The effects of electrical stimulation of the nucleus basalis magnocellularis/the reuniens thalamic nucleus on memory function in a rat model of dementia

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Directed by Professor Jin Woo Chang

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June 2013

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ABSTRACT

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Deep brain stimulation has been used to treat various neurological disorders. Recently, some studies suggest that deep brain stimulation can treat Alzheimer's disease. Memory deficits associated with a reduction in cholinergic projections in the cortex and hippocampus are the one of the characteristics of Alzheimer's disease. This study was designed to determine the effect of electrical stimulation of the nucleus basalis magnocellularis or the reuniens thalamic nucleus on spatial memory using a rat model mimicking the basal forebrain cholinergic deficits of Alzheimer's disease. We damaged basal forebrain cholinergic neurons using 192 IgG-saporin. Rats in the stimulation group received stimulation of the nucleus basalis magnocellularis and the reuniens thalamic nucleus daily beginning one week after surgery until the start of behavioral testing. The Morris water maze and the object in place were used to evaluate visuo-spatial and visuo-working

memory 2 weeks after surgery. Choline acetyltransferase immunohistochemistry was performed to examine 192 igG-saporin-induced cholinergic lesions in the medial septum, and acetylcholinesterase assay was used to evaluate acetylcholinesterase activity in the medial prefrontal cortex and hippocampus. Also, we used western blot analysis to examine changes in GABAergic and glutamatergic systems. The stimulation group of the nucleus basalis magnocellularis showed excellent performance in the probe trial of the Morris water maze task. In the stimulation group of the nucleus basalis magnocellularis, the reduction of the glutamate and glutamic acid decarboxylase was induced in the medial prefrontal cortex. The stimulation group of the reuniens thalamic nucleus showed slightly higher performance in the object in place task. In the stimulation group of the reuniens thalamic nucleus, the reduction of the glutamate in the medial prefrontal cortex and glutamic acid decarboxylase in the hippocampus was induced. Also, the acetylcholinesterase activity was significantly increased as compared to the implantation group of the reuniens thalamic nucleus. The present study demonstrates that stimulation of the nucleus basalis magnocellularis appears to predominately facilitate visuo-spatial memory, whereas stimulation of the reuniens thalamic nucleus appears to predominately facilitate visuo-working memory.

Key words: Deep brain stimulation(DBS), Reuniens thalamic nucleus(RE), Nucleus basalis magnocellularis(NBM), Glutamic acid decarboxylase(GAD), Acetylcholinesterase(AChE), GABA

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I. INTRODUCTION

As an effective neurosurgical method, deep brain stimulation (DBS) has provided beneficial effects for various movement disorders ranging from Parkinson's disease (PD) to essential tremor since the late 1980s [1]. Although DBS has been used for a long time and has therapeutic effects, the specific mechanisms underlying DBS are still under debate [2]. However, evidence supporting the possibility that DBS may work by modifying pathological activity within neural circuits has increased, and studies are now proceeding for a variety of disorders. In particular, many recent studies have aimed to test the effects of DBS on cognitive disorders observed in Alzheimer's disease (AD) and idiopathic PD with dementia.

Although AD is a rapidly growing geriatric disease, we still not have a full cure. Drugs such as Memantine, Tacrine, and Donepezil are currently used for treatment. However, their effectiveness is limited because they only delay

the onset of dementia. Therefore, it is necessary to find other treatment options. Toward this aim, some studies report that DBS can improve cognitive abilities in patients with AD. One study reports that stimulation of the entorhinal region during learning enhances visuo-spatial memory [3]. Another study reports that stimulation of the fornix and hypothalamus can delay or even reverse the progression of AD [4].

A characteristic pathologic feature of AD is the selective loss of cholinergic neuron in the basal forebrain (BF) [5]. The BF cholinergic complex, which comprises the medial septum (MS), horizontal and vertical diagonal band of Broca, and nucleus basalis magnocellularis (NBM), provides the major cholinergic projections to the cerebral cortex and hippocampus [6,7]. The cortex is a part of the nervous system that controls working memory, and the hippocampus plays an important role in memory consolidation [8,9]. Therefore, degeneration of the BF and its cholinergic projections is believed to be an important factor contributing to the cognitive decline and functional impairment in AD [7,10].

The NBM, located in the ventromedial region of the globus pallidus, and the anterior nucleus basalis project mainly to frontal, temporal, and parietal cortices [11]. The NBM is a complex and heterogeneous structure consisting of mainly cholinergic neurons and at least 20%–30% non-cholinergic neurons (GABA-ergic, glutamatergic, and peptidergic) [11,12]. The NBM is the major source of cortical acetylcholine (Ach) and has been shown to provide the major cholinergic innervation to the cerebral cortex [13]. The NBM in rodents is involved in a variety of learning and memory tasks, including the water maze and temporal discrimination learning tasks [14-16]. A study describing the effects of NBM lesion on Y-maze performance reports that lesions of the NBM may impair working memory performance due to a loss of NBM cholinergic neurons [17]. Another study suggests that stimulation of the NBM during an avoidance task could facilitate memory during the acquisition phase but not during the retention phase [13].

The reuniens thalamic nucleus (RE) lies ventrally to the midline of the

thalamus, above the third ventricle, and extends longitudinally throughout the thalamus [18]. The hippocampus densely projects to the medial prefrontal cortex (mPFC), although the mPFC has no direct projections returning to the hippocampus. However, the mPFC projects to the RE of the ventral midline thalamus, and the RE is the major source of afferents to the hippocampus. Therefore, the RE is a critical link between the mPFC and the hippocampal formation (HF), completing an important HF→mPFC→RE→HF loop [18]. In a recent study of this pathway, role of the RE in acquisition, consolidation, and retrieval of spatial memory in the Morris water maze was examined using a reversible inactivation approach [19].

In the present study, we examined whether unilateral electrical stimulation of the RE or NBM improves memory in a rat model mimicking the basal forebrain cholinergic deficits of AD.

