Amelioration of Spatial Learning and Memory Impairment By Foetal Cholinergic Neuronal Grafts In Rats With Lesions of the Nucleus Basalis Magnocellularis

S. MELTEM CAN, ORHAN BARLAS, ALİ SAVAŞ

İstanbul University, School of Medicine, Department of Neurosurgery, Çapa, İstanbul, Türkiye

Abstract : The acethylcholinergic afferents of the neocortex from subcortical areas participate in learning and memory. Autopsy studies in cases of Alzheimer's disease have shown that most of the neurons of the nucleus basalis magnocellularis are atrophic or decreased in number. In this study, the nucleus basalis magnocellularis (nucleus of Meynert), an important nucleus of the basocortical cholinergic system, was destroyed by the injection of kainic acid stereotactically so as to make a model of Alzheimer's disease, and cell suspension grafts prepared from acethylcholine rich foetal ventral forebrain neurons were implanted stereotactically to the frontal cortex. The results of Morris' water maze task showed that neurotoxic lesions of the nucleus basalis magnocellularis disrupt spatial learning and memory, and ventral forebrain grafts to the neocortex can restore the spatial learning impairment of lesioned rats.

Key words : Neural transplantation, Nucleus basalis magnocellularis, Spatial learning and memory.

INTRODUCTION

Since the end of the last century many investigations have pointed out that some of the neurons in the mammalian central nervous system (CNS) have the capacity to regenerate. In view of these studies, grafting neuronal tissue to the mammalian CNS has been attempted for the re-establishment of severed connections and the substitution of lost pathways in the treatment of degenerative disorders (3-9,11-19,21). It is now well established that cholinergic fibers from the nucleus basalis magnocellularis-NBM (nucleus of Meynert) to the neocortex is mainly involved in learning and memory processes (20). Electrolytic and neurotoxic lesions of the NBM result in an approximately 70% decrease in the acetylcholinesterase (AchE) activity of the neocortex causing Alzheimer-type learning and memory impairment in experimental animals (9,11,20). Biochemical and histological studies have shown that Ach-rich foetal ventral forebrain (VFB) grafts can establish functional synaptic contacts with host neurons in the neocortex. Additional confirmation of the functional effects of these grafts has been obtained in behavioural tests of learning and memory (9,11).

MATERIALS AND METHODS

A total of eighteen young adult male Wistar-Albino rats (İstanbul University, Centre for Experimental Medical Research and Application, Turkey) weighing about 230-300 g at the time of surgery served as experimental subjects. They were housed in groups with ad. lib. access to food and water throughout the experiments. Grafts were obtained from 14-16 day (crown-rump length 12-16 mm) foetuses of the same genus.

Unilateral neurotoxic lesions with kainic acid were made stereotactically in 11 rats, 3 rats were grouped as sham- operated controls, and 4 rats serving as normal controls were not treated surgically. Ten days after surgery spatial learning and memory

functions were tested by Morris' water maze tasks. At the end of the test, 6 of the 11 rats with NBM lesions received stereotaxic injections of cell suspensions containing tissue dissected from VFB of 14-16 day foetuses into the ipsilateral frontal cortex. After the implantation procedure water maze tasks were repeated.

Surgery: A mixture of ketamine (Ketalar, Parke-Davis, 10 mg/kg) and xylazine (Rompun, Bayer, 5 mg/kg) was used as anaesthetic during surgery. Rats were placed in the stereotaxic apparatus with the incisor bar at a level of 5 mm above the interaural line. A burr hole 2 mm in diameter was made, the 24 gauge needle of a 5 ul Hamilton microsyringe was inserted from two different coordinates to reach the NBM: A_1: 0.2mm anterior to the bregma, L_1: 3.4 mm lateral to the midline, V_1: 7.0 mm below the dura; A_2: 1.0 mm, L_2: 2.6 mm, V_2: 7.3 mm. Kainic acid (Sigma) was dissolved in distilled water in a concentration of 1%. From each coordinate 2 ul kainic acid 1% was injected at a speed of 1 ul/min. The sham-operated group received no injection. Fifteen days after lesion surgery, foetuses of 14-16 days' gestation were removed from pregnant rats of the same genus. Solid tissue pieces 2x2x2 mm in size were dissected microsurgically from the VFB region as described by Björklund (3). This procedure was carried out in a glass slide containing sterile ringer lactate solution. Tissue pieces from 8 foetuses were collected in sterile 0.6% glucose-0.9% NaCl at room temperature, incubated in crude trypsin (Sigma type II: 0.1% in the glucose-saline medium) for 20 min at 37°C, washed 4-5 times with fresh glucose-saline and mechanically dissociated by Pasteur pipette to form a milky suspension. For implantation, rats were placed in the stereotaxic apparatus with the incisor bar at a level of 5 mm above the interaural line. 3 ul of cell suspension was injected into the right frontal cortex through the previously opened burr hole at a speed of 1 ul/min at the following coordinates: A: 1.0 mm, L: 3.0 mm, V: 2.0 mm.

