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Deep Brain Stimulation of the Medial Septum Improves Okadaic Acid-Induced Impairment of Spatial Working Memory and Restores the Cholinergic Activity in the Hippocampus

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In the present study, we evaluated the effect of medial septum (MS) deep brain stimulation (DBS) on okadaic acid (OA) induced impairment of spatial working memory and neuropathological changes in the hippocampus in rats. Rats were divided in four groups: rats in the normal group had no surgical procedure (group – N); rats in the lesion group had ICV administration of OA (group – O); rats in the implantation group had ICV administration of OA and implantation of an electrode in the MS (group – O/I); rats in the stimulation group had ICV administration of OA and electrical stimulation of the MS (group – O/S). In behavioral experiments the possible beneficial effect of chronic DBS of MS on the OA-induced spatial short-term memory impairment was examined in spontaneous alternation task in the plus-maze. The present results demonstrate that MS DBS improves OA-induced impairment of spatial working memory and restores acetylcholinesterase (AChE) immune-stained cells numbers in the hippocampal CA1 and CA3 fields. We hypothesize that the enhancement of working memory function may be associated with an increase in hippocampal neurogenesis and hippocampal cholinergic activity induced by MS DBS in a rat model of AD. These results allow us to identify the medial septum as one of the stimulation sites for improving memory function. *© 2023 Bull. Georg. Natl. Acad. Sci.*

okadaic acid, deep brain stimulation, working memory, hippocampus, rat

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive memory loss and cognitive decline, with hallmark pathologies related to amyloid beta (Aβ) and tau phosphatase [1]. To study the mechanism and progression of a disease of animal models for the specific disease are needed. Intracerebroventricular (ICV) injection of okadaic acid (OA), a protein phosphatase 2A (PP2A) inhibitor, in rats causes neurotoxicity associated with neurofibrillary degeneration. However, this model lacks amyloid pathology as observed in AD [2].

Degeneration of cholinergic basal forebrain neurons, including those in the medial septum (MS), is a common feature of AD and vascular dementia, and has been correlated with cognitive decline [3,4]. Lesions or inactivation of MS neurons impair hippocampal-dependent forms of learning and memory [5,6]. One key modulator of the hippocampal neural network is the MS, a part of the basal forebrain [7,8]. In our previous work [9], we have shown that ICV microinjection of OA induces impairment of spatial short-term memory, assessed in the spatial alternation paradigm, and reduces the number of surviving pyramidal neurons in the hippocampus. In another work, we showed that ICV microinjection of OA induces spatial memory impairment assessed in the water maze and reduces the number of acetylcholinesterase (AChE) sensitive neurons in various regions of the hippocampus [10]. We hypothesized that OA-induced memory impairment may be related, at least in part, to OA-induced hippocampal cell death and changes in cholinergic activity.

Deep brain stimulation (DBS), which involves delivering a therapeutic electrical current to specific areas of the brain through implanted electrodes, has been used for treatment of numerous diseases of the central nervous system [11-13]. One of the most exciting emerging frontiers is the possibility of using DBS in the context of memory and cognitive disorders. There is growing evidence from laboratory and clinical trials that DBS at memory associated structures enhances cognitive functions, and has been considered as a potential therapy because of its recent effects in improving memory function [14]. The best site for memory enhancing DBS is still unclear, but pilot data suggest that the basal forebrain might be a key target to accomplish therapeutic efficacy in memory impaired patients [15, 16]. Research is needed to characterize not only the effective regions for DBS inducing improvement in various forms of memory, but also the mechanisms linking memory improvement and stimulation-induced neural circuit changes.

The present study was designed to investigate the effect of MS DBS on okadaic acid-induced impairment of spatial working memory and neuropathological changes in the hippocampus. Such studies highlight potential experimental target areas of DBS for the treatment of AD.

Methods

Subjects. A total of 40 male rats, approximately 4 months of age and weighing 220-250 g at the start of experimentation served as subjects. All animal experiments were conducted in accordance with the European Communities Council Directive Guidelines for the care and use of Laboratory animals (2010/63/EU – European Commission) and approved by the animal care and use committee at the Ivane Beritashvili Center of Experimental Biomedicine. The rats were kept in a polyacrylic cage (22.5×37.5 cm^2) in-group of four rats per cage and maintained under standard housing conditions (room temperature 22-25°C and humidity 60-65%) with a 12-hour light and dark cycle. Food and water were available ad libitum.

