Spatial Memory Impairments Following Excitotoxic Lesions of the Basal Forebrain Noncholinergic Neuronal Ensembles

P.1.j.016

Lali Kruashvili[1], Maia Burjanadze[1], Maia Demurishvili[2], Nino Chkhikvishvili[1], and Khatuna Rusadze[3]

1.I.Beritashvili Center of Experimental Biomedicine
2. St.Andrew the Firs-Calld Georgian University of the Patriarchy of Georgia
3. Akaki Tsereteli State University, Kutaisi, Georgia

Inrtrodaction: : Basal forebrain (BF) afferents represent one of the largest cortical-projecting neuromodulatory systems in mammals. Recent studies have demonstrated that AD patients show significant BF volume loss compared to age-matched controls. Postmortem assessment has revealed significant degeneration of BF neurons as an early pathological feature of AD patients The BF cholinergic system has at the extremes 2 relatively distinct subcomponents, the nucleus basalis magnocellularis (NBM; Meynert in humans and primates) and the medial septal area (MS), which have projections to the frontal cortex and hippocampus, respectively.

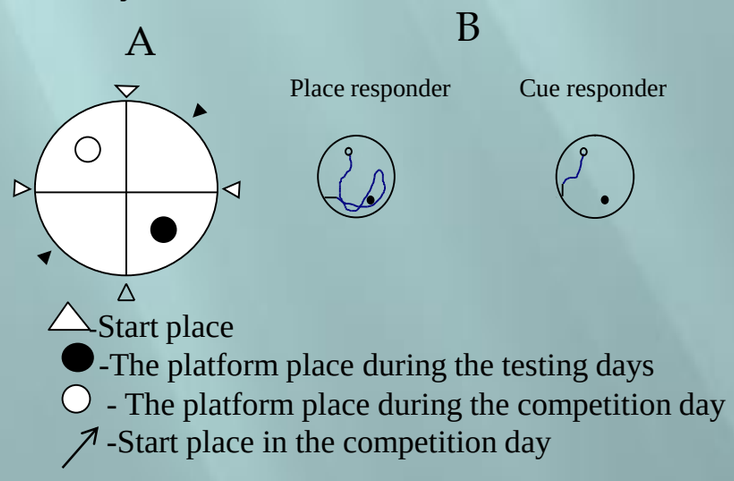
Objective: The central aim of the present study was to investigate the modulation of spatial memory function by the noncholinergic cells of the MS and NBM using the neurotoxin ibotenic acid (IBO).

Method and Materials: A total used of 36 male outbred albino rats. The animals were randomly assigned to MS (n = 12) and NBM (n = 12) IBO lesioned and MS (n = 6) and NBM (n = 6) sham-lesioned groups. The experiments have been conducted in accordance with the Guide for the Care and Use of Laboratory Subjects.Experimental protocol was approved by Animal Studies Committee of I. Beritashvili Center of Experimental Biomedicine.

All injection were performed stereotaxically. Rats received bilateral infusions (0.2 µl per side, 0,05 µl/min) into the NBM (AP -(-1,3): ML – 2,5: DV – 7,7) and infusions into the MS (AP – 0.7; ML – 0; DV – 6,2 and 7.8mm) at two positions (0,2 and 0,3 µl; 0,05 µl/min).

Animals were tested in a standard Morris water-maze.s On days 1–9, rats received four trials per day, The trial ended when the rat climbed on the available platform or until 60 s had elapsed. If a rat could not find the platform after 60 s, it was placed on the platform by the experimenter. Rats were left on the platform for 15 s. On days 1 and 2, rats were trained to locate a visible platform ,followed by a third day in which the platform was submerged at the same location. This 3-day sequence was repeated twice , On day 10, a competition test was given in which the visible platform was moved to theopposite quadrant to its placement on the training.

The location of the IBO lesions was determined in control and experimental rat brain by microscopic examination of serial coronal sections (30 µm) stained with cresyl violet.



