Akaki Tsereteli State University
Faculty of Exact and Natural Sciences

On the Manuscript Rights

Khatuna Rusadze

Modulation of hippocampal cholinergic transmittion and memory function by septohippocampal projections

05 Sciences/Natural Sciences
0504 Biology/Life Sciences
050404 Phyziology (humans and animals)

ABSTRACT

of the dissertation for the academic degree
of Doctor of Biology

Kutaisi
2016

This scientific paper has been performed in the Akaki Tsereteli State University Department of Biology and the Ivane Beritashvili Center of Experimental Biomedicine.

Scientific Supervisor: **Temur Naneishvili**
Doctor of Biological Sciences,
Academician

Scientific Co-Supervisor: **Reniko Sakandelidze**
Doctor of Biological Sciences,
Professor of Akaki Tseretely
State University

Reviewers: **1. Elene Abzianidze**
Doctor of Biological Sciences,
Professor of Tbilisi State
Medical University

2. Mzia Zhvania
Doctor of Biological Sciences,
Professor of Ilia State University

The Defense of the Dissertation will be held on 17 July 2016, at 13:00 at the meeting of dissertation commission created by dissertation board of the Faculty of Exact and Natural Sciences of Akaki Tsereteli State University.

Address: Block I, room 1114, 59 Tamar Mepe St., Kutaisi 4600.

The Dissertation will be available in the Akaki Tsereteli State University Library (59 Tamar Mepe St., Kutaisi, 4600)

The abstract of the Dissertation is dispatched on 16.06.2016.

The Secretary of the
Dissertation Council
Professor Z. Sokhadze

Short Description of the Work

Topicality of Research

The septum and the hippocampus are heavily interconnected through the fimbria-fornix and are functionally coupled, often referred to collectively as the septohippocampal (SH) system. The SH projection includes well-known cholinergic and GABAergic components and a subpopulation of septal glutamatergic neurons. Given the central role the hippocampus plays in declarative memory formation and the strong input to the hippocampus from the SH pathway, it is tempting to hypothesize that this input is critical for memory processes. Extensive data from a number of different experimental approaches suggest that the septohippocampal cholinergic system is sufficient for normal memory function.

Traditionally, most basal forebrain (BF) functions have been attributed to its cholinergic neurons. However, an increasing body of evidence suggests that behavioral deficits after lesions of cholinergic nuclei are not entirely due to destruction of cholinergic cells. Selective damage of cholinergic medial septal (MS) neurons using 192-IgG saporin has generated equivocal results with both impairments and no impairments reported. Analyzing bulk of scientific literature devoted to the issue, it became clear that the question of how SH projections can modulate memory and learning process is far from resolved. In order to clarify further the role of the cholinergic and noncholinergic projections from the MS, the effects of electrolytic, selective immunotoxic lesions of cholinergic or GABAergic neurons of this nuclei will be compared in the same subjects in tests taxing different forms of memory.

A loss of cholinergic or GABAergic MS neurons can affect the activity of neurotransmitters and their receptors in the hippocampus. Study of

modulation of complex synaptic mechanisms and neural circuits of the hippocampus by cholinergic and noncholinergic SH projections, which would need detailed and multidisciplinary studies to be completely understood, might be crucial step for the better understanding of the fundamental neurobiology of memory and for understanding the role of SH projections in disorders of memory. Pharmacological or genetic inhibition of acetylcholine receptors cause memory deficits but it is often unclear which receptor subtypes are involved.

Acetylcholine release within hippocampal circuits results in the activation of both muscarinic (mAChRs) and nicotinic (nAChRs) acetylcholine receptors, causing the subsequent modulation of cellular excitability and synaptic transmission. These two types of receptors are differentially expressed across the hippocampus and fulfill different functions.

The purpose of research is to obtain information for understanding the role of the medial septal cholinergic and GABAergic projections in memory and learning process. The idea that cholinergic and GABAergic SH projections can modulate memory was tested by comparing effects of electrolytic, selective ACh or GABA immunotoxic lesions of MS on different form of memory and learning process and on expression of hippocampal cholinergic receptors.

