ORIGINAL RESEARCH

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Improvement of Spatial Learning and Memory Impairments by Fetal Neural Tissue Transplantation in Experimental Rat Model of Alzheimer's Disease

Sıçanlarda Deneysel Alzheimer Hastalığı Modelinde Hacimsel Öğrenme ve Bellek Bozukluklarının Fetal Nöral Doku Transplantasyonu ile Düzelmesi

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Abstract

Objective: It is known that the acetylcholinergic afferents of the neocortex from subcortical areas participate in learning and memory. Autopsy studies in cases of Alzheimer's disease (AD) have shown that most of the neurons of nucleus basalis magnocellularis (NBM) are atrophic or decreased in number. In this study, we searched for whether or not it was possible to improve the impaired learning and memory functions with foetal neural tissue transplantation in an experimental model of AD.

Method: A total of thirty seven young adult male Wistar albino rats were served as experimental subjects. NBM on the right side was destroyed by the injection of kainic acid stereotactically so as to make a model of AD. The grafts were obtained from 14-16 day foetuses of the same genus. After the tissue with cholinergic neurons dissected from ventral forebrain and tissue with non-cholinergic neurons dissected from telencephalic vesicle, cell suspensions were prepared and injected stereotactically to the ipsilateral frontal cortex. Spatial learning and memory functions were tested by Morris' water maze tasks.

Results: Spatial learning and memory functions in rats were impaired by unilateral lesions of nucleus basalis magnocellularis. The impairment observed during the early period partially improved by the time. It was observed that this amelioration was accelerated with both cholinergic and non-cholinergic foetal neural tissue implantation.

Öz

Amaç: Subkortikal alanlardan neokortekse uzanan asetilkolinerjik nöronların öğrenme ve bellek süreçlerinde rol aldığı bilinmektedir. Alzheimer hastalığında (AH) yapılan otopsi incelemeleri bazal magnoselüler çekirdekteki nöronlarda atrofi veya azalma olduğunu göstermiştir. Bu çalışmada deneysel AH modelinde bozuk öğrenme ve bellek fonksiyonlarının, fötal nöral doku transplantasyonu ile düzelmesinin mümkün olup olmadığı araştırıldı.

Yöntem: Deneysel çalışmada 37 adet Wistar albino cinsi genç erişkin erkek sıçan kullanıldı. AH modeli sağ bazal magnoselüler çekirdeğe stereotaktik yöntemle verilen, nörotoksik bir ajan olan kainik asit ile oluşturuldu. Greftler aynı cins sıçanların 14-16 günlük fetuslarından alındı. Kolinerjik nöronları içeren doku ventral ön beyinden, non-kolinerjik nöronların olduğu doku ise telensefalik vezikülden elde edilerek, hücre süspansiyonu haline getirildi ve stereotaktik yöntemle ipsilateral frontal kortekse implante edildi. Hacimsel öğrenme ve bellek fonksiyonları Morris'in "water maze" testi ile değerlendirildi.

Bulgular: Tek taraflı bazal magnoselüler nükleus lezyonları ile sıçanlarda hacimsel öğrenme ve bellek fonksiyonları bozuldu. Erken dönemde bozulan bu fonksiyonlar geç dönemde kısmen düzeldi. Hem kolinerjik hem de non-kolinerjik fötal doku implantasyonu ile bu düzelmenin hızlandığı saptandı.

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Abstract

Conclusion: In our study, improvement of spatial learning and memory impairment with both cholinergic and non-cholinergic foetal neural tissue implantation can be explained by re-establishment of impaired connections via proliferation of limited number of surviving cholinergic neurons creating new synapses, as a result of upregulation of endogenous neural stem cells and activation of trophic mechanisms by implantation, rather than creation of functional synapses between the graft and the recipient tissue.

Keywords: Alzheimer's disease, neural transplantation, nucleus basalis magnocellularis, spatial learning and memory

Öz

Sonuç: Çalışmamızda hem kolinerjik hem de non-kolinerjik fötal nöral doku implantasyonu ile hacimsel öğrenme ve bellek fonksiyonlarının düzelmesi, greftin alıcı doku ile fonksiyonel sinapslar oluşturmasından ziyade, implantasyonun endojen nöral kök hücrelerini regüle etmesi ve trofik mekanizmaları harekete geçirmesi sonucu, sağ kalan az sayıdaki kolinerjik nöronların çoğalması ve yeni sinapslar oluşturarak bozulmuş bağlantıları yeniden kurması ile açıklanabilir.