A - Schematic illustration of the water maze and location of platform in training (black) and in competition (white) trials. B - Representative swim path of place and cue responders on the 10th day of the behavioral protocol (competition test).

All statistical analyses were conducted with a significance level of $P < 0.05$.

Results

Based on Nissl staining neurotoxic lesion destroyed most of the MS and NBM neurons and sham lesion left these structures intact (Fig. 1.): there was significant difference among control and neurotoxic MS or NBM lesioned groups (MS - p=0.015; NBM - p=0.025).

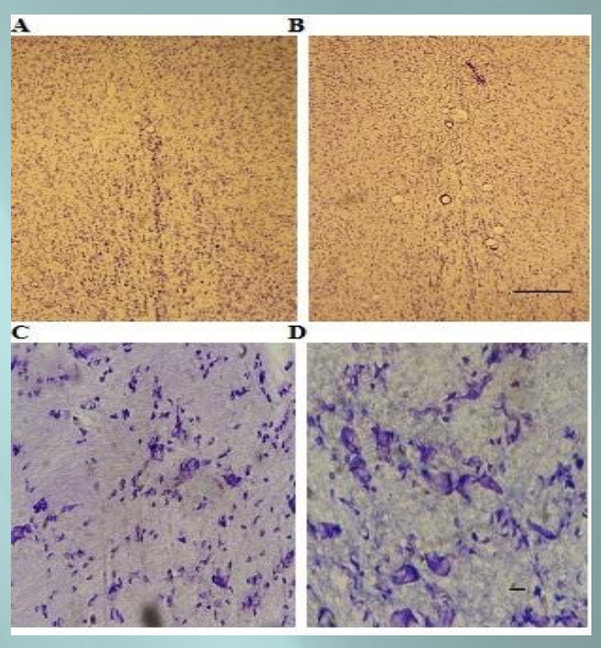


Fig. 1. A, C - a sham lesion that left the MS andNBM intact, **B, D -** a representative IBO lesion that destroyed most of the MS and NBM neurons, (Scale bar: 100 µm for A and B; 70 µm for C and D).

The MS and 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. 2). For training trials, a two way ANOVA [group X testing condition (visible/unvisible platform)] indicated statistically significant effect of group ($F(2, 323) = 4{,}418$; $P = 0.013$) and testing condition ($F(1, 323) = 21{,}879$; $P < 0{,}001$) and there is a statistically significant interaction between group and testing condition ($F(2, 323) = 5{,}372$; $P = 0.005$). The effect of different group depends on what testing condiiont is present. There is a statistically significant interaction between group and testing condition ($P = 0.005$). Post Hock analysis (Tukey Test) showed no significant difference between groups ($P>0{,}05$) in visible platform trials and significant difference between NBM and control ($P<0{,}001$), also between MS and control ($P=0{,}044$) groups in hidden platform trials.

