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Transplantation of Hippocampal Cell Lines Restore Spatial Learning in Rats With Ventral Subicular Lesions

J. Rekha
National Institute of Mental Health and Neuro Sciences (NIMHANS Deemed University), India

Sridhara Chakravarthy
National Centre for Biological Sciences (NCBS), Tata Institute of Fundamental Research (TIFR), India

L. R. Veena
National Institute of Mental Health and Neuro Sciences (NIMHANS Deemed University), India; People's Education Society Institute of Technology, India

Vani P. Kalai
National Institute of Mental Health and Neuro Sciences (NIMHANS Deemed University), India

Rupam Choudhury
National Centre for Biological Sciences (NCBS), Tata Institute of Fundamental Research (TIFR), India

Harsha N. Halahalli, Phalguni Anand Alladi, Anandh Dhanushkodi, and M. Nirmala
National Institute of Mental Health and Neuro Sciences (NIMHANS Deemed University), India

Geetha M. Swamilingiah
National Centre for Biological Sciences (NCBS), Tata Institute of Fundamental Research (TIFR), India

Maulishree Agrahari
People's Education Society Institute of Technology, India

T. R. Raju
National Institute of Mental Health and Neuro Sciences (NIMHANS Deemed University), India

M. M. Panicker
National Centre for Biological Sciences (NCBS), Tata Institute of Fundamental Research (TIFR), India

Bindu M. Kutty
National Institute of Mental Health and Neuro Sciences (NIMHANS Deemed University), India

We have demonstrated in our previous studies that ventral subicular lesion induces neurodegeneration of the hippocampus and produces cognitive impairment in rats. In the present study, the efficacy of transplanted green fluorescent protein (GFP)-labeled hippocampal cell line (H3-GFP) cells in establishing functional recovery in ventral subicular lesioned rats has been evaluated. The survival of H3-GFP transplants and their ability to express trophic factors in vivo were also investigated. Adult male Wistar rats were subjected to selective lesioning of ventral subiculum and were transplanted with H3-GFP cells into the cornu ammonis 1 (CA1) hippocampus. The transplants settled mainly in the dentate gyrus and expressed neurotrophic factors, brain-derived neurotrophic factor (BDNF), and basic fibroblast growth factor (bFGF). The ventral subicular lesioned (VSL) rats with H3-GFP transplants showed enhanced expression of BDNF in the hippocampus and performed well in eight-arm radial maze and Morris water maze tasks. The VSL rats without hippocampal transplants continued to show cognitive impairment in task learning. The present study demonstrated the H3-GFP transplants mediated recovery of cognitive functions in VSL rats. Our study supports the notion of graft meditated host regeneration and functional recovery through trophic support, although these mechanisms require further investigation.

Keywords: brain lesion, neurodegeneration, learning impairment, hippocampal transplants, trophic factors, functional recovery

J. Rekha, Vani P. Kalai, T. R. Raju, Harsha N. Halahalli, Phalguni Anand Alladi, Anandh Dhanushkodi, M. Nirmala, and Bindu M. Kutty, Department of Neurophysiology, National Institute of Mental Health and Neuro Sciences (NIMHANS Deemed University), Bangalore, India; Sridhara Chakravarthy, Rupam Choudhury, Geetha M. Swamilingiah, and M. M. Panicker, National Centre for Biological Sciences (NCBS), Tata Institute of Fundamental Research (TIFR), GKVK Campus, Bangalore, India; L. R. Veena, Department of Neurophysiology, National Institute of Mental Health and Neuro Sciences (NIMHANS Deemed University), Bangalore, India; Maulishree Agrahari, Department of Biotechnology, People's Education Society Institute of Technology, Bangalore, India.

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Correspondence concerning this article should be addressed to Bindu M. Kutty, Department of Neurophysiology, National Institute of Mental Health and Neuro Sciences (NIMHANS Deemed University), Bangalore 560029, India. E-mail: bindu.nimhans@gmail.com

