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Effects of medial septum deep brain stimulation for memory recovery in a memory impaired rat model established by 192 IgG-saporin injection

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Effects of medial septum deep brain stimulation for memory recovery in a memory impaired rat model established by 192 IgG-saporin injection

Directed by Professor Jin Woo Chang

The Doctoral Dissertation
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

Da Un Jeong

June 2015

This certifies that the Doctoral Dissertation of Da Un Jeong is approved.

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ABSTRACT

Effects of medial septum deep brain stimulation for memory recovery in a memory impaired rat model established by 192 IgG-saporin injection

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(Directed by Professor Jin Woo Chang)

The possibility of using deep brain stimulation (DBS) for memory enhancement was recently reported; however, the mechanisms underlying its effects are not precisely understood. This study was performed to find the therapeutic effects and mechanism of DBS using a memory impaired rat model. First, a spatial memory impaired rat model mimicking the cholinergic deficit in Alzheimer's disease was established. Second, the effects of medial septum (MS)-DBS on this memory impaired rat model were investigated. Furthermore, to detect the stage of the memory process that was affected, DBS was delivered at different

times.

In the first experiment, 192 IgG-saporin was infused into the cerebral ventricle to cause selective cholinergic damage. Two weeks later, cholinergic denervation caused spatial memory impairment in the Morris water maze test and glucose hypometabolism of the cingulate cortex in the [F-18] fluorodeoxyglucose PET study. In the second experiment, a 60-Hz stimulation was delivered to the MS to confirm the therapeutic effects of MS-DBS. During the 2-week stimulation, MS-DBS enhanced spatial memory and increased the number of doublecortin immunopositive cells in the hippocampus. Next, to detect the stage of the memory process affected by MS-DBS, stimulation was delivered at different times: pre-stimulation, 5days of before the water maze test; training-stimulation, 5days after the start of the training phase for the water maze; probe-stimulation, 2 h of stimulation shortly before the probe test of the water maze. The most effective memory restoration occurred in the pre-stimulation group in which the latency of the training phase was similar to that of the normal group. Moreover, the pre-stimulation group had a better recalled of the platform position than the other stimulation groups. An increase in the level of brain derived neurotrophic factor (BDNF) was observed in the pre-stimulation group

that was maintained in both the frontal cortex and hippocampus. The level of glutamic acid decarboxylase 65/67, an enzyme that catalyzes the decarboxylation of glutamate to GABA, was reduced in the hippocampus. However, acetylcholinesterase activity in the pre-stimulation group was not different from the lesion group. These results suggest that the spatial memory improvement associated with MS-DBS is mainly correlated with increased BDNF expression in the frontal cortex and hippocampus, rather than with direct electrical stimulation of cholinergic or GABAergic neurons in the hippocampus.

Taken together, the memory impairment caused by cholinergic denervation could be improved by MS-DBS (60Hz). Additionally, it has a significant correlation with the up-regulation of BDNF expression and neurogenesis. Based on the results of this study, the use of MS-DBS during the early stage of disease is thought to restore spatial memory impairments.

Key words: deep brain stimulation, spatial memory, medial septum, brain derived neurotrophic factor, neurogenesis

Effects of medial septum deep brain stimulation for memory recovery in a memory impaired rat model established 192 IgG-saporin injection

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I. INTRODUCTION

The prevalence of dementia is consistently growing, particularly among the elderly. Dementia is a syndrome affecting multiple functions, such as memory, thinking, calculation, learning, language, and judgment. It is caused by a variety of brain diseases including Alzheimer's disease (AD) and vascular dementia (the most common diseases associated with dementia).[1] Several therapies have been investigated for the treatment of dementia,

including deep brain stimulation (DBS).

DBS is a neurosurgical procedure involving the implantation of electrodes into the brain to stimulate subcortical structures. It changes brain activity not only in the target volume, but also in distant brain areas that originate in the target volume. DBS has been used to treat various types of movement disorders and psychiatric disorders.[2-4] Food and Drug Administration approval was granted for thalamic DBS for treating essential tremor in 1997, subthalamic nucleus and globus pallidus internus DBS for treating Parkinson's disease in 2002, dystonia in 2003, and obsessive-compulsive disorder in 2009. Recently, DBS of memory-associated brain structures was tested as a possible treatment for Alzheimer-type dementia, with some studies providing promising results. DBS of the hypothalamus modulates limbic activity and improves certain memory functions,[5] and DBS of the nucleus basalis magnocellularis (NBM) improved cognitive functioning in patients with Parkinson's disease-related dementia.[6] Furthermore, stimulation of the entorhinal region enhanced memory for spatial information when applied during learning.[7] In animal studies, high frequency stimulation led to production of specific amino acids in the hippocampus that may be involved in the enhancement of short-term memory formation.[8] In addition, formation of water maze memory was facilitated after bilateral stimulation of the entorhinal cortex.[9] Clinical evidence obtained from patients with dementia or other neurological disorders showed that DBS could be used as a tool to enhance memory function. However, this evidence is not strong, as above clinical trials were either not randomized and

case-controlled or did not employ large sample sizes. Moreover, the therapeutic mechanism of memory enhancement in these clinical studies is not clear. Therefore, disease-specific animal experiments are necessary to advance the clinical application of DBS.

In this study, a memory impaired rat model mimicking the cholinergic deficit in patient with AD was made using 192 IgG-saporin. 192 IgG-saporin, composed of a monoclonal antibody, has low affinity to the rat nerve growth factor receptor p75 located on cholinergic cell bodies in the basal forebrain, and contains a ribosomal inactivating protein called saporin. 192 IgG-saproin was infused into the cerebroventricle to damage basal forebrain cholinergic neurons. Degeneration of cholinergic basal forebrain neurons is one of the common features of AD and vascular dementia and has been correlated with cognitive decline.[10,11]

Memory, as measured by changes in an animal's behavior, is the process including acquisition, consolidation, retention, and retrieval. In a contextual fear condition, for example, memory acquisition occurs as the animal learns an association between a context and a shock. During consolidation, this memory is strengthened from an unstable to a more stable state. During retrieval, the animal shows freezing behavior when it is returned to the conditioning context.[12] The hippocampus is required for different types of memory, such as declarative or episodic memory and it might be engaged in different memory processes.[13,14] Animal studies provide support for the transient requirement of the hippocampus in the process of memory.[15,16] The medial septum (MS) was chosen as a stimulation site. Projections from the

MS (a part of the basal forebrain) to the hippocampus consist of more than three types of fibers, including cholinergic, GABAergic and glutamatergic,[17-19] and are the primary source of cholinergic input to the hippocampus.[20] Furthermore, the MS is reported to regulate hippocampal activity through acetylcholine, GABA, and glutamate.[21-23]

The most effective stimulation site and parameters for DBS therapy for dementia has not yet established. In general, high frequency stimulation (> 30 Hz) leads to long term potentiation (LTP), and low frequency stimulation (< 10 Hz) induces long term depression.[24,25] This study was performed to understand the mechanisms of therapeutic effects of MS-DBS in memory impairment due to a cholinergic deficit. To detect the affected stage of memory, stimulation was delivered before or during the training phase (right after training trails), or 2 h of stimulation was delivered shortly before the probe test.

II. MATERIALS AND METHODS

1. Memory impaired rat model

A. Animals

Sprague-Dawley rats, weighing 200 – 250 g, were used. This study was conducted according to the guidelines of the Institution Animal care and Use Committee of Yonsei University. All efforts were made to minimize animal suffering, and reduce the number of rats used. Three rats were housed per cage with free access to food and water. The cages were kept in a temperature- and humidity-controlled room with a 12-12 h light–dark cycle.

B. Surgical procedure

The rats were anesthetized with a mixture of ketamine (75 mg/kg), acepromazine (0.75 mg/kg), and rompun (4 mg/kg) and secured in a stereotaxic frame. After scalp incision, rats were injected bilaterally with 8 μl of 192 IgG-saporin (0.63 μg/μl, Chemion, Temecula, CA) at the cerebroventricle following Bregma coordinates: AP:-0.8 mm, ML: ± 1.2 mm, DV:-3.4 mm. 192 IgG-saporin was delivered at a rate of 1 μl/min. The syringe was left in place for 5 min after injection.

2. [F-18]FDG and micro-PET imaging

In the first experiment, metabolic images were obtained using [F-18] fluorodeoxyglucose (FDG) in micro-positron emission tomography (PET). Two weeks after surgery, five rats each from the normal or lesion groups were deprived of food for 12–15 h prior to the experiments to enhance [F-18]FDG uptake in the brain. Each animal was warmed and adapted to a sound-attenuated booth (temperature maintained at 30°C) with 60-dB white noise for at least 30 min before the [F-18]FDG injection. [F-18]FDG (500 mCi/100 g body weight) was injected through the tail vein without anesthesia. After [F-18]FDG uptake (40 min), imaging was performed using a Focus 120 MicroPET system (Concorde Microsystems, Knoxville, TN). During the PET scans (40 min), the animals were maintained under isoflurane inhalation anesthesia (2% in 100% oxygen; IsoFlo; Abbott Laboratories, Quebec, Canada).

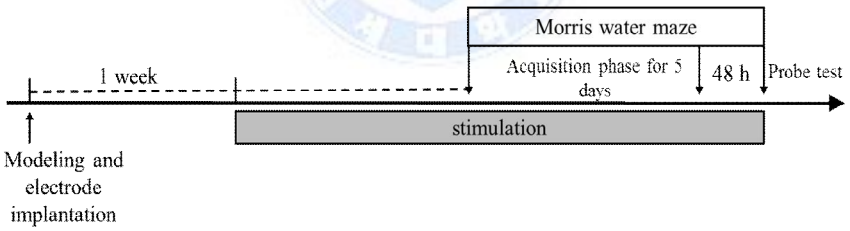

Figure 1. Schematic diagram of the [F-18]FDG-PET and behavioral test.

3. DBS

A. Surgical procedure

After the administration of 192 IgG-saporin, the rats underwent an additional procedure for electrode implantation. A hole was drilled in the skull at the level of the MS, and a tungsten electrode (254 μm diameter, A-M systems, Sequim, WA) was implanted in the MS (AP + 0.6 mm, ML 0.1 mm, DV – 6 mm).The stimulation electrode was fixed with dental cement (Long Dental Manufacturing, Wheeling, IL) and two screws.

B. Stimulation parameter and schedule

The electrode was connected to a stimulator (Pulsemaster A300, stimulus isolator A365, WPI). Electrical stimulation consisted of pulses (120 μs, 100 μA) delivered at 60 Hz.

In the second experiment, the rats were randomly assigned to one of the four groups. Rats in the normal (n=11) group underwent no surgical procedure. Rats in the lesion (n=15) group received intracerebroventricle (ICV) administration of 192 IgG-saporin. Rats in the implantation (n=14) group received ICV administration of 192 IgG-saporin and implantation of an electrode in the MS. Rats in the stimulation (n=10) group received ICV administration of 192 IgG-saporin and electrical stimulation of the MS. Stimulation period on the second experiment was maintained for 2 weeks. Rats were stimulated daily beginning a week after surgery until the end of behavioral testing (5 hours per day, every day for 2 weeks). Stimulation was

delivered after the daily water maze training session.

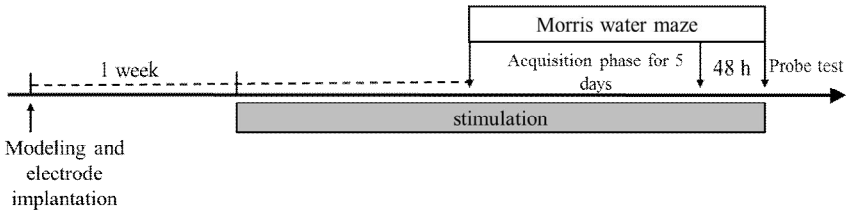

Figure 2. Schematic diagram of the electrical stimulation and behavioral test.