II. MATERIALS AND METHODS

1. Dementia rat model

A. Animals

Thirty-eight male Sprague-Dawley (SD) rats weighing between 190 and 210 g were used at the beginning of this experiment. They were housed in groups of three to four in clear plastic cages with food and water, and they were maintained in a temperature- and humidity-controlled room with a 12-h light/12-h dark cycle.

Surgery was performed after a 1-week period of acclimation to the animal facility environment. Rats were randomly assigned to one of six experimental groups before surgery. The normal group (n=14) underwent no surgical procedures. The lesion group (n=7) had intraventricular administration of 192 IgG-saporin. The NBM implantation group (n=7) and NBM stimulation group (n=5) had intraventricular administration of 192 IgG-saporin and implantation of an electrode in the NBM. The RE implantation group (n=7) and RE stimulation group (n=5) had intraventricular administration of 192 IgG-saporin and implantation of an electrode in the RE. All experiments were conducted according to the guidelines of the Institutional Animal Care and Use Committee of Yonsei University. All efforts were made to minimize animal suffering and to reduce the number of rats used.

B. Induction of dementia rat model using 192 IgG-saporin

Rats in the five experimental groups, but not the normal group, 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. 8 μl

(0.63 μg/μl) of 192 IgG-saporin (Chemicon, Temecula, CA, USA) was bilaterally injected into the lateral ventricle (AP - 0.8 mm, ML ± 1.2 mm DV - 3.4 mm. The solutions were delivered at a rate of 1 μl/min and diffused for 5 min after each injection.

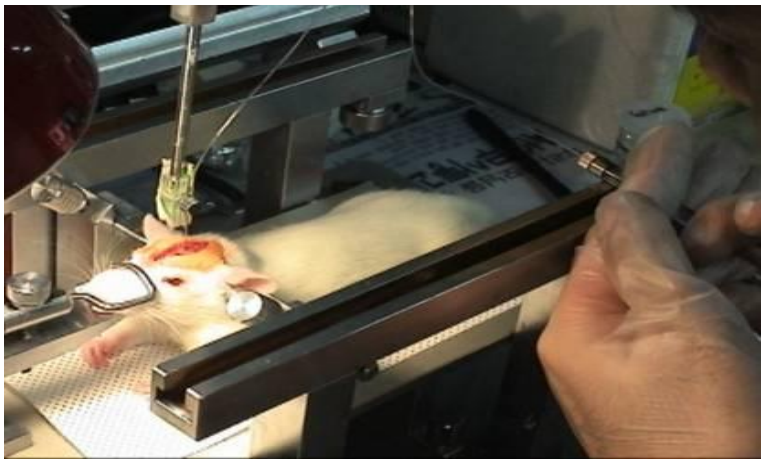

Fig 1. Dementia rat model By Injection of 192 IgG-saporin.

2. Deep brain stimulation

A. Electrode implantation

After the administration of 192 IgG-saporin, an additional hole was drilled in the skull at the level of the NBM (AP -1.32 mm, ML + 2.8 mm, DV -7.4 mm) of the rats in the NBM implantation and NBM stimulation groups, and an electrode (SNEX-100, contact diameter 0.1 mm, shaft diameter 0.25 mm, David Kopf Instruments, Tujunga, CA, USA) was inserted into the NBM. Dental cement (Long Dental Manufacturing, Wheeling, IL, USA) was used to firmly fix the stimulation electrode in

place to the skull surface, to help the scalp close easily, and to reduce the scar size after implantation of the electrode. Similarly, an additional hole was drilled in the skull at the level of the RE (AP -1.92 mm, ML + 0.2 mm, DV -7.2 mm) in rats in the RE implantation and RE stimulation groups. An electrode was inserted into the NBM and fixed in place with dental cement.

Fig 2. Electrode implantation into the rat brain.

B. Electrical stimulation

A stimulating electrode was implanted in the ipsilateral (right) NBM or RE. The electrode was a concentric bipolar 7.2 mm- and 7.4 mm-long stainless steel electrode (SNEX-100, contact diameter 0.1 mm, shaft diameter 0.25 mm, David Kopf Instruments, Tujunga, CA, USA). The stimuli were bipolar and electrical stimuli (120 Hz, 90 μs, 1v) delivered via a Grass A-300 pulsemaster (Grass instrument, Quincy, MA, USA). Stimulation parameters were monitored in real time at the beginning and end of stimulation using an oscilloscope (HDS 1022M, Owon, Korea). Rats were stimulated daily beginning one week after surgery until the start of behavioral testing (1 h per day, 1 week in total).

3. Behavior tests

A. Morris water maze

Apparatus

A circular dark water maze tank (200 cm diameter, 50 cm deep) located in a dimly lit room that was furnished with several extramaze cues, such as racks, a door, shelves, and posters mounted on the wall, was filled to a depth of 40 cm with water (maintained at 23℃ and dyed with nontoxic paint). A circular escape platform (10 cm diameter) was submerged 2 cm below the water surface in the center of one of the arbitrarily designated northeast (NE), southeast (SE), southwest (SW) or northwest (NW) orthogonal quadrants. The position of the animal was monitored by a camera mounted above the center of the pool. It was possible to record, in real time, the swim path during each trial and estimate escape latency, time spent in any designated area of the pool, and swimming speed.

Procedures

The Morris water maze test began 2 weeks after surgery. The test consisted of 5 consecutive days of training trials and 1 day of a probe trial. In the training trials, rats were given four acquisition trials per day with four different starting positions that were equally distributed around the perimeter of the maze. The task requires rats to swim to the hidden platform guided by distal spatial cues. The platform was located in a fixed position in the center of the SW quadrant of the pool throughout acquisition. For each trial, rats were released randomly from each of the four cardinal points from the edge of the pool and gently released facing the wall. Rats were given a maximum of 60 s to find the platform. After finding the platform, rats were allowed to remain on the platform for 10 s and placed in their home cages until the start of the next trial. Animals failing to find the platform in 60 s were guided by an experimenter, placed on the platform, and allowed to rest for 10 s. After completion of training, rats were returned

to their home cages for 2 days until the probe trial. The latencies to reach the platform were recorded and subsequently computed by a video-tracking system. The probe trial consisted of a 60-s free-swim period without a platform, during which the time spent in the target quadrant and the number of platform crossings was recorded.