Anahtar kelimeler: Alzheimer hastalığı, nöral transplantasyon, nükleus basalis magnoselüler, hacimsel öğrenme ve bellek

Introduction

Many investigations have emphasized that some of non-myelinated and scarcely myelinated neurons in mammalian central nervous system (CNS) have regeneration capacity since the late 19th century (1-10). Inspired by these studies, fetal tissue graft models have been studied for the restoration of damaged circuits in the treatment of degenerative disorders. (4,6,7,10-24).

Neuroanatomy and neurophysiology of learning and memory functions have not been clearly defined yet. It is believed that they are regulated by a complex system including anatomic associations and chemical pathways (25,26). It has been shown that nucleus basalis magnocellularis (NBM), which was first microscopically defined by Meynert in 1872, and located in both anatomic and chemical pathways, plays an important role in learning and memory functions by acting as a bridge between cortex and limbic system (25-27). Autopsy studies performed on neurodegenerative diseases progressing with Alzheimer disease (AD) and dementia indicated that there was atrophy or decrease in neurons in NBM (28-32). Decreases were determined in cortical cholinesterase (ChAT), acetylcholine esterase (AchE) activities with acetylcholine (Ach) levels in these patients, and it was shown that these were directly correlated with the damage in NBM (28-33). As the result of histological, pharmacological, and biochemical studies, it is believed that clinical picture of dementia in AD is due to decreased acetylcholinergic inputs in cortex which is commonly associated to the degeneration in cholinergic neurons in nucleus of Meynert (25,30,31). In experimental animals, neurotoxic or electrolytic lesions in NBM causes 70-80% decrease in neocortical ChAT, and AchE activities along with similar learning and memory disorders in subjects with AD model (19,20). Both histological and biochemical studies have documented that Ach-rich ventral forebrain (VF) grafts can set up functional synaptic connections with host neurons in the neocortex. Besides, it was determined that clinical picture in sensorimotor and learning tests were recovered (19,20). The aim of the present study was to establish an experimental AD model by disrupting learning and memory functions by a unilateral NBM lesion in rats, and to investigate effects of fetal neural grafts on spatial learning and memory functions in this experimental model of AD.

Material and Methods

Experimental study was performed at İstanbul University, Center for Experimental Medical Research and Application (DETAM). Animal rights were followed according to Helsinki Declaration, and handling and use of laboratory animals were managed according to institution and national guidelines. The study was approved by the Local Ethics Committee (DETAM project number: 1990/31).

A total of 37 young adult male Wistar albino rats, which were grown up at DETAM and weighing about 200-300 g at time of surgery served as experimental subjects. Grafts were obtained from 14-16-day old fetuses of the same genus (Figure 1). Coordinates in stereotactic interventions were calculated by using Pellegrino's rat stereotaxis map.

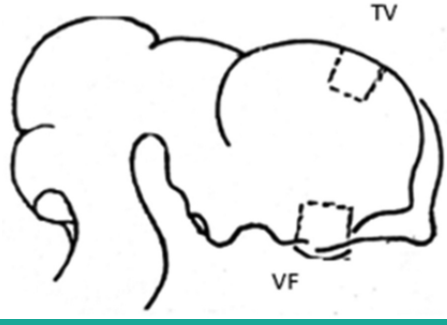

Figure 1. Location of grafts obtained in 14-16 days old fetus

VF: Ventral forebrain, TV: Telencephalic vesicle

A two-staged experiment was performed in the investigation (Table 1). Eighteen rats were used in the first experiment. Four of them had no surgical intervention and were included in the normal rat group. Neurotoxic lesions with kainic acid were induced stereotactically in the right NBM in 11 rats. In three rats, Hamilton needle was introduced into NBM, but no lesion was performed, so they were defined as the sham-operated (self help and actualization movement) group. Ten days after NBM lesion, spatial learning and memory functions were assessed by Morris' water maze tasks. At the end of this test, six out of 11 rats with NBM lesions received stereotaxic injections of cell suspensions containing tissue dissected from VF area of fetuses into the ipsilateral frontal cortex. At day 100 after NBM lesion, water maze tasks were repeated in all groups.

In the second experiment performed after the first one, 19 rats were used. Of them, four constituted normal group, whereas three formed the sham-operated group. In 12 rats, neurotoxic lesions of NBM with kainic acid were made stereotactically. Ten days after the NBM lesion, spatial learning and memory functions were assessed by Morris' water maze tasks. At the end of this test, cholinergic cell suspensions prepared from tissue obtained from VF area of fetuses were implanted stereotactically into the ipsilateral frontal cortex in five out of 12 rats with NBM lesions. In four out of 12 lesioned rats, cell suspensions prepared from the tissue dissected from the telencephalic vesicle (TV) of the same fetuses, which did not contain cholinergic neurons, were implanted stereotactically into the ipsilateral frontal cortex. At day 100 after NBM lesion, water maze tasks were repeated in all groups.

