საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 10, №3, 2016
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 10, no. 3, 2016

Human and Animal Physiology

Effects of Medial Septal Lesions on Learning Strategy Selection in Plus-Shaped Maze

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ABSTRACT. In the present study electrolytic and immunotoxic lesions of the medial septum (MS) were used to investigate the importance of septo-hippocampal projections in place or response learning strategy selection in plus-shaped maze. In current study rats were trained in the dual-solution plus-maze task. In this task, rats are trained to retrieve food from a consistently baited arm, starting always from the same start box. Using this procedure, they may learn to find food in a particular place in space (place strategy) or to make a particular body turn (response strategy). But the strategy they use can be resolved by submitting them to a probe test from the opposite arm. In behavioral experiments no differences in speed of learning were found between control and MS lesioned groups ($DF_{(3,35)}$= 0.209, P = 0.889). Using a multiple memory systems approach, findings from our experiments show that control and 192 IgG saporin treated rats trained on an appetitive dual-solution plus-maze task use spatial or place strategies effectively. On the other hand, MS electrolytic and GAT1- SAP treated rats tend to use response strategies. Decreased place-bias in MS electrolytic lesioned and GAT1-SAP treated rats compared to the control rats was significant (t_d = 3.8 P<0.001; t_d =1.99, P<0.02, respectively). Results of our study suggest that the MS is essential for spatial learning and suggest its role in processing information about the spatial environment, but deficits observed after septal electrolytic lesions cannot be accounted to the loss of MS cholinergic neurons and suggest a role for GABAergic MS neurons in spatial memory. © *2016 Bull. Georg. Natl. Acad. Sci.*

Key words: learning strategy, medial septal nucleus, plus-shaped maze, rat

Accumulating evidence suggest that the septo-hippocampal (SH) system is sufficient for normal memory function. The interconnections between the septum and the hippocampus are reciprocal. The ascending connections from the septum to the hippocampus includes well-known cholinergic and GABAergic components [1] and a subpopulation of septal glutamatergic neurons [2]. About 90 % of the cholinergic innervation of the hippocampus comes from the medial septum (MS). Lesions of the fimbria-fornix, electrolytic or neurotoxic lesions in the MS impair hippocampus-dependent learning and memory [3, 4]. More specifically, the role of the MS in learning and memory appears to involve an influence on

cholinergic processes in the hippocampus [5]. Lesions of the SH pathway decrease acetylcholinesterase staining intensity [6] and extracellular acetylcholine levels in the hippocampus [7], a property that is the best described neurotransmitter-defect in Alzheimer's disease (AD).

AD is a progressive and irreversible neurodegenerative disease accompanied by decline of memory and cognitive function [8]. Degeneration of cholinergic basal forebrain (BF) neurons is one of the common features of AD [9]. Postmortem assessment has revealed significant degeneration of BF neurons as an early pathological feature of AD patients [10 - 12]. It has been reported that degeneration of BF cholinergic neurons and the decrease of cholinergic projections could be an important factor characterizing the cognitive decline and functional impairment that characterizes this disorder [9, 13].

The animals with MS lesions and resultant learning impairments were offered as models of AD [14] and can help understand neurotransmitter systems involved in the AD pathology and identify cognition-enhancing drugs that might be useful in AD. Spatial memory is the most universally accepted critical function of the hippocampus in rodents, monkeys and humans. In addition, the mechanisms of spatial learning are thought to be similar in rodents and humans. This makes the study of spatial memory a good model for the study of human diseases affecting cognitive processes. However when spatial learning deficits are observed, the impairments with selective cholinergic lesions are generally smaller than those observed with nonselective MS lesions, suggesting a role for noncholinergic MS neurons in spatial memory. Many studies using the cholinergic immunotoxin 192 IgG-saporin have demonstrated that selective removal of cholinergic neurons in the BF does not disrupt simple place learning [15, 16]. The most important SH noncholinergic neurons are the GABAergic neurons. The involvement of GABAergic SH projections in hippocampal-based spatial learning remains unspecified. However, a new more selective toxin for GABAergic neurons would facilitate research.

It is possible that the absence of impairment in spatial learning tasks following lesions of the MS cholinergic neurons reflects the fact that there are multiple strategies available for correct solving of these tasks, only some of which are affected by removal of hippocampal cholinergic input. Examination of particular strategies used by MS-lesioned animals to solve spatial problems might represent a fruitful avenue of investigation.