B. Object in place task

Apparatus

The object-in-place (OIP) task occurred in an open-topped acrylic box with one blue inner wall and three black inner walls. An overhead camera and a video recorder were used to monitor and record rats' behavior for subsequent analysis. The objects presented were varied in shape, color, and size (10 × 10 × 10 cm to 8 × 8 × 8 cm) and too heavy for rats to displace.

Procedures

In this task, rats were placed in the center of the arena and allowed to explore the objects for a certain length of time. The rats were habituated to the arena without objects for 5 min a day before the commencement of behavioral testing. The test was comprised of an exposure phase and a reintroduction phase. During the exposure phase, rats were presented with three different objects (A, B, and C) placed in the corners of the arena 10 cm from the walls for 5 min. During the reintroduction phase, one of the three objects, the position of an object has been changed, and rats were allowed to explore the objects for 3 min. Discrimination was assessed by comparing the time spent exploring the object that changed positions with the time spent exploring the two objects that remained in the same positions. If OIP memory is intact, rats spend more time exploring the object that changed location compared with the two objects that remained in the same locations.

4. Choline Acetyltransferase Immunohistochemistry

After behavioral testing, five rats of each group were perfused with cold 4% paraformaldehyde. Their brains were removed, post-fixed, and transferred to 30% sucrose for 3 days. The brains were cut into 30-µm coronal sections using a freezing microtome and stored in a cryoprotectant solution at -20℃. The cryoprotectant solution consisted of 0.1M phosphate buffer (pH 7.2), 30% sucrose, 1% polyvinylpyrrolidone, and 30% ethylene glycol. Tissue was stained with cresyl violet to confirm correct placement of the needle tracks in the NBM and RE. To detect cholinergic cells, tissue was immunohistochemically processed using polyclonal antibodies against choline acetyltransferase (ChAT; 1:100; cat# AB144P, Chemicon, Temecula, CA, USA). The sections were stained using the avidin-biotin complex method (Vector Labs, Burlingame, CA, USA) with diaminobenzidinetetrahydrochloride as the substrate. Anatomical landmarks from a stereotaxic atlas (Paxions and Watson, 2007) were used to localize the medial septum (MS).

5. Acetylcholinesterase assay

The enzymatic activity of acetylcholinesterase (AChE) was determined using the method of Ellman et al. with some modifications. Briefly, 20 µl triplicate samples identical to those used in western blot analyses were mixed with a reaction mixture [0.2 mM dithiobisnitrobenzoic acid (Sigma, St. Louis, MO, USA), 0.56 mM acetylthiocholine iodide (Sigma), 10 µM tetraisoprpylpyrophosphoramide (Sigma), 39 mM phosphate buffer; pH 7.2] at 37℃. After 30 min, the optical density was measured at 405 nm.

6. Western blot

The other half of each group of rats was anesthetized with a mixture of ketamine (75 mg/kg), acepromazine (0.75 mg/kg), and rompun. To acquire western blot data, rats were decapitated with a guillotine, and their brains were quickly removed. The frontal cortex and hippocampus were dissected with fine forceps from 1-mm-thick coronal brain slices. The samples were homogenized in lysis buffer (Intron, Seongnam, Korea) and centrifuged for 10 min at 12,000 rpm, and the protein in the supernatant was measured using the bicinchoninic acid protein assay reagent kit (Pierce, Rockford, IL, USA). 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% nonfat dry milk in phosphate-buffered saline containing 0.05% Tween-20 (PBST)] for 1 h at room temperature. They were incubated with the indicated antibodies overnight at 4℃ and washed three times (each for 8 min) with PBST. The membranes were incubated with corresponding secondary antibodies for 1 h at room temperature. After washing with PBST, proteins were detected with enhanced chemiluminescence solution (Pierce) 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 GAD 65/67 (1:500; Millipore, Temecula, CA, USA), glutamate transporter (1:4000; Abcam, Cambridge, UK), and β-actin (1:1000; Sigma, St. Louis, MO, USA).

7. Statistical analysis

The results of all experiments were expressed as a percentage of the values for the normal group.

The results of the Western blots were normalized to β-actin for each sample and expressed as a percentage of the normal values. One-way ANOVA was used for most analysis of experiments. ANOVA followed by least significant difference was used as a post hoc test at each time point for statistical analysis. Also, repeated measures ANOVA with conservative Bonferroni corrections for multiple comparisons was used for analysis of training trial in Morris water maze. p values less than 0.05 were considered statistically significant. All statistical analyses were performed by PASW (version 18; SPSS Inc., Chicago, Ill., USA).

III. RESULTS

1. Degeneration of ChAT-immunopositive neurons in the Medial Septum by 192 IgG-saporin

In this experiment, we evaluated ChAT-immunopositive neurons in the MS in all groups after intraventricular 192 IgG-saporin injection.

Intraventricular 192 IgG-saproin injections produced denervation of ChAT-immunopositive neurons in the MS, which is thought to be part of the BF complex (Fig. 3). In normal rats, the ChAT-immunopositive neurons were evenly distributed in the MS, and the structure of the cell bodies and dendrites were wholly intact as shown in Figure 3a. By contrast, lesion, implantation, and stimulation groups, which were injected with 192 IgG-saporin, showed a remarkable decrease in the numbers of ChAT-immunopositive neurons. Also, they showed marked damage of cell bodies and dendritic structures.

