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Spatial Memory Impairments Following Immunotoxic Lesion of GABAergic Neurons of the Nucleus Basalis Magnocellularis

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ABSTRACT. The central aim of the present study was to investigate the modulation of spatial memory function by the GABAergic cells of the nucleus basalis magnocellularis (NBM) using immunotoxin GAT1-SAP for immunolesion of GABAergic neurons. 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. Of the 24 rats that underwent behavioral testing, 12 received immunotoxic GAT1-SAP injections and 12 received mouse saporin (control group) injections to the NBM. The NBM lesioned rats as control rats rapidly learned to escape to the visible platform and reached the 6-7 s asymptote on day 2. But statistical analysis showed no significant difference between groups (P = 0.954) in visible platform trials and significant difference between NBM and control (P < 0.001) groups in hidden platform trials. The data obtained in the control and NBM-lesioned animals in the present study, demonstrate that decreased place-bias in NBM-lesioned rats compared to the control rats was significant. The NBM lesioned rats acquired the visible platform version of the water maze task but failed to learn the platform location in space. When the visible platform was moved to a new location they often swam directly to it. These findings suggest the role of NBM GABAergic cortical projection neurons in processing information about the spatial environment. *© 2017 Bull. Georg. Natl. Acad. Sci.*

Key words: learning strategy, medial septal nucleus, water maze, rat

The basal forebrain (BF) is composed of an affiliation of heterogeneous structures and includes the medial septum, ventral pallidum, diagonal band nuclei, substantia innominata/ nucleus basalis magnocellularis, and peripallidal regions. This highly complex brain region has been implicated in cortical activation, attention, motivation, memory, and neuropsychiatric disorders such as Alzheimer's disease

(AD), Parkinson's disease, schizophrenia, and drug abuse [1-9]. Part of the difficulty in understanding the role of the BF in these functions, as well as the processing characteristics of these disease states, lies in the anatomical complexity of this region.

The BF contains a diverse population of neurons, including cortically projecting cholinergic and noncholinergic neurons as well as various interneurons [10]. The most prominent noncholinergic component of the BF corticopetal projection system are the GABAergic corticopetal projections.

Substantial evidence suggests that the nucleus basalis magnocellularis (NBM) plays an important role in learning and memory [11,12]. In contrast to research on the cortical cholinergic input system, little is known about the functions corticopetal GABAergic neurons, largely due to the absence in the past of specific research tools to manipulate selectively this projection. Recently, a more specific lesion technique was developed that allows selective lesioning of BF GABAergic neurons with immunotoxin - GAT1-SAP [13]. Pang et al [13], characterized the effects of GAT1-saporin on the medial septal (MS) neurons and showed that intraseptal GAT1-SAP preferentially reduced GABAergic neurons as compared to ChAT-ir neurons in the MS.

The central aim of the present study was to investigate the modulation of spatial memory function by the GABAergic cells of the NBM using immunotoxin GAT1-SAP. 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.

Materials and Methods

A total of 24 male outbred white rats weighing between 200 and 250 g at the beginning of the experiment were used in the present study. The rats were housed in standard cages at a natural light/dark cycle and were tested during the light period. All animals were given access to food and water *ad libitum*. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (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. All injections of GAT1-SAP (325ng/ml) 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 according to Paxinos and Watson [14]. Rats received bilateral infusions (0.2 µl per side, 0,05ml/min) into the NBM (AP -(-1.3): ML – 2.5: DV – 7.7).

The needle was left in place for an additional 10 min after completion of the injection, to allow the toxin to diffuse from the injection site. All injections were made with a1-µl Hamilton syringe with a microinjection pump (CMA 402 Syringe Pump, Sweden). For analgesia the rat was given a 0.1 mg/kg injection of buprenorfin after the surgery. The rats were allowed to recover from the surgery for two weeks before starting the behavioral experiments.