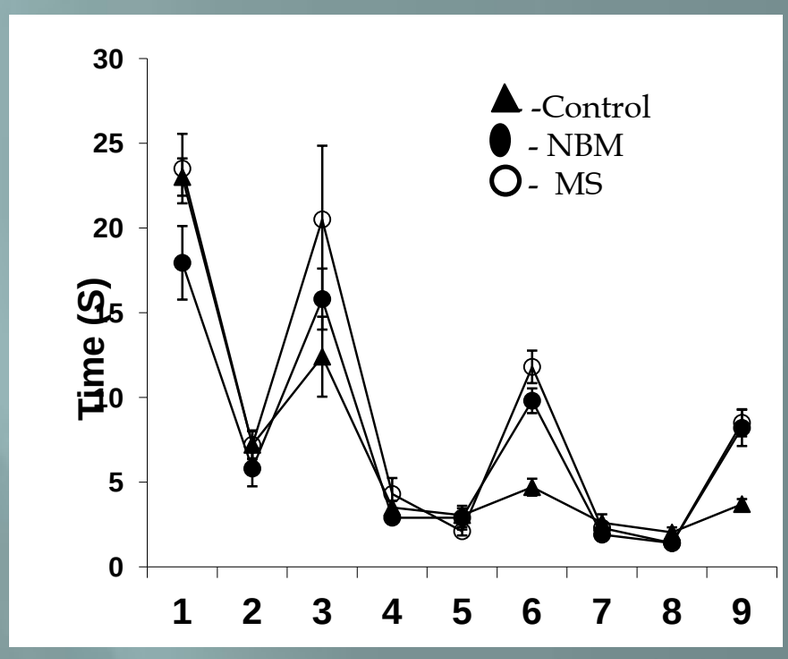


Fig. 2. Water maze acquisition. Mean escape latency for the visible (days: 1,2,4,5,7,8) and hidden (days: 3,6,9) platform tasks.

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 and MS lesioned rats used a place strategy compared with NBM lesioned rats. There was no difference in strategy between control and MS lesioned groups ($t_d = 1{,}09$, $P>0{,}1$). The increased cue-bias in NBM lesioned rats compared with control and MS lesioned rats was significant ($t_d = 2{,}8$, $P< 0{,}01$; $t_d = 2{,}16$, $P< 0{,}05$, respectively). On the second trial, the majority of MS and NBM lesioned rats used a cue strategy. There was no difference in strategy between lesioned groups ($t_d = 1{,}2$, $P>0{,}1$). The majority of the control animals used a place strategy. There was significant difference in strategy between control and lesioned groups (contr/MS: $t_d = 2{,}5$, $P< 0{,}02$; contr/NBM: $t_d = 2{,}16$, $P< 0{,}05$). Table 1 and Fig. 3 summarizes the rats' performance across both trials of the competition test.

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

First trial	Second trial	control	MS lesioned	NBM lesioned
Place	Place	9 (75 %)	1 (8,3 %)	-
Place	Cue	2 (16,66 %)	8 (66,7%)	3 (25 %)
Cue	Place	-	1 (8,3 %)	3 (25%)
Cue	Cue	1 (8,33 %)	2 (16,7 %)	6 (50 %)

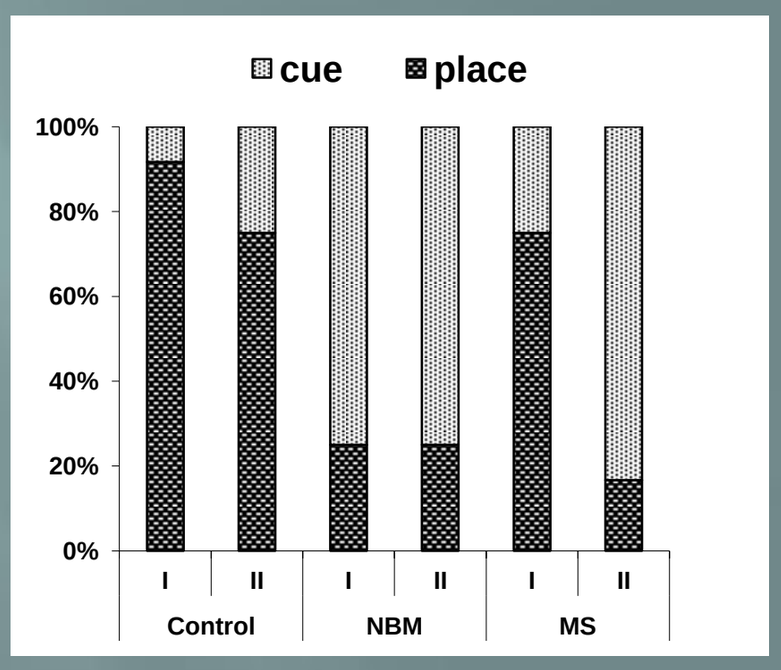


Fig. 3. Exhibiting place or cue strategies (%) on two competition trials in different groups of rats

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