The third experiment was performed to identify the stage of memory affected by the MS stimulation. Rats were randomly assigned to one of the five groups. Rats in the normal (n=8) group had no surgical procedure. Rats in the lesion (n=8) group and all stimulation groups had intraventricular administration of 192 IgG-saporin. Rats in all stimulation groups had an electrode implanted in their MS. Rats in the pre-stimulation (n=9) group received stimulation for 5 days prior to the water maze training. Rats in the training-stimulation (n=9) group received stimulation for 5 days during the water maze training phase. Rats in the probe-stimulation (n=7) group received stimulation for 2 hours shortly before the probe test of the water maze.

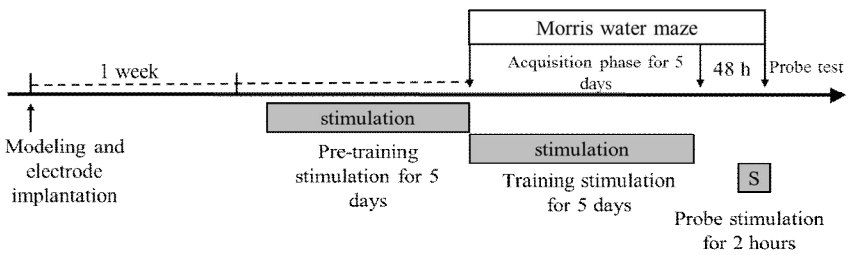

Figure 3. Schematic diagram of the timely electrical stimulation and behavioral test.

4. Morris water maze

Two weeks after 192 IgG-saporin administration, the rats were trained on the Morris water maze. The water maze consisted of a circular pool, 2 m in diameter and 0.5 m in depth, filled with opaque tap water (23 °C); a circular (0.15 m in diameter) black escape platform was submerged 2 cm below the water surface. The rats were trained in four trials per day for 5 consecutive days with a fixed platform. For each training trial, the rat was semi-randomly placed into the pool at one of the four start points and given 60 sec to reach the platform. Any rat that did not reach the platform within 60 sec was led to the platform by the experimenter and allowed to remain on the platform for 10 sec. Forty-eight hours after the last training trial, the rats were given probe trials, lasting 60 sec. The platform was removed from the pool. Swimming distance, time, path, and speed were recorded using a video tracking system.

5. Histology

Immediately after behavioral testing (probe test), rats were anesthetized with a mixture of ketamine, acepromazine, and rompun and perfused with normal saline and cold 4% paraformaldehyde. The brains were removed, post-fixed, and transferred to 30% sucrose for 3 days. The brains were sectioned into 30 μm sections using a freezing microtome and stored in a cryoprotectant solution (0.1M phosphate buffer, pH 7.2, 30% sucrose, 1% polyvinylpyrrolidone, and 30% ethylene glycol) at -20°C. Anatomical landmarks from a stereotaxic atlas[26] were used to localize the MS and hippocampus.

Cresyl violet staining was performed to confirm the location of the electrode. To perform immunohistochemistry, sections were incubated in 0.3% H_2O_2 for 30 min to inactivate endogenous peroxidase activity. They were blocked with 5% normal serum and incubated with polyclonal antibodies against choline acetyltransferase (1:200, ChAT, Chemicon, Temecula, CA) or doublecortin (DCX, 1:200, Santa Cruz Biotechnology Inc., Santa Cruz, CA) overnight at 4°C. The sections were incubated with biotinylated secondary antibodies, followed by the avidin-biotin complex method (ABC Elite, Vector Labs, Burlingame, CA). They were visualized with diaminobenzidine (DAB) using a DAB substrate kit (Thermo, Rockford, IL). To perform immunofluorescence, sections were blocked with 10% normal horse serum (Vector Labs, Burlingame, CA) and incubated with primary antibodies at following dilutions: Sex determining region Y-Box2 (Sox2, 1:50, Santa Cruz Biotechnology Inc., Santa Cruz, CA), DCX(1:50,

Santa Cruz Biotechnology Inc., Santa Cruz, CA). After primary immunoreaction, sections were incubated with secondary antibodies conjugated with Cy3 (1:400, Jackson Immuon Reserch,West grove, PA) or fluorescein (1:400, Thermo, Rockford, IL). Staining on sections was visualized with LSM 700 (Carl Zeiss, Jena, Germany) confocal microscope.

6. Western blot

The brains of the anesthetized rats were quickly removed to acquire protein. The frontal cortex (FC, including cingulate cortex and prelimbic cortex), MS, diagonal band (DB) and hippocampus were dissected with fine forceps from 1 mm coronal brain slices. The samples were homogenized in lysis buffer (Intron, Seongnam, Korea) and placed in ice for 30min. They were then centrifuged for 20 min at 12,000 rpm, and the protein in the supernatant was measured using the bicinchoninic acid protein assay reagent kit (Pierce, Rockford, IL). The protein samples were stored at -70°C. Proteins were separated using a 10-15% sodium dodecyl sulfate-polyacrylamide gel and transferred onto polyvinylidene fluoride membranes. The membranes were incubated with blocking buffer (5% non-fat dry milk in phosphate buffered saline containing 0.05 % Tween 20, PBST) for an hour at room temperature. The membranes were then incubated with the indicated antibodies overnight at 4°C, and washed three times (each for 5 min) with PBST. The membranes were incubated with corresponding secondary antibodies for 1 hour at room temperature. After washing with PBST, proteins were detected with enhanced chemiluminescence solution (Pierce, Rockford, IL) and LAS-4000

(Fujifilm, Tokyo, Japan). The intensity of each band was determined using an analysis system (Multi Gauge version 3.0, Fujifilm, Tokyo, Japan). The membranes were incubated with antibodies to BDNF (1:1000; Millipore, Temecula, CA), glutamate decarboxylase 65/67 (GAD, 1:1000; Millipore, Temecula, CA) and ß-actin (1:5000; Sigma, Louis, MO).

7. Acetylcholinesterase (AChE) assay

The protein sample was same as the sample used for western blotting. The enzymatic activity of AChE was determined using the method of Ellman et al.[27] with some modifications. In brief, 20 µl triplicate samples were mixed with the reaction mixture of 0.2 mM dithiobisnitrobenzoic acid (Sigma, Louis, MO), 0.56 mM acetylthiocholine iodide (Sigma, Louis, MO), 10 µM tetraisopropylpyrophosphoramide (Sigma, Louis, MO), and 39 mM phosphate buffer (pH 7.2) at 37°C. After 30 min, the optical density was measured at 405 nm.

8. Statistical analysis

A. Analysis of Morris water maze

An independent *t*-test was used to analyze data from the water maze performed by normal and lesion groups, to test for memory impairment by cholinergic denervation. Any *p* values less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS (version 21; IBM). Other water maze training trials were analyzed with a

Linear Mixed-Effects Model. Statistical analyses were performed using SPSS and SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

Performance indices for the water maze probe test were expressed as a percentage of the values of the normal group. One-way analysis of variance (ANOVA) was used to analyze data from all trials except training trials. ANOVA was followed *post hoc* by a least significant difference test or Tukey's honestly significant difference test at each time point. An experiment which had same number of rats among the group was followed Tukey's honestly significant difference test, whereas other experiment which had different number of rats among the groups was followed a least significant different test. *P*-values less than 0.05 were considered statistically significant.

B. Analysis of immunohistochemistry, immunofluorescence and Western bolt

The number of DCX immunopositive cells was counted in 10 coronal sections per group, located 3.0 to 3.6 mm posterior to bregma (immunohistochemistry). The number of Sox2 and DCX immunopositive cells was counted in 8 coronal sections per group, located 3.0 to 3.6 mm posterior to bregma (immunofluorescence). The number of DCX and Sox2 immunopositive cells was presented as mean ± standard error of the mean (SEM). The results of the western blots were normalized to β-actin for each sample and expressed as a percentage of the control values. One way ANOVA followed by a *post hoc* least significant difference test was used at

each time point for statistical analysis. *P*-values less than 0.05 were considered statistically significant. All statistical analyses were performed with SPSS.

C. Analysis of PET imaging

For statistical analysis, brain regions in the [F-18]FDG microPET images were manually extracted. All individual images were normalized using the [F-18]FDG rat brain template. To make the [F-18] FDG rat brain templates, normal rat brain images (n=12) were coregistered to the respective images and resliced with trilinear interpolation ($0.4\times0.4\times0.4$ mm^3) using SPM5 (http://www.fil.ion.ucl.ac.uk/spm). Individual images were averaged to create the [F-18]FDG rat brain template. Next, the template was normalized into the MRI template for accurate anatomical information, which was placed into stereotaxic space.[28] Voxel-based statistical analyses were carried out with statistical parametric mapping. The statistical threshold was set at $p < 0.05$ (family-wise error correction) with an extent threshold of 100 contiguous voxels. T value maps of the results were overlaid on transverse views of the MRI template to define voxels that showed significant changes. For the analysis of correlations among the brain areas with significant metabolic changes, average glucose metabolism in each activated or deactivated brain region was calculated with all voxels at the Paxinos position of each region. Pearson's correlation coefficients were calculated for regions of interest ($p < 0.01$, two-tailed).

III. RESULTS

1. Spatial memory impairment and glucose hypometabolisam by cholinergic denervation

A. Spatial memory impairment by intraventricular 192 IgG-saporin injection

Two weeks after 192 IgG-saporin administration, spatial memory was tested with the Morris water maze. On the first day of the training trials, the latencies of the normal and lesion groups to reach the platform were 34.9 s and 38.1 s, respectively. There was no significant difference between the latencies of the two groups (Figure 4 A). Both groups showed a similar latency of 12 s on the last day of the training trial, suggesting that, as latency to reach the platform declined progressively across training days, progressive learning of the hidden platform location occurred. The water maze probe test results are expressed as a percentage of the values for the normal group (Figure 4 B). The lesion group showed no differences from the normal group in motor-related behaviors, as evidenced by similar swim distances and speeds. These findings suggest there are no effects of cholinergic lesions on motor function. However, the amount of time spent by the lesion group in the target quadrant and platform zone decreased across training days to 60% (*$p < 0.05$) and 36% (*$p < 0.05$) of normal group values, respectively. The difference between time spent by the lesion group in the target and platform zone was significant. Moreover, the number of

platform crossings was markedly reduced to 38% compared with the normal group, although there were no statistically significant differences between groups.