To determine more definitively whether septohippocampal projection neurons are required for the spatial memory it would be ideal to compare the effect of electrolytic - nonselective and selective immunolesions of the cholinergic or GABA-ergic septohippocampal projection neurons on spatial memory and learning process. In current study rats were trained in the dual-solution plus-maze task developed by Tolman et al. [17]. In this task, rodents are trained to retrieve food from a consistently baited arm, starting always from the same start box. Using this procedure, they may learn to find food in a particular place in space (place strategy) or to make a particular body turn (response strategy). However, by testing them from the start box used during training does not allow a discrimination of the kind of strategy used by the animals. But the strategy they use can be resolved by submitting them to a probe test from the opposite arm.

Materials and Methods

A total of 36 male outbred albino rats were used in the present study. The animals were randomly assigned to control (n = 10), electrolytic (n = 10), selective cholinergic (n = 8) or GABAergic (n = 8) immunotoxin MS-lesioned groups. At the time of surgery, their weights ranged from 230 to 280 g. The rats were housed in standard cages at a natural light/dark cycle and were tested during the light period. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Ani-

mals (Eighth Edition, 2011) and were approved by the Animal Care and Use Committee of the I. Beritashvili Center of Experimental Biomedicine.

Surgery. Rats were anaesthetized with i.p. injection of 4**%** chloral hydrate (9 ml/kg) and placed in a stereotaxic apparatus. For electrolytic lesions a stainless steel electrode (0.15 mm in diameter), insulated except at the tip, was inserted in the MS (AP – 0.7; ML – 0; DV – 6.5), according to Paxinos and Watson [18] sterotaxic atlas; A 1.0 mA anodal current was passed through the electrode twice for 30 s. Sham operations (n = 4 rats) were performed by inserting the electrode at the same coordinates except that the depth was only 0.5 mm and electrolytic lesion was not produced. Injection of GAT1-SAP (0.5 μl; 0.05μjl/min) for selective immunolesions of GABAergic neurons or mouse saporin (this product serves as a control for the immunotoxin) for control surgeries (Advanced Targetting System, San Diego, USA) were performed from the side by a 15 degree angle with the following coordinates AP- 0.4; M L - 1.7; DV - 6.4. Injection of 192 IgG-saporin to produce selective lesion of MS cholinrgic neurons 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 8 min and 5 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.

Maze Training. One week before training, rats were food-restricted to 85% of their ad libitum body weight plus 5 g for normal growth. Rats were handled daily for 3 min beginning 1 wk before training and given three piece of cereal, which later served as the food reward during training.

Rats were trained to find a food reward in a four-arm plus-shaped maze with floor and walls made of black Plexiglas. The arms of the maze (12.5 cm wide by 46 cm long by 7 cm high) extended radially from a central square platform (sides = 13 cm); the floor of the maze was positioned 0.7 m above the floor. One of the four arms blocked so that the maze formed a "T" shape throughout training. At the end of each arm was a container that contained inaccessible piece of cereal to eliminate the use of olfactory cues to find the reward. Food reward was placed in the container in the goal arm such that it was accessible to the rat. The training room (3 m × 4 m) contained a moderate density of cues including high-contrast posters and dark-colored three-dimensional objects set against a light-colored wall.

Before training, a habituation trial was given to allow all rats to encounter the food reward on the first trial. If the rat did not enter the goal arm within 2 min during the habituation trial, it was placed in the goal arm. For the training trials, the maze was configured into a T with the start and goal arms remaining in the same relative position throughout training. At the start of a training trial, the rat was placed in the start arm facing the choice point. If no choice was made within 2 min, the rat was removed from the maze and placed in the holding cage for 30 sec before another trial was begun. On trials in which the rat chose the goal arm, it was allowed to eat the reward and it was removed from the maze after 10 sec or after it turned to exit the goal arm. On trials in which the rat did not choose the goal arm, the rat was removed from the arm after 10 sec or after it turned to exit the arm. The intertrial interval was 30 sec, during which the rat was placed in the holding cage. Training was completed within a single session. All rats received one day session which consists of 100 training trials and 5 prob trials. Probe trials were administered after each 20 training trials in which the start arm was rotated 180° relative to its position during training and both choice arms were baited.

Use of the place strategy was indicated when rats went to the arm that was in the same location of the room as it was during training. Use of the response strategy was indicated when rats turned in the same

direction (left or right) as they did during training. Rats had a maximum of 2 min to enter an arm during the probe trial. For all trials correct or incorrect choice were recorded.

Histology. After termination of the experiments, in order to examine localization and volume of the electrolytic lesion in the brain, the Nissl-stained slices of the brain were studied under the light microscope. At the end of behavioral testing a random sample of rats from control (n=4) and immunolesioned (n=4+4) groups were killed and their brains collected in order to verify lesion effects. The immunotoxic (GAT1-SAP or 192 IgG-saporin) 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. Totally 6-10 sections of MS level within experimental and control animals were selected and were used to assess the effect of MS lesion on ChAT and PV-stained neurons.