Morris water-maze. Animals were tested in a standard Morris water-maze (MWM), consisting of a circular tank (1.5-m in diameter and 0.5 m height) filled with opaque (white-colored) water. Escape platform (10 cm in diameter) was located 2 cm beneath the surface on hidden platform training days and raised 2 cm above the water surface on visible platform training days. The room, in which the tank was stationed, had sufficient number of the cues (door, window, furniture, posters on the walls, *etc*.) in order to provide spatial cues. The task was adapted from Bizon, at al [15]. On days 1–9, rats received four trials per day, one from each of four equidistantly located start locations (N, S, E, W). On both visible- and hidden platform days, the rats were placed into the water facing the wall of the maze. The trial ended when the rat climbed on the available platform or until 60 s had elapsed. If a rat could not found the platform after 60 s, it was placed on the platform by the experimenter. Rats were left on the platform for 15

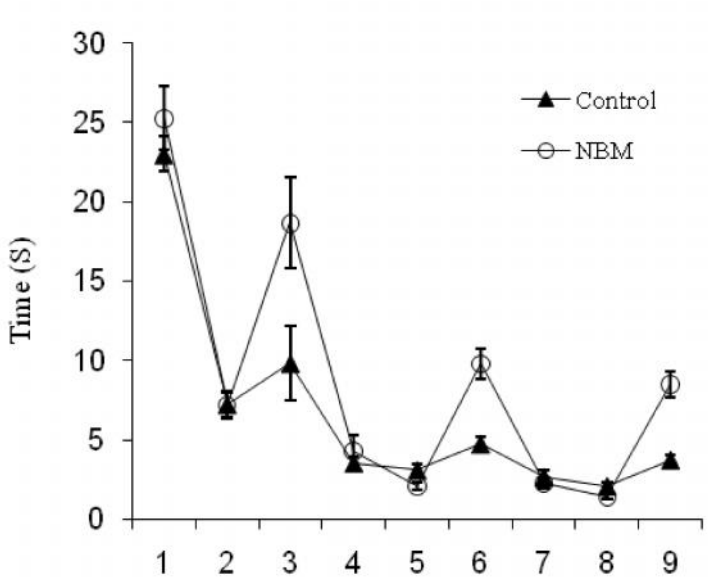

Fig. 1. Water maze acquisition. Mean±SEM escape latency for the visible (1,2,4,5,7,8 day) and hidden (3,6,9 day) platform tasks.

s and were then moved to a holding cage for a 2-min inter trial interval. On days 1 and 2, rats were trained to locate a visible platform in the southeast quadrant of the pool, followed by a third day in which the platform was submerged at the same location. This 3-day sequence was repeated twice on days 4–6 and 7–9 for a total of 36 trials (24 visible and 12 hidden). On day 10, a competition test was given in which the visible platform was moved to the northwest quadrant (opposite to its placement on the training days). Two trials were given with start points equidistant from the two platform locations (SE and NW). Video recordings were analyzed to determine whether rats swam within southeast quadrant before escaping to the visible platform in the northwest quadrant. Tracking the animal movements in water-maze, also collection of other numeric data (time in zone, escape latency, and so on) were made with an aid of video tracking system.

Histology. At the end of behavioral testing a random sample of rats from each group (six control and six GAT1-SAP medial septal lesioned) were killed and brains collected in order to verify lesion effects. The immunotoxic GAT1-SAP lesions of GABAergic neurons of the NBM were verified by observing decreased parvalbumine (PV) staining of the NBM. The 20 м thick coronal sections using freezing microtome were stained with PV primary antibody and ABC Staining System. All necessary reagents and buffers were received from Santa Cruz Biotechnology, Inc. (USA). The NBM sections were analyzed with a microscope Leica MM AF.

Statistical Analysis. Statistical analysis was performed using the SigmaStat statistical software. To determine the effect of group and testing condition (visible/invisible platform) on escape latency in MWM training task two-way ANOVA were used. Further *post hoc* comparisons were made using Tukey's tests, where appropriate. Group differences in frequencies of strategies in the competition trials between groups were assessed by Student's *t*-test. Two sample *t*-test was used to compare histological data between control and lesioned groups. All statistical analyses were conducted with a significance level of $P < 0.05$.