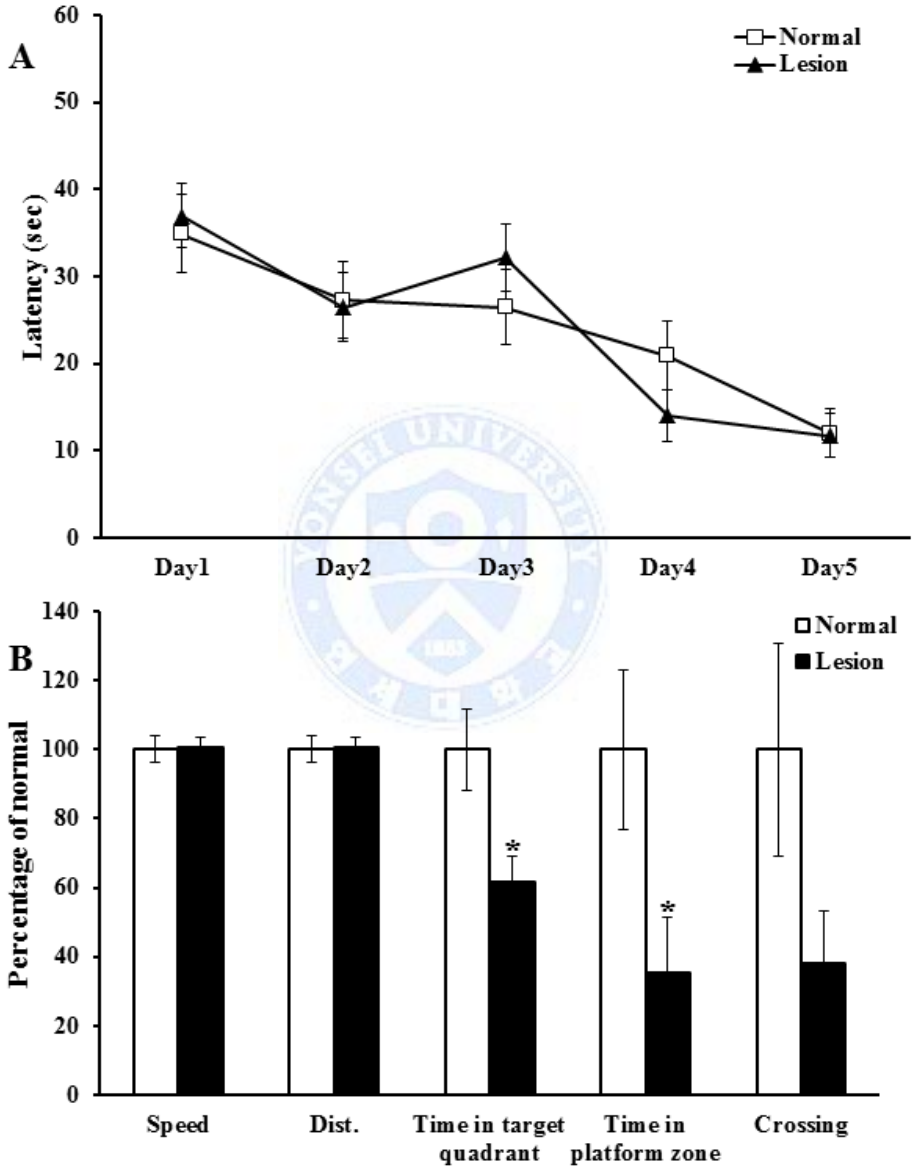

Figure 4. Effects of cholinergic deficits on spatial memory. (A) Training

trails of water maze. Latency indicates the time required for the rat to find the escape platform during training trials. Both groups showed a similar latency of 10 s on the last day of the training trial, suggesting that they acquired the location of the platform. Data are shown as mean ± standard error of the mean. (B) Probe test of water maze. During the probe test, the time spent in the target quadrant (*$p < 0.05$) and in the platform zone (*$p < 0.05$) were significantly different between the lesion and normal groups. Indices are expressed as percentages of the normal group values.

B. Cholinergic denervation by intraventricular 192 IgG-saporin injection

Intraventricular 192 IgG-saproin injections produced denervation of ChAT immunopositive neurons in the MS. ChAT immunopositive neurons in normal rats were evenly distributed in the MS, and the structure of the cell bodies and dendrites were intact (Figure 5 A). In contrast, the lesion group showed a remarkable decrease in the number of ChAT immunopositive neurons as well as severe damage of the cell bodies and dendrites (Figure 5 B).

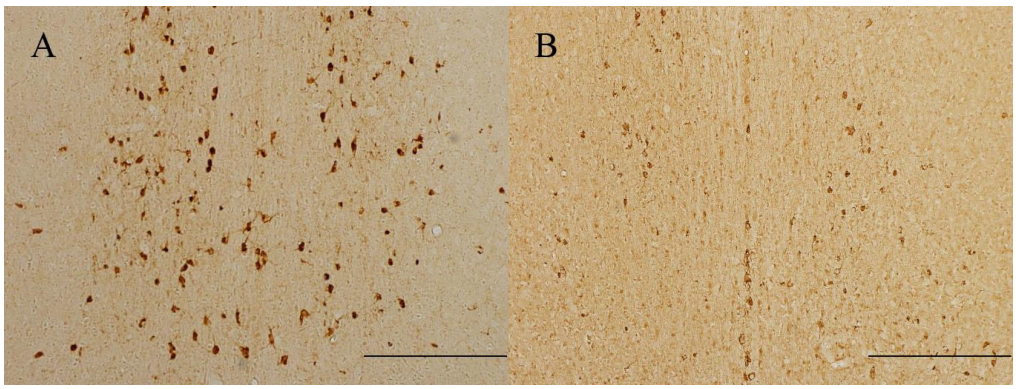

Figure 5. Representative images showing effects of the cholinergic lesion

after intraventricular injection of 192 IgG-saporin. (A) The normal group. It had numerous ChAT immunopositive neurons in the MS. (B) The lesion group. It displayed a loss of cholinergic neurons in the MS. Scale bar represents 500 μm.

C. Changes in glucose metabolism induced by intraventricular 192 IgG-saporin injection

Figure 6 shows changes in glucose metabolism in the coronal (A-F), horizontal (G) and sagittal (H) sections in rats. Coronal sections indicating glucose hypometabolism were located 0.6 mm to -1.4 mm from bregma (A-F). There was a significant decrease ($p < 0.05$) in glucose metabolism in both the bilateral cingulate and motor cortices of the lesion group compared with the normal group. However, there were no significant differences in glucose metabolism in the other regions, including the hippocampus and basal forebrain.

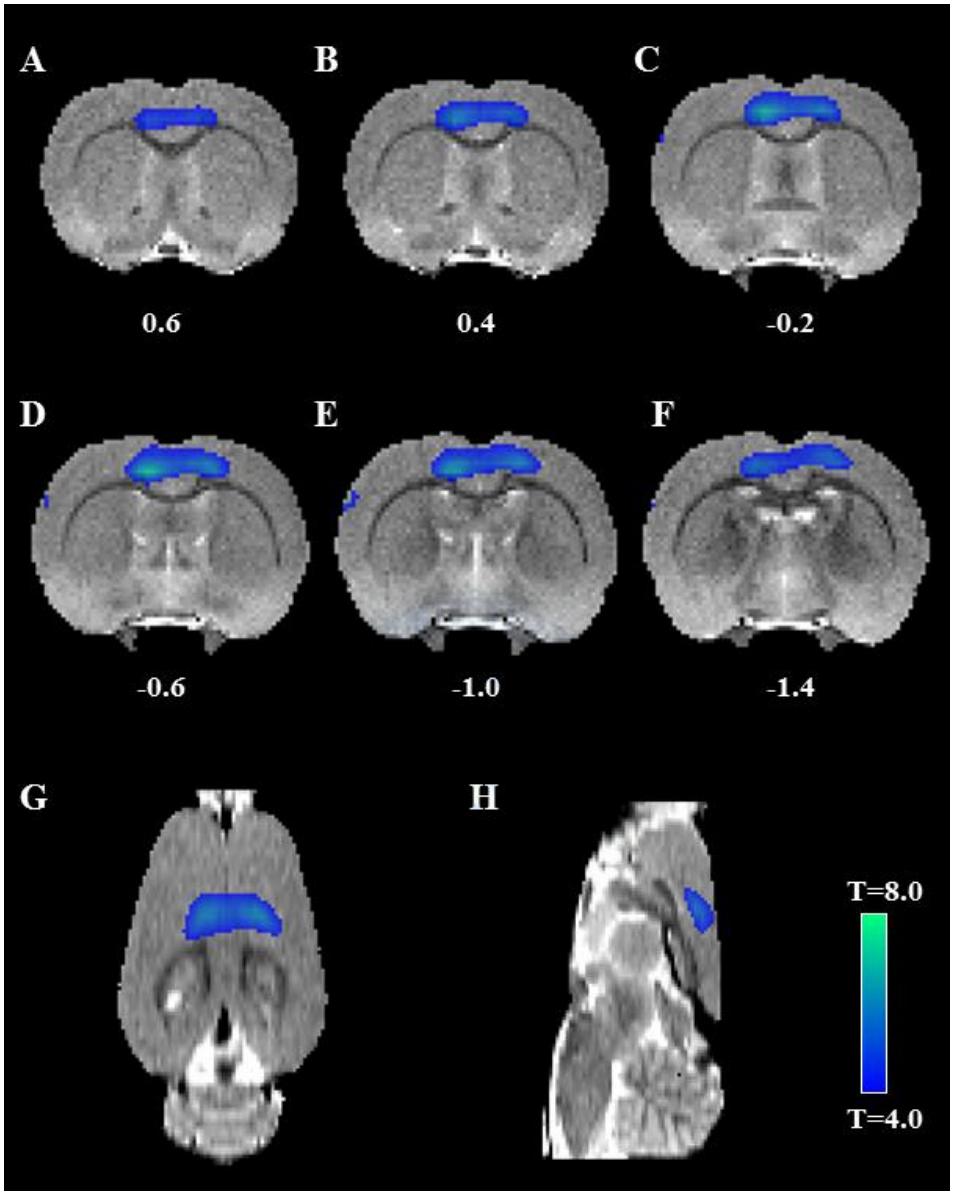

Figure 6. Changes in glucose metabolism in coronal (A-F), horizontal (G), and sagittal (H) sections in rats. Significant reductions ($p < 0.05$) in glucose metabolism in the bilateral cingulate and motor cortices of the lesion group are shown compared with the normal group.

D. Changes in AChE activity by intraventricular 192 IgG-saporin injection

In the normal group, AChE activity in the cingulate cortex was measured at an optical density (OD) of 0.56. In contrast, the lesion group had an OD value of 0.54. This difference was significant (*p < 0.05, Figure 7), suggesting that 192 IgG-saporin-induced damage to cholinergic neurons in the basal forebrain reduced the cholinergic activity of the cingulate cortex.

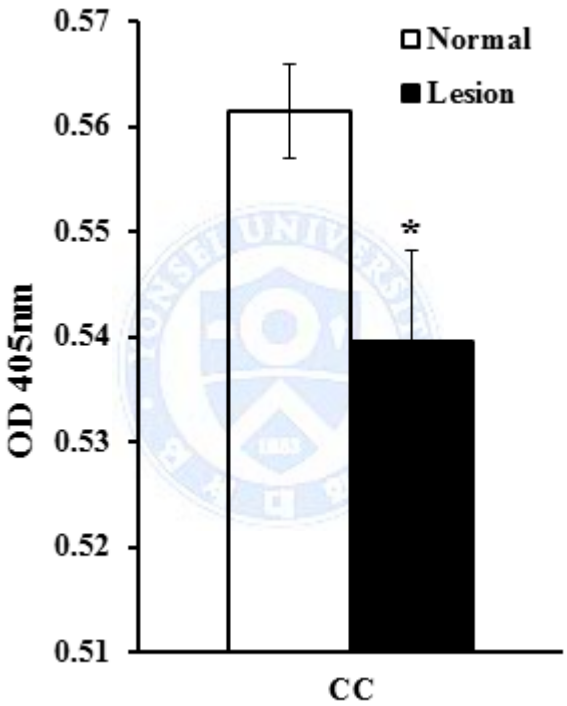

Figure 7. Effects of cholinergic deficits on AChE activity in the cingulate cortex. AChE activity of the lesion group was significantly decreased compared with the normal group (*p < 0.05). AChE activity was expressed as optical density at 405 nm. Values are the mean ± standard error of the mean.

2. Effects of the MS stimulation

A. Cholinergic damage by intraventricular 192 IgG-saporin injection

Intraventricular 192 IgG-saporin injections produced denervation of ChAT immunopositive neurons in the MS (Figure 8). The ChAT immunopositive neurons in normal rats were evenly distributed in the MS. Additionally, the structure of the cell bodies and dendrites were wholly intact as shown in Figure 8 A. In contrast, the lesion (Figure 8 B), implantation (Figure 8 C), and stimulation groups (Figure 8 D) showed noticeable damage to cell body and dendrite structures.