At the baseline, every rat was given a number by marking their tails. Procedures during surgical interventions were recorded for each rat. Until the end of the study, all rats were kept together in big rat cages located at the floor of experimental animals in DETAM. Subjects performing Morris' water maze tasks were blinded for groups of rats while performing the test.

Graft preparation: For each experiment, a total of four fetuses 14 to 16 day-old (crown-rump leng 12-16 mm) were dissected out by caeserian section from a deeply anesthetized pregnant rat under aseptic conditions. After a fetus was removed from the uterus, the brain tissue was submerged in 0.6% glucose-saline solution on a glass slide, and the area of interest was dissected, 2x2x2mm in size, under a operating microscope using a pair of iridectomy scissors and watchmaker's forceps. To prepare a cell suspension, the dissected regions were cut into smaller pieces and transferred to a test tube containing a trypsin-glucose-saline solution (0.1% trypsin, Sigma crude type II and 6% d-glucose in sterile saline) for 20 minutes incubation at 37 °C. Following the enzyme incubation, the trypsin solution was then rinsed off by 4-5 times with the glucose-saline solution. A cell suspension was made by mechanical dissociation, which was performed by gentle pipetting 15 times using a Pasteur pipette. A total of 3 µL cell suspension was injected through Hamilton needle no 24 with a speed of 1 µ/L/minute.

Morris' water maze test: A cylindrical tank, 100 cm in diameter and a depth of 40 cm, was filled to a depth up to 30 cm, and the water was made turbid by using milk powder. The pool was divided four equal imaginary quadrants. In the middle of a fixed quadrant, a glass platform with 10x10 cm was placed 1 cm under the water level, so that rats could not see the platform. The pool was placed in a fixed place in the experiment room with fixed objects such as wall, mirror, or hanger around. Rats, facing to borders of the pool, were placed in the water from four corners twice a day for 4 days with 24-hour intervals and their time to find the platform within 120 seconds was calculated by using a chronometer. In order to observe the surroundings, rats were kept on the platform for 30 seconds after each trial. A total of 32 trials were performed within 4 days. The platform was removed on the 5th day, and rats were placed in the pool from four different corners. Time spent in the place where platform was previously located was measured within 60 seconds.

Statistical Analysis

Results were assessed by using ANOVA statistically analysis program for Macintosh, repeated one-way variation analysis was used for each graphic, and the level of significance was determined at $p<0.05$.

Table 1. Study groups in two-staged experiments

	A Normal	B Sham-operated	C NBM lesions	D VF grafts	E TV grafts	Total
Experiment 1	4	3	5	6	-	18
Experiment 2	4	3	3	5	4	19

NBM: Nucleus basalis magnocellularis, VF: Ventral forebrain, TV: Telencephalic vesicle

Results

Experiment 1

Figure 2 shows the graphic of arithmetic means of the results of 8 trials in 4 consecutive days starting 10 days after lesion surgery. There was no significant difference between groups A (normal) and B (Sham-operated), whereas group C (NBM lesion) statistically differed from the others ($p<0.001$). Similar results were determined in assessment of day 5 results ($p<0.05$) (Figure 3).

The results of the first four days of the tasks performed 100 days after NBM lesions are shown in Figure 4. The statistically analyzes between all groups (A-normal, B-sham-operated, C- NBM lesion, D- VF implantation) revealed no difference between groups A and B, A and D, B and D; but significant difference was determined between groups A and C; B and C; C and D ($p<0.0001$). At the 5th day, the results between the same groups were again significant (Figure 5) ($p<0.001$).

The results of water maze tasks performed on lesioned rats 10 (group C1 n=11) and 100 days after lesion surgery (group C2 n=5) and on implanted rats 100 days after lesion surgery (group D n=6) were compared (Figure 6). The time-to-find the platform was shorter at day 100 than at day 10 among lesioned rats, but it was observed that learning was slower than the rats receiving implantation. Statistically significant differences were determined in this assessment between groups C1 and C2; C1 and D; C2 and D ($p<0.0001$).