Figure 3. Representative pictures showing the effect of cholinergic lesion on the BF. The normal group had numerous ChAT-immunopositive neurons in the MS. The lesion, NBM implantation, RE implantation, NBM stimulation, and RE stimulation groups displayed a loss of cholinergic neurons in the MS. Scale bar represents 500 µm. Abbreviations: ChAT, choline acetyltransferase; MS, medial septum; NBM, nucleus basalis magnoceelularis; RE, reuniens thalamic nucleus; impl, implantation; stim, stimulation.

2. Confirmation of the location of the electrode in the NBM or the RE

We confirmed the location of the inserted electrode in the NBM and RE using cresyl violet staining.

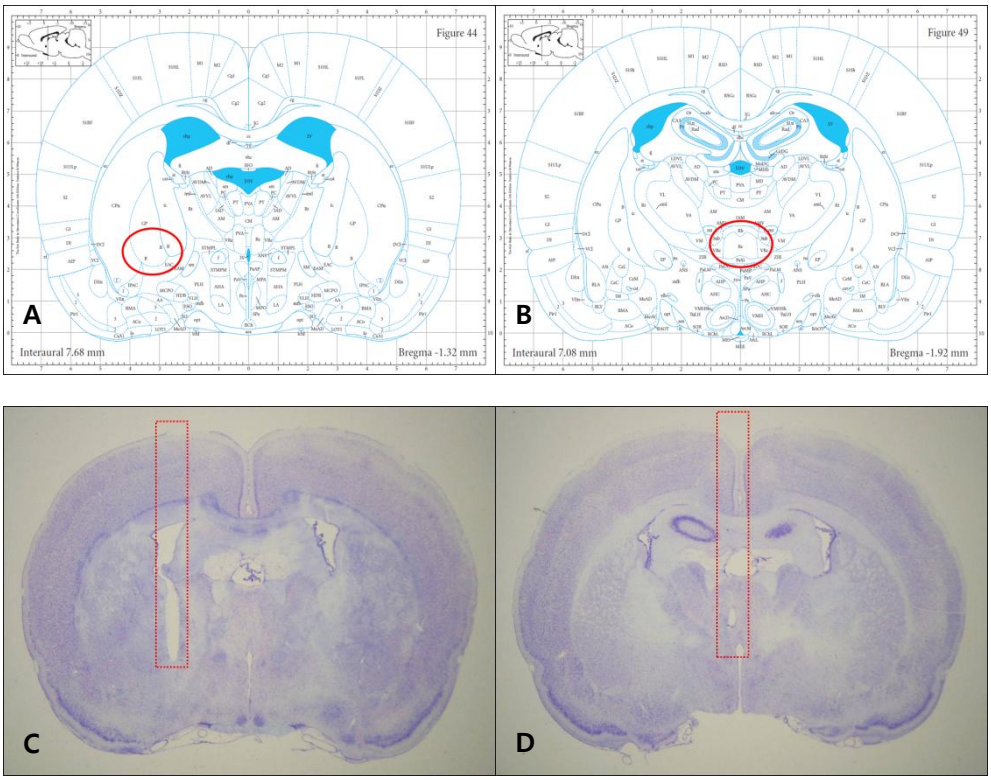

Fig 4. Illustrations of NBM (A) and RE (B) locations according to a rat brain atlas. Photomicrographs show brain slices stained with cresyl violet from the NBM group (C) and the RE group (D). The red dotted box demonstrates the insertion track of the electrode, showing the depth of electrode insertion and the end places in the NBM and RE.

3. The effect of NBM or RE stimulation in Behavior tests

In this experiment, we measured average time to reach the platform during water maze training trials (Fig. 5A). On the first day of training trials, all groups required approximately 40 s on average to find the hidden platform. All groups appeared to learn the location of the platform across 5 consecutive days, and there were no significant differences among groups. On the last day of training trials, all groups found the platform in 20 s, which was evidence of their learning the location of the platform.

During the probe trial, all groups showed similar motor-related behavior, evidenced by equivalent swim distances and speeds. These findings suggest no effect of cholinergic lesion, electrode implantation, or electrical stimulation on motor function (Fig. 5B).

Compared to the normal group, the lesion group showed less time in the target quadrant, less time in the platform zone, and fewer platform crossings. There was statistically significant difference in the platform crossings between the normal and lesion group. The RE implantation and RE stimulation group showed better performances compared to the lesion group. However, there was no significant difference between two groups. Interestingly, the NBM stimulation group showed significantly better performance than the other groups in terms of time spent in the target quadrant and number of platform crossings.

Especially, there were statistically significant difference between NBM implantation group and NBM stimulation group in the time in the platform zone ($p<0.05$), and platform crossings ($p<0.05$).

