Combined effects of biperiden and acute tryptophan depletion on memory in healthy volunteers: an EEG study

Introduction

Research on the neurobiological foundations of memory has shown that both acetylcholine and serotonin (5-HT) are involved in memory processing. However, little research has been carried out to discover the way in which these two neurotransmitters interact during these kinds of processes. Examinations like this can provide crucial information on memory *per se*, but also diseases such as AD.

In AD extensive cholinergic cell loss exists parallel with cognitive deficits. The same deficits can be artificially induced by blocking cholinergic mechanisms in young subjects (Bartus et al.1982). On the other hand, cholinergic stimulation improves memory in aged people. This proves that the cholinergic system is involved in cognitive processes. Moreover, decreases in cholinergic function in patients with AD is also correlated with the aggressive behavior which supports a dual role for the cholinergic system both in cognitive and in non-cognitive disturbances associated with this illness (Bartus et al.1982).

Degeneration of cognition in the later phases of human life appears as senile dementia and is shared by many other mammalian species, including mice, rats and monkeys (Bartus et al.1982). These kinds of disturbances in cognition are probably also connected with the cholinergic system. They are reflected in decreased neuronal activity of cholinoreceptive neurons. We can easily associate them with the loss of memory that occurs with age.

Some of the patients also suffer from other syndromes of dementia such as over activity, depression, or psychosis, which indicates how relevant the role of the cholinergic system in non-cognitive disturbances can be. However, these disturbances are too complex, so some scientists argue that imbalances between various neurotransmitters may be the cause (Chen et al.2005). They suggest involvement of the serotonergic system which is relevant for higher cognitive processes such as memory and learning and there is evidence suggesting that changes in levels in this neurotransmitter are correlated also with non-disease aging.

In addition, cholinergic and serotonergic pathways interact with cholinergic systems (Chen et al.2005). It is also worth mentioning, that “interact” does not mean “connected anatomically”.

Evidence for the involvement of serotonergic and cholinergic systems in cognitive and non-cognitive processes is indicated by the fact that we can observe depletions in several cholinergic markers and serotonin in the frontal and temporal cortex of AD patients in *post-mortem* studies (Chen et al.2005). This hypothesis is controversial because it is widely believed that cognitive impairment in AD is only caused by neurodegeneration.

To examine the issues described above scientists commonly used two kinds of anticholinergic substances on healthy volunteers: the muscarinic antagonists - biperiden and scopolamine. They used these treatments to uncover how cholinergic antagonists may influence memory and exactly what sorts of disturbances they produce (Curran et al.1998). If scientists want to examine older people they should keep in mind that there is a significant decrease in the binding of muscarinic antagonists (biperiden) in the cortex with age (Bartus et al.1982), which makes those type of participants unsuitable for this research. To examine the serotoninergic system scientists often used acute tryptophan depletion (Evers et al.1998).

Biperiden is a selective, long-half life drug used for treatment of the Parkinson syndrome. It decreases the level of acetylcholine because of its antagonistic effect on muscarinic 1 (M1) cholinergic receptors. Selectivity is important in this case because M1 receptors are mainly located in the cerebral cortex and hippocampus (Bartus et al.1982). Studies show that many cognitive processes are impaired by biperiden. There are not only episodic memory deficits in immediate recall, delayed recall and recognition of verbal material, but also in working and short-term memory (Pickles et al.2000). Moreover, motor learning as well as visuospatial processes are impaired by this selective drug (Hulstijn et al. 2005). These data show that acetylcholine is not only involved in memory processing, but is also important for visuospatial abilities.

Both human and animal data showed rapid absorption of biperiden. Healthy volunteers responded to this treatment just 1-2 hours after intake and showed a significant central nervous system reaction (for instance: fatigue, dizziness) up to 6 hours (Hollmann et al. 1984). At this time biperiden reached 12% of it‘s peak value. After 48 hours its concentration is always close to or below the detection limit (Hulstijn et al. 2005). Sometimes intramuscular administration of biperiden can cause

delirium (Shinohara,2010). Nevertheless, biperiden can also be used as an effective remedy – for instance it could be successfully used in the treatment of severe dyskinesia, which is one of symptom of Parkinson disease (Anlar et al.2003).