- **Principal Goals and Tasks of the Reserch**

Different forms of memory and development of learning process were studied and the immunocytochemical determination of expression level of cholinergic (α 7 nAChRs and M1) hippocampal receptors were carried out in MS electrolytic, selective cholinergic and/or GABAergic immunotoxic lesioned and sham-operated rats. Investigation the effects of antidementic drug - memantine treatment on memory in MS lesined rats also were carried out .

The objectives:

- Study of different forms of memory and learning process in MS electrolytic, selective ACh or GABA immunotoxic lesioned and sham-operated rats.
- Immunocytochemical determination of expression level of cholinergic (α7 nAChRs and M1) receptors in MS electrolytic, selective ACh or GABA immunotoxic lesioned and sham-operated rats.
- Study of different forms of memory and learning process in MS ACh and GABA conjoint immunotoxic lesioned and sham-operated rats.
- Study of antidementic drug - memantine treatment on memory in MS lesined rats

The scientific novelty

It is the first time to found that:

Expression of α-7 nACh receptors in the hippocampus is reduced by either nonselective or immunotoxic lesions of medial septal GABAergic, but not cholinergic neurons; expression of M_1 mACh receptors in the hippocampus is unchanged by nonselective - electrolytic lesion of the medial septal necleus;

There is dissociation between the two major components (cholinergic and GABAergic) of the septohippocampal pathway in hippocampal dependent spatial memory assessed in Morris water maze – the hippocampal dependent spatial memory is affected by either nonselective or immunotoxic lesions of GABAergic, but not cholinergic medial septal neurons;

There is correlation between spatial memory impairment and the reduction of α7 nACh receptors expression in the hippocampus induced by medial septal lesions. Lesion-induced spatial memory impairment

lesion was not produced. All injections of 192 IgG-saporin (1 μg/μl) and GAT1 SAP (325ng/μl) for immunolesion surgeries or mouse saporin (this product serves as a control for the immunotoxin) for control surgeries (Advanced Targetting System, San Diego, USA) were performed stereotaxically. Injection of GAT1-SAP were performed from the side by a 15 degree angle with the following coordinates AP- 0.4; M L -1.7; DV - 6.4 (0.5 μl; 0.05 μl/min). Injection of 192 IgG-saporin were performed bilateraly at two depths on each side: AP - 0.45; ML - 0.25; DV - 7.8 (0.3 μl; 0.05 μl/min) and DV - 6.2 (0.2 μl; 0.05 μl/min). After injection the needle was left in place for an additional 9 min and 6 min, respectively, to allow the toxin to diffuse from the injection site. All injections were made with a 1-μl Hamilton syringe with a microinjection pump **(CMA 402 Syringe Pump, Sweden).** The rats were allowed to recover from the surgery for two weeks before starting the behavioral experiments.

Immunohistochemistry. The size and location of the electrolytic lesions were determined by microscopic examination of serial coronal sections (25 μm) stained with cresyl violet. The immunotoxic 192 IgG-saporin lesions of MS were verified by observing decreased Acetylcholinesterase (AChE) staining of the MS and hippocampal sections (hippocampal AChE is used as a quantitative measure of lesion extent). The immunotoxic GAT1-SAP lesions of MS were verified by observing decreased Acetylcholintransferase (ChAT) and parvalbumine (PV) staining of the MS. The 20 μ thick coronal sections using freezing microtome were stained with ChAT and PV primary antibody and ABC Staining System. All necessary reagents and buffers were received from Santa Cruz Biotechnology, Inc. (USA). Totally 30 fields per animal were analyzed and average of immunostained cells per field was used to assess the effect of MS lesion on AChE, ChAT and PV-stained neurons. The sections were analyzed with a microscope Leica MM AF.

Electrophoresis and immunoblotting. After decapitation hippocampus was removed and frozen immediately on dry ice. Tissue from hippocampus and single rat formed a single sample and standard immunochemical procedures were carried out.