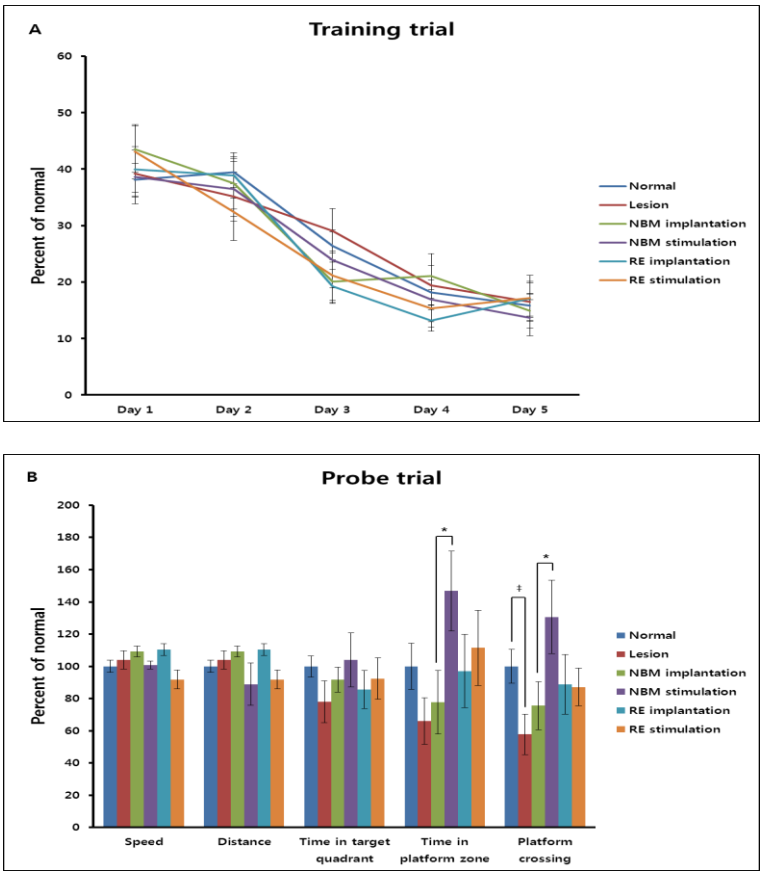

Fig 5. Spatial memory performance during training trials

(A) and the probe trial (B) in the Morris water maze

The normal group spent most of their time in the target quadrant and appeared to remember the location of the platform (Fig. 6A). Also, the implantation and stimulation groups showed swim paths that were similar to those of the normal group (Fig. 6C, D, E, F). However, the lesion group spent little time in the target quadrant and did not appear to remember the location of the platform (Fig. 6B).

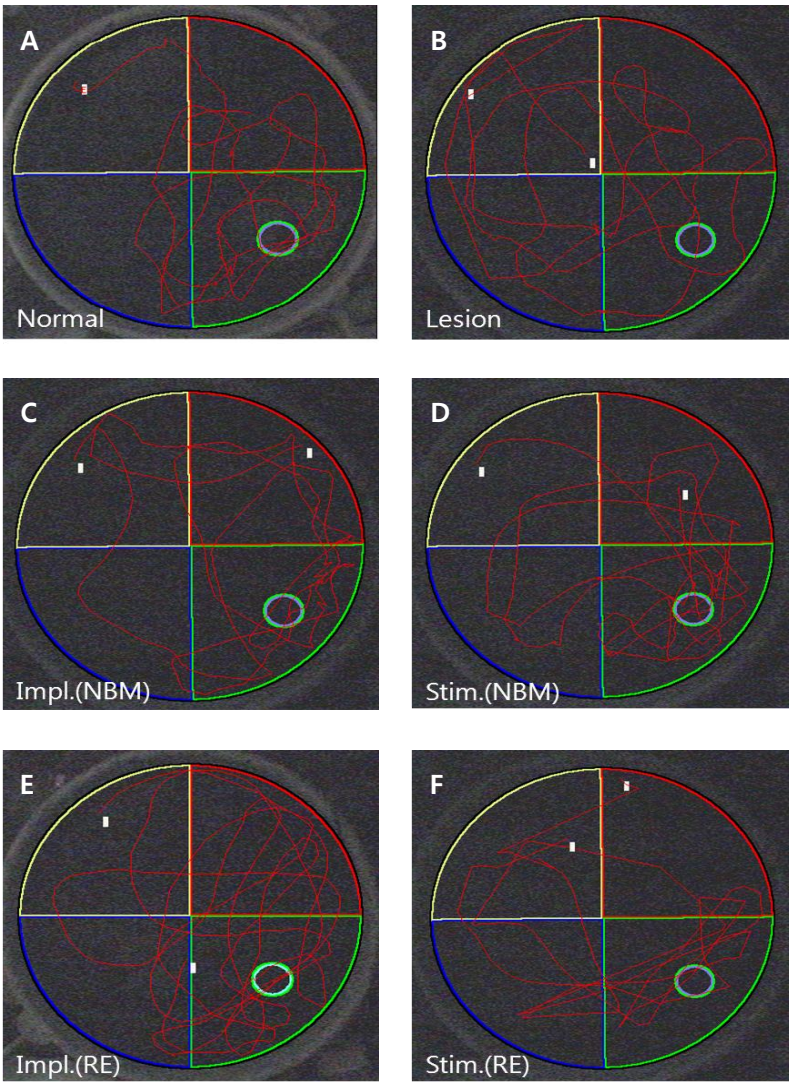

Fig 6. Detailed swim paths of rats in each group during the probe trial of the Morris water maze. Swim paths showed that all groups of rats, except for the lesion group, spent significantly more time in the target quadrant versus the three other quadrants, indicating that they remembered the platform location. Normal (A), lesion (B), NBM implantation (C), NBM stimulation (D), RE implantation (E), and RE stimulation (F) groups.

4. The effect of NBM or RE stimulation in Object in place task

In this experiment, we evaluated time spent exploring the object that changed position in all groups. In the lesion group, novel object preference percentage declined to 40% of normal group values (Fig. 7) and there was statistically significant difference between two groups. The NBM implantation and NBM stimulation group showed same percentage as compared to the normal group. RE implantation group showed slightly higher percentage than RE implantation.

