compared to symptoms of adult patients, and it is usually frontotemporal, while occipital headache in children is rare. The manifestation of migraine may also be connected to children's lifestyle, psychological stress, excessive physical fatigue and exposure to strong light effects.

Several imaging studies examined **the effects of migraine on the brain**. Enhanced or decrease grey matter volume was described in some brain regions. Increased cortical excitability, altered brain blood flow and changes in the pain modulatory system were observed, too. One study reported abnormal volume and resting-state networks of the left caudate body and right nucleus accumbens in the migraineurs. Furthermore, other imaging studies have found decreased activation in the basal ganglia due to migraine and increased blood flow during the episodes. Decreased volume was observed in the hippocampus in newly diagnosed migraineurs and one study reported larger hippocampal nociceptive activation during thermal pain stimulation in the migraine using fMRI.

Cognitive and memory functions in adult migraineurs, as well as the visuo-cognitive processing have been found not to be working properly in patients suffering from migraine, both with and without aura. Braunitzer et al. (2010) have found that migraine-suffering children exhibited a slower development of visual contour integration. Moreover, in paediatric migraine the contrast sensitivity is mostly affected at low spatial frequencies. It has been also observed that motion coherence processing capacity was reduced. The impairment of short-term and long-term memory in children suffering from migraine and the significant

differences in the mean total and verbal intelligence quotient scores between the child migraineurs and the control children were previously discovered. Furthermore, in the previous studies the impairment of psychomotor abilities, attention and verbal memory, have been also found, which may be due to the damage caused by migraine. Not all studies have described the cortical deficits or deterioration in cognitive performances.

In our previous study, a poorer performance in associative learning and an extensive impairment in generalization were observed in adult migraineurs without aura, which findings seem to support the involvement of the basal ganglia and the hippocampi in the pathomechanism of migraine. We also investigated a cohort of 265 healthy subjects with an age range of 3-52 years how performance in different tasks of the RAET changes with age and found a significant effect on associative learning and on retrieval of the learned pairs, but not on generalization.

2. *Aims of the study*

The aim of our study was to investigate whether the differences found in the RAET performance among adults suffering from migraine compared to healthy age-matched controls can be observed in children migraineurs as well. We also intended to investigate the pediatric patients' performance compared to the control children groups in the auditory and multisensory guided equivalence learning developed by our lab, based on AET.

3. Methods

Participants

A total of 54 children between the ages of 8 and 17.5 years and 44 adult subjects between the ages of 20 and 65 years were enrolled in our study (published earlier were reanalyzed). The child migraineur group consisted of 27 participants and the child control group involved 27 healthy subjects matched on age, gender and level of intelligence tested by Raven's Progressive Matrices. The mean age in the child migrainer group (±SD) was 14.1±3.0 years and in the child control group was 14.2±3.0 years. The female-male ratio was 15 girls and 12 boys. The adult migraineur group consisted of 22 adult patients, and the adult control group involved 22 healthy individuals matched on age, gender and level of education. In the adult migraineur group, mean age (±SD) was 40±11.76 years, in the adult control group it was 40±11.76 years and in both groups the female-male ratio was 20 women and 2 men.

The child migraineur patients were recruited from the Department of Pediatrics, University of Szeged, Hungary, while adult migraineurs were patients of the Neurology and Stroke Department of the Hospital of Kecskemét, Hungary. The diagnosis of migraine was established according to the International Classification of Headache Disorders, 3rd edition (ICHD-3beta). All children patients were diagnosed by the same pediatric neurologist, and also, all adult cases were diagnosed by the same neurologists. The inclusion criterion was the diagnosis of migraine without aura, while exclusion criteria were the presence of other neurological, psychiatric or ophthalmological disorders. At least five days had passed since the last attack at the time of testing for all patients and no attack occurred in the 24 hours following the testing.

The child controls were recruited from different primary schools and secondary schools in Szeged, Hungary. The participants in the child control group had no history of any kind of headache, and they were also free of any kind of neurological, psychiatric or ophthalmological disorder as well. Only participants without colour vision deficiency tested by Ishihara plates were eligible for this study in all investigated groups. The adult controls were recruited from the employees working at the Faculty of Medicine, University of Szeged, Hungary. The exclusion criteria were the same as in the child control group.

The study protocol conformed to the ethical principles of the Declaration of Helsinki in all aspects. All recruitment and protocols were conducted with written informed consent

and with the approval of the Regional Research Ethics Committee for Medical Research at the University of Szeged, Hungary (52/2015).

The visually guided associative learning paradigm

The visual stimuli referred to as antecedents were cartoon faces of a woman (A1), a girl (A2), a man (B1) and a boy (B2). The responses referred to as consequents were yellow (X1), red (X2), green (Y1) and blue (Y2) fish. The test was arranged into trials. During a trial the participant saw an antecedent stimulus (a face) and two possible consequents (a pair of fish of different colour) and asked to give a choice by pressing on one of two buttons corresponding to the two fish of different colour. The trials were structured into two phases: 1. the acquisition phase, 2. the test phase which has two parts, the retrieval and generalization parts.