The rats were randomly assigned to one of the following groups: (1) rats in the normal group had no surgical procedure (group – N); (2) rats in the lesion group had ICV administration of OA (group – O); (3) rats in the implantation group had ICV administration of OA and implantation of an electrode in the MS (group – O/I); (4) rats in the stimulation group had ICV administration of OA and electrical stimulation of the MS (group – O/S). The number of rats in the groups in which the statistical analysis was carried out was: N - $n = 12$, O - $n = 12$, O/I - $n = 11$ and O/S - $n = 10$.

Surgery. Rats were anaesthetized with an intraperitoneal injection of ketamine-xylazine (80 mg/kg and 10 mg/kg, respectively) and placed in a stereotaxic apparatus (Stoelting. Co, USA) with a rat adaptor and lateral bars. Okadaic acid was dissolved in artificial cerebrospinal fluid (aCSF) and injected ICV (A: 0.2 mm from bregma, L: 1.1

mm and V: 3.6 mm) 200 ng in avolume of 10 µl bilaterally in rats that formed groups O, O/I and O/S).

After the administration of OA, 21 rats had an additional procedure for electrode implantation in rats that formed groups O/I and O/S. A hole was drilled in the skull and a bipolar stimulating electrode was implanted in the MS from the side by a 15 degree angle with the following coordinates AP - 0.4; ML -1.7; DV - 6.4 based on the rat brain atlas of Paxinos and Watson [17] and were corrected in our pilot experiments. The stimulation electrode was fixed with dental cement. The rats were allowed to recover from the surgery for one week before starting the DBS procedures.

Deep brain stimulation. For stimulation, a bipolar stimulating electrode was constructed using two individually insulated (insulation material – teflon), platinum iridium wire electrodes (Plastics One). A 9-channel programmable pulse stimulator Master 9 and stimulus isolators ISO-Flex (A.M.P. Instruments, Jerusalem, Israel) were used to deliver the electrical stimuli. Rats were stimulated daily beginning a week after surgery (2 consecutive hours per day, every day for 3 weeks). Chronic DBS parameters are: 60 Hz, 120 μs, 50 μA [15]. Stimulation parameters were monitored in real time at the beginning and end of stimulation with an oscilloscope. The rats of O/I group were concurrently placed into stimulation chambers for 2 hr daily but did not receive electrical stimulation. A schematic representation of the experimental design is shown in Fig. 1.

Spontaneous alternation behavior in a plus-shaped maze. Spontaneous alternation (SA) behavior was assessed using a four-arm plus-shaped maze test, as previously detailed [9]. In brief, the rats were placed at the center of the maze and allowed to explore the area for 20 min. The maze had four identical arms interconnected with a central platform. The number and sequence of arms entered were recorded to determine alternation scores. The alternation percentage was calculated as follows: (actual alternation/possible alternation) × 100; the possible alteration sequences are equal to the number of arm entries minus four.

Histological evaluation. At the end of the behavioral experiments, a random sample (n = 6) for each group of animals was used for the histological studies as described previously [10]. In brief, rats were deeply anesthetized with ketamine-xylazine and perfused through the ascending aorta with 300 ml saline followed by 600 ml 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed and postfixed. Quantification of hippocampal pyramidal cells was performed by microscopic examination of serial coronal sections stained with cresyl violet.

In the immunohistochemical study, specific antibodies were used: for immunostaining of AChE-sensitive neurons – AChE (H-134) Rabbit polyclonal antibody (Santa Cruz Biotechnology; Inc. USA). Their detection was performed by Rabbit specific secondary antibody/HRP and ABC Staining System. Stained sections were analyzed with fluorescence optic microscope Leica MM AF. The cell counting was performed in the CA1 and CA3 areas of the hippocampus. For this purpose the systematic random sampling was employed. The 2-dimensional counting grid (250 µm x 250 µm) at the magnification 400x was used. Totally 6 - 10 sections of hippocampal levels from each group of animals were selected.

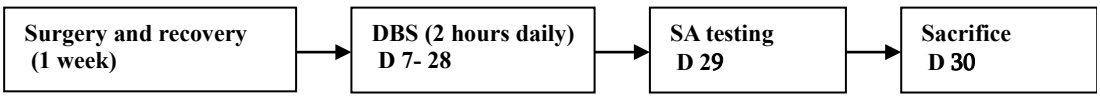

Fig. 1. Schematic representation of the experimental design for chronic stimulation and behavioral testing of memory functions in the rats of O/S group.

Cresyl violet staining was also performed to confirm the location of electrode.

Statistical analysis. Statistical analysis was performed by one-way analysis of variance (ANOVA; SigmaStat statistical software) followed by post hoc comparisons where necessary. All data are presented as mean ± standard error of the mean. Differences were considered significant when $p < 0.05$.