It is also worth mentioning, that selective muscarinic M1 receptor antagonists do not always cause impairment of cognitive processes. One such substance - trihexyphenidyl – causes retrograde facilitation of words learned prior to oral administration of this treatment. But even this improved recall of learned information prior to drug intervention is associated with anterograde amnestic effect (Belzer et al.2010).

Scopolamine is a non-selective muscarinic receptor antagonist which has large effects on memory (Pickles et al.2000). Items which were studied under the influence of this drug are harder to recall after a delay. Furthermore, it is interesting that scopolamine does not disrupt the recall of information (either separate or episodic) studied prior to the administration of this treatment. Neither scopolamine effect on recollection nor recognition is well established. Especially data concerning recognition are mixed. There is evidence that scopolamine significantly impairs recognition. On the other hand some research has shown that this drug has little or no effect on recognition memory (Pickles et al.2000). Being aware that these data could be confusing for many people, in 2003, Atri and colleagues decided to examine this issue. They assumed that recognition performance is supported by two separate processes – recollection and familiarity. They used a task in which subjects saw two sets of pictures: the second set was two times bigger than the first and contained all the items from the first presentation and with the same amount of additional, new pictures. Under the influence of scopolamine subjects correctly recognized new items but made mistakes with old items. This shows that recollection can be more sensitive to scopolamine than familiarity (Atri et al. 2003).

There is some evidence that serotonin may modulate cholinergic function in many regions of the mammalian brain (Cassel et al.1995). Serotonin’s synthesizing neurons are primarily found in the rostral and the caudal raphe nuclei. Axons of these neurons create descending pathways through the brain and spinal cord (Evers et al.2010). Due to the neuroanatomy and development of its neurons serotonin can strongly modify most other neurotransmitter systems in the central nervous system. Serotonergic projections are known to alter the response threshold to glutamate, to facilitate the dopaminergic mesolimbic reward pathway, to inhibit the noradrenergic

locus ceruleus system and to inhibit release of acetylcholine in the hippocampus and cortex (Bhatti et al.2000). Serotonin is involved in many functions of brain, including: cognition, learning, sleep, social behavior, mood, impulsive emotion, appetite, anxiety and sexual behaviors. Lower levels of serotonin are always associated with depression. Evidence for the involvement of serotonin in social communication and behavior come not only from human studies but also from studies with rodents and primates (Brammer et al.2010). It is worth mentioning that it plays a crucial role in emotional behavior – especially the ability to recognize emotional facial expressions (Deutz et al.2007) and the interpretation of emotional stimuli (Evers et al.2010). However, a recent fMRI study showed that serotonergic modulation has an effect on the processing of fearful, disgusted, and happy but not sad expressions across differing intensities (Brammer et al.2010).

To examine the serotoninergic system scientists often used acute tryptophan depletion (Evers et al.2010) – a noninvasive technique for reducing serotonin levels (Brammer et al.2010). It is well established that serotonin cannot cross the blood-brain barrier. Its synthesis depends on precursors: L-tryptophan and 5-hydroxytryptophan. L-tryptophan is first hydroxylated to 5-hydroxytryptophan which is then synthesized to serotonin. By ingesting a mixture containing large neutral amino acids and not containing L-tryptophan we can observe tryptophan depletion in blood (Evers et al.2010). Central depletion after this mixture can be achieved by the effects of: a decrease in the ratio of tryptophan to other larger neutral amino acids, competition between large amino acids and tryptophan when crossing the blood-brain barrier due to using the same carrier and increased protein synthesis incorporating tryptophan in organs outside the central nervous system that reduces the available precursor in the plasma (Evers et al.2010). The amount of tryptophan in plasma declines to 10-50% of baseline levels within 4-12 hours after intake of a drink containing large amino acids. During research, intake of drink always occurs together with reduced dietary intake of tryptophan which reduces its level in the plasma and diminishes its transport from plasma into brain (Bhatti et al.2000).