Statistical Analysis. The effects of MS treatment on trials to criterion were assessed with one-way ANOVA. Differences in strategy use between treatment groups were evaluated with the Student's *t*-test. 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 and Discussion

Overall, in our experiments electrolytic lesions destroyed on average 75% of the intact MS. Intraseptal GAT1-SAP reduced the number of PV-ir neurons, representing GABAergic septohippocampal neurons by 73%. Counts of ChAT-ir neurons made in the same rats used to assess PV-ir neurons demonstrated a mild reduction following GAT1-SAP. The reduction of cholinergic neurons represented a loss of only 23%. On the contrary intraseptal 192 IgG saporin reduced the number of ChAT-ir neurons and spared most of PV-ir neurons representing GABAergic septohippocampal neurons.

Some animals were found to have extraseptal damage or died before the end of the experiment and were excluded from the analysis. In the remaining cohort the number of animals in each group was as follows: MS electrolytic (MSel, n = 10), MS immunotoxin 192 IgG saporin [MS(sap), n = 8] and GAT1-SAP, [MS(GAT), n = 8] lesioned. Since there were no significant differences ($P > 0.05$) between sham-operated (5 rats) and vehicle-injected rats (5 rats) these groups were combined into a single one, designated as control (n = 10).

This experiment compares three types of MS lesions: electrolytic lesions that destroy cells and fibers of passage, GAT1-SAP lesions that spare fibers of passage but predominantly affect the septal GABAergic neurons, and immunotoxin – 192 IgG saporin infusions that eliminate cholinergic neurons. The rats learned to approach the correct arm quite rapidly. Accuracy improved from 50% during the first 10 trials to 87-92% in the second 10 trials, and then reached and stayed at ≈95% throughout the rest of training. The mean number of trials taken before beginning a run of 9 of 10 correct choices was 20.8 ± 1,15. The learning measures for the different groups are shown in Figure 1. As shown no differences in speed of learning were found between control and MS lesioned groups. The control rats reached the criterion of 9/10 correct in means of 20 trials, MS electrolytic lesioned rats in means of 20,9 trials and MS cholinergic and GABAergic immunotoxic lesioned rats in means of 19.6 and 22.5 trials, respectively. There is not a statistically significant difference between groups ($DF_{(3,35)} = 0.209$, $P = 0.889$). The present results of the training trials demonstrate that there were no obvious differences between the groups in perception, motivation, or motor abilities that could differentially influence acquisition of task.

The task used in the current study can be solved by using two different effective strategies, place and

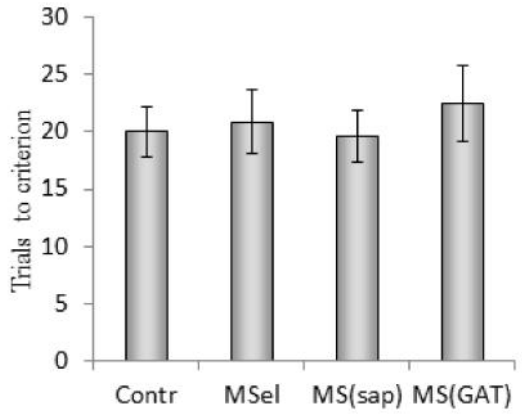

Fig. 1. Main effects of MS treatment on learning speed measured as trials to reach criterion.

response, that have been mapped onto the hippocampus and striatum, respectively [19]. Using a multiple memory systems approach, findings from our experiments showed that control and 192 IgG saporin treated rats trained on an appetitive dual-solution plus-maze task use spatial or place strategies effectively. On the other hand, MS electrolytic and GAT1-SAP treated rats tend to use response strategies. Specifically, an overview of the data from prob trials for each group show that the control rats in 34 (68%) trials out of 50 prob trial and 192 IgG-saporin treated rats in 26 (65%) trials out of 40 prob trial used place strategy, while MS electrolytic lesioned ones used this strategy in 16 (32%) trials only. GAT1-SAP treted rats in 25 (62.5%) trials out of 40 prob trial used response strategy (Fig. 2). Decreased place-bias in MS electrolytic lesioned and GAT1-SAP treated rats compared to the control rats was significant (t_d = 3.8 P<0.001; t_d =1.99, P<0.02, respectively).

It is interesting to note that both control and MS lesioned rats using response strategies took significantly more trials to reach criterion than did rats using place strategies regardless of MS treatment. Interestingly, a different pattern of results has been obtained in the previous study [20] using the same task. Rats using response strategies solved the task significantly faster than did those using place strategies. Behavioral discrepancies in studies designed to examine the use of different learning strategies are not unprecedented. It has been known for many decades that the nature of the spatial environment (e.g., extra and intra-maze cues) influences the choice of "place" or "response" strategies [21]. The learning speed difference between rats using place and rats using response strategies may result from the possibility that distribution of the room cues in the current experiment favored place learning. Although other differences across investigations cannot be excluded: the male outbred albino male rats were used in the present study and Sprague-Dawley female rats used by McElroy and Korol [20].