Results

Spontaneous alternation behaviour. The one-way ANOVA for the number of arms entered during the testing session showed a significant effect of group factor ($F\ 3.44 = 7.089$; $P < 0.001$). Post hoc (Tukey test) analysis revealed a significant difference between the O/I and O/S ($P = 0.001$) and O and O/S ($P = 0.001$) groups. There was no significant difference between the N and O/I groups ($P = 0.064$), O/I and O ($P = 0.951$), N and O ($P = 0.176$), or between the N and O/S groups ($P = 0.369$; Fig.2A). The results showed that the rats of O/S group, relative to OA treated and rats of O/I group, had a significantly lower level in the number of arms entered during the testing session; there was no significant difference between the N and O/S groups.

As shown in Fig. 2B, ICV injection of OA significantly impaired SA performance and MS DBS improves it. The one-way ANOVA for the SA score revealed a significant effect of group ($F\ 3.44 = 17.843$; $P < 0.001$). The post hoc (Tukey test) analysis uncovered a significant difference between the normal and O or O/I groups, also between the O/S and O or O/I groups ($P < 0.001$, in all cases). Also, there was significant difference between the N and O/S groups ($P = 0.043$).

The Nissl stained cells numbers in the hippocampal CA1 and CA3 fields. The results revealed that the number of pyramidal cells in N group are significantly higher than those in the O, O/I and O/S groups ($P < 0.001$, in all cases). In rats of the O/S groups, there was a trend towards an increase in the number of pyramidal neurons in the hippocampus, but the difference between the O/S and O or O/I groups was not significant. These results indicated that MS DBS had no effect on the recovery of OA-induced cell loss in the hippocampus.

The AChE immune-stained cells numbers in the hippocampal CA1 and CA3 fields. The one-way ANOVA for the AChE immune-stained cell numbers in the hippocampal CA1 and CA3 fields of the hippocampus showed a significant effect of group factor (CA1 - $F\ 3.23 = 231.712$; $P < 0.001$; CA3 - $F\ 3.23 = 178.890$; $P < 0.001$). Post hoc (Tukey test) analysis revealed that the number of the AChE immune-stained cells in N group is significantly higher than that in the O or O/I groups (CA1 - P <

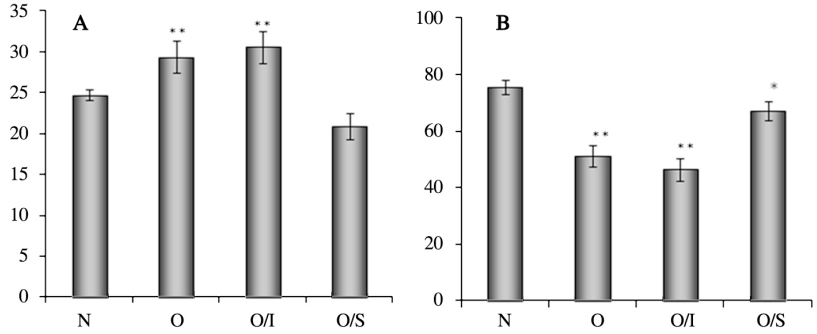

Fig. 2. Effects of MS DBS on spatial working memory, assessed in spatial alternation testing procedures in a four-arm plus-shaped maze. Groups: N – normal, O – with administration of OA, O/I - with administration of OA+implantation of electrodes, O/S – stimulation.
(A) Ordinate: number of arm entries; ** $P < 0.001$ *v.s.* O/S
(B) Ordinate: alternation score (%). *$P < 0.05$; **$P < 0.001$ *v.s.* N

0.001; CA3 - $P < 0.01$), also in the O/S group, it is higher than in the O or O/I groups ($P < 0.01$, in all cases). There was no significant difference between O/S and N groups (CA1 - $P = 0.08$; CA3 - $P = 0.075$). These results show that MS DBS effectively restores OA induced reduction of cholinergic activity in the hippocampus.

Discussion

The present results demonstrate that the MS DBS improves OA-induced impairment of spatial working memory and restores AChE immune-stained cells numbers in the hippocampal CA1 and CA3 fields.

In behavioral experiments the possible beneficial effect of chronic DBS of MS on the OA-induced spatial short-term memory impairment was examined in SA task in the plus-maze. The present results demonstrate that MS DBS improves OA-induced impairment of spatial working memory. It should be note that, findings showed that rats of stimulation group had a significantly lower level in the number of arms entered during the testing session, relative to OA injected rats or with implantation of electrode (group O/I). However, there was no significant difference between normal rats and OA injected rats, which showed impaired working memory or the stimulation group, which showed improved alternation behavior. Accordingly, it can be suggested that changes in the number of arms entered (i.e. locomotor activity) are not the cause of changes in alternation scores.