It is quite well established that women are more predisposed to the effects of acute tryptophan depletion than men (Abrams et al.2001). Also, these effects probably have no influence on mood, but some data are inconsistent with this statement. The discrepancy between findings is explained by differences in the baseline mood states of subjects in the studies. However, it is well established that

rapid tryptophan depletion led to a return of depressive symptoms in patients who had responded to treatment with antidepressants (Abrams et al.2001) and induce little mood changes in patients who were untreated (Bhatti et al.2000).

Studies that examined the effects of tryptophan depletion on memory and related cognitive processes have provided some evidence that reducing serotonin function through this technique impairs episodic memory consolidation (Evers et al.2010). This may be of particular relevance to disorders where a deficit of serotonin is known to be linked with cognitive effects – for instance in AD, where diminished serotonin levels were found in *post-mortem* studies (Abrams et al.2001). Also, links between memory deficits and depression are well established – serotonergic antidepressants have been found to improve memory before mood improved (Metter et al.2006).

Some studies suggest that acute tryptophan depletion impairs cognitive flexibility which refers to the ability to switch between behavioral strategies, when changes in the environment lead to alternations in the outcome of actions. It impairs decision making and reversal learning (Evers et al.2010). fMRI studies show that acute tryptophan depletion decreased the BOLD response in the left medial frontal gyrus and in the precuneus during word generation (compared to word repetition) (Evers et al.2010).

Rapid tryptophan depletion does not always impair performance in cognitive tasks. fMRI data shows that it does not have any effect on the Stroop task. Moreover, effects on motor responses depend on the kind of task (Evers et al.2010).

There aren't a lot of studies which use electroencephalography to examine the effect of rapid tryptophan depletion on cognitive functions. One ERP study showed that acute tryptophan depletion impaired memory recall, but in the absence of any significant change in either the magnitude or topography of the electrophysiological correlates of episodic retrieval. The authors concluded that effects of rapid tryptophan depletion on recall may reflect an impairment of memory encoding and/or consolidation unrelated to specific aspects of retrieval processing (Allen et al.2006). These results are somewhat contradictory to most of the previous fMRI findings where neurophysiological changes were observed in the absence of any behavioral effects.

The effect of acute tryptophan depletion on attention is usually examined with auditory stimuli. In an experiment carried out by Ahveninen and colleges subjects

were instructed to discriminate equiprobable 200- and 400-ms tones by pressing one of two buttons rapidly while being monitored by E.E.G. Occasionally, the frequency of the tones changed, causing involuntary attention shifting. Compared to the control condition, acute tryptophan depletion reduced the amplitude of the deviant-tone N200 wave, including overlapping mismatch negativity (MMN) and N200b subcomponents, which are suggested to reflect changes of detection in the brain. These results suggest that decreased levels of central serotonin function after acute tryptophan depletion may decrease involuntary attention shifting to task-irrelevant sound changes and thus modulate resource allocation to the task-relevant activity (Ahveninen et al.2001).

Analysis of the literature leads to the conclusion that both cholinergic and serotoninergic systems are involved in memory processing. Therefore it's expected that impairment in both of these systems will produce worse memory performance than impairment in these systems separately. We can assume that in ERP studies of memory recognition we will be able to observe bigger amplitude of P150 component in properly recognized trials compare with incorrectly recognized trials (Li H et al. 2010). We can predict the same phenomenon for the N200 component (Berti et al.2010). During the P600 component analysis we have to keep in mind that this peak follows distinct patterns of activation in the anterior, central, and posterior brain areas and gender differences are observed simultaneously at several electrodes within these areas (Capsalis et al.2010).

In our experiment we hypothetise to observe combined effect of biperiden and ATD treatments. We assume that it will cause decreasing of amplitude and increasing of latency of particular components which we are suppose to analyze: P150, N200, P300 and P600. This effect should be bigger than separate effects of biperiden and acute tryptophan depletion. We assume that effects of kind of stimuli and electrodes can also be found.