Experiment 2

In Figure 7, the mean of groups within the first four days of water maze tasks performed on day 10-14 after NBM lesion is shown. Among rats in normal (A), sham-operated (B), and with NBM lesions (C), no significant difference between

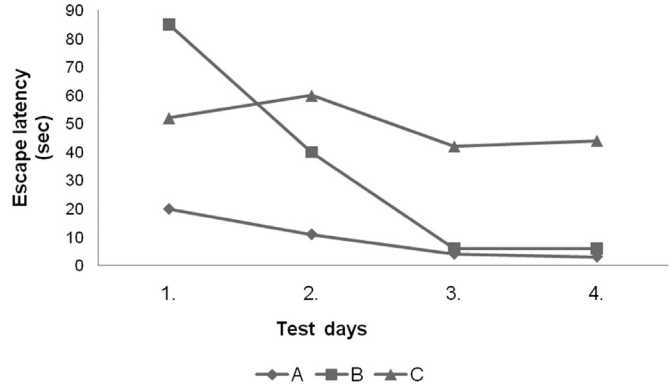

Figure 2. The results of the first 4 days of water maze tasks 10 days after lesion surgery. A: Normal, B: Sham-operated, C: NBM lesion

NBM: Nucleus basalis magnocellularis

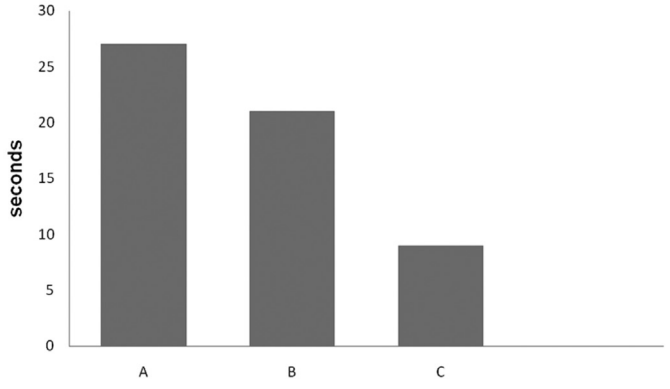

Figure 3. The results of the 5th day of water maze tasks 10 days after lesion surgery. A: Normal, B: Sham-operated, C: NBM lesion

NBM: Nucleus basalis magnocellularis

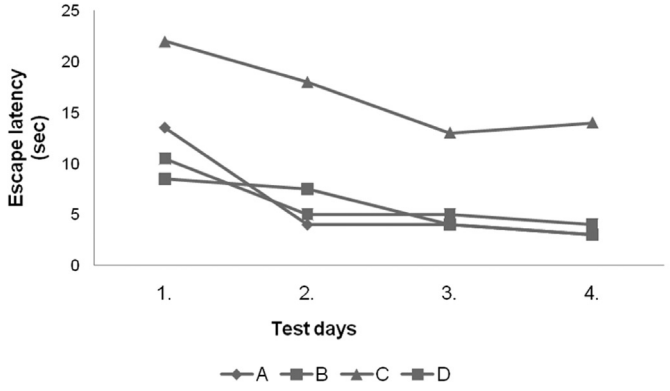

Figure 4. The results of the first 4 days of water maze tasks 100 days after lesion surgery. A: Normal, B: Sham-operated, C: NBM lesion, D: VF implantation

NBM: Nucleus basalis magnocellularis, VF: Ventral forebrain

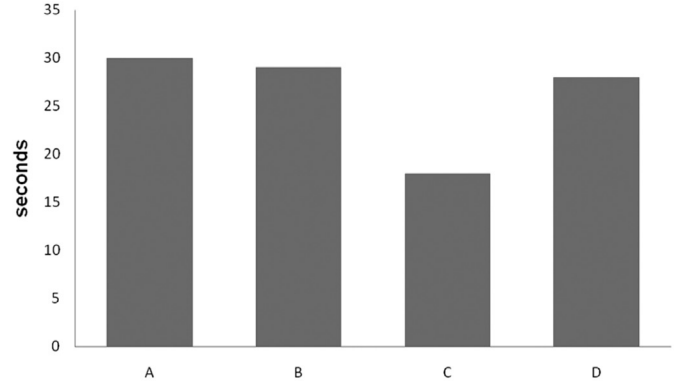

Figure 5. The results of the 5th day of water maze tasks 10 days after lesion surgery. A: Normal, B: Sham-operated, C: NBM lesion, D: VF implantation

NBM: Nucleus basalis magnocellularis, VF: Ventral forebrain

group A and B was found, but significance was determined in results between groups A and C; B and C ($p<0.01$). The results were similar in the assessment of 5th day ($p<0.001$) (Figure 8).

The means of water maze tasks in the first four days, which was performed between days 100 and 104 after formation of the NBM lesions, are shown in Figure 9. Among rats in normal group (A), sham-operated group (B), group with NBM lesion (C), group transplanted with VF grafts (D) and group transplanted with TV grafts (E), there were statistically significant differences between group C and other groups ($p<0.001$). The same results were determined in assessment of the 5th day's test results ($p<0.01$) (Figure 10).