Results and Discussion

Infusions of GAT1-SAP into the BF resulted in loss of PV- positive neurons in the GP, mostly in the medial and ventral aspects including the nucleus basa-

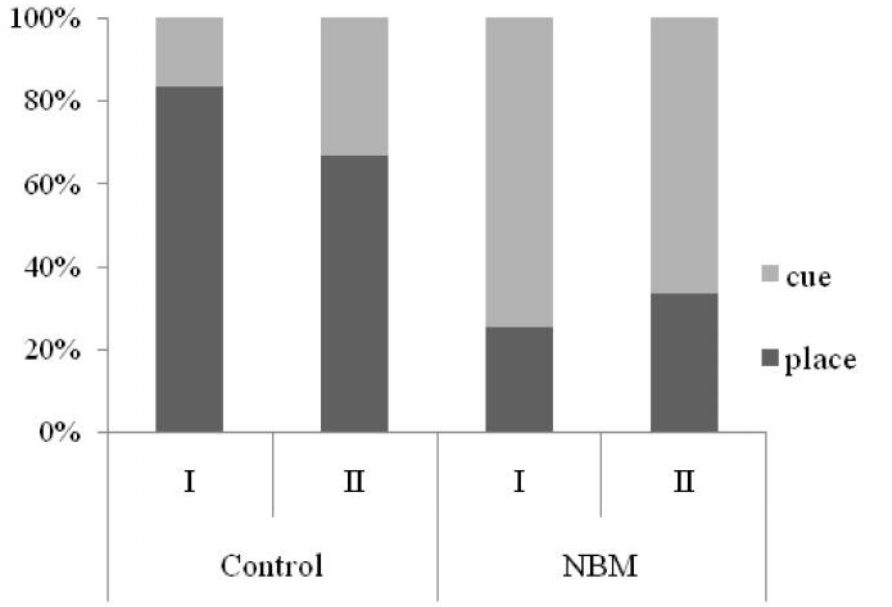

Fig. 2. Exhibiting place or cue strategies (%) on two competition trials in different groups of rats.

Table 1. Number of rats (and percentage of group) exhibiting place or cue strategies on the two competition trial

First trial	Second trial	control	NBM lesioned
Place	Place	7 (58,31%)	0
Place	Cue	3 (25%)	3 (25%)
Cue	Place	1 (8.33%)	4 (33.32%)
Cue	Cue	1 (8.33%)	5 (41.65%)

lis regions and the SI. The GAT1 SAP-induced loss of PV-positive cells was large and statistically significant ($P < 0.001$). Thus, GAT1-SAP when infused into the NBM extensively damaged GABAergic NBM neurons.

Of the 24 rats that underwent behavioral testing, 12 received immunotoxic GAT1-SAP injections and 12 received mouse saporin (control group) injections to the NBM. The NBM lesioned rats as control rats rapidly learned to escape to the visible platform and reached the 6-7 s asymptote on day 2 (Fig. 1). For training trials, a two way ANOVA [group x testing condition (visible/invisible platform)] indicated statistically significant effect of group ($F(1, 215) = 7,624$; $P = 0.006$) and testing condition ($F(1, 215) = 8.373$; $P = 0,004$) and there is a statistically significant interaction between group and testing condition ($F(1, 215) = 7.996$; $P = 0.005$).The effect of different group depends on what testing condiiont is present. Post Hock analysis (Tukey Test) showed no significant difference between groups ($P = 0.954$) in visible platform trials and significant difference between NBM and control ($P < 0.001$) 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 control rats used a place strategy compared with NBM lesioned rats. The increased cue-bias in NBM lesioned rats compared with control rats was significant ($t_d = 2.8$; $P < 0.01$). On the

Table 2. Number of rats' classified on the basis of their performance across both trials of the competition test using established criteria.