Materials and methods

Participants

17 healthy males and females between 18 and 35 years of age participated in the experiment. All of them were healthy, had normal static binocular acuity and body

mass index between 18.5 and 30. They were recruited via advertisements at Maastricht University. All subjects submitted a screening protocol which included a standard medical questionnaire on physical and mental health. Additionally, they received a medical examination.

Those volunteers who suffered from or have a history of cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, haematological or psychiatric illness were excluded. Other exclusion criteria were excessive drinking (>20 glasses of alcohol containing beverages a week), pregnancy or lactation, use of medication other than oral contraceptives, use of recreational drugs from 2 weeks before until the end of the experiment, and any sensory or motor deficits which could reasonably be expected to affect test performance. Those volunteers who had a first-degree relative with a psychiatric disorder or a history of a psychiatric disorder were excluded as well.

Subjects could leave the study at any time for any reason if they wished to do so without any consequences. The investigators could decide to withdraw a subject from the study if he/she did not comply with the rules of the experiment.

Stimuli

A ‘continuous recognition memory test’ was used, which assesses recognition memory and can be seen as a model of time-dependent forgetting. In the task, a series of 400 pictures (black and white line drawings) were presented on a computer screen with a duration of 500 ms and an inter-stimulus interval of 2500 ms. Ten pictures were presented ten times and occurred randomly in the series. The other pictures were only repeated once in the series. The latter occur 1, 3, or 10 words after the first occurrence of the picture. An equal number of stimuli were presented for each of the different presentation delays. The participants’ task was to rate each of the pictures as ‘new’ or ‘old’, according to whether they were presented for the first or as a repetition. The stimuli were blocked in two series with a short break in between.

Procedure

The study was conducted according to a double-blind, placebo-controlled, 4-way cross-over design. The order of treatments (biperiden, ATD, a combination, and a placebo) was balanced over four test days, which were separated by a washout

period of at least 7 days. The balancing of the treatment order was accomplished by counterbalancing.

Subjects were not allowed to use any psychoactive medication within 5 days before drug intake. Use of drugs like paracetamol, ibuprofen, aspirin, and oral contraceptives were allowed during the study.

Treatments consisted of:

- Biperiden hydrochloride (Akineton®), instant release, 2 mg orally
- Acute tryptophan depletion, 100 g gelatine-powder in 200 ml water
- A combination of biperiden and ATD
- A placebo

Treatments were administered in identical appearing capsules and drinks to ensure blinding.

After enrolment in the study, the participants first underwent a training session. During the session, all cognitive tests were practiced to familiarize the participants with the study procedures and minimize any procedural learning effects.

Each test day started with the assessment of the general health's status and participants filled in the questionnaires. Next, a blood sample was taken, which was followed by them receiving either the ATD or the balanced drink. This was followed by a waiting period, in which the participant could read a book or study. Three hours after the intake of the drink, the participants received the capsule containing either biperiden or a placebo. Next, they could have some low-protein lunch, containing protein free bread, some jam, a tomato, an apple, and some soda without caffeine. Four hours after intake of the drink, the experiment started.

EEG activity was recorded during 'continuous recognition memory task' performance in every condition (ATD, biperiden, combination and placebo).

An EEG cap was used to place a set of 32 EEG electrodes according to the international 10-20 system (Jaskowski, 2004). A reference and a ground were placed at the linked mastoids and at the forehead, respectively. Eye movements were detected by horizontal and vertical electro-oculogram (EOG) recordings. Before electrode attachment, the positions were cleaned with alcohol and slightly scrubbed

with a gel in order to provide a good measurement. Both EEG and EOG were filtered between 0.01 and 100 Hz and sampled at 1000 Hz.

Offline, the EEG was checked for EOG activity and other artifacts. The EEG data that contained artifacts were excluded from the analysis. Frequency analyses were performed on epochs between 100 before and 1000 ms after the onset of stimuli in the tasks (event-related frequency analysis). Event-related potentials were extracted by averaging the responses within an epoch of 100 ms before and 1000 ms after stimulus onset. Separate averages were made for correct and incorrect responses within a task.