Results of the test done on 10 days after NBM lesion were evaluated within the group (C1, n=12). Results of water maze test, which was repeated 100 days after the lesion were evaluated within the group (C2, n=3). Results of rats transplanted with VFGs (D, n=5), and transplanted with TVG (E, n=4) were also evaluated within their groups (Figure 11). Rats with lesion found the platform on day 100 (C2) in a shorter period of time than on the 10th day (C1). However, the learning among them was slower than the rats in groups D and E. For this assessment, there were statistically significant differences between C1 and C2 ($p<0.05$); C1 and D, E ($p<0.001$); C2 and D, E ($p<0.001$).

Discussion

It is believed that impairments of learning and memory functions in AD and other neurodegenerative diseases progressed with dementia are mainly due to decreased Ach, serotonin, and noradrenalin levels in the cortex in association with damage in the basal nuclei (25,28-30,32). AH-like learning and memory dysfunctions may be established in experimental animals by forming NBM

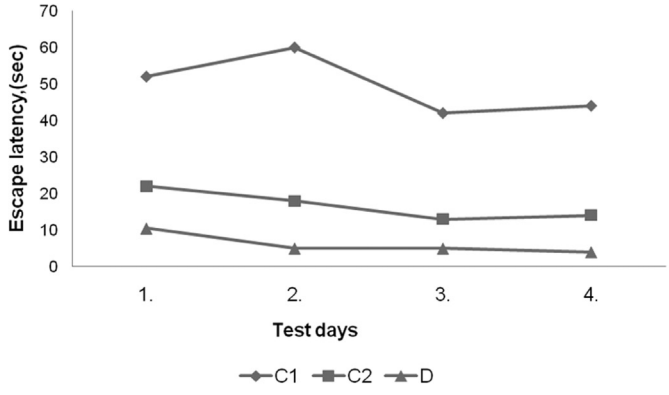

Figure 6. C1: NBM lesion (10th day), C2: NBM lesion (100th day), D: VF implantation (100th day)

NBM: Nucleus basalis magnocellularis, VF: Ventral forebrain

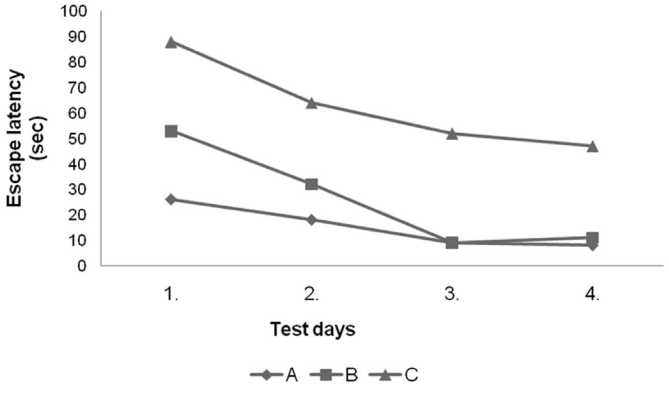

Figure 7. The results of the first 4 days of water maze tasks 10 days after lesion surgery. A: Normal, B: Sham-operated, C: NBM lesion

NBM: Nucleus basalis magnocellularis

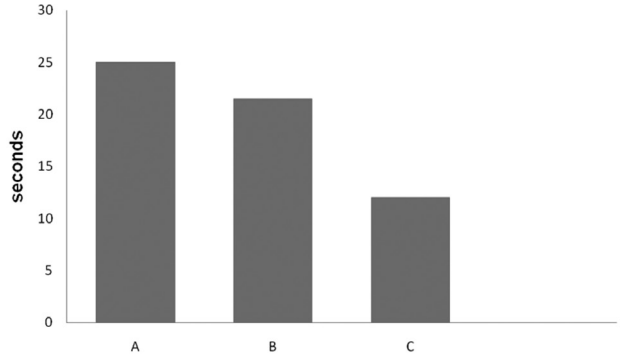

Figure 8. The results of the 5th day of water maze tasks 10 days after lesion surgery. A: Normal, B: Sham-operated, C: NBM lesion

NBM: Nucleus basalis magnocellularis

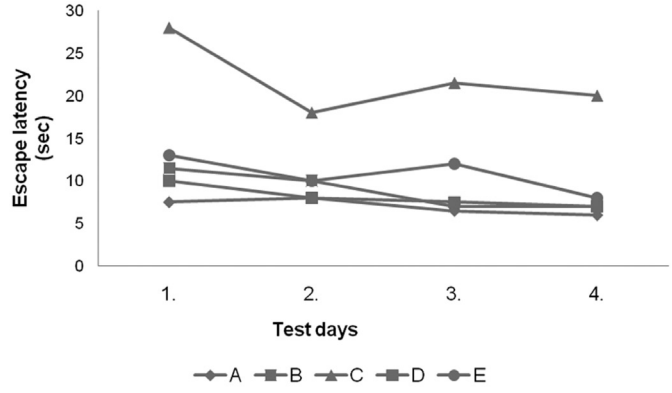

Figure 9. The results of the first 4 days of water maze tasks 100 days after lesion surgery. A: Normal, B: Sham-operated, C: NBM lesion, D: VF implantation, E: TV implantation

NBM: Nucleus basalis magnocellularis, VF: Ventral forebrain, TV: Telencephalic vesicle

or medial septal area lesions (34,35). In the present study, NBM lesion is selected, because it is the most debatable method.