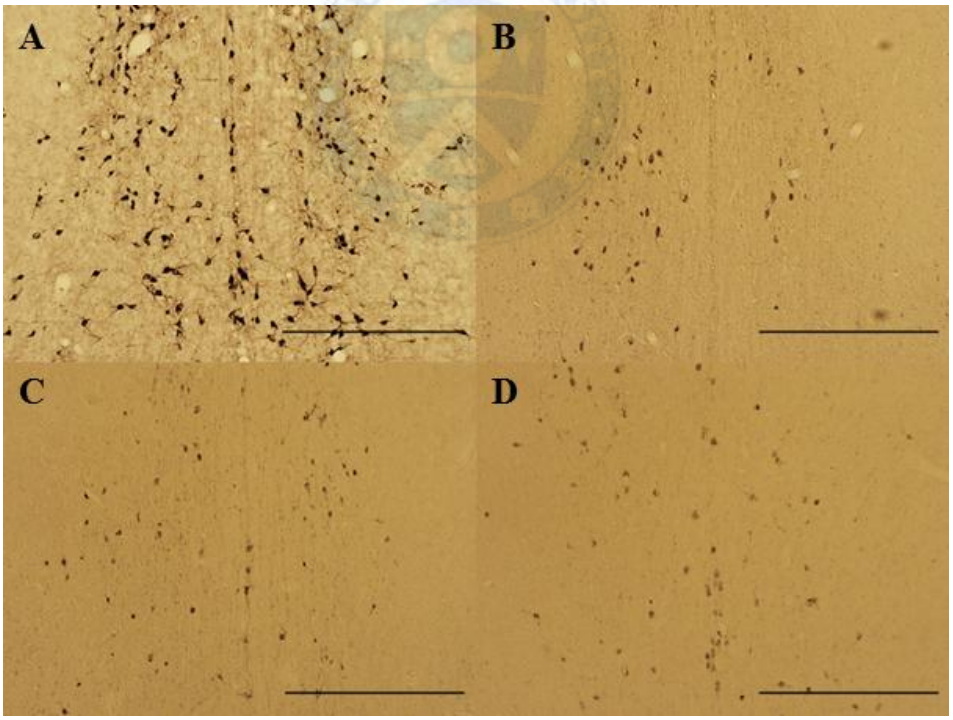

Figure 8. Representative pictures showing effects of the cholinergic lesion

on the basal forebrain. (A) The normal group. It had numerous ChAT immunopositive neurons in the MS. The lesion (B), implantation (C), and stimulation (D) groups displayed a loss of cholinergic neurons in the MS. Scale bar represents 500 μm.

B. Verification of electrode insertion site

The location of stimulating electrodes in the MS was confirmed with cresyl violet staining (Figure 9). After the electrode implantation, there were no abnormal behavioral changes.

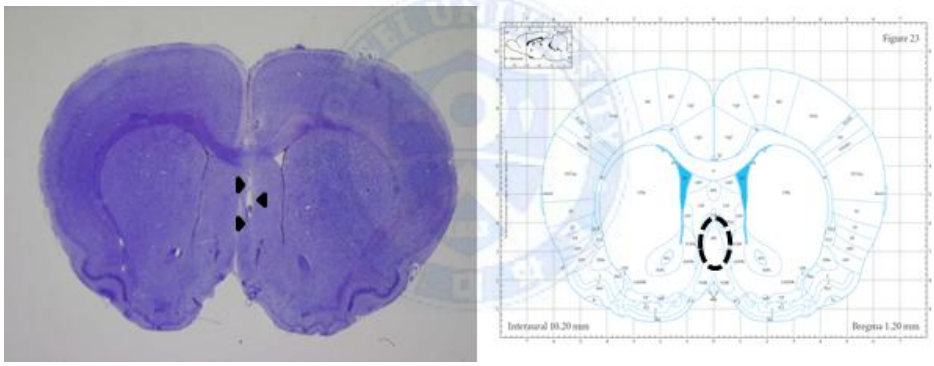

Figure 9. Cresyl violet stained coronal section at the level of the MS and representation of the slice on atlas.[26] Arrowheads show the tract of the electrode in the MS.

C. Spatial memory improvement by MS-DBS

The results of water maze training are shown in Figure 10 A. Latencies were decreased to less than 20 seconds on the final day of the training trails. The differences in latencies between the first and fifth days were statistically

significant ($p < 0.001$) for all groups. However, there were no significant differences among any group depending on time passage ($p = 0.3897$). Taken together, these data show that latency to reach the platform declined progressively across training days for all groups, indicating progressive learning of the hidden platform location. Water maze probe test indices were expressed as a percentage of values for the normal group (Figure 10 B). The lesion, implantation, and stimulation groups showed no differences from the normal group in motor-related behavior, evidenced by similar swim distances and speeds. These findings suggest no effect of cholinergic lesion, electrode implantation, or electrical stimulation on motor function. However, the amount of time that the lesion group spent in the target quadrant was decreased to 70% of normal group values. In addition, the amount of time that the lesion group spent in the platform zone was significantly decreased to 26% of normal group values (**$p < 0.01$), whereas it was only decreased to 70% ($p = 0.472$) and 98% ($p = 0.965$) for the implantation and stimulation groups, respectively. Moreover, the number of platform crossings was significantly reduced to 27% (***$p < 0.001$) and 61% (*$p < 0.05$) for the lesion and implantation groups, respectively, whereas it was only decreased to 95% of normal group values for the stimulation group ($p = 0.805$).

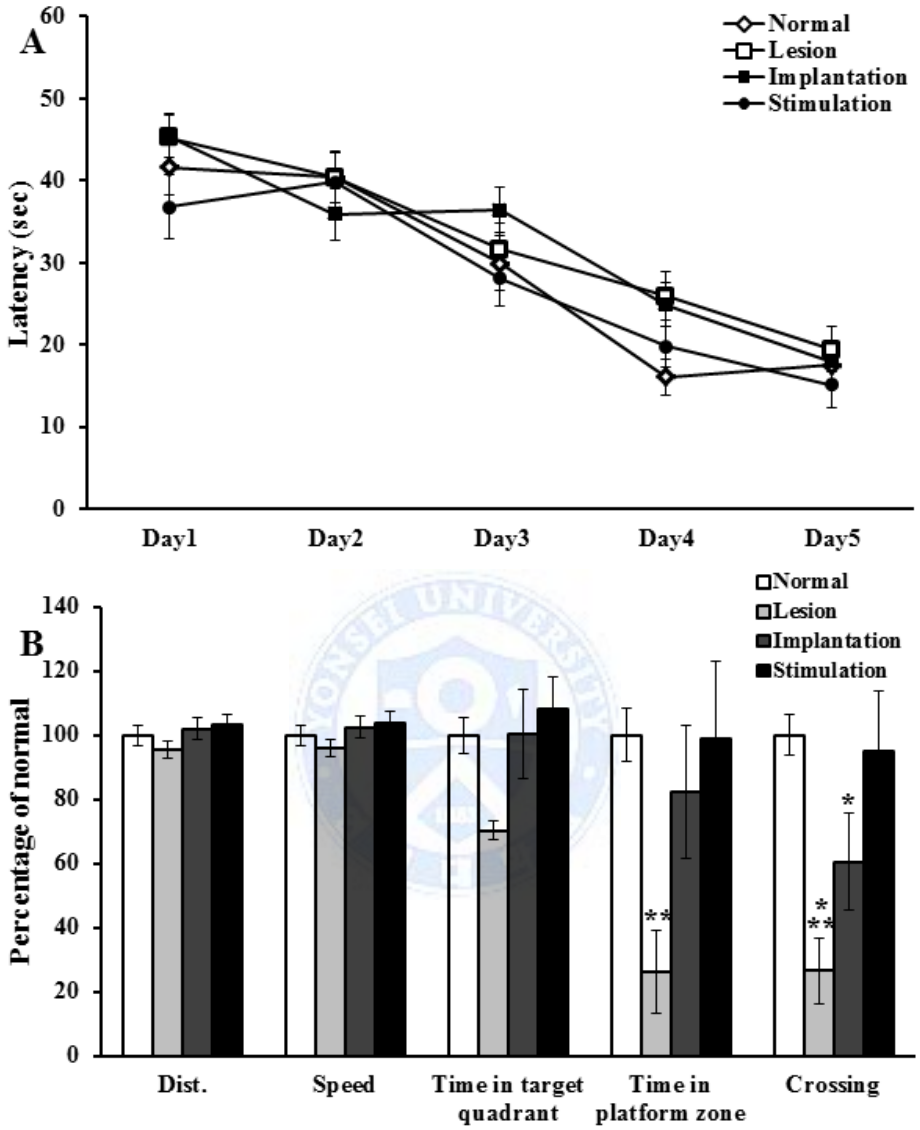

Figure 10. Effect of the MS-DBS on spatial memory. Latency indicates the time required for the rat to find the escape platform during training trails. (A) Training phase of water maze. During training trails, all groups gradually acquired the location of the platform. (B) Probe test of water maze. After a

delay of 2 days, spatial memory was improved by MS-DBS. Time spent in the platform zone (**$p < 0.01$) and number of crossings (***$p < 0.001$) were significantly different between lesion and normal groups. However, the stimulation group did not differ from the normal group. There was no disruption of motor function in any group. (A) Data are shown as mean ± SEM. (B) Indices are expressed as the percentage of normal group values. One-way analysis of variance was used to analyze data from probe test. Data are shown as mean ± SEM. *P*-values were considered statistically significant form the normal group. Abbreviations: MS, medial septum; DBS, deep brain stimulation: Dist, distance.

D. Changes in AChE activity induced by MS-DBS

In the medial prefrontal cortex, AChE activity declined in the lesion and implantation groups (Figure 11), although there were no statistically significant differences from the normal group. AChE activity in the stimulation group was higher than that in the normal group, and was significantly increased compared with that in the implantation group (*$p <$ 0.05). There was a statistically significant decline in hippocampal AChE activity in the lesion group (*$p < 0.05$) and implantation group (***$p <$ 0.001) compared with the normal group. Interestingly, hippocampal AChE activity in the stimulation group was similar to that in the normal group and significantly higher than that in the lesion ($p = 0.05$) and implantation groups ($p = 0.001$).

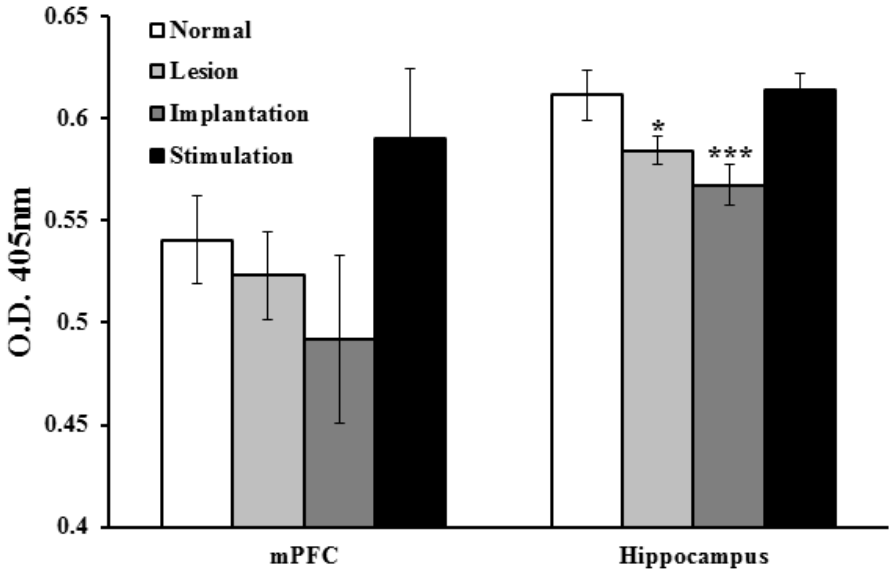

Figure 11. Effect of MS-DBS on AChE activity. In the prefrontal cortex, AChE activity of the stimulation group was increased compared with that in the implantation group. Hippocampal AChE activity in the lesion (*$p < 0.05$) and implantation (***$p < 0.001$) groups was significantly less than that in the normal group. However, AChE activity in the stimulation group was equivalent to that in the normal group. The AChE activity was expressed as the optical density of the colorimetric reading at 405 nm. Values are mean ± SEM. Abbreviations: OD, optical density.