During the acquisition phase, participants learned a series of antecedent-consequent pairs via trial and error. When face A1 or face A2 were shown, the correct choice was X1 fish over Y1 fish; however, when B1 or B2 appeared on the screen, the correct answer was Y1 fish, instead of X1 fish. Thereby beside the face-fish associations, participants have also learned that the A1 face is equivalent with A2 face in relation to the associated fish. New associations were introduced gradually, and they were presented mixed with trials of previously learned associations. A certain number of consecutive correct answers had to be accomplished after the presentation of each new association, therefore the number of trials required to finish the acquisition phase depends on the performance of the subjects. In detail, four consecutive correct responses were required after the presentation of the first two associations (A1 and X1, B1 and Y1), and an increasing number of consecutive correct choices (6, 8, 10, 12) were needed after the presentation of each new association in order to make sure that the participant successfully acquired each association before proceeding to the test phase.

In the test phase, the task remained the same but feedback was no longer provided. The test phase consisted of 48 trials. The participants got 48 tasks showing already known pairs (retrieval) mixed with tasks presenting new pairs (A2 and X2; B2 and Y2) testing the generalization of the learned equivalence. Subjects were not informed about the appearance of new associations.

The auditory and multisensory guided associative learning paradigm

Our laboratory have developed and validated an auditory and audio-visual (multisensory) guided acquired equivalence learning test. The structure of the paradigm was the same as the visually guided associative learning test. In the auditory paradigm, in contrast the visual and multisensory paradigm, every sound had to be associated with the corresponding button (left or right button), not with the other sound. Four pairs of sounds (eight stimuli: two animal sounds (a cat meowing and a dog barking), two different gender voices (a woman and a man said a Hungarian word), two instrumental sounds (a note played by a guitar and a piano), two sounds of vehicle (sounds of an ignition key and a motorcycle)) were used, and each sound lasted 1.5 sec long, and had same intensity. During the multisensory test, the participants had to learn to associate four antecedent sounds (one of the pairs used in the auditory paradigm) and the consequents four faces (as in the visual paradigm; a woman, a girl, a man and a boy).

Statistical analysis

Statistical analysis was performed in SPSS 21.0 (IBM, USA). The level of significance was set at $p=0.05$. Before the hypothesis tests, extreme outliers were removed from the dataset as suggested by Tukey (for each variable in each group, the 25th and 75th percentile limits (Q1 and Q3) were calculated, the value of Q1 was subtracted from the value of Q3, the result was multiplied by 1.5, and the product was subtracted from Q1 and added to Q3 to modify the limits (Q1' and Q3'). Values under Q1' and over Q3' were removed.). As the Shapiro-Wilk normality test was not fulfilled in all groups and all parameters, we decided to use the Mann-Whitney U test for hypothesis testing.

4. Results

Visual guided associative learning paradigm

The **child migraineur and the child control groups** did not differ significantly regarding the median number of the trials required for completing the acquisition phase (*p=0.395)*. The median error ratios during the acquisition phase was similar in the two investigated child groups (*p=0.369*). Statistical analysis of the median error ratios in the retrieval part of the test phase did not show significant difference between the two investigated groups (*p=0.621*). There was also no significant difference between children patients and controls in terms of the median error ratios in the generalization part of the test phase (*p=0.484*).

In the **adult migraineur group** the median number of the trials required for completing the acquisition phase was not significantly higher than in the **adult control population** (*p=0.080*). The median error ratio during the acquisition phase was significantly increased in the adult migraineur group as compared to the adult control group (*p=0.043*). The median error ratios during the retrieval part of the test phase were similar in the adult migraineur and adult control groups/adult cases vs. controls (*p=0.395*). However, a significantly higher median error ratio in the generalization part of the test phase was found among the adult patients as compared to the adult controls (*p<0.001*).

Auditory guided associative learning paradigm

The **child migraineur and the child control groups** did not differ significantly regarding the median number of the trials required for completing the acquisition phase (*p=0.123*). There was not significantly higher among the child patients as compared to the children controls in term of the median error ratios in the acquisition phase (*p=0.06*). The median error ratios in the retrieval part of the test phase did not differ significantly between the two investigated child groups (*p=0.06*). Statistical analysis of the median error ratios in the generalization part of the test phase did not show significant difference between the child migraneur and the child control populations (*p=0.79*).

Audiovisual guided associative learning paradigm

The median number of the trials needed to complete the acquisition phase was similar between child migraineurs and child controls (*p=0.29*). There was also no significant difference between the two investigated control groups in term of the mean error ratios in the acquisition phase (*p=0.32*). The median error ratios during the retrieval part of the test phase

were similar in the child cases compared to controls ($p=0.98$). However, a significantly higher median error ratio in the generalization part of the test phase was found among the child patients as compared to the child controls ($p=0.002$).