Lesion should be extensive as neurons in NBM are scattered, and this may damage the adjacent structure. Surrounding fasciculi are also damaged along with neurons in electrolytic lesions formed by radiofrequency. Therefore, neurotoxic lesion is selected in the present study. To form neurotoxic lesions, different excitotoxic amino acids are used (27). Kainic acid, used in the present study, is the analogue of glutamic acid, which is an excitatory amino acid. In intraparenchymal injection, it binds nonspecifically to receptors on dendrites of neurons carrying excitatory amino acids, and it ruptures cell body and dendrites without damaging axons by overstimulation. As it is nonspecific, it ruptures other neurons along with

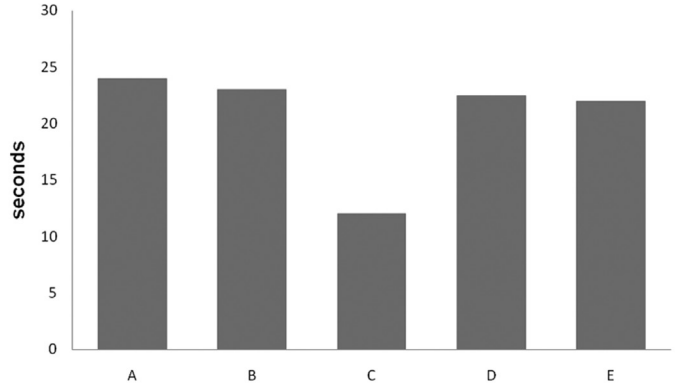

Figure 10. The results of the 5th day of water maze tasks 10 days after lesion surgery. A: Normal, B: Sham-operated, C: NBM lesion, D: VF implantation, E: TV implantation

NBM: Nucleus basalis magnocellularis, VF: Ventral forebrain, TV: Telencephalic vesicle

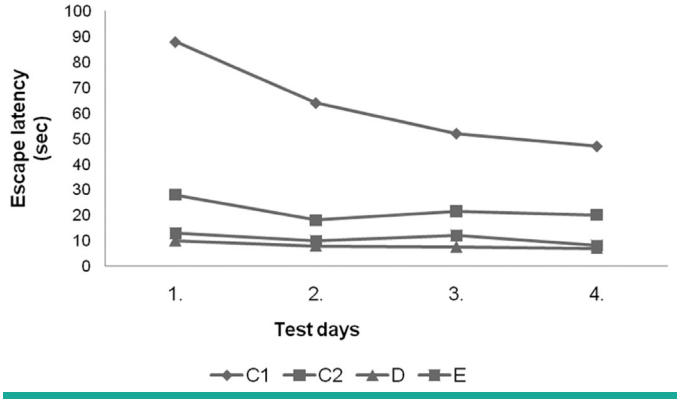

Figure 11. C1: NBM lesion (10th day), C2: NBM lesion (100th day), D: VF implantation (100th day), TV implantation (100th day)

NBM: Nucleus basalis magnocellularis, VF: Ventral forebrain, TV: Telencephalic vesicle

cholinergic neurons. In lesions formed by using kainic acid, medial globus pallidus, hippocampus, and ventrolateral thalamus are also partially damaged other than NBM. Since these areas are determined as partially damaged in AD, it is preferred to establish the model (33). In previous preliminary studies, bilateral NBM formation caused problems in water drinking, feeding, and related survival issues, unilateral lesion was formed in the present study. Since the right hemisphere is dominant in spatial learning (36), the lesion is formed on the right side.

Water maze test, which was originally created by Morris et al. (34), is excellent to assess spatial learning and memory performance in experimental animal models. In this test, the ability of rats to learn and remember the location of the hidden platform in a fixed quadrant by using the fixed surrounding objects is investigated. During the first 4 days normal rat learns to find the hidden platform quickly and directly by using the fixed cues around the tank. The rat spends more time in the same quadrant on the 5th day when the platform is removed. This reflects its ability to learn and remember the location of the platform.

Solid grafts and cell suspensions are used in transplantation studies. In studies by Björklund et al. (14,18), it was demonstrated that cell suspensions provided better results when compared to solid grafts. In studies by Plunnkett (37,38), it was observed that cells did not rupture if the suspension was given at 1 micro/min rate by using no 22-26 Hamilton needle. Administration of cell suspension by using stereotactic method eliminates neural tissue damage and high infection risks, which may be encountered during craniotomy, and it provides easy implantation.