E. Increase in DCX immunopositive cells induced by MS-DBS

The number of hippocampal cells containing DCX was quantified using immunohistochemistry, and expressed in various neurogenesis stages from the differentiation phase to the axonal and dendrite targeting phase (Figure 12). The lesion group showed a significant decline in the numbers of DCX immunopositive cells to 77.6% of normal group values (**$p < 0.01$), confirming the effect of the damaged basal forebrain cholinergic neurons on hippocampal neurogenesis. The implantation group did not show a significant difference from the lesion group ($p = 0.142$). However the stimulation group showed a significant increase in number of DCX immunopositive cells compared with the lesion group ($p = 0.002$).

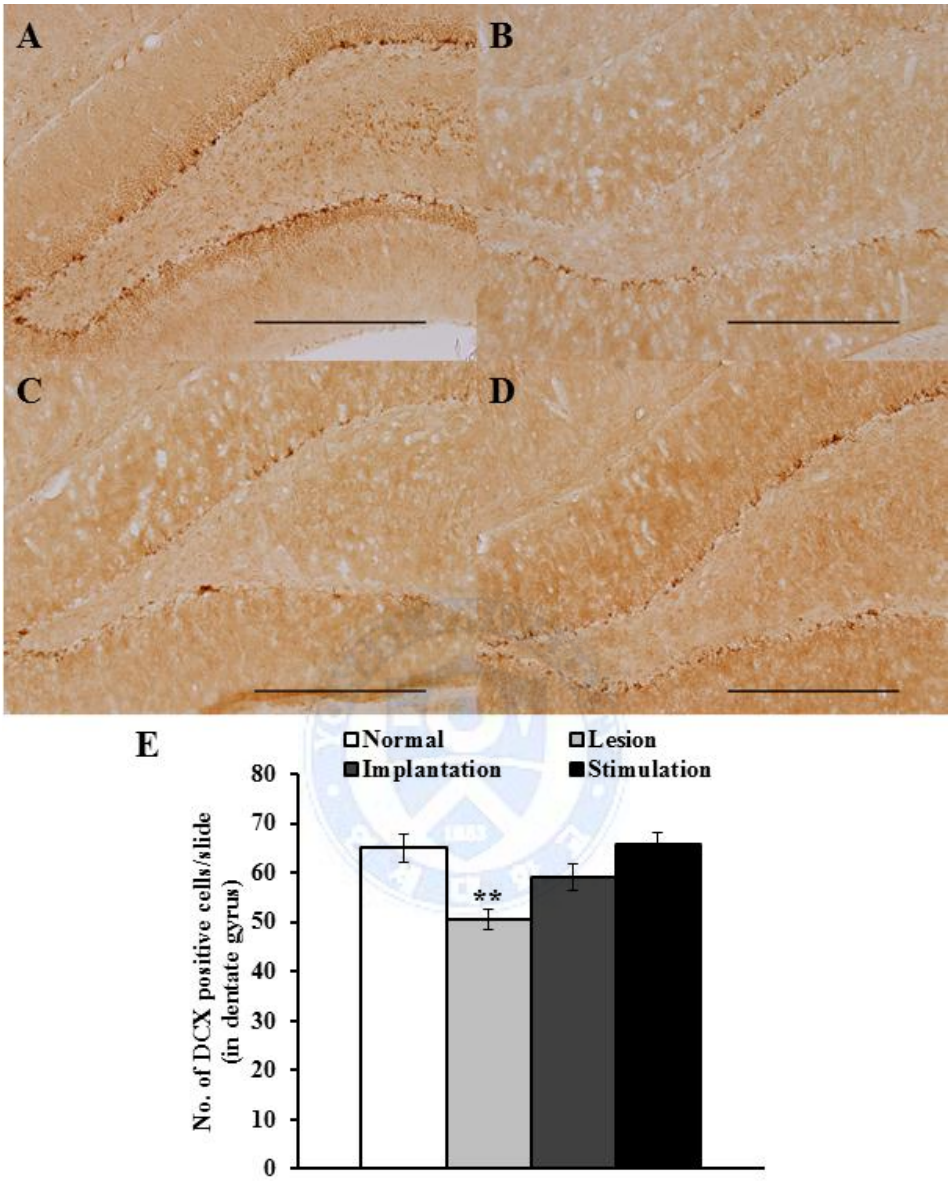

Figure 12. Effects of MS-DBS on adult hippocampal neurogenesis revealed by DCX immunohistochemistry. Representative pictures show the effects of basal forebrain cholinergic deficits and MS-DBS on hippocampal neurogenesis (A-D). (A) The normal group. Many DCX immunopositive

cells were observed in the normal group. However, the number of these cells was decreased in the lesion (B) and implantation (C) groups, in which vasal forebrain cholinergic neurons were damaged, but not in the stimulation group (D). The number of the immunopositive cells (E) in the lesion group was significantly different from that in the normal group (**$p < 0.01$). However, there was no difference between the lesion group and the implantation group. The number of DCX immunopositive cells was significantly increased in the stimulation group from the lesion group. Scale bar represents 500 μm.

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3. Effects by MS stimulation time

A. Changes in spatial memory acquisition and consolidation induced by MS stimulation time

The results of water maze training are shown in Figure 13. In all groups, the latencies on the first day (over 30 seconds) were decreased to less than 17 seconds on the final day of the training trails. These data show progressive learning of the hidden platform location. The pre-stimulation group performed like the normal group and probe-stimulation group performed like the lesion group. The latencies of the training-stimulation group were slightly longer that of the normal group; however, these differences were not significant.

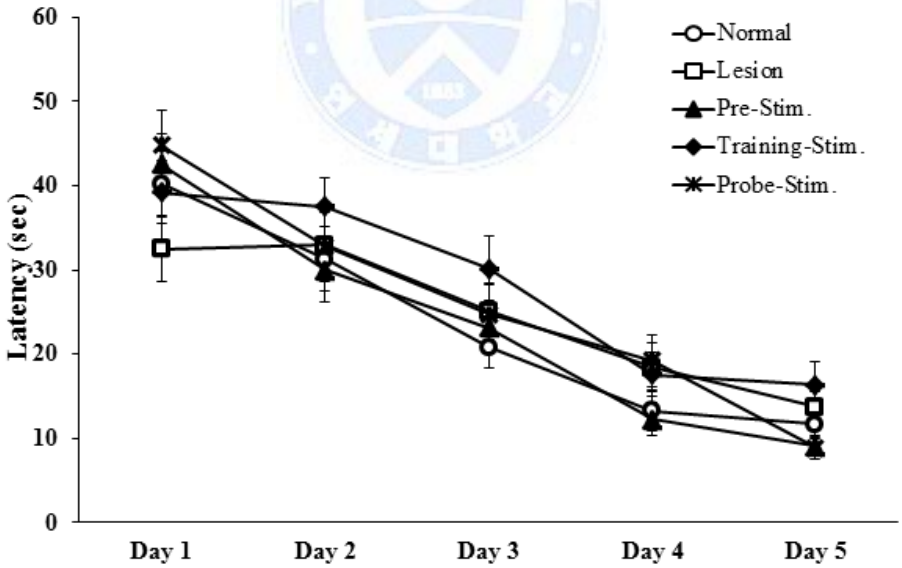

Figure 13. Effect of MS-DBS on spatial memory acquisition depending on stimulation time. Latency indicates the time required for the rat to find the

escape platform during training trials. All groups gradually acquired the location of the platform. The training-stimulation group shows slightly delayed memory acquisition. Data are shown as mean ± standard error of the mean.

B. Changes in spatial memory recall induced by MS stimulation time

In the probe test of the water maze (Table 1), the speed, distance, and time spent in the target quadrant were not significantly different between the groups. However, it is appears that there was spatial memory impairment associated with cholinergic deficit, as evidenced by the time spent in the target quadrant. On average, the normal group spent more than 10 seconds longer than chance level (15 seconds), whereas the other groups spent only 5 seconds longer than chance. The amount of time in the platform zone of for lesion group was significantly decreased to 15% of normal group values (*$p < 0.05$), whereas it was only decreased to 72% ($p = 0.442$) and 66% ($p = 0.389$) for the training-stimulation and probe-stimulation groups, respectively. Moreover, the pre-stimulation group spent a similar amount of time as the normal group (0.99 seconds and 1.04 seconds, respectively) in the platform zone. The mean number of platform crossings was 2.25 in the normal groups, whereas it was 0.37 (**$p < 0.01$) in the lesion group was. The number of platform crossing was recovered in order of pre-stimulation (2.11), training-stimulation (1.88) and probe-stimulation (1.28) groups.

Table 1. Effects of MS-DBS on spatial memory by stimulation time.

	Speed (cm/sec)	Distance (cm)	Time in target quadrant (sec)	Time in platform zone (sec)	Number of crossing
Normal	21.2 ± 0.7	1272.1 ± 42.0	24.8 ± 2.4	0.99 ± 0.2	2.25 ± 0.5
Lesion	21.1 ± 0.9	1269.4 ± 55.9	19.4 ± 0.7	0.15 ± 0.1 *	0.37 ± 0.3 **
Pre-Stim.	19.7 ± 0.6	1185.8 ± 38.9	20.5 ± 2.8	1.04 ± 0.2	2.11 ± 0.4
Training-Stim.	21.3 ± 0.6	1282.0 ± 39.3	21.1 ± 3.1	0.71 ± 0.1	1.88 ± 0.5
Probe-Stim.	20.3 ±0.7	1222.2 ± 44.1	22.1 ± 3.4	0.66 ± 0.2	1.28 ± 0.5

One-way analysis of variance was used to analyze data from probe test. Data are shown as mean ± SEM. *P*-values were considered statistically significant form the normal group. *$p < 0.05$, **$p < 0.01$.

C. Changes in AChE activity indeced by MS stimulation time

There was no restoration of AChE activity associated with MS-DBS, except in the MS and DB of the probe-stimulation group as shown in Figure 14. In the FC, AChE activity was significantly reduced in the lesion (**p < 0.01), pre-stimulation (***p < 0.001), training-stimulation (***p < 0.001), and probe-stimulation groups (***p < 0.001), compared with the normal group (Figure 14 A, p < 0.005). In the MS and DB, AChE activities of the lesion (**p < 0.01), pre-stimulation (**p < 0.01) and training-stimulation groups (**p < 0.01) were significantly decreased, whereas AChE activities were similar to the normal group in the probe-stimulation groups (Figure 14 B). In the hippocampus, AChE activities of the lesion and all stimulation groups were significantly decreased compared with the normal group (Figure 14 C, ***p < 0.001).