Previous studies have shown that peak plasma levels of biperiden are reached 1-2 h after a single dose (Gammans, Mayol, & LaBudde, 1986; Hollmann, et al., 1987). Peak plasma levels after ATD are reached around 4-6 hours after treatment (Sambeth et al., in press). Therefore, measurement started 4 hours after intake of the ATD drink. One hour before testing, the participants received the capsule containing biperiden or a placebo.

Blood samples (10 ml) were collected in sodium heparin tubes at baseline (t-4) and before the cognitive tests (t0) by venipuncture.

Data Analysis

EEG signal was analyzed using Vision Analyzer software. After data preprocessing the EEG fragments within an epoch of 100ms before onset of response and 1000ms after response onset were averaged for all trials, per type of stimuli (old, new). The mean amplitude of the 100ms before stimulus onset was used as a baseline value (baseline correction). High-pass filter 1Hz, with 12 dB slope was used to filter out very slow activity. Low-pass filter 30Hz, with 12dB slope was used to filter out the very fast frequencies. The Gratton&Coles algorithm was used to reduce the effect of eye movements.

P150, N200, P300 and P600 components were determined on the basis on the individual (single subject) and grand average (over all subjects). The amplitude of P150 component was defined as the maximum positive amplitude between 120 and 200 ms after stimulus onset, N200 as most negative value between 190 and 300 ms after picture onset, P300 as most positive between 270 and 400 ms and P600 as most positive between 440 and 680 ms.

General Linear Model was used for the analyses of each component. 4 within subject design was performed. ATD (2 levels) was compared to biperiden using

treatment (2 levels), kind of stimuli (2 levels – old and new) and electrodes (5 levels) for amplitude and latency as within subject factors per each of every four component. Bonferroni correction was used in all analyses.

Results

All subject's data were used for the analysis. GLM showed no significant effect of combined ATD and biperiden. However, P600 voltage showed an effect of biperiden ($F=4,540$ $P<0.05$) and P600 latency showed effect of ATD ($F=4,401$ $P<0.05$) but only on stimulus type. Latency of P150 showed combined effect of both kind of stimuli and ATD ($F=8,570$ $P<0,01$).

Both N200 latency and voltage showed significant effect of kind of stimuli ($F=5,354$ $P<0.034$ and $F=23,346$ $P<0.000$). Furthermore, in this case old kind of stimuli showed significant effect of electrodes ($F=13,337$ $P<0.001$). We could observed the same effect for new kind of stimuli ($F=12,123$ $P<0.001$).

Kind of stimuli also had influence on latency of P600 ($F=16,192$ $P<0.001$). We could observed it also with combination with effect of electrodes ($F=4,344$ $P<0.009$). Just electrodes also had influence on P600 latency ($F=6,373$ $P<0.003$).

Both kind of stimuli and electrodes showed effect on P300 voltage ($F=10,850$ $P<0.000$). We could also observed separate effect of kind of electrodes ($F=11,162$ $P<0.003$). Effect of electrodes was also observed on old kind of stimuli in this case ($F=21,864$ $P<0.000$).

Kind of electrodes had statistically significant effect on P300 latency ($F=8,333$ $P<0.001$). Considering this peak's latency we could also observe effect of electrodes on old stimuli ($F=6,137$ $P<0.006$) and on new kind of stimuli ($F=4,727$ $P<0.008$).

Electrodes had significant effect on P150 voltage ($F=7,628$ $P<0.009$). Electrodes had significant effect on old stimuli which were presented ($F=10,086$ $P<0.003$) and on new stimuli ($F=5,266$ $P<0.027$).

Kind of electrodes showed effect on P600 voltage ($F=95,978$ $p<0.000$). Electrodes had significant effect on P600 voltage when old stimuli were being presented ($F=92,976$ $P<0.000$) and also on new stimuli ($F=60,976$ $P<0.000$).