Morris' Water Maze Test: A cylindrical tank (100 cm in diameter, 40 cm deep) was filled to a depth of 30 cm at room temperature, and the water made opaque by the addition of powdered milk. Four starting points, 90° apart, were marked on the edge of the tank. A transparent glass platform (10x10 cm) was placed into a constant quadrant of the tank so that it was 1-2 cm below the water's surface. The pool was located in a corner of a room containing many fixed extra-maze cues (i.e. bright green flag, window, mirror, etc.). Rats were given 2 blocks of 4 trials on each day of 4 consecutive days. For each trial, the rat was placed in water facing the tank wall at each of the starting points and was given 120 sec to find the hidden platform and climb onto it, and was allowed to rest for 30 sec. Time to find the platform (escape latency) was measured by chronometer. After 32 trials in 4 days, the platform was removed. On the 5th day, the rat was placed in the tank at each of the starting points and allowed to swim for 60 sec, and the time spent in the quadrant in which the platform was placed previously was measured.

RESULTS

Arithmetic means of the results of eight trials in 4 consecutive days starting on the 10th day after the lesion surgery, are shown graphically in Figure 1. Repeated measures ANOVA on all groups (A-normal n:4, B-sham-operated n:3 and C- NBM lesion n:11) were performed. No difference was found between groups A and B, but group C differed markedly from the others ($p<0.001$). The results on the 5th day were similar ($p<0.03$) (Figure 2).

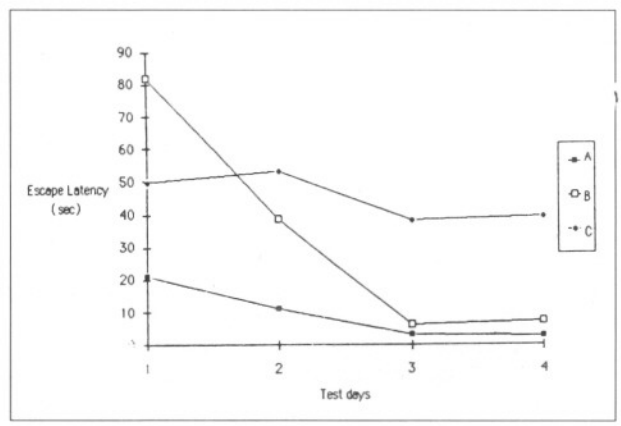

Fig. 1 : The results of the first 4 days of water maze tasks 10 days after lesion surgery
A: Normal, B: Sham-operated, C: NBM lesion

The results of the first four days of the tests done on the 100th-104th days after the implantation surgery, are shown in Figure 3. Repeated measures ANOVA on all groups (A-normal n:4, B-sham-operated n:3, C-NBM lesion n:5 and D-VFB implantation n:6) showed no difference between groups A and B, A and D, B and D; but a statistically significant difference was present between group C and the

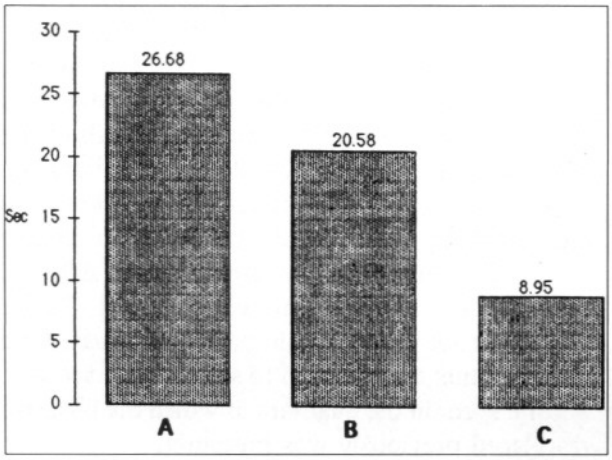

Fig. 2 : The results of the 5th day of water maze tasks 10 days after lesion surgery
A: Normal, B: Sham-operated, C: NBM lesion

others ($p<0.0001$). Figure 4 shows the results of the 5th days on this test. These were again significant between the same groups ($p<0.001$).