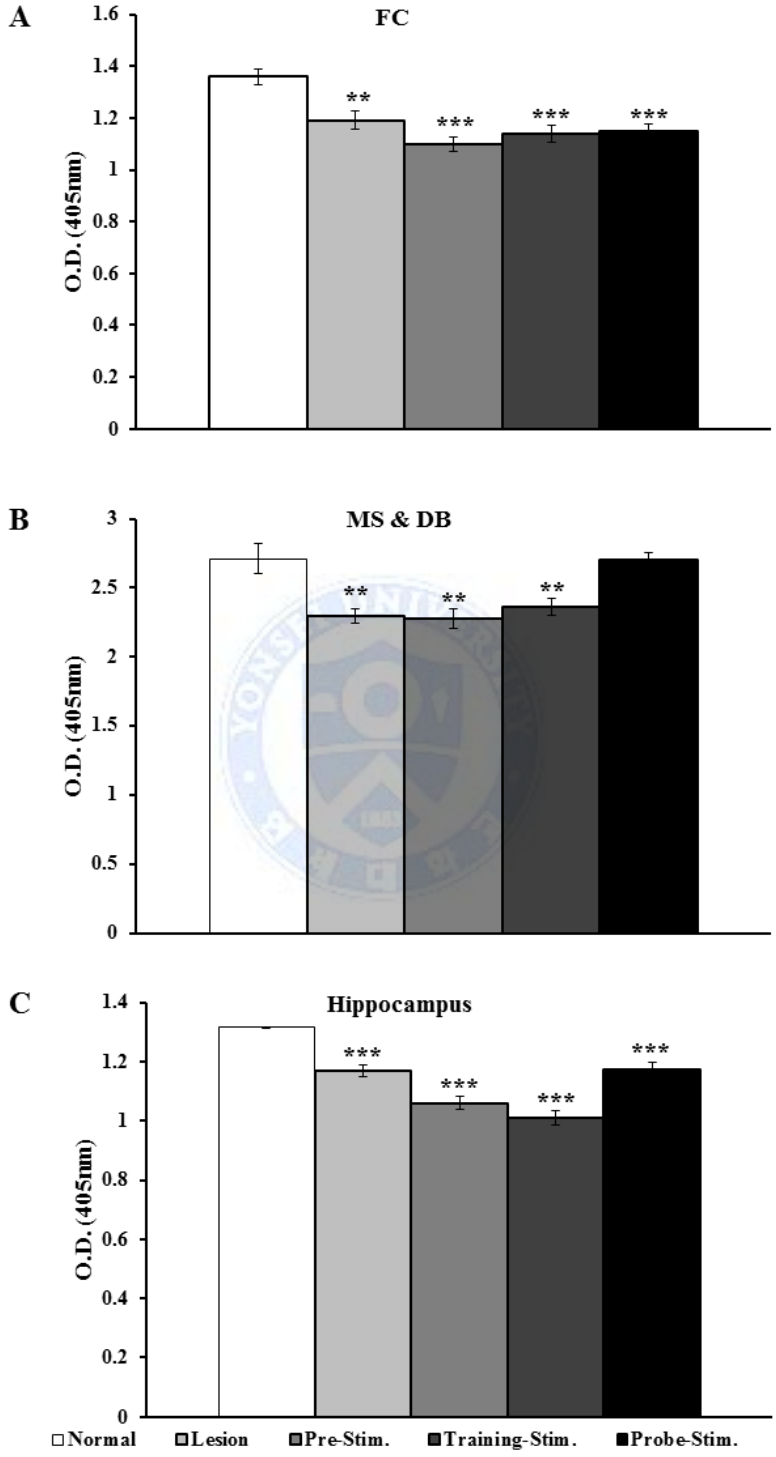
A
FC
O.D. (405nm)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
***
***
***
B
MS & DB
O.D. (405nm)
3
2.5
2
1.5
1
0.5
0
**
**
**
C
Hippocampus
O.D. (405nm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
***
***
***
***
Normal
Lesion
Pre-Stim.
Training-Stim.
Probe-Stim.

Figure 14. Effect of MS-DBS on AChE activity. (A) AChE activity in the FC. AChE activities of the lesion and all stimulation groups were significantly decreased compared with the normal group (B) AChE activity in the MS and DB. It was restored only in the probe-stimulation group. (C) AChE activity in the hippocampus. Hippocampal AChE activities in the lesion and all stimulation groups were significantly lesser than that in the normal group. The AChE activity was expressed as the optical density at 405 nm. Values are mean ± SEM. *P*-values were considered statistically significant form the normal group. $^{**}p < 0.01$, $^{***}p < 0.001$

D. Changes in GAD 65/67 expression induced by MS stimulation time

The level of GAD 65/67 was measured to determine the activity level of GABAergic neurons, which are one of the main components in the projection from the basal forebrain to the hippocampus (Figure 15). The expression level of GAD 65/67 was not significantly different from the normal in the FC (Figure 15 A), MS, and DB (Figure 15 B) in rats with cholinergic lesions or MS-DBS. However, the hippocampal expression level of GAD 65/67 was markedly decreased compared with the normal group in the pre-stimulation ($^{***}p < 0.001$), training-stimulation ($^{**}p < 0.01$), and probe-stimulation groups ($^{*}p < 0.05$).

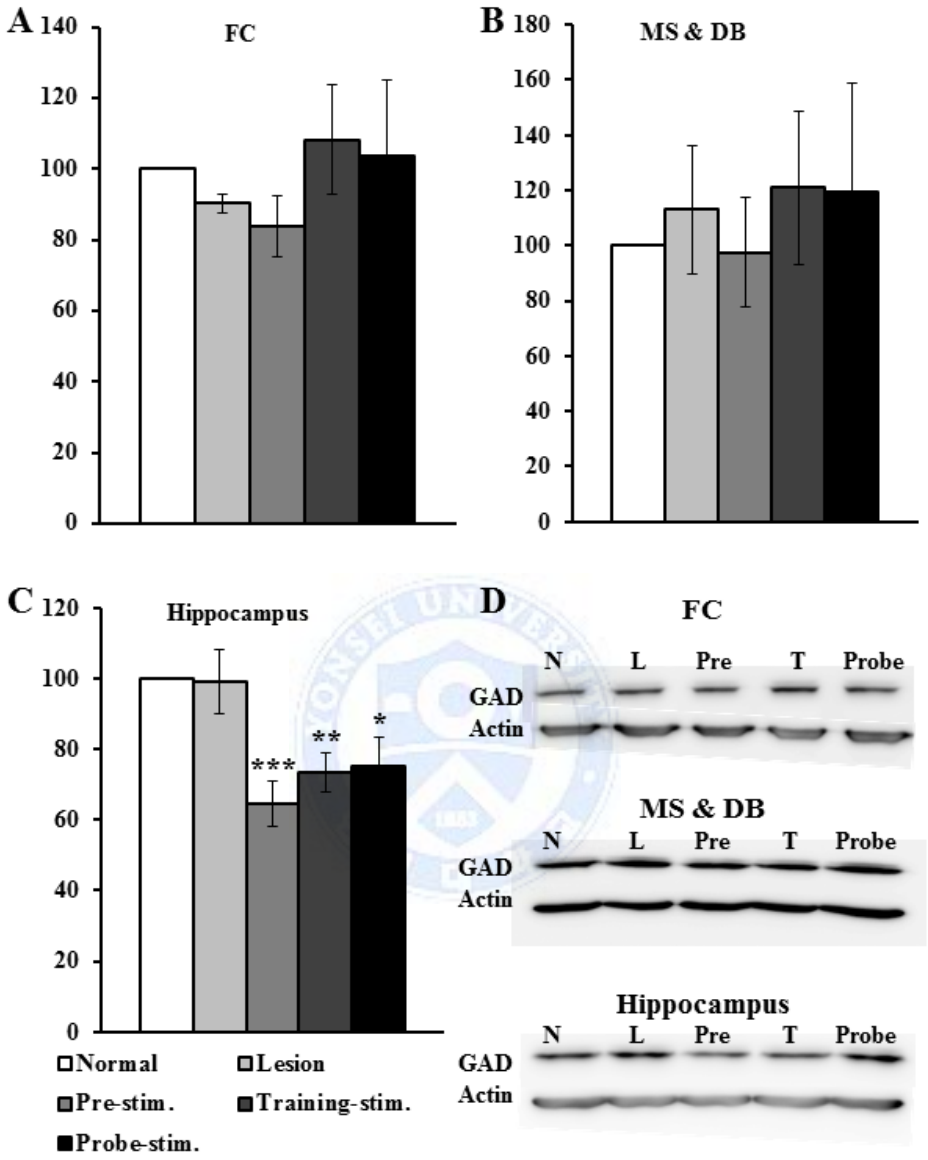

Figure 15. Expression levels of GAD 65/67 associated with MS-DBS. The expression level of GAD 65/67 was not different in the FC (A), and MS and DB (B) for all groups. (C) The hippocampal level of GAD 65/67 was significantly decreased relative the normal group, regardless of stimulation

time (Pre-stimulration, ***$p < 0.001$; training stimulation, **$p < 0.01$; probe stimulation, *$p < 0.05$). (D) Representative results of western blotting. Indices are expressed as a percentage of the values for the normal. Values are mean ± SEM. *P*-values were considered statistically significant form the normal group.

E. Changes in BDNF expression induced by MS stimulation time

Western blotting was also performed to measure the changes in expression of BDNF as a function of stimulation time (Figure 16). The expression level of BDNF tended to increase in all groups that received stimulation. In the FC, the level of BDNF was significantly increased regardless of time of stimulation (pre-stimulation, *$p < 0.05$; training-stimulation, *$p < 0.05$; probe-stimulation, **$p < 0.01$). The highest levels of BDNF in the MS and DB, and the hippocampus were expressed in the probe-stimulation group. Then, the expression level was increased in order to training-stimulation than pre-stimulation. However, these differences were not significant.

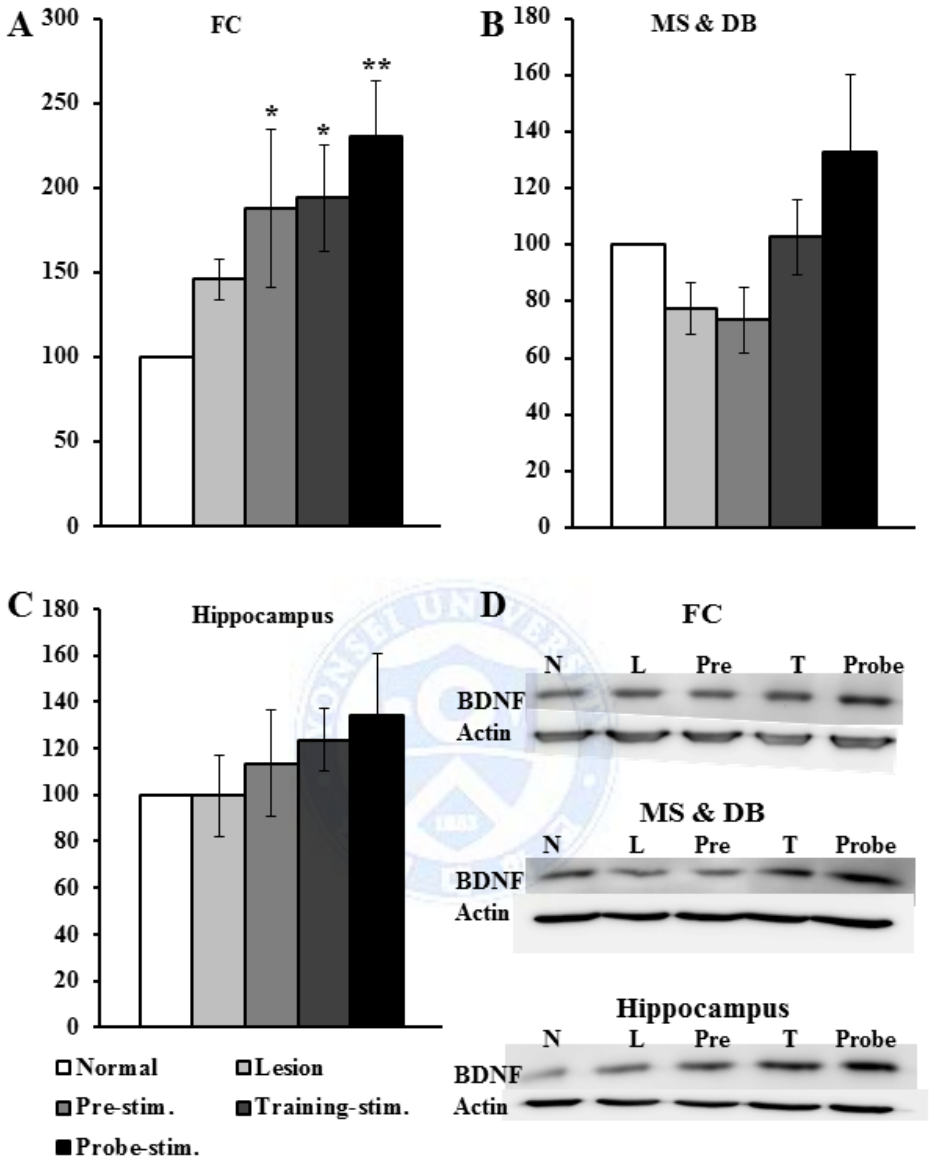

Figure 16. Expression levels of BDNF associated with MS-DBS. (A) The expression level of BDNF. It was significantly increased in all stimulation groups in the FC. And it shows an increasing trend in the MS and DB (B) and hippocampus (C). (D) Representative results of western blotting. Indices

are expressed as a percentage of values for the normal group (mean ± standard error).