Statistical Analysis. Differences between groups were determined by ANOVA (SigmaStat statistical software). Two-sample t-test was used to compare immunohistological data between control and lesioned groups. All data are presented as mean ± standard error of the mean. Differences were considered significant when $p < 0.05$.

Results

3.1. Effects of medial septal selective and nonselective lesions on hippocampal cholinergic activity and memory

Acetylcholine release within hippocampal circuits results in the activation of both muscarinic (mAChRs) and nicotinic (nAChRs) acetylcholine receptors, causing the subsequent modulation of cellular excitability and synaptic transmission. These two types of receptors are differentially expressed across the hippocampus and fulfill different functions.

The idea that cholinergic and GABAergic SH projections can modulate memory was tested by comparing effects of electrolytic, selective ACh or GABA immunotoxic lesions of MS on different form of memory and learning process and on expression of hippocampal cholinergic receptors.

3.1.1. Effects of medial septal selective and nonselective lesions on hippocampal cholinergic receptors expression

The aim of this study was to investigate the effects of medial septal selective (by GAT1-SAP and 192 IgG saporin) and nonselective (electrolytic) lesions on hippocampal cholinergic α7 and M_1 receptors expression.

In our experiments electrolytic lesions destroyed on average 69% (range - 50%–88%) of the intact MS. Examination of the ChAT stained sections showed that after injections of 192 IgG saporin into the MS, animals exhibited significantly less ChAT staining in MS ($P<0.001$) as compared to sections obtained from control animals. Counts of PV-ir neurons neurons made in the same rats used to assess ChAT-ir neurons demonstrated a mild reduction following 192 IgG saporin. Intraseptal GAT1-SAP preferentially reduced GABAergic neurons as compared to cholinergic neurons in the MS ($P<0.001$). Counts of ChAT-ir neurons made in the same rats used to assess PV-ir neurons demonstrated a mild reduction following GAT1-SAP ($P<0.05$). The reduction of cholinergic neurons represented a loss of only 26%. Thus, GAT1-SAP when infused into the MS extensively damaged GABAergic MS neurons and spared most cholinergic neurons. The results showed that after injections of 192 IgG saporin into the MS, animals exhibited significantly less AChE staining in hippocampus as compared to sections obtained from control (CA1 - $P < 0.01$, CA3 –$P<0.01$) and GAT1-SAP treated (CA1 - $P<0.01$, CA3 –$P<0.01$) animals.

Data for the amounts of $\alpha7$ and M_1 were analyzed by the One-way analysis of variances (factor type of treatment). For $\alpha7$ the One way ANOVA indicated statistically significant effect of group ($P<0.001$). Post Hock analysis (Tukey Test) showed significant difference between control and MS GAT ($P<0.01$) and El lesioned ($P>0,05$) groups and no significant difference between control and MS SAP lesioned ($P=0,094$) groups. or GAT lesioned ($P<0,001$), but no significant difference between control and SAP treated rats ($P=0,286$). Analysis for hippocampal cholinergic M_1 receptors showed no significant difference between control and El lesioned ($P=1,000$) groups.

3.1.2. Effects of electrolytic and immunotoxic lesions of medial septal neurons on spatial long-term memory

The aim of this study was to investigate the role of the MS cells in hippocampal dependent spatial learning using the electrolytic and immunotoxic (GAT1-SAP and 192 IgG saporin) to produce nonselective and selective lesions of MS neurons, respectivey. In current study rats were trained in a visible platform version of the Morris water maze in which either a place or cue strategy could be used to escape successfully.

The number of animals in each group was as follows: MS electrolytic ($n = 8$), MS immunotoxic (192 IgG saporin, $n = 8$ and GAT1-SAP, $n = 8$) lesioned. Since there were no significant differences ($P >0.05$) between sham-operated (4 rats) and vehicle-injected rats (4 rats) these groups were combined into a single one, as of now designated as control ($n = 8$).

The control and SAP treated rats rapidly learned to escape to the visible platform and reached the 6-7 s asymptote on day 2. The MS electrolytic and GAT lesioned rats were significantly impaired on the first 4 days and improved in their ability to escape to the platform at slower rate.