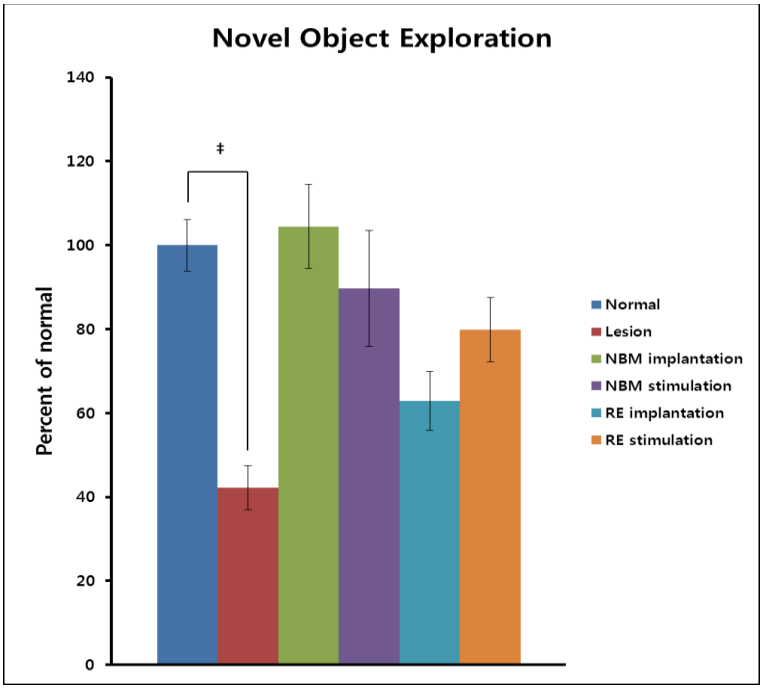

Fig 7. The Novel Object Preference percentage for each group. The lesion group showed half the percentage value of that of the normal group.

5. Glutamate and GAD expression in the mPFC and the Hippocampus

In this experiment, we examined changes in the expression of GAD and glutamate in the mPFC and hippocampus. In the mPFC and hippocampus, the expression of GAD and glutamate in the lesion group was increased compared with that in the normal group. In the mPFC, although the expression of glutamate in the NBM implantation and RE implantation groups was slightly decreased compared with the lesion group, expression of glutamate in the NBM stimulation and RE stimulation groups was similar to that in the normal group (Fig. 8A).

The implantation and stimulation groups showed a significant decrease in expression of glutamate in the hippocampus (Fig. 8B). However, we could not confirm whether this decrease was caused by implantation or stimulation.

There were differences between implantation and stimulation groups in the expression of GAD in the mPFC (Fig. 8C). The NBM stimulation group showed a marked decrease in GAD compared with the NBM implantation group. By contrast, there was no difference in GAD expression between RE implantation and RE stimulation groups.

The expression of GAD in the hippocampus was similar to the expression of GAD in the mPFC for all groups (Fig. 8D). GAD expression in all implantation groups were slightly decreased compared with that in the lesion group, and GAD expression in all stimulation groups was similar to that in the normal group.

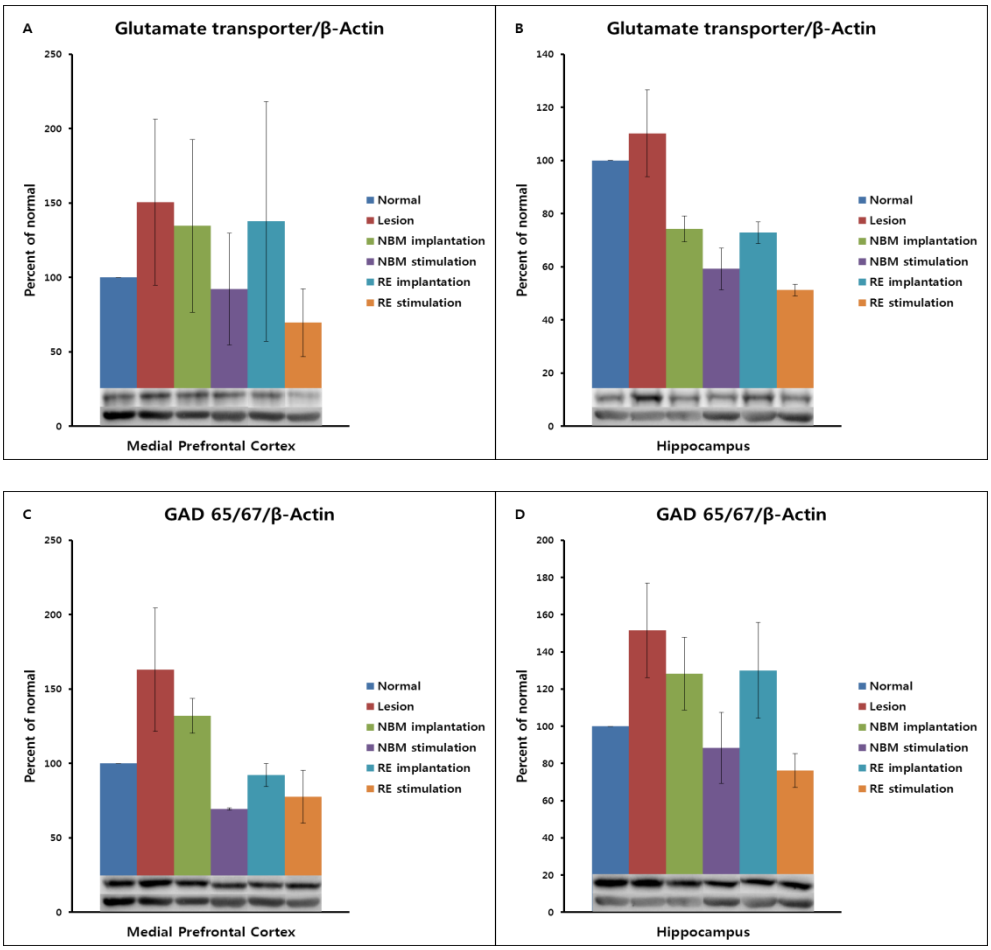

Fig 8. The expression of GAD and glutamate in the mPFC and hippocampus in all groups. The expression of glutamate transporter in the mPFC (A). The expression of glutamate transporter in the hippocampus (B). The expression of GAD in the mPFC (C). The expression of GAD in the hippocampus (D). The results of the western blots analyses were normalized to β-actin for each sample and expressed as a percentage of normal group values.