F. Changes in the number of DCX and Sox2 immunopositive cells induced by MS stimulation time

To evaluate the effect of time dependent MS-DBS on neurogenesis and differentiation, the neuronal progenitor cells (Sox2, red) and neuroblasts or post-mitotic immature neurons (DCX, green) were quantified (Figure 17). The lesion group showed a significnat decrease in the number of Sox2 (***$p < 0.001$) and DCX (*$p < 0.05$) immunopositive cells dompared to the normal group (57.3% and 65.7%, respectively). The pre-, training-stimulation groups showed a slight decline in the number of Sox2 and DCX cells compared to the normal group. However, there was no significant difference. The normal percentage values of Sox2 and DCX immunopositive cells were: pre-stimulation group, 90.9% and 78.4%, respectively; training-stimulation group, 85.5% and 75.5%, respectively. However, the probe-stimulation groups showed significant decreases in the number of Sox2 and DCX immunopositive cells compared with normal group values (72.9% and 60.5%, respectively,* $p < 0.05$).

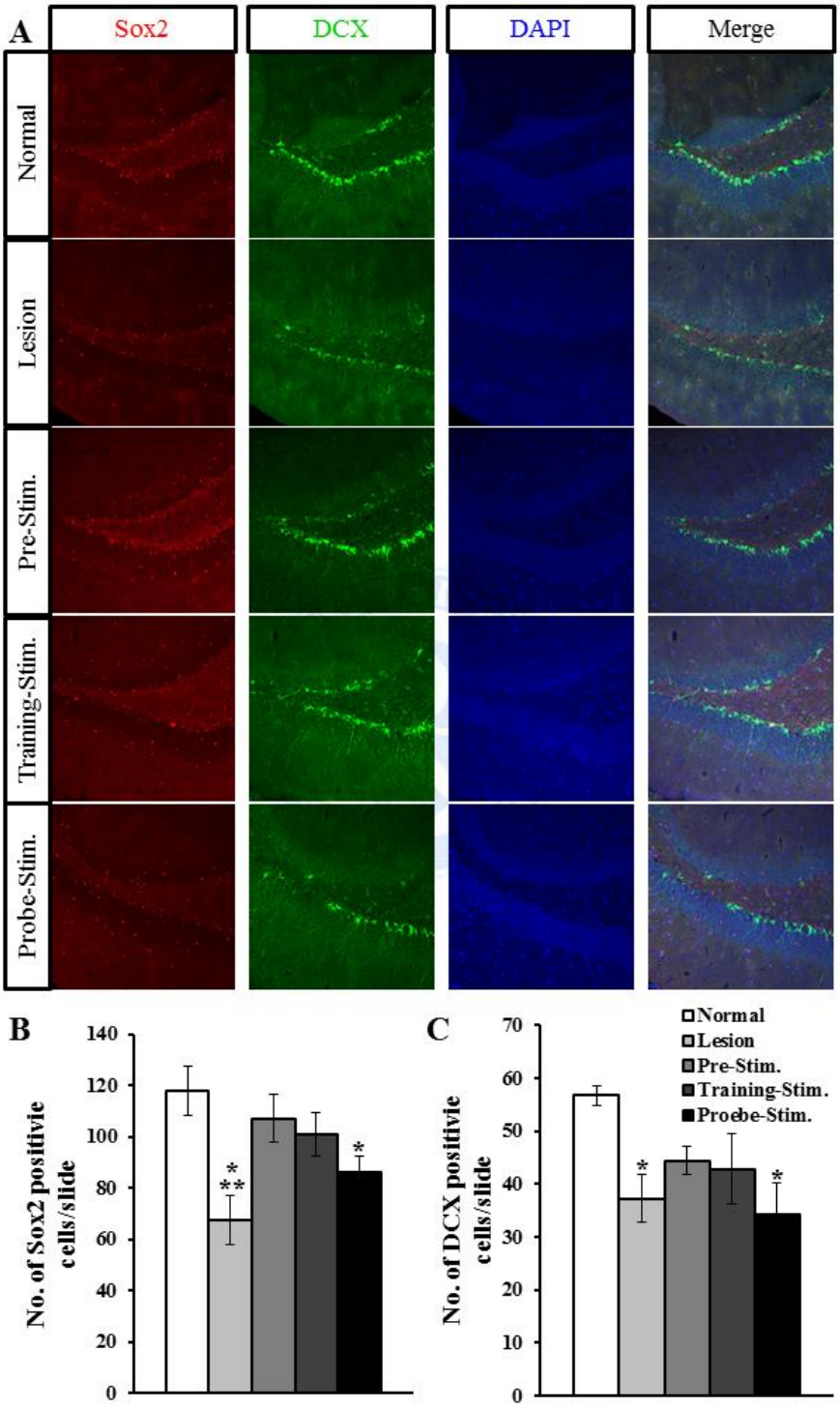
A
Sox2
DCX
DAPI
Merge
Normal
Lesion
Pre-Stim.
Training-Stim.
Probe-Stim.
B
No. of Sox2 positivie cells/slide
140
120
100
80
60
40
20
0
**
**
*
C
No. of DCX positivie cells/slide
70
60
50
40
30
20
10
0
*
*
Normal
Lesion
Pre-Stim.
Training-Stim.
Proebe-Stim.

Figure 17. Effects of time dependent MS-DBS on adult hippocampal neurogenesis. (A) Representative immunofluorescence images showing the effects of time dependent MS-DBS on neurogenesis and differentiation. Hippocampal dentate gyrus sections stained for Sox2 (red), DCX (green), and DAPI (blue) are shown. The number of Sox2 (B) and DCX (C) immunopositive cells in the lesion and probe-stimulation groups was significantly lower than that in the normal group. However, the number of Sox2 immunopositive cells in the pre- and training-stimulation groups was slightly higher than that in the lesion group. Values are mean ± SEM. *p < 0.05, ***p < 0.001

IV. DISCUSSION

The possibility that DBS can enhance memory function has been reported in some clinical cases and experimental studies.[5-7,9] DBS therapy is a minimally invasive and reversible method. However, the mechanisms of DBS are unclear, and effective stimulation parameters, such as duration and location, are still not well defined. Thus, this study was designed to clarify the effects of MS-DBS on memory performance. This study was divided into three sub-experiments: establishment of a memory impaired rat model, confirmation of the effects of MS-DBS on spatial memory, and confirmation of the stage of memory affected by MS-DBS.

In the first experiment, ICV 192 IgG-saporin injection led to a decrease in the number of ChAT positive cholinergic cells in the in the basal forebrain. The cholinergic damaged rats showed impaired spatial memory and reduced glucose metabolism and cholinergic activity in the cingulate cortex. In addition, AChE activity was decreased by the cholinergic lesion in the FC and hippocampus (third experiment).

The results suggest that basal forebrain cholinergic neurons could regulate the cingulate cortex. Basal forebrain cholinergic projections to the cingulate cortex have been verified by histologic studies. Electrolytic lesions of the MS and diagonal band region reduce diffuse AChE staining in the cingulate cortex.[29] A study using a fluorescent AChE tracer showed that pathways to the cingulate cortex are derived from all subdivisions of the diagonal band and the nucleus basalis of Meynert (NBM, a part of basal forebrain cholinergic complex).[30] Therefore, it is possible that basal forebrain

cholinergic denervation caused by 192 IgG-saporin affects the activity of the cingulate cortex. Moreover, animal tests examining the relationship between basal forebrain cholinergic neuron deficits and cerebral glucose metabolism have also been conducted. Bilateral damage to the NBM caused a persistent suppression of cortical glucose metabolism.[31] An experimental study of damaged cholinergic neurons using bilateral intraventricular injection of 192 IgG-saporin reported sustained hippocampal and cortical hypometabolism for 4.5 months.[32] In this study, cholinergic neurons damaged using bilateral intraventricular injection 192 IgG-saporin showed a significant reduction of cortical glucose metabolism in the cingulate cortex that was maintained for 3 weeks. However, in a study of damage to the left NBM in baboons, glucose metabolism slowly recovered to within almost normal limits within 6-13 weeks,[33] even though significant metabolic depression of the entire ipsilateral cingulate cortex was observed on the fourth day after lesion. Glucose metabolism of the hippocampus was profoundly suppressed in the third week, but returned to control values after 3 months following damage of the MS.[34] After unilateral or bilateral lesion of the NBM with ibotenic acid, glucose metabolism decreased on the third day and recovered between 3 weeks to 3 months.[35,36] Some studies reported recovery of hypometabolism, following a partial lesion of the cholinergic system, thus; it is possible to spare cholinergic function of intact areas by damaging only the NBM or MS. On the other hand, intraventricular injection of 192 IgG-saporin damages cholinergic cells expressing nerve growth factor receptor p75, likely damaging most of the cholinergic projections from the basal forebrain to the

cortex or hippocampus. Differences in hypometabolism recovery could arise from differences in methods of cholinergic lesion.

The results also demonstrate that spatial memory impairment is related to cingulate cortical dysfunction. Degeneration of basal forebrain cholinergic neurons and reduction of cholinergic markers in both the hippocampus and cortex have been observed and correlated with cognitive decline in patients with AD.[11,37] It is well documented that the hippocampus plays an essential role in the formation of memory.[38,39] However, extra-hippocampal structures, especially neocortical regions, have been investigated for crucial roles in long-term memory.[40] Animal studies showed that a the anterior cingulate cortex is associated with remote memory.[41,42] The cingulate cortex is connected to the hippocampus both directly and indirectly through the retrosplenial cortex.[43,44] Additionally, there were evidence to suggest that the cingulate cortex forms a functional circuit, which is associated with spatial learning and memory. Lesions of the fornix, one of the major connections of the hippocampus, induce a decrease in fos expression in the entire circuit including the hippocampus, anterior cingulate cortex, retrosplenial cortex and anterior thalamic nuclei.[45]

Taken together, these results indicate that the spatial memory impairment in the animal model mimicking cholinergic denervation in patients with AD results from cholinergic dysfunction in the hippocampus and cingulate cortex, and glucose hypometabolism in the cingulate cortex.