Discussion

The aim of this study was to investigate of combining effect of acute tryptophan depletion and biperiden on human memory. These medicines were chosen because of their effect on either the serotoninergic or cholinergic system which we know are involved in cognitive processes.

Biperiden and ATD separately had effect on the P600 peak which is a late component of electrophysiological response and does not depend on automatic

reactions but rather depends on internal rules, kind of task which is performed and memory. We can conclude that this peak actually refers to cognitive processes which takes part during task. Abnormal peak or/and latency of P600 can be a sign of abnormalities that concern AD or another kinds of cognitive disturbances.

Kind of presented stimuli had effect on all components what showed that new stimuli and stimuli, which were seen before undergo another cognitive processes. The latter are elaborated deeper and are remembered better, than new. Old/new effect was also present. This phenomenon states that correct responses to targets or old stimuli elicited significantly more pronounced positive waveform in comparison to correct rejections of distractors or new stimuli between 400 and 700 ms after stimulus onset on all left and central electrode positions (Daum et al.2006).

All peaks vary in latency and/or voltage according to type of electrodes. We can conclude that all of them have different sources in structures of the brain and they are signs of different cognitive processes.

There was no significant effect of both treatments on peaks voltage or latency. However, effect of treatments was observed separately on P600 component. We can hypothetize that cholinergic and serotoninergic systems work separately to some extension. This sentence can be established because combined effect of two treatments which impair both system was not bigger that effect of these treatments separately.

This subject require further and deeper analysis. First of all, biperiden blocked only M1 receptors and we can argue that another combined effects can be observed if we use more selective cholinergic receptors blockers. Furthermore, combined effect of biperiden could be observed in another memory tasks, like this which require recalling information not just recognition.

References

Abrams, J., Bell, C., Nutt, D. (2001). Tryptophan depletion and its implications for psychiatry. *British Journal of Psychiatry,178,399-405.*

Ahveninen, J., Ilmoniemi, R.J., Jaaskelainen, I.P., Liesivuori, J., Kahkonen, S. (2002). Tryptophan depletion effects on EEG and MEG responses suggest serotoninergic modulation of auditory involuntary attention in humans. *NeuroImage,16,1052-1061.*

Allen, P., Cortesi, M., Fusar-Poli, P., McGuire, P., Perez, J., Placentino, A. (2006). Neuroimaging and electrophysiological studies of the effects of acute tryptophan depletion: a systematic review of the literature. *Psychopharmacology,188,131-143.*

Anlar, O., Caksen, H., Odabas, D. (2003). Use of biperiden hydrochloride in a child with severe dyskinesia induced by phenytoin. *Journal of Child Neurology,18(7),494-496*

Bartus, R.T., Dean, R.L., 3rd, Beer, B., & Lippa, A.S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science*, *217*(4558), 408-14.

Belzer, K., Facelle, T.M., Pomara, N., Sidtis, J.J., Willoughby, L.M., Yi L. (2010). Retrograde facilitation of verbal memory by trihexyphenidyl in healthy erderly with and without the APOE e4 allele . *European Neuropsychopharmacology,20,467-472.*

Berti, S., Gamer, M. (2010). Task relevance and recognition of concealed information have different influences on electrodermal activity and event-related brain potentials. *Psychophysiology,42(2),355-364.*

Bhatti, T., Clark, C., Gillin, C.J., Kelsoe, J., Landolt, H.P., Moore, P., Seifritz, E. (2000). Clinical and physiological consequences of rapid tryptophan depletion. *Neuropsychopharmacology,23(6),601-622.*

Blokland, A., Jans, L.A.W., Lieben, C.K.J., Smits, L.T. (2009). Pharmacokinetics of acute tryptophan depletion using a gelatin-based protein in male and female Wistar rats. *Amino Acids,37,349-357.*

Brammer, M., Cleare, A., Craig, M., Daly, E., Deeley, Q., Ecker, C., Hallahan, B., Lamar, M., Murphy, D.G.M., Phillips, M.L., Surguladze, S., Toal, F. (2010). Effects of acute tryptophan depletion on neural processing of facial expression of emotion in humans. *Psychopharmacology,210,499-510.*

Capsalis, C.N., Hountala, C.D., Kyprianou, M.A., Maganioti, A.E., Parageorigiou, C.C., Rabacilas, A.D. (2010). Principal component analysis of the P600 waveform: RF and gender effects. *Neuroscience letters,478(1),19-23*

Cassel, J.C., & Jeltsch, H. (1995). Serotonergic modulation of cholinergic function in the central nervous system: cognitive implications. *Neuroscience, 69*, 1-41.