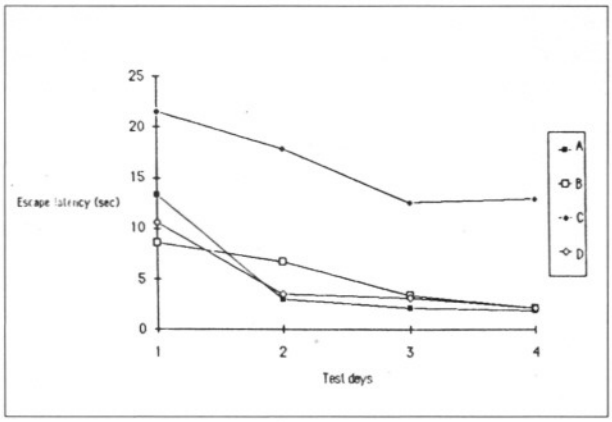

Fig. 3 : The results of the first 4 days of water maze tasks 100 days after the implantation of VFB
A:Normal, B:Sham-operated, C:NBM lesion, D:VFB implantation

The results of the water maze test performed on lesioned rats 10 (group C_1 n:11) and 115 days after lesion surgery (group C_2 n:5) and on implanted rats 100 days after implantation (group D n:6) were compared. Group C_2 found the platform quicker than group C_1 but slower than group D. A statistically significant difference was found between groups C_1 and C_2, C_1 and D, C_2 and D ($p<0.0001$) (Figure 5).

No difference was observed between their swimming speed.

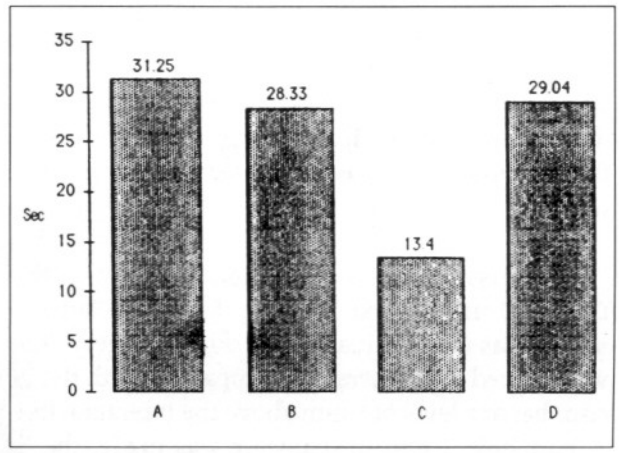

Fig. 4 : The results of the 5th day of water maze tasks 100 days after the implantation of VFB
A:Normal, B:Sham-operated, C:NBM lesion, D:VFB implantation

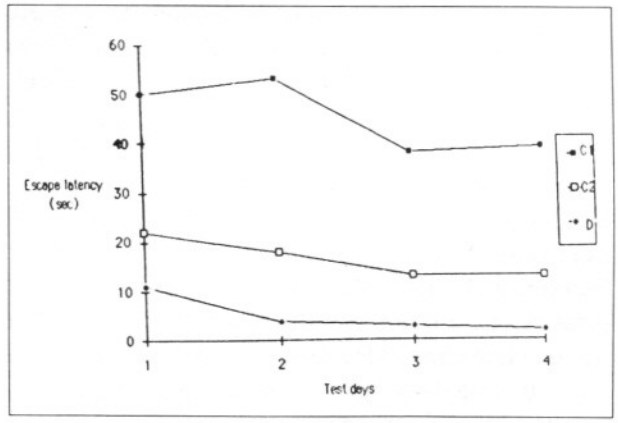

Fig. 5 : C_1:NBM lesion (10th day), C_2:NBM lesion (100th day), D:VFB implantation (100th day)

DISCUSSION

Since the end of the 19th century, regeneration, trophic mechanisms and axonal development of neuronal connections in mammalian CNS have been studied in a series of neuronal grafting experiments by many investigators. The poor results of early studies (Thomson 1890, Saltykow 1905) impeded further investigations (7,17). The first investigator to try grafts of foetal tissue to the brains of mammals, Del Conte (1907), concluded that the CNS was an unfavourable transplantation site (7,17). Although Dunn (1917) had reported survival of four of 44 grafts of neonatal cerebral cortex, the first successful results were obtained by Clark in 1940 (7,13,17). Since the

early 1970's Björklund, Stenevi, Das, Altman, Gage, Perlow and other investigators have shown that neural grafts can survive and be anatomically and functionally incorporated in the mammalian CNS (3-9,11-13,15-19,21). In 1983 Björklund and Stenevi (3-6) developed the cell suspension technique which allows stereotaxic graft implantations into any brain site without craniotomy. In these studies donor grafts were harvested from adrenal medulla or foetal brains, better results being reported with foetal grafts (1,2,4,7,8,13,15,19). The development and success of neural grafting techniques have suggested alternative ways to treat currently incurable degenerative disorders such as Parkinson's disease and Alzheimer's disease (1,2,14).