For training trials, a two way ANOVA [group X testing condition (visible/unvisible platform)] indicated statistically significant effect of group ($F(4,287) = 12.569$; $P < 0.001$) and testing condition ($F(1,287) = 28.556$; $P< 0,001$) and there is a statistically significant interaction between group and testing condition ($F(4,287) = 3.744$; $P = 0.006$). The effect of different group depends on what testing condiiont is present. Post Hock analysis (Tukey Test) showed no significant difference between groups ($P>0,05$) in visible platform trials and significant difference between control and MS el lesioned ($P<0,001$) or GAT lesioned ($P<0,001$), but no significant difference between control and SAP treated rats ($P=0,286$).

On the competition trials, a greater number of control and SAP treated rats used a place strategy. The rats of EL lesioned and GAT treated groups, in contrast of control and SAP treated rats preferentially perform the test trials, using single visual cues.

The rats of Control and SAP treated groups, had significantly more accurate searches on hidden platform days, providing an additional evidence of their effective use of a place learning strategy rather than the rats of EL or GAT treated groups exhibiting a cue or place strategy in competition trials.

3.1.3. Effects of electrolytic and immunotoxic lesions of medial septal neurons on spatial short-term memory

In the present study electrolytic and immunotoxic (GAT1-SAP and 192 IgG saporin) lesions of MS were used to investigate the importance of SH projections in spatial working memory. Spatial alternation (SA) is assumed to be a hippocampal-dependent measure of spatial working memory.

The number of animals in each group was as follows: MS electrolytic (n = 8), MS immunotoxic (192 IgG saporin, n = 8 and GAT1-SAP, n = 8) lesioned. Since there were no significant differences (P >0.05) between sham-operated (4 rats) and vehicle-injected rats (4 rats) these groups were combined into a single one, as of now designated as control (n = 8).

The electrolytic lesion of MS significantly impaired SA performance. Specifically, the percent alternation scores of MS lesioned rats were significantly lower than control rats ($P < 0.05$). However, the groups did not differ in the number of arms entered during the testing session. According to the data obtained it could be suggested that the MS is necessary for normal short-term spatial memory function.

3.2. Effect of chronic memantine treatment on different forms memory in rats with conjoint immunolesions of GABAergic and cholinergic MS neurons

In the present study the effect of conjoint immunolesions of GABAergic and cholinergic MS neurons on spatial long-term and short-term memory is and investigated the effects of chronic memantine treatment in control and MS lesioned rats are evaluated. A total of 32 male outbred white rats were used in the present study. Animals were divided into four groups with eight rats in each group: control rats injected i.p. with saline - Contr(S) or memantine - Contr(M) and immunolesioned rats injected i.p. with saline - MS_{IMM}(S) or memantine - MS_{IMM}(M). Memantine (5 mg/kg i.p.) or saline were given daily for two weeks starting from the day of immunotoxins injection.

3.2.1. Effect of chronic memantine treatment on spatial long-term memory in rats with conjoint immunolesions of GABAergic and cholinergic MS neurons

In current study rats were trained in a visible platform version of the Morris water maze. The rats of Contr(M) and MS_{IMM}(M) groups as control rats rapidly learned to escape to the visible platform and reached the 6-7 s asymptote on day 4. Saline treated MS immunolessioned rats were significantly impaired on the first 6 days and improved in their ability to escape to the platform at slower rate. Thus, the learning was slower in this group.

For training trials, a two way ANOVA [group X testing condition (visible/unvisible platform)] indicated statistically significant effect of group ($F(3, 287) = 19,986$; $P < 0.001$) and testing condition ($F(1, 287) = 7,706$; $P = 0,006$) and there is no significant interaction between group and testing condition ($F(3, 287) = 1,782$; $P = 0.151$). The effect of different group depends on what testing condiiont is present. Post

Hock analysis (Tukey Test) showed no significant difference between Contr(S) and Contr(M) or MS_{IMM}(M) groups (P=0,88; P=0,082, respectively) in visible platform trials and significant difference between Contr(S) and MS_{IMM}(S) (P<0,001), also between MS_{IMM}(S) and MS_{IMM}(M) (P<0,001) groups in hidden platform trials.