6. AChE activity in the Medial Prefrontal Cortex and the Hippocampus

We investigated changes in AChE activity in the mPFC and hippocampus in all groups. AChE activity in the mPFC of the lesion group declined to 60% of normal group values (Fig. 9A). Interestingly, the NBM stimulation and RE stimulation groups showed differences in AChE activity. AChE activity in the NBM implantation group declined to 80% of that in the normal group, and AChE activity in the NBM stimulation group declined further to 30% of that in the NBM implantation group. The RE stimulation group showed more than twice the level of AChE activity in the RE implantation group. AChE activity in the lesion, implantation, and stimulation groups declined to 25-30% of that in the normal group (Fig. 9B). However, there were no differences among groups in AChE activity in the hippocampus except for the normal group.

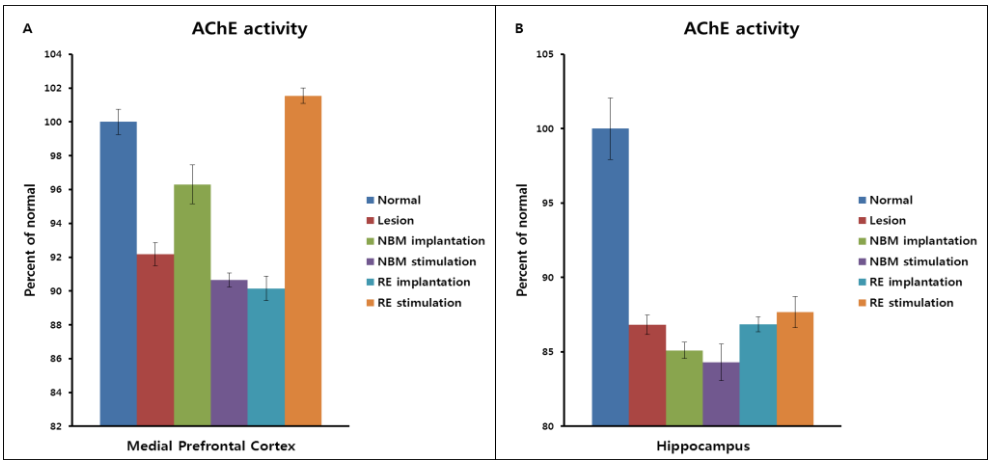

Fig 9. AChE activity of each group in the mPFC (A) and hippocampus (B). NBM groups and RE groups different levels of AChE activity in the mPFC. (A) However, there were no significant differences in AChE activity in the hippocampus among groups except for the normal group. (B) Indices are expressed as the percentage of normal group values (mean ± standard error of the mean).

IV. DISCUSSION

In the present study, we analyzed changes in memory function using behavioral tests and changes in Ach, GABA, and glutamate neurotransmitter systems after stimulation of the NBM or RE.

We conducted the MWM and OIP task to evaluate different types of memory. The MWM is a particularly useful tool for assessing visuo-spatial memory, and the OIP task assesses visuo-working and recognition memory for multiple items and their association with particular locations in rats [20 21].

We confirmed that stimulation of the NBM or RE appears to improve memory, as performance of the stimulation groups was similar to that of the normal group in two object tests. Interestingly, the NBM stimulation group showed better performance than the RE stimulation group in probe trial of spatial memory in the water maze test. However, there were significant differences among groups in the training trials of spatial memory in the water maze test.

We found that stimulation of the NBM or RE inhibited the GABA and glutamate systems in the mPFC and hippocampus using western blot analysis.

In the AChE assay, survival of Ach in the mPFC by inactivating AChE after NBM stimulation appeared to improve memory. On the other hand, less survival of Ach in the mPFC by activating AChE after RE stimulation appeared to have little effect on memory.

The effect of NBM stimulation in behavioral tests

The NBM stimulation group showed better performance in terms of time spent in the target quadrant, time in the platform zone, and platform crossings in the probe trial of the MWM as compared with other groups.

Extrapolating to findings that the NBM implantation group showed performance similar to that of the normal group in the water maze test, we confirmed that NBM implantation had little effect on memory.

The NBM stimulation group showed better performance than the NBM implantation group, showing a recovery of memory similar to that in the normal group. This suggests that NBM stimulation could help recover memory.

On the other hand, we could not confirm differences between NBM implantation and NBM stimulation groups in the OIP task, as the normal, NBM implantation, and NBM stimulation groups showed similar performance. Therefore, we need further evidence to determine why there were no differences between NBM implantation and NBM stimulation groups in the OIP task.

The results obtained in the two behavior tests after stimulation of the NBM suggest that NBM stimulation appears to have a greater effect on visuo-spatial memory than visuo-working memory.

The effect of RE stimulation in behavioral tests

The RE implantation and RE stimulation groups showed performance similar to the normal group in the probe trial of the water maze test. The RE implantation group showed a decrease in novel object preference percentage to 60% of the normal group value in the OIP task. However, novel object preference percentage of the RE stimulation group increased by 20% as compared to the RE implantation group in the OIP task.

The results obtained in the two behavioral tests after stimulation of the RE suggest that RE stimulation appears to have a greater effect on visuo-working memory than visuo-spatial memory.

Changes in GABA and glutamate systems in the mPFC and hippocampus after stimulation of the NBM or RE

We measured changes in AChE, GAD, and glutamate to evaluate changes in these neurotransmitter systems after implantation or stimulation of the NBM or RE. It has been reported that increasing GABA could cause memory impairment [22]. It has also been reported that increasing glutamate could cause memory enhancement [23]. However, a recent study reports that excessive activation of glutamate could instead cause memory impairment [24].

Some studies report that the NBM accounts for 70-80% of cholinergic innervation and at least 20-30% of non-cholinergic (GABAergic and glutamatergic) innervation to the cortex [11].

We examined expression of GAD and glutamate using western blot analysis. Expression of GAD and glutamate in the mPFC and hippocampus was decreased after NBM stimulation. Expression of GAD in the mPFC was particularly decreased. Although GAD and glutamate have relatively few projections from the NBM to the mPFC, further reduction of projections to the mPFC by NBM stimulation could be one of the factors contributing to memory improvement. A study reports that the RE has glutamatergic projections to the hippocampus [25]. Taken together with the other studies mentioned above, the reason why glutamate expression is decreased in the mPFC and hippocampus after RE stimulation may be that RE stimulation activates the glutamate system through RE→HF→mPFC projections.