Subcortical cholinergic afferents of the neocortex involve many circuits of the CNS, and play a crucial role in learning and memory processes (10,20,22). The histochemical studies of Schute and Lewis (20) show that acethylcholinergic afferents of neocortex from subcortical areas which participate in learning and memory mainly originate in the NBM. Autopsy studies in cases of Alzheimer's disease have shown that most of the neurons of the NBM are atrophic or decreased in number. Associated with degenerative changes of the NBM is a profound reduction of cortical AchE activity (9-11). The most important sign observed in Alzheimer's disease is impairment of learning and memory performance.

Spatial learning and memory performance is best assessed with the water maze tasks originally developed by Morris (17). In this test, the ability of rats to learn and remember the location of the hidden platform placed in a constant quadrant by using extra-maze cues is observed. During the first 4 days normal rats learn to find the hidden platform quickly and directly by using the cues around the tank. This reflects their ability to learn and remember the location of the platform. On the 5th day, when the platform is removed, normal rats swim primarily in the training quadrant spending more time there.

An experimental model of Alzheimer's disease is provided by neurotoxic lesions of the NBM, which decreases neocortical AchE activity. In 1985 Fine and Dunnet (9,11) investigated the effects of neuronal grafts in the basocortical cholinergic pathways. They showed the formation of histologically and biochemically effective synaptic connections between graft and host tissue, and observed a significant recovery of spatial learning, as shown in the spatial probe trial on the 5th day of the water maze test.

The cholinergic septo-hippocampal system has also been shown to be involved in learning and memory processes. Björklund, Stenevi, Nilsson and co-workers (5,6,17,18) have investigated the effects of intrahippocampal foetal neuronal implants, and have shown that the Ach-rich grafts reinnervated the denervated hippocampus and formed AchE positive synapses with the hippocampus. They have also observed amelioration of spatial learning and memory impairment by neuronal grafting. Korfalı and co-workers (1990) (14) were the first to transplant foetal cholinergic grafts to the fimbria in a case of Alzheimer's disease, and improvement of behavioural and memory impairment was observed for a period of 8 to 9 months.

As bilateral lesions of the NBM cause severe feeding problems that can be fatal, we performed unilateral lesions. Stereotaxic methods were used both for neurotoxic lesions and transplantation. This technique minimized the risks of unwanted destruction of the host tissue and infections.

The results of implantation of neuronal tissue in the form of a dissociated cell suspension have been shown to be more successful than the results of solid grafts (4-8,12,17). The main advantages of this technique are that it induces minimal destruction of the host tissue, and allows for precise and easier placement of the implanted cells. In the imprint smear of the cell suspension, the cells are shown to be intact.

Since it has been observed that the basocortical cholinergic system will regenerate spontaneously 3-6 months after unilateral lesions of the NBM (9,11), we performed the water maze test 10 days after lesion surgery and repeated it 100 days after implantation.

Recovery of learning and memory impairment in rats with NBM lesions by grafts could be construed as indicative of graft survival and reinnervation, but this must be corroborated by anatomical and histochemical confirmation. Improvement of clinical impairment could be caused by reinnervation of the basocortical cholinergic system by grafts, but the

release of nerve growth factor by the host tissue or stimulation of unknown mechanisms could also be the reason. Further understanding of mechanisms of recovery may lead to successful treatment of Alzheimer's disease patients with implantation techniques as shown by Korfalı and co-workers (14).

Acknowledgements : We wish to thank Prof. Korfalı for kindly reviewing the manuscript, and to Prof. Bayındır for preparing the imprint smear of our cell suspension. The study was performed in the laboratories of İstanbul University, Centre for Experimental Medical Research and Application (DETAM), and supported by DETAM (project no:1990/31).