The rats' responses on the competition test were classified as either cue or place, based on the swim path for those trials. On the first competition trial, a greater number of contr(S), contr(M) and MS_{IMM}(M) rats used a place strategy compared with MS_{IMM}(S) rats. The increased cue-bias in MS_{IMM}(S) rats compared with contr(S), contr(M) and MS_{IMM}(M) rats was significant (P< 0,05 for all groups). On the second trial, the majority of MS_{IMM}(S) rats used a cue strategy. The majority of rat of the contr(S), contr(M) and MS_{IMM}(M) **groups** used a place strategy. There was no difference in strategy between these groups (P>0,1).

An overview of the data from both competition trials for each group show that the saline treated control rats in 16 trials out of 14 competition test trial used place strategy, while Saline treated MS immunolesioned ones used this strategy in 6 trials only. Decreased place-bias in MS lesioned rats compared to the control rats was significant (P<0,02). Notably, the Saline treated MS immunolesioned rats, exhibited corresponding differences in performance during training trials. The majority of rat of the contr(S), contr(M) and MS_{IMM}(M) **groups,** identified as place responders, had significantly more accurate searches on hidden platform days, providing an additional evidence of their effective use of a place learning strategy rather than the MS_{IMM}(S) rats exhibiting a cue strategy in competition trials.

3.2.2. Effect of chronic memantine treatment on spatial short-term memory in rats with conjoint immunolesions of GABAergic and cholinergic MS neurons

In the present study the effect of chronic memantine treatment on spatial alternation behavior (SA) in control and MS immunolesioned rats are evaluated.

The one way ANOVA for the number of arms entered during the testing session showed significant effect of group ($F_{3,31}$=5.962, P=0.003). Post hoc (Tukey Test) analysis showed a significant difference between the saline and memantine treated control rats (P =0.008). The significant difference revealed between the saline treated control and saline or memantine treated immunolesioned rats (P = 0.007; p =0.016, respectively). There was no significant difference between the saline and memantine treated immunolesioned rats (P=0.983).

Behavioral study showed that memantine treated control rats, relative to saline treated control rats, had a significantly lower level in the number of arms entered during the testing session.

Immunolesion of MS significantly impaired SA performance. The one way ANOVA for spatial alternation score showed significant effect of group ($F_{3,31}$=20.449, P=0.001). Post hoc (Tukey Test) analysis showed a significant difference between the saline treated control and immunolesioned rats (P < 0.001), but there was no significant difference between the saline and memantine treated control (P = 0.993) rats and between saline treated control and memantine treated immunolesioned (P = 0.454) rats. Memantine treatment causes improvement of spontaneous alternation performance; the difference between saline and memantine treated immunolesioned rats is significant (P < 0.001).

Conclusions:

Our results indicate that:

1. expression of α-7 nACh receptors in the hippocampus is reduced by either nonselective or immunotoxic lesions of medial septal GABAergic, but not cholinergic neurons;
2. expression of M_1 mACh receptors in the hippocampus is unchanged by nonselective - electrolytic lesion of the medial septal necleus;
3. the deficits after nonselective damage of medial septum are limited to a subset of cognitive processes dependent on the hippocampus;
4. medial septum is substantial for declarative form of spatial memory, but not for nondeclarative form of spatial memory - nondeclarative form of spatial memory can be supported outside the septohippocampal system;
5. there is dissociation between the two major components (cholinergic and GABAergic) of the septohippocampal pathway in hippocampal dependent spatial memory assessed in Morris water maze – the hippocampal dependent spatial memory is affected by either nonselective or immunotoxic lesions of GABAergic, but not cholinergic medial septal neurons;
6. there is correlation between spatial memory impairment and the reduction of α7 nACh receptors expression in the hippocampus induced by medial septal lesions. Lesion-induced spatial memory impairment may be attributed, at least in part, to the reduction of hippocampal α7 nACh receptors expression caused by the lession of GABAergic medial septal neurons;
7. the selective loss of septohippocampal cholinergic or GABAergic projections does not disrupt the function of the hippocampus to a sufficient extent to impair spatial short-term spatial memory;