In summary, stimulation of the NBM or RE appears to affect neurotransmitter systems in the mPFC and hippocampus, causing improvements in memory above those of the implantation groups.

Changes in AChE activity in the mPFC and hippocampus after stimulation of the NBM or RE

As mentioned briefly above, GABA and glutamate have relatively few

projections from the NBM to the mPFC as compared with Ach. Also, it is known that the RE has mostly glutamatergic projections to the hippocampus [25]. Therefore, we postulated that Ach could be one of the most important factors in the mPFC after NBM stimulation. However, there are not enough previous studies supporting effects of Ach in the mPFC and hippocampus after RE stimulation on memory. Therefore, we conducted an experiment to gather more evidence for these effects.

There was no change in AChE activity in the hippocampus after stimulation of the NBM. These results support the observation that the NBM has little or no projections to the hippocampus. On the other hand, it appears that stimulation of the RE was not sufficient to activate the Ach system in the hippocampus, although the RE has connections with the hippocampus.

Interestingly, we observed that AChE activity was decreased in the NBM stimulation group compared with the NBM implantation group. By contrast, it was increased in the RE stimulation group compared with the RE implantation group.

In conclusion, stimulation of each region caused similar changes to GABA and glutamate systems but caused opposite changes in AChE activity.

We suppose that the decrease in AChE activity in the mPFC after stimulation of the NBM could be related to memory improvement. To be specific, it appears that NBM stimulation inactivated the breakdown of Ach by AChE and promoted the survival of Ach in the mPFC.

Good performance of the NBM stimulation group in the Morris water maze may be a result of a reduction of AChE activity after NBM stimulation.

Therefore, an abundance of Ach appears to improve memory. On the other hand, it appears that RE stimulation activates the breakdown of Ach by AChE, leading to less survival of Ach in the mPFC. Although AChE was significantly increased in the mPFC, it did not cause memory improvement. This may be because the cholinergic system in the RE is not extensive.

In conclusion, RE stimulation decreased GABA and glutamate system and inactivated AChE more than RE implantation. However, changes in neurotransmitter systems after RE stimulation appear to have less of an effect on memory compared with NBM simulation.

V. CONCLUSION

Although the same stimulation conditions were applied to the NBM and RE, the proportion of neural fibers and the regulation of neurotransmitter systems in each target region appeared to have different effects on memory function.

Our results provide additional evidence supporting the hypothesis that electrical stimulation in some brain areas may play an important role in memory improvement. This study attempted to examine correlations between behavior and neurotransmitter systems after stimulation of these regions.

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ABSTRACT (IN KOREA)

치매 모델 쥐에서 nucleus basalis magnocellularis/reuniens thalamic nucleus의 전기적 자극이 기억 기능에 미치는 영향

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이지은

뇌 심부 자극은 다양한 신경질환을 치료하기 위하여 사용 되어 왔다. 최근, 몇몇 연구에서 뇌 심부 자극이 알츠하이머를 치료할 수 있다고 보고 되었다. 피질과 해마에서의 콜린성 연결의 감소와 연관된 기억 손상은 알츠하이머의 특징 중 하나이다. 본 연구는 알츠하이머의 뇌 기저 전외 콜린성 손상을 모방하는 쥐 모델을 이용하여 공간 기억에서의 nucleus basalis magnocellularis 또는 reuniens thalamic nucleus의 전기적 자극의 효과를 알아보았다.

192 IgG-saporin을 이용하여 기저 전뇌의 콜린성 뉴런을 손상하였다. 자극 그룹의 쥐들은 수술 1주 후 매일 nucleus basalis magnocellularis 또는 reuniens thalamic nucleus에 전기적 자극을 받았다. 수술 2주 후 Morris water maze task와 object in place task를 이용하여 쥐들의 visuo-spatial memory와 visuo-working memory를 평가하였다. 면역염색을 이용하여 192 IgG-saporin으로 인하여 내측 중격의 콜린성 뉴런 손상

을 확인하였고, 아세틸콜린에스테라제 분석을 이용하여 내측 전전두피질과 해마에서의 아세틸콜린 변화를 평가하였다. 또한 GABAergic과 glutamatergic 체계의 변화를 알아보기 위하여 단백질 발현 분석법을 시행하였다.

Nucleus basalis magnocellularis의 자극 그룹은 Morris water maze task의 probe trial에서 우수한 수행 능력을 나타내었고, 그들의 내측 전전두피질에서의 glutamate와 glutamic acid decarboxylase의 감소가 유도되었다. Reuniens thalamic nucleus의 자극 그룹은 object in place task에서 전극 삽입만을 실시한 그룹보다 위치 변경 물체에 대한 약간 더 높은 탐색률을 나타내었고, 그들의 내측 전전두피질에서의 glutamate와 해마에서의 glutamic acid decarboxylase의 감소가 유도되었다. 특히 내측 전전두피질에서 높은 아세틸콜린에스테라제 활성이 유도되었다.

본 연구의 결과를 통하여 nucleus basalis magnocellularis와 reuniens thalamic nucleus의 전기적 자극은 기억이 손상된 치매 모델 쥐에서의 공간 기억력 향상에 도움을 주는 것을 관찰하였다.

특히 nucleus basalis magnocellularis의 전기적 자극은 주로 visuo-spatial memory의 향상에, reuniens thalamic nucleus의 전기적 자극은 visuo-working memory의 향상에 영향을 주는 것으로 보인다.

핵심되는 말: 뇌 심부 자극술, nucleus basalis magnocellularis, reuniens thalamic nucleus, 공간 기억력, glutamic acid decarboxylase(GAD), glutamate