In the second experiment, 2 weeks of electrical stimulation (60Hz) in the MS was delivered to examine the effects of MS-DBS on spatial memory

recovery. In the probe test of the water maze, the performance of the stimulation group was not different to the normal group. Additionally, DCX positive cells in the dentate gyrus (DG) were recovered by MS-DBS. It is not clear which stage of memory processing was affected; however, interestingly the impairment in spatial memory by 192 IgG-saporin was rescued by the MS-DBS. A single shock or brief tetanic stimulation of the MS was found to sharply enhance population spikes evoked in the hippocampal CA1 pyramidal cell layer, and a comparable facilitation of population spikes was produced by microapplication of acetylcholine at the same site.[46] The cholinergic and GABAergic septal drive plays a role in the tuning of signal conversion within the hippocampus.[47] The results of the second experiment demonstrated an increase in, hippocampal AChE activity induced by MS-DBS for 2 weeks. This shows that the changes in hippocampal function induced by the MS-DBS could involve a cholinergic role. Electrical stimulation of the MS could possibly affect these systems, although we did not investigate glutamatergic changes in this study. Moreover, the increase in hippocampal acetylcholine associated with MS-DBS could affect the hippocampal theta rhythm and spatial memory. Considerable research has demonstrated correlations between hippocampal theta rhythm and learning and memory,[48-50] and positive correlations between hippocampal acetylcholine and theta rhythm have been reported.[51,52] However, unless changes in the levels of other neurotransmitters are directly confirmed, their effects cannot be excluded. Low-frequency stimulation of the MS and commissural fibers induces NMDA-dependent, long term potentiation (LTP)

of hippocampal synapses.[53]

The other potential mechanism through which MS-DBS could enhance spatial memory is via hippocampal neurogenesis. Adult hippocampal neurogenesis is restricted to the subgranular zone of the DG, and new neurons continue to be generated throughout life. Hippocampal neurogenesis is thought to be associated with hippocampus-dependent memory. Knockdown of adult hippocampal neurogenesis impairs spatial memory,[54] and treatments that disrupt hippocampal neurogenesis impair hippocampus-dependent memory.[55,56] DCX is a protein that promotes microtubule polymerization and can serve as a marker of adult neurogenesis in late mitotic neuronal precursors and early post-mitotic neurons.[57,58] DCX is expressed specifically in newly generated neurons and not expressed in GFPA-expressing astrocytes. Additionally, there is an absence of DCX expression during neuronal regeneration or lesion induced gliogenesis.[59] Sox2 is expressed in the adult brain in proliferating precursor cells.[60] And it is observed mainly by type 1 and type 2a cells not by type 2b or type 3 cells.[61] Stone at al. reported that electrical stimulation of the entorhinal cortex promotes proliferation in the DG and integration of these neurons into hippocampal circuits, thus supporting spatial memory.[9] It has been reported that cholinergic forebrain lesions decrease neurogenesis.[62] The results of the current study are similar to the above report. The number of DCX-positive neurons reduced by the administration of 192 IgG-saporin. Interestingly, stimulation of the MS after cholinergic damage recovers DCX immunopositive cells, suggesting that MS-DBS promotes neurogenesis in

the DG, which, in turn, may promote hippocampus-dependent learning and memory. Additionally, in the third experiment, the number of Sox2 and DCX immunopositive cells decreased after cholinergic lesion but recovered after the application of MS-DBS for 5 days (pre- and training-stimulation). However, MS-DBS for 2 h was not sufficient to improve neurogenesis. This could be caused by the short time interval between stimulation and sacrifice.

Unexpectedly, the behavioral test deficits of lesioned rats seen during the probe trials appear to be partially reversed by the electrode implantation, even in the absence of electrical stimulation. Insertion effect is a common phenomenon after deep brain stimulation surgery for Parkinson disease or neuropathic pain. In a clinical study, motor symptoms of patients with Parkinson's disease improved without stimulation in the early postoperative period.[63] In another clinical study, 43% of patients with neuropathic pain had a substantial reduction in pain scores in the absence of stimulation.[64] However, the underlying mechanisms for this are still unclear. A previous study indicated that levels of glucose metabolism could be changed in the absence of stimulation after the insertion of an electrode into the subthalamic nucleus of patient with PD.[65] In the unstimulated hippocampus, enzyme activity can be changed in the narrow area surrounding the electrode.[66] Because the insertional effects are still unclear, relevant studies are needed in the future.

The results of the second experiment suggest that spatial memory improvement by MS-DBS could be associated with cholinergic regulation and neurogenesis. However, the affected stage of memory among acquisition,

consolidation, and retrieval was not clear because the stimulation was delivered during the all memory processes. Therefore, the third experiment was performed to detect the stage of memory affected by MS-DBS. In this experiment, the most effective memory enhancement occurred in the pre-stimulation group. The memory enhancement may be mainly due to the increasing of BDNF expression by the stimulation.

In this experiment, MS-DBS increased BDNF expression not only in the MS (stimulated site) but also in the hippocampus and FC. In addition, the increased BDNF expression was maintained for 1week after the cessation of stimulation. BDNF release associated with electrical stimulation has been reported.[67,68] BDNF has a critical role in modulation a variety of neural function such as membrane excitability, activity dependent synaptic plasticity and neurogenesis. Therefore, increasing the level of BDNF by MS-DBS could improve spatial memory. Memory formation is thought to involve both short-term change such as variations in electrical properties and long term structural changes in synapses. A significant role of BDNF in LTP in the hippocampus and cortex has been revealed. Immediate release of BDNF modulates the induction and early maintenance phase of LTP.[69] Additionally, sustained BDNF release and activation of tyrosine kinase B receptors are involved in the formation of stable LTP.[70] The increase in BDNF level induced by MS-DBS could influence spatial memory improvement through the modulation of hippocampal LTP. Additionally, activity-dependent morphological changes have been shown to be regulated by BDNF. $TrkB^{-/-}$ knockout mice had lower densities of synaptic contacts

and important structural alterations in presynaptic aoutons, such as decreased density of synaptic vesicles.[71] BDNF is associated with increases in the spine density of CA1 pyramidal neurons.[72] Additionally, BDNF is involved in both spatial memory formation and maintenance.[73] Moreover, elevation of hippocampal BDNF is associated with increase numbers of newly generated cells in the granule cell layer.[74] The results of this study also suggest that increasing the BDNF level by stimulation could improve spatial memory acquisition and maintenance through hippocampal neurogenesis. However, differences in BDNF expression levels at the stimulation site could be induced by altering the interval between stimulation and sampling. However, the increase in BDNF expression was maintained in the FC and hippocampus. Therefore, the pre-stimulation group could have had the most enhanced memory because it had the longest period of increased BDNF.

It is also assumed that hippocampal GABAergic suppression by MS-DBS is involved in memory restoration. Two major neurotransmitter systems of the MS, GABA and ACh regulate hippocampal activity and memory.[75,76] The memory impaired rat model in this experiment had selectively damaged cholinergic neurons. Therefore, the intact GABAergic system could be relatively hyper-active. MS-DBS might regulate the balance between damaged cholinergic and intact GABAergic neurons. Additionally, GABAergic regulation of neuronal architecture was reported. A hippocampal $GABA_A$ receptor agonist impairs spatial memory[77] and mutant mice in which enhanced $GABA_BR$ activity reduced the expression of immediate-early genes encoded the protein Arc.[78] Arc is essential for the

consolidation of synaptic plasticity and memories.[79] Furthermore, Arc knockout mice fail to form long lasting memories.[80] Therefore, spatial memory restoration might change synaptic plasticity by suppressing GABAergic activity.

In results of training phase, pre stimulation was more effective than right after training stimulation. Therefore memory acquisition could be associated with MS-DBS, rather than memory consolidation is. For the memory retrieval, the memory was more enhanced in the pre-stimulation group than probe-stimulation. Therefore, the enhanced spatial memory associated with MS-DBS might mainly result from increased BDNF, rather than from direct electrical stimulation of cholinergic or GABAergic neurons.

V. CONCLUSION

Deficit of basal forebrain cholinergic neurons using 192 IgG-saporin induced spatial memory impairment. Additionally, the deficit of these cholinergic neurons was associated with not only hippocampal dysfunction but also glucose hypometabolism in the cingulate cortex.

MS-DBS (60 Hz, 120 μs, 100 μA) for 2 weeks restored spatial memory impairment by increasing neurogenesis. In the time dependent simulation, it appeared that increased BDNF associated with MS-DBS could enhance spatial memory formation and maintenance. Therefore the pre-stimulation group could have had the most enhanced memory because it had the longest period of increased BDNF. The enhanced spatial memory associated with MS-DBS might mainly result from increased BDNF in the frontal cortex and hippocampus, rather than from direct electrical stimulation of cholinergic or GABAergic neurons.

Based on the results of this study, I propose the use of MS-DBS during the early stage of disease in order to restore spatial memory impairments.

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ABSTRACT(IN KOREAN)

192 IgG-saporin을 이용한 흰쥐 기억력 손상 모델에서 내측 중격 심부뇌자극이 기억력 회복에 미치는 효과

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정 다 운

심부뇌자극에 의한 기억력 향상의 가능성이 최근 보고되고 있지만 그 효과의 기초가 되는 기전은 정확하게 알려져 있지 않다. 이 연구에서는 기억손상 동물 모델을 이용하여 심부뇌자극의 치료효과와 그 기전을 이해하고자 수행하였다. 첫 번째로, 알츠하머 병의 콜린성 부족을 모방하며 공간기억이 손상된 흰쥐 모델을 확립하였다. 두 번째로, 그 흰쥐 모델에서 내측 중격 심부뇌자극에 의한 효과를 연구하였다. 또한 세

번째로, 영향을 받은 기억의 단계를 확인하기 위해 각기 다른 시간에 자극을 하였다.

첫 번째 실험에서, 선택적인 콜린성 손상을 유발하기 위해 192 IgG-saporin을 대뇌 뇌실에 주입하였다. 2주후, 콜린성 세포의 손상에 의해 모리스 수중미로 실험에서 공간기억의 손상과 [F-18] 플루오로데옥시글루코스 양전자 방사 단층 촬영에서 대상피질의 포도당 대사 저하가 나타났다. 두 번째 실험에서는 내측 중격 심부뇌자극의 치료효과를 확인하기 위해 60Hz 주파수의 자극을 내측 중격에 전달하였다. 2주의 자극 동안, 내측 중격 심부뇌자극은 공간기억을 향상시켰고 해마의 DCX 면역양성 세포를 증가시켰다. 내측 중격 심부뇌자극에 의해 영향을 받은 기억의 과정을 찾기 위해 자극은 다음과 같은 각기 다른 시간에 전달되었다: 사전-자극, 수중미로 시작 전 5일; 훈련-자극, 수중미로 훈련기간 5일; 프로브-자극, 수중미로 프로브 테스트 바로 전의 2시간 자극. 가장 효과적인 기억 회복은 사전-자극군에서 나타났다. 훈련 기간 동안 사전-자극군의 플랫폼까지 이른 시간은 정상군과 비슷한 시간을 나타냈다. 또한, 사전-자극군은 다른 자극군 보다 플랫폼의

위치를 더 잘 상기하였다. 사전-자극군의 BDNF는 전뇌 피질과 해마에서 증가하고 유지되었다. 글루타메이트를 GABA로 탈카르복실화 시키는 효소인 GAD 65/67은 해마에서 저하되었다. 하지만 사전-자극군의 아세틸콜린콜린 가수분해 효소는 손상군과 차이가 없었다. 이러한 결과는 내측 중격 심부뇌자극에 의한 공간기억력 향상은 직접적인 콜린성 혹은 GABA성 신경세포의 전기자극에 의한 것 보다는 주로 전뇌 피질과 해마에서 올라간 BDNF에 영향을 받은 것으로 보여진다.

종합하면, 콜린성 신경손상에 의한 기억력 저하는 내측 중격 심부뇌자극 (60Hz)에 의해 향상될 수 있다. 그리고 이는 BDNF발현의 증가와 신경발생과 유의한 상관 관계를 보였다. 이 연구의 결과에 기초하여, 공간기억 손상을 회복시키기 위한 내측 중격 심부뇌자극의 질병 초기 사용을 제안한다.

핵심되는 말 : 심부뇌자극, 공간기억, 내측 중격, 뇌 유래 신경 성장인자, 신경발생

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