Comparing results observed across immuno- and electrolytic lesion techniques in current study demonstrates a dissociation between the two major components (cholinergic and GABAergic) of the SH pathway

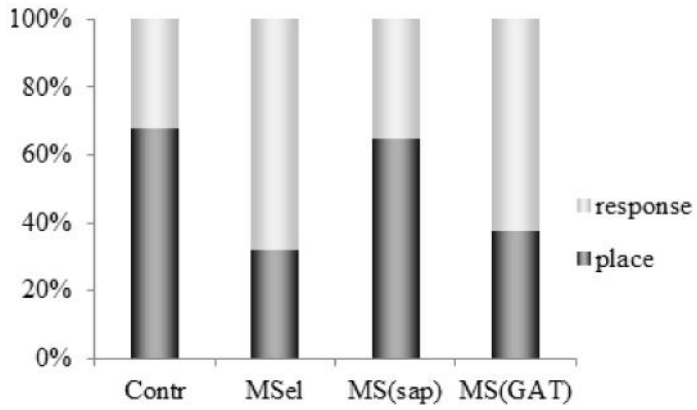

Fig. 2. Exhibiting place or response strategies (%) on prob trials in different groups of rats.

in spatial memory assessed in the plus-maze task. The control and 192 IgG-saporin treated rats exhibited their effective use of a place learning strategy rather than the MS electrolytic and GAT1- SAP treated rats exhibiting a response strategy in prob trials. These results suggest that the MS is essential for spatial learning and suggest its role in processing information about the spatial environment, but deficits observed after septal electrolytic lesions cannot be accounted to the loss of MS cholinergic neurons and suggest a role for GABAergic MS neurons in spatial memory.

Our results are consistent with many other studies that suggest that GABAergic SH projection neurons may be involved in memory. Some studies have reported that ibotenic and kainic acid lesions, which primarily affect GABAergic SH neurons, impair learning [22]. Selective lesions of cholinergic SH neurons do not prevent the memory-impairing effects of muscimol [23], further suggesting that GABAergic SH projection neurons are involved in memory. Moreover, electrophysiological studies show that MS administration of ACh agonists, which can have memory-enhancing actions within a certain range [24], selectively excites GABAergic SH projection neurons, but not cholinergic SH projection neurons [25]. Similarly, the muscarinic ACh receptor antagonist scopolamine, a drug that impairs memory when infused into the MS [23, 26], also influences GABAergic SH projection neurons selectively [27].

Finaly, the present results demonstrate that MS GABAergic neurons are essential for the choice or expression of a place response, even in situations in which an alternative (i.e., response) strategy could be used to solve the task successfully and suggest a role of MS GABAergic neurons in processing information about the spatial environment.

ადამიანისა და ცხოველთა ფიზიოლოგია

მედიალური სეპტუმის დაზიანების ეფექტები დასწავლის სტრატეგიის არჩევაზე ჯვრისმაგვარ ლაბირინთში

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წარმოდგენილ ნაშრომში მედიალური სეპტუმის (MS) ელექტროლიზური დაზიანების, ასევე ქოლინერგული ან GABA-ერგული ნეირონების იმუნოტოქსინებით (GAT1-SAP, 192 IgG saporin) სელექტიური დაზიანების მეთოდის გამოყენებით, შეისწავლებოდა სეპტოჰიპოკამპური პროექციების მნიშვნელობა ადგილის ან პასუხის დასწავლის სტრატეგიის არჩევაზე ჯვრისმაგვარ ლაბირინთში. ქცევით ექსპერიმენტებში გამოვლინდა, რომ ამოცანის დასწავლისათვის საჭირო სინჯების რაოდენობა საკონტროლო და დაზიანების მქონე ცხოველებში არ განსხვავდება. ამასთან, საკონტროლო და 192 IgG saporin-ით დაზიანების მქონე ცხოველები სატესტო სინჯებში ეფექტურად იყენებენ სივრცის, ანუ ადგილის დასწავლის სტრატეგიას. MS-ის ელექტროლიზური ან GAT1- SAP-ით დაზიანების მქონე ცხოველები სატესტო სინჯებში უპირატესად პასუხის დასწავლის სტრატეგიას იყენებენ; განსხვავება საკონტროლო ჯგუფის ცხოველებთან შედარებით სარწმუნოა. მიღებული შედეგები ადასტურებს MS-ის GABA-ერგული ნეირონების ჩართულობას სივრცითი მეხსიერების პროცესებში.

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Received July, 2016