It was observed that basocortical cholinergic system regenerated in unilateral lesions after 3-6 months (20). Therefore, Water maze test was performed 10 days later lesion formation, and it was repeated within the first 3 months after implantation.

In the first experiment, learning and memory functions, which were damaged after NBM lesion formation, were recovered after cholinergic neuron implantation. It was thought that primarily the graft survived, and might provide basocortical cholinergic system reconstruction by making functional synapses with recipient neural tissue. In the second experiment, which was conducted to investigate whether or not functional improvement resulted from cholinergic neuron reinnervation by the graft; similar recovery was observed in rats with noncholinergic neuron implantation as well as rats with cholinergic neuron

implantation. In both experiments, rats with lesion recovered partially in the late phase.

Underlying causes of functional recovery after neural tissue transplantation has not been clearly defined yet. Researches indicate four probable mechanisms.

The first mechanism is that graft tissue survives, and functions as a store releasing deficient neurotransmitters. Partial recovery observed in sensorimotor and behavioral disorders after adrenal medulla grafts in Parkinson disease (PD) model, and peripheral cholinergic ganglion grafts in AD model reinforces these mechanisms (39-54).

The second possibility is that neurons remain alive, and make functional synapses with recipient neural tissue, thus re-establishing the severed circuit. In studies with experimental animals, dopaminergic and cholinergic synapses were determined after transplantation in PD and AD models, respectively (5,10-13,15,16,19,20,23,41,46,52,55-59). It was observed that learning and memory functions were better in older rats after intrahippocampal fetal cholinergic grafts, and axons extending from the graft tissue to hippocampus were determined in histochemical studies (8,60).

It was postulated that locomotor activity recovery after adrenal medulla and fetal mesencephalon grafts used in PD model was associated to dopamine, norepinephrine, and epinephrine secreted from the graft in the early phase. The recovery was associated to dopaminergic connections, which were built up between the graft and striatum, in the late phase (21,41,42,47,48,50,54,61). Although partial recovery was detected in clinical picture in some Parkinson patients after adrenal medulla transplantation, few living cells or necrosis was observed in autopsy examination of these patients (53,62). In some studies, conducted on experimental animals, limited number of synapses was observed between the graft and recipient neural tissue in subjects with functional recovery (63). In the autopsy study of a patient, who received adrenal medulla transplantation in the striatum, and had poor recovery, it was determined that graft tissue remained alive and formed synapses (64).

It is thought provoking that bilateral response is gained with unilateral transplantation in Parkinson disease, and experimental PD models (65). If recovery is associated to functional synapses between the graft and striatum, then the response should have been unilateral. If the graft acts as a store secreting dopamine, then it should not be liable for bilateral recovery, because dopamine diffusion is limited to a few millimeters in the tissue (66).

In the present study, it has been shown that the recovery of learning and memory functions with both cholinergic and noncholinergic grafts cannot be explained by providing deficient neurotransmitters or cholinergic synapse formation from the graft to recipient neural tissue that are proposed in both mechanisms for functional recovery.

The third probability is stimulation of neurotrophic mechanisms. In recent 40 years, various neurotrophic factors, which prevent death of damaged neurons, and facilitate reinnervation of damaged circuits in CNS, have been defined (67-74). Nerve growth factor (NGF) is the most studied of all. It was reported that NGF pump implantation together with adrenal medulla implantation into putamen improved clinical recovery partially (75). It was observed in *in vivo* and *in vitro* studies that NGF prevented cell death in central cholinergic neurons and peripheral sympathetic ganglions, and also accelerated axonal growth (70,74). It was observed in tissue culture that NGF increased proliferation and differentiation of fetal basal forebrain neurons (76). In autopsy study on Alzheimer patients, Mufson et al. (77) observed in NGF-immunoperoxidase examinations that neurons carrying NGF receptor, especially in NBM, were quite decreased in basal forebrain neurons. Nieto-Sampedro et al. (78) showed that the graft tissue was alive 3-6 days after the lesion, and an NGF-like liquid, stimulating multiplication, accumulated in the cavity opening to the cortex. Korfalı et al. (79) reported that neurotrophic factors provided by gelfoam implantation in the cavitation ensured survival of adrenal medulla grafts implantation. In solid graft transplantations, grafts that were implanted after cavitation had better outcomes, and this was explained by activation of lesion-related trophic mechanisms (80,81). In the study performed with hemiparkinsonism model in monkeys, Bankiewicz et al. (23) reported that locomotor activity was recovered by both cavitations opened in caudate nucleus and dopaminergic and nondopaminergic tissue transplantations. In the study, dopaminergic axons extending from recipient neural tissue to the graft and cavity were detected (46,51,80). These findings indicated that functional recovery was obtained as the result of recipient neural tissue, itself, started to build up the damaged connections by using trophic mechanisms stimulated by cavitation and implantation. Bohn and Kanuicki (65) determined macrophages intensely around adrenal medulla grafts, and some of other investigators also reported similar findings. They proposed that recipient neural tissue developed an immunologic response against the graft, and macrophages secreted substances such as interleukins, which activated neurotrophic mechanisms