5. Discussion

The Rutgers Acquired Equivalence Test is a visually guided equivalence learning paradigm involving associative learning and implicit rule extraction in the acquisition phase and application of the acquired knowledge in the test phase.

Migraine is a primary headache, one of the most prevalent chronic neurological disorder with extremely incapacitating symptoms affecting about 1.04 billion people worldwide that corresponds to a prevalence of 11 %. Migraine is most common between the ages of 20 and 45, but affects children as well. Adult migraineurs often report headaches as early as childhood. Migraine is characterized by significant negative effects on quality of life in childhood and the risk of chronic and persistent headaches in adulthood.

In our previous study, a poorer performance in associative learning and an extensive impairment in generalization was observed in adult migraineurs without aura, which findings seem to support the involvement of the basal ganglia and the hippocampi in migraine. We also investigated a cohort of 265 healthy subjects with an age range of 3-52 years how performance in different tasks of the RAET changes with age and found a significant effect on associative learning and on retrieval of the learned pairs, but not on generalization.

Our study tested a simple hypothesis. We hypothesized that the pediatric patient population would show significantly poorer performance in at least one phase of our learning paradigms than age- and sex-matched controls. The current study failed to support the hypothesis. The difference between the pediatric groups was not significant in any of the studied parameters (except in GER in the multisensory equivalent learning test).

Recent results of our research group revealed no significant difference among the performance (error ration) in the unimodal visual, unimodal auditory and the combined audio-visual paradigms in the acquisition phase in healthy humans. Similarly, we have found no differences between the equivalence learning of pediatric patients and control children irrespectively of the stimulus modality. Thus, the modality of the stimuli does not affect the performance in this phase of the behavioral test. Thus, the feedback-based pair learning a very old and conserved function, which can be linked to an ancient structure, the basal ganglia is not affected by pediatric migraine. In contrast, the generalization part of the test phase, which is modality dependent seems to be affected in pediatric patients. The only significant difference, which was found in the comparison between the pediatric patients and healthy

controls in our research, was in the generalization error ratio of the multisensory test. This is the most complicated part of the applied three paradigms and if there are any alteration in the sensory guided associative learning it is the easiest to detect because of its complexity. This interesting finding could shed light on the altered multisensory integration even in pediatric migraine patients, which can be elicit a significant decrease in the performance of the generalization part of the multisensory paradigm.

6. Conclusion

Our results demonstrated no significant deficit of equivalence learning and the connected memory processes in pediatric migraine patients in visual and auditory learning paradigms and in the acquisition and the retrieval parts of the multisensory paradigm. Our results suggest that the loss of the visual associative learning function in adult patients is not an inherent feature of the migrainous cognitive profile rather the result of the attacks ’interference with the development / function of the underlying structures. The only significant deficit in the performance of pediatric patients was in the generalization part of the multisensory learning paradigm. The altered generalization of multisensory stimuli because of most probably from the altered multisensory integration could be an early signal of the loss of associative learning function in children and adolescent patient suffering in migraine.

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Publication connected to the thesis

1. **Zsófia Giricz**, Ákos Pertich, Attila Őze, András Puszta, Ágnes Fehér, Gabriella Eördegh, Jenő Kóbor, Katalin Bihari, Éva Pálinkás, Gábor Braunitzer, Attila Nagy (2020) Visually guided associative learning in pediatric and adult migraine without aura. Cephalalgia DOI: 10.1177/0333102420958388

Other publications:

1. Ágnes Fehér, **Zsófia Giricz**, Anna Juhász, Magdolna Pákáski, Zoltán Janka, János Kálmán (2018) ABCA1 rs2230805 and rs2230806 common gene variants are associated with Alzheimer's disease. Neurosci Lett. 2018 Jan 18;664:79-83.
2. András Puszta, Ákos Pertich, Xénia Katona, Balázs Bodosi, Diána Nyujtó, **Zsófia Giricz**, Gabriella Eördegh & Attila Nagy (2019) Power-spectra and cross-frequency coupling changes in visual and Audio-visual acquired equivalence learning. Scientific Reports volume 9, Article number: 9444 (2019).
3. Gabriella Eördegh, Ákos Pertich, Zsanett Tárnok, Péter Nagy, Balázs Bodosi, **Zsófia Giricz**, Orsolya Hegedűs, Dóra Merkl, Diána Nyujtó, Szabina Oláh, Attila Őze, Réka Vidomusz, Attila Nagy (2020) Impairment of Visually Guided Associative Learning in Children With Tourette Syndrome. PLoS One 2020 Jun 16;15(6):e0234724.
4. András Puszta, Ákos Pertich, **Zsófia Giricz**, Diána Nyujtó, Balázs Bodosi, Gabriella Eördegh, Attila Nagy (2020) Predicting stimulus modality and working memory load during visual- and audiovisual-acquired equivalence learning. Frontiers in Human Neuroscience, DOI: 10.3389/fnhum.2020.569142