8. spatial short-term memory is affected by conjoint immunolesions of GABAergic and cholinergic medial septal neurons;
9. chronic administration of memantine at doses which is therapeutic relevance for its use in AD patients significantly attenuated lesion-induced spatial memory impairment;

In conclusion, selective cholinergic lesion of medial septal neurons did not affect expression of α-7 receptors, although medial septal ChAT and hippocampal AChE immunolabeling were significantly reduced. In contrast, GAT1 SAP lesions of the medial septum, more selective for GABAergic projection neurons, produced significant reductions in α-7 receptors expression. These findings are the first to show changes in α7 receptors expression in association of memory deficit following hippocampal denervation and suggest that medial septal hippocampal innervation regulates some functional aspects of hippocampal α7 receptors. The results confirm hippocampal α7 nicotinic receptors as viable therapeutic targets in diseases that involve degradation of the septohippocampal pathway and may indicate that GABAergic medial septal hippocampal input plays a more substantial role in the regulation of α7 nicotinic receptor function than medial septal hippocampal cholinergic input.

The list of papers published by the author on the theme of dissertation:

Articles:

- Dashniani M., Kruashvili L ., Rusadze Kh., Mataradze S., Beselia G. Efects of immunotoxic and electrolytic lesions of medial septal area on spatial short-term memory in rats. Georg. Med. News. N2 (239) Georgia, 2015;

- Burjanadze M,. Mataradze S., Rusadze Kh., Chckikvishvili N., Dashniani M. Selective lesion of GABA-ergic neurons in the medial septum by GAT1-saporin impairs spatial learning in a water-maze. Georg. Med. News. N3 (240) Georgia, 2015;
- Naneishvili T., Rusadze Kh., Dashniani M., Burjanadze M., Chkhikvishvili N., Beselia G., Kruashvili L., Pochkhidze N. Chronic Memantine Treatment Prevents Short-Term Memory Impairment Caused by Conjoint Immunolesions of GABAergic and Cholinergic Medial Septal Neurons in Rats" Bulletin of Georgian national academy of science. vol.10, no.2, 2016;

Abstracts:

- Rusadze Kh., Meparishvili M., Naneishvili T., Beselia G., XII National Scientific Conference of Georgia "Actual Problems of Physiology and Biomedicine" Spatial short-term memory and modulation of NMDA receptor subunit expression in medial septal immunolesioned rats. Kutaisi, Georgia 2014
- Mataradze S., Naneishvili T., Demurishvili M., Chigladze M.,. Rusadze KH., 9th Fens Forum of Neuroscience "Study of selective immunolesions of medial septal cholinergic and GABA-ergic neurons on hippocampal dependent memory and learning process" Milan, Italy 2014
- Rusadze Kh., Georgian National Academy of Sciences, Conference of Young Scientists "modulation of spatial short-term memory and NMDA receptors expression by septohippocampal projection". Tbilisi, Georgia 2015
- Rusadze Kh., "Actual problems of biology and medicine" Effects of selective and nonselective lesions of medial septum on learning strategy in morris water maze. Telavi, Georgia 2015

- Rusadze Kh., Georgian National Academy of Sciences, Conference of Young Scientists "Effects of immunotoxic and electrolyc lesions of medial septal area on spatial short-term memory in rats" Bakuriani, Georgia 2016
- Lali Kruashvili, Maia Burjanadze, Maia Demurishvili, Nino Chkhikvishvili and Khatuna Rusadze, 29th ECNP Congress of Applied and Translational Neuroscience. Spatial memory impairments following excitotoxic lesions of the basal forebrain noncholinergic neuronal ensembles, Vienna, Austria 2016
- T. Naneishvili, Kh. Rusadze, R. Sakandelidze, 29th ECNP Congress of Applied and Translational Neuroscience. Chronic memantine treatment prevents short-term memory impairment caused by conjoint immunolesions of GABAergic and cholinergic medial septal neurons in rats. Vienna, Austria 2016