Chen, C.P.L.-H., Diez-Ariza, M., Francis, P.T., Garcia-Alloza, M., Gil-Bea, F.J., Lasheras, B., Ramirez, M.J. (2005). Cholinergic – serotonergic imbalance contributes to cognitive and behavioral symptoms in Alzheimer's disease. *Neuropsychologia,43,442-449.*

Curran, H.V., Pooviboonsuk, P., Dalton, J.A., & Lader, M.H. (1998). Differentiating the effects of centrally acting drugs on arousal and memory: an event-related

potential study of scopolamine, lorazepam and diphenhydramine. *Psychopharmacology (Berl)*, *135*(1), 27-36.

Daum, I., Wiese, A.(2006). Frontal positivity discriminates true from false recognition. *Brain Research,1075,183-192*

Deutz, N.E.P., Evers, E.A.T., Schmitt, J.A.J., van der Veen, F.M. (2007). Effects of acute tryptophan depletion on mood and facial emotion perception related brain activation and performance in healthy women with and without a family history of depression. *Neuropsychopharmacology,32,216-224.*

Evers, E.A.T., Ramaekers, J.G., Riedel, W.J., Sambeth, A., van der Veen, F.M. (2010). The effects of acute tryptophan depletion on brain activation during cognition and emotional processing in healthy volunteers. *Current Pharmaceutical Design,16,1998-2011.*

Hollmann, M., Brode, E., Greger, G., Muller Peltzer, H., & Wetzelsberger, N. (1984). Biperiden effects and plasma levels in volunteers. *Eur J Clin Pharmacol*, *27*(5), 619-21.

Hulstijn, W., Ruigt, G.S.F., Sabbe, B.G.C., Wezenberg, E., Verkes, R.J. (2005). Modulation of memory and visuospatial processes by biperiden and rivastigmine in elderly healthy subjects. Psychopharmacology,181(3),582-594.

Jaskowski, P. (2004). Zarys psychofizjologii. Wyzsza Szkola Finansow i Zarzadzania, Warszawa.

Li, H., Li, S., Liu, Q., Long, C., Shen, X., QIU, J. (2010). Neural signs of flexible categorization: evidence from the flexibility of inclusion of humans in animal/non-animal categorization. *Brain Research,1337,64-73*

Meeter, M., Talamini, L., Schmitt, J.A., & Riedel, W.J. (2006). Effects of 5-HT on memory and the hippocampus: model and data. *Neuropsychopharmacology, 31*, 712-720.

Pickles, C.D., Potter, D.D., Roberts, R.C., Rugg, M.D. (2000). Scopolamine impairs memory performance and reduces frontal but not parietal visual P3 amplitude. *Biological Psychology,52,37-52.*

Schmitt, J.A., Jorissen, B.L., Sobczak, S., van Boxtel, M.P., Hogervorst, E., Deutz, N.E., & Riedel, W.J. (2000). Tryptophan depletion impairs memory consolidation but improves focused attention in healthy young volunteers. *Journal of Psychopharmacology, 14*, 21-29.

Sherman, S.J., Atri, A., Hasselmo, M.E., Stern, C.E., & Howard, M.W. (2003). Scopolamine impairs human recognition memory: data and modeling. *Behav Neurosci*, *117*(3), *526-39.*

Shinohara, Y. (2010). Delirium caused by intramuscular administration of biperiden. *The Journal of Neuropsychiatry and Clinical Neurosciences,22(2)E15.*