Correspondence : S. Meltem Can,
M. D., İ. Ü. İstanbul Tıp Fakültesi
Nöroşirurji Anabilim Dalı, Çapa, İstanbul.
Tel : (1) 525 99 98
Fax : (1) 521 60 81

REFERENCES

1. Backlund EO, Granbergi PO, Hamberger B, et al: Transplantation of adrenal medullary tissue to striatum in Parkinsonism. First clinical trials. J. Neurosurg. 62: 169-173, 1985.
2. Backlund EO, Olson L, Seiger A, et al: Toward a transplantation therapy in Parkinson's disease. Ann NY Acad Sci 495: 658-673, 1987
3. Björklund A, Stenevi U, Schmidt RH, et al: Intracerebral grafting of neuronal cell suspensions. I. Introduction and general methods of preparation: Acta Physiol Scand Suppl 522: 1-7, 1983
4. Björklund A, Stenevi U, Schmidt RH, et al: Intracerebral grafting of neuronal cell suspensions. II. Survival and growth of nigral cell suspensions implanted in different brain sites. Acta Physiol Scand Suppl 522:9-18, 1983
5. Björklund A, Gage FH, Stenevi U, et al: Intracerebral grafting of neuronal cell suspensions. VI. Survival and growth of intrahippocampal implants of septal cell suspensions. Acta Physiol Scand Suppl 522: 49-58, 1983
6. Björklund A, Gage FH, Schmidt RH, et al: Intracerebral grafting of neuronal cell suspensions. VII. Recovery of choline acetyltransferase activity and acetylcholine synthesis in the denervated hippocampus reinnervated by septal suspension implants. Acta Physiol Scand Suppl 522: 59-65, 1983
7. Björklund A, Stenevi U: Intracerebral neural implants. Annv. Rev. Neurosci 7: 279-308, 1986
8. Dunnet SB, Björklund A, Schmidt RH,et al: Intracerebral grafting of neuronal cell suspensions. IV. Behavioral recovery in rats with unilateral 6-OHDA lesions following implantation of nigral cell suspensions in different forebrain sites. Acta Physiol Scand Suppl 522: 29-37, 1983
9. Dunnet SB, Toniolo G, Fine A, et al: Transplantation of embrionic ventral forebrain neurons to the neocortex of rats with lesions of nucleus basalis magnocellularis. II. Sensorimotor and learning impairments. Neuroscience 16: 787-792, 1985
10. Fibiger HC, Damsma G, Day JC: Behavioral pharmacology and biochemistry of central cholinergic neurotransmission, in Nagier TC (ED): The basal forebrain. New York: Plenum press, 1991, pp. 399-414
11. Fine A, Dunnet SB, Björklund A, et al: Transplantation of ventral forebrain neurons to the neocortex of rats with lesions of nucleus basalis magnocellularis. I. Biochemical and anatomical observations. Neuroscience 16: 769-786, 1985
12. Gage FH, Björklund A, Stenevi U, et al: Intracerebral grafting of neuronal cell suspensions. VIII. Survival and growth of implants of nigral and septal cell suspensions in intact brains of aged rats. Acta Physiol Scand Suppl 522: 67-75, 1983
13. Korfalı E: Sıçanlarda denerve edilmiş korpus striatumun fötal dopaminerjik nöron greftleri ile innervasyonu. Doçentlik tezi, Uludağ Üniversitesi 1981
14. Korfalı E, Doygun M, Balkır N, ve ark: Alzheimer Hastalığında fimbriya'ya fötal kolinerjik greft implantasyonu (Bir olgunun klinik sonuçları). Türk Nöroşirurji Dergisi Ek 4: 118, 1991
15. Lindvall O, Björklund A: Transplantation strategies in the treatment of Parkinson's disease, experimental basis and clinical trials. Acta Neurol Scand 126: 197-210, 1989
16. Mampalan TS, Gonzales MG, Weinstein P, et al: Neuronal changes in fetal cortex transplanted to the ischemic adult rat cortex. J. Neurosurg. 69: 904-912, 1988
17. Nilsson OG: Intracerebral neuronal transplantation: Growth and function of cholinergic neurons transplanted to the hippocampus. University of Lund press, 1990, pp. 1-12
18. Nilsson OG, Shapiro ML, Gage FG, et al: Spatial learning and memory following fimbria-fornix transsection and grafting of fetal septal neurons to the hippocampus. Exp Brain Res 67: 195-215, 1987
19. Perlow M: Brain grafting as a treatment of Parkinson's disease. Neurosurgery 20: 335-342, 1987
20. Schute CCD, Lewis PR: The ascending cholinergic reticuler system, neocortical, olfactory and subcortical projections. Brain 90: 497-522, 1967
21. Sunde N, Laubery S, Zimmer J: Brain grafts can restore irradiation damaged neuronal connection in newborn rats. Nature 310: 51-53, 1984
22. Wilson FAW: The relationship between learning, memory and neuronal responses in the primate brain,in Napier TC, et al (ED): The basal forebrain. New York: Plenum press, 1991, pp. 253-266