	Control	NBM lesioned
Place responder	7	0
Cue responder	1	7
Cue/place responder	4	5

second trial, the majority of NBM lesioned rats used a cue strategy and the control animals used a place strategy. There was significant difference in strategy between control and lesioned group ($t_d = 2.16$; $P < 0.05$). Table 1 and Fig. 2 summarize the rats' performance across both trials of the competition test.

An overview of the data from both competition trials for each group show that the sham-operated rats in 24 trials out of 18 competition test trial used place strategy, while NBM lesioned ones used this strategy in 7 trials only. Decreased place-bias in NBM lesioned rats compared to the sham-operated rats was significant ($t_d = 3.19$, $P < 0.001$).

The information obtained from a discrete trial, such as the competition test, is limited; therefore, we also analyzed the rats' performance by combining data across the two competition trials. Rats were designated as 'place responder', if they swam within 10 cm of the previous platform location on two competition trial or as 'cue responders', if they swam toward visible platform location across both trials of the competition test. Rats were designated as 'cue/place responder', if they exhibited different strategy in two competition trials. Table 2 summarizes the rats' performance across both trials of the competition test, using established criteria.

As expected, escape latency averaged across both competition trials, on day 10 was significantly greater for control rats (place responders) as compared with NBM lesioned rats (cue responders), confirming the more indirect path taken by the place responders.

Notably, the control and NBM-lesioned rats, exhibited corresponding differences in performance during training trials. The control rats, 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 NBM-lesioned rats exhibiting a cue strategy in competition trials.

The data obtained in the control and NBM-lesioned animals in the present study, demonstrate that decreased place-bias in NBM-lesioned rats compared to the control rats was significant. The NBM lesioned rats acquired the visible platform version of the water maze task but failed to learn the platform location in space. When the visible platform was moved to a new location they often swam directly to it. A similar pattern of the results was obtained in rats with selective lesions of cholinergic NBM neurons [12] using the same task. These latter findings combined with the present results suggest the role of NBM GABAergic as well as cholinergic cortical projection neurons in processing information about the spatial environment.

ადამიანისა და ცხოველთა ფიზიოლოგია

სივრცითი მეხსიერების დარღვევები მსხვილუჯრედოვანი ბაზალური ბირთვის GABA-ერგული ნეირონების იმუნოტოქსიკური დაზიანების პირობებში

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კვლევის მიზანს წარმოადგენდა მსხვილუჯრედოვანი ბაზალური ბირთვის (**nucleus basalis magnocellularis - NBM**) GABA-ერგული ნეირონების დაზიანების ეფექტების შესწავლა სივრცით მეხსიერებაზე. კვლევაში გამოიყენებოდა წყლის აუზის ამოცანა, რომლის წარმატებით შესრულება შესაძლებელია როგორც ადგილის, ასევე ერთეულ სიგნალზე ორიენტაციის სტრატეგიის გამოყენებით. კვლევა ჩატარებულია 24 ვირთაგვაზე, მათგან 12-ს ჩაუტარდა NBM-ში GAT1-SAP-ის, ხოლო 12-ს თაგვის საპორინის (საკონტროლო ჯგუფი) მიკროინექცია. ორივე ჯგუფის ცხოველები სწრაფად სწავლობდნენ ხილული ბაქნის მოძიებას და 6-7 წმ-იან ზღვარს მეორე დღეს აღწევდნენ. სტატისტიკურმა ანალიზმა აჩვენა ბაქნის მოძიების ლატენტური დროის არასარწმუნო განსხვავება ($P = 0,954$) ჯგუფებს შორის ხილულბაქნიან სინჯებში, ხოლო სარწმუნო განსხვავება ($P < 0,001$) უხილავი ბაქნის პირობებში. ეს ფაქტი იმუნოტოქსინით დაზიანების მქონე ცხოველებში ადგილის დასწავლის სტრატეგიის გამოყენების დეფიციტზე მიანიშნებს. მიღებული მონაცემების თანახმად, NBM-ის GABA-ერგული ნეირონები მნიშვნელოვანია სივრცითი ინფორმაციის გადამუშავების პროცესებისათვის.

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