(46,65,80,82). It is believed that bilateral recovery after unilateral implantation may occur through this mechanism (54). Transplantation results are better in young experimental animals and young Parkinson patients, and it is explained by less trophic activity in elderly people (9,54).

The fourth probability is activation of neurogenesis by stimulation of endogenous stem cells. Stem cell is defined as cells, which can regenerate and have the potential to differentiate into different cell types. Stem cells do not have any predefined functions, and they provide *in vivo* functional reconstruction of a specific tissue by differentiating into different cell types according to signals they receive. They are classified as embryonic and adult according to their origin. Since the first embryonic stem cell growth in culture medium in 1998, experimental studies and investigations related to stem cell treatment have been conducted on many neurological degenerative disorders mainly spinal cord injuries, cerebral infarct, Parkinson disease, AD (83-91). Mainly two stem cell types are used: pluripotent stem cell/induced pluripotent stem cell originated from embryonic stem cell, and adult stem cells (neuronal stem cells, hematopoietic stem cells, mesenchymal stem cells). Neural stem cells are primarily located in hippocampal dental gyrus and subventricular zone in CNS (83-85,89). These cells are activated during conditions such as cerebral infarct, and facilitate recovery. However, their effects are limited, because of their inadequate number and gliosis. Therefore, administration from outside is considered as the suitable option. It was designated that neural stem cells decreased with age; hippocampal neurogenesis rate was slowed down with age; and there was progressive and diffuse cell death in these areas in AD (89). It was indicated that endogenous neural stem cells were regulated by various chemical agents and growth factors (89,90). In recent years, studies about prevention of death of neurons and glial cells, produced by the stem cells by the stimulation and regulation of primarily endogenous neural stem cells, have been performed (89,90). It is a known fact that in case of damage, endogenous neural stem cells prevent apoptosis in damaged cells by producing and secreting various trophic factors (90). In AD, experimental study findings indicated that neural stem cell transplantations, and neural progenitors might stimulate endogenous neural precursors and sinaptogenezis; and damaged cells might be rescued by secreted local neurotrophic and neuroprotective factors; and also, amyloid beta and tau protein accumulations might be diminished by therapeutic gene transfers (89,90). It is believed that symptoms and signs associated to cognitive disorders in AD may be improved by using these treatments.

The partial recovery in the late phase among rats with lesion may be explained as multiplication of limited number of alive cholinergic neurons as a result of lesion-related trophic mechanisms, and endogenous stem cell stimulation; thus, partial reconstruction of basocortical cholinergic system by re-establishing new axonal connections. Amelioration of spatial learning and memory impairments by both cholinergic and noncholinergic fetal neural tissue implantations may reflect that lesion stimulated trophic mechanisms and endogenous neural stem cells, namely hippocampal neurogenesis, become active after transplantation leading to acceleration of reconstruction.

When mechanisms playing a role in functional recovery are explained more clearly by anatomical and histochemical examinations, studies associated to neurogenesis activation will be a candidate treatment for many CNS disease involvements.

Conclusion

In rats, experimental AD model may be set up by performing unilateral NBM lesion, and damaging cortical cholinergic innervation, which leads to deterioration of learning and memory functions. İmpaired learning and memory functions in the early phase can recover in the late phase, which indicates that trophic mechanisms and hippocampal neurogenesis are stimulated due to the lesion itself. It is determined that improvement is accelerated both with cholinergic and noncholinergic fetal neural tissue implantations. Rather than functional synapses between the graft and recipient tissue, this condition can be explained by multiplication of limited number of living cholinergic neurons, and re-establishment of damaged connections by forming new synapses, as a result of endogenous neural stem cell regulation and activation of trophic mechanisms after implantation.

Ethics

Ethics Committee Approval: The study was approved by the local ethics committee (DETAM project number: 1990/31)

Informed Consent: This study does not include patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.M.C., O.B., Concept: S.M.C., O.B., Design: S.M.C., O.B., Data Collection or Processing: S.M.C., O.B., Analysis or Interpretation: S.M.C., O.B., Literature Search: S.M.C., O.B., Writing: S.M.C., O.B.

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