It should be noted that SA is assumed to be a measure of spatial working memory [18]. The underlying assumption is that in order to alternate successfully between locations, the rat must remember its visits to previous places. Interestingly, Jeong and colleagues [15] showed that MS DBS restores spatial memory in the water maze after selective immunolession of cholinergic neurons in MS, and this effect is associated with increased hippocampal cholinergic activity and neurogenesis. The present results showed that ICV injection of OA significantly impairs SA performance and MS DBS causes an improvement in SA performance that occurs in parallel with an increase in cholinergic activity in the hippocampus. It can be assumed that in this study, the enhancement of working memory function by MS DBS in a rat model of AD may be associated with an increase in hippocampal neurogenesis. Our hypothesis about neurogenesis, on the one hand, is related to the data that MS DBS promotes hippocampal neurogenesis, and, on the other hand, to the data that newborn dentate gyrus granule cells are continuously generated from neural progenitor cells in the dentate gyrus of the adult hippocampus and can make functional synapses within a week of their terminal mitosis, and already propagate neural inputs to the hippocampal network [19]. However, further studies are needed to confirm this opinion.

Recently, we have shown that ICV microinjection of OA decreases the number of cholinergic and gamma-aminobutiric acid-ergic (GABAergic) neurons in MS [10]. The specific degeneration of basal forebrain neurons takes place in AD and contributes to the memory loss exhibited by AD patients [20]. It can be assumed that cholinergic and GABAergic deficits in MS after OA administration are compensated by stimulation of surviving MS neurons and that the effect of MS DBS on memory improvement is associated with the modulation of septo-hippocampal activity.

In conclusion, we suggest that the enhancement of working memory function may be associated with an increase in hippocampal neurogenesis and recovery of the AChE immune-stained cells numbers in the hippocampus induced by MS DBS in a rat model of AD. These results allow us to identify the medial septum as one of the stimulation sites for improving memory function.

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ადამიანისა და ცხოველთა ფიზიოლოგია

მედიალური სეპტუმის ელექტრული სტიმულაცია აუმჯობესებს ოკადაის მჟავას ზემოქმედებით გამოწვეულ სივრცითი მუშა მეხსიერების დარღვევას და აღადგენს ქოლინერგულ აქტიურობას ჰიპოკამპში

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წინამდებარე ნაშრომში შეისწავლებოდა მედიალური სეპტუმის (medial septum - MS) ტვინის ღრმა სტიმულაციის (deep brain stimulation - DBS) ეფექტები ოკადაის მჟავათი (okadaic acid - OA) გამოწვეულ სივრცითი მუშა მეხსიერების დარღვევასა და ჰიპოკამპში აცეტილქოლინ-ესთერაზა იმუნო-მგრძნობიარე ნეირონების დაკარგვაზე ვირთაგვებში. ახალგაზრდა ზრდასრული ვირთაგვები განაწილებული იყვნენ შემდეგ ჯგუფებში: (1) ნორმალური ცხოველები, რომლებსაც არ უტარდებოდათ რაიმე ქირურგიული ჩარევა; (2) OA-ს ინტრაპერიტონიალური შეყვანით; (3) OA-ს შეყვანით და ელექტროდის იმპლანტაციით MS-ში; (4) OA-ს შეყვანით და MS-ის ელექტრული სტიმულაციით. ქცევით ექსპერიმენტებში MS-ის ქრონიკული DBS-ის შესაძლო გამაუმჯობესებელი ეფექტი OA-თი გამოწვეული სივრცითი მოკლევადიანი მეხსიერების დაქვეითებაზე შესწავლილ იქნა ჯვრისმაგვარ ლაბირინთში სპონტანური მორიგეობის ამოცანით. მიღებულმა შედეგებმა აჩვენა, რომ MS-ის DBS აუმჯობესებს OA-თი გამოწვეულ სივრცითი მუშა მეხსიერების დარღვევას და აღადგენს აცეტილქოლინესთერაზა იმუნო-მგრძნობიარე ნეირონების რაოდენობას ჰიპოკამპში. ჩვენი ვარაუდით მედიალური სეპტუმის სტიმულაციით ვირთაგვების AD-ის მოდელში მუშა მეხსიერების ფუნქციის გაუმჯობესება შესაძლოა ასოცირებული იყოს ჰიპოკამპის ნეიროგენეზის ზრდასა და ქოლინერგული აქტიურობის აღდგენასთან ჰიპოკამპში. მიღებული შედეგები საშუალებას გვაძლევს, მედიალური სეპტუმი განვიხილოთ სტიმულაციის ერთ-ერთ სამიზნედ მეხსიერების გაუმჯობესებისათვის.

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