The subiculum constitutes the major output structure of hippocampal formation and projects to various neocortical and subcortical structures (Lopes da Silva, Witter, Boeijinga, & Lohman, 1990; Swanson & Cowan, 1997, 1979). Studies indicate that the structures of hippocampal formation (the subiculum, hippocampus, and entorhinal cortex) are closely associated with each other both anatomically and functionally (Sharp, 1999; Sharp, Kubie, & Muller, 1990) and constitute the hippocampal learning system. Earlier studies support the role of the hippocampus to construct a neurocognitive map (Morris, Garrud, Rawlins, & O'Keefe, 1982; O'Keefe & Nadel, 1978; Rawlins & Tsaltas, 1983); however, recent studies emphasize the role of the entorhinal cortex and subiculum along with the hippocampus in spatial map construction (Cho & Jaffard, 1995; Morris et al., 1982; Oswald & Good, 2000; Taube, Kesslak, & Cotman, 1992). We have demonstrated in our previous studies that selective lesioning of the ventral subiculum induces neurodegeneration of the hippocampus and impairs spatial learning in rats (Anandh, Bindu, Raju, & Kutty, 2007; Bindu, Rekha, & Kutty, 2005; Devi, Diwakar, Raju, & Kutty, 2003; Govindaiah, Rao, Raju, & Meti, 1997; Laxmi, Bindu, Raju, & Meti, 1999). Selective lesioning of the ventral subiculum reduces the dendritic arborization of both cornu ammonis 1 (CA1) and cornu ammonis 3 (CA3) hippocampal neurons (Bindu et al., 2007; Nutan & Meti, 1998; Shankaranarayana Rao, Govindaiah, Laxmi, Meti, & Raju, 2001); induces neurodegeneration of the entorhinal cortex (Devi et al., 2003; Govindaiah et al., 1997); and causes spatial learning impairment in T maze (Laxmi et al., 1999), eight-arm radial maze (Devi et al., 2003), and water maze (Bindu et al., 2005) tasks. The behavioral impairment was attributed to the combined effect of subiculum lesion and associated neurodegeneration of hippocampal neurons (Anandh et al., 2007; Bindu et al., 2005; Devi et al., 2003; Govindaiah et al., 1997). Other studies also reported the role played by the subiculum alone or together with other hippocampal structures in spatial representation and spatial navigation (Cho & Jaffard, 1995; Jarrard, 1986; Jarrard, Kant, Meyerhoff, & Levy, 1984; Morris, Schenk, Tweedie, & Jarrard, 1990; Oswald & Good, 2000; Schenk & Morris, 1985; Taube et al., 1992). Electrophysiological studies have also reported the spatial characteristics of subicular place cells, which fire in both a location- and direction-specific manner (Sharp et al., 1990).

Neural transplantation studies (Corti et al., 2002; Deng, Guo, Yuan, & Li, 2003; Eaton, Santiago, Dancausse, & Whittemore, 1997; Saporta, Borlongan, & Sanberg, 1999; Sinden, Helen-Hodges, Jeffery, & Gray, 1995; Zigova, Pencea, Sanberg, & Luskin, 2000) have demonstrated the graft survival and integration leading to central nervous system (CNS) repair and cognitive recovery (Hodges et al., 2000; Lunberg et al., 1997; Virley et al., 1999). Sinden et al. (1997) demonstrated the recovery of spatial learning in ischemic rats following transplantation of MHP36 cell lines into the damaged CA1 regions. Wong, Hodges, and Horsburgh (2005) demonstrated a reduction of ischemic damages in the caudate nucleus following transplantation of MHP36 cell lines. The transplants integrated well within the damaged caudate nucleus and differentiated mainly into neurons. Extensive migratory properties of both neural and embryonic stem cells were also reported following transplantation into contralateral sites (Modo et al., 2002a, 2002b; Veizovic, Beech, Stroemer, Watson, & Hodges, 2001) or vascular compartments (Chu, Kim, Jeong, Kim, & Yoon, 2003). Hodges et al. (2000) demonstrated extensive migration of neuroepithelial stem cell grafts (MHP36 cells) in aged rats. The grafts appeared to ameliorate spatial learning deficits associated with widespread degenerative changes in aged rats.

Neurodegeneration of hippocampal structures together with cognitive impairment is reported in Alzheimer's disease (Struble et al., 1991). Developing strategies to promote functional recovery using appropriate animal models may be of great therapeutic importance. In the present study, we have therefore evaluated the efficacy of green fluorescent protein (GFP)-labeled hippocampal cell line (H3-GFP) transplants in establishing functional recovery in ventral subicular lesioned (VSL) rats. The survival of the hippocampal cell line transplants and their ability to express trophic factors in vivo have also been investigated to throw light on potential repair mechanisms.

Method

Subjects

Two-month-old male Wistar rats were obtained from the Central Animal Research Facility of National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore, India. The rats were housed in polypropylene cages (dimensions 22.5 × 35.5 × 15 cm) at room temperature (26 ± 2 °C) and were maintained on a 12-hr light–dark cycles. Food and water were provided ad libitum.

The rats were divided randomly into normal control (NC) and VSL groups. The NC rats were not subjected to any surgical interventions, whereas the VSL rats were subjected to chemical lesioning of ventral subiculum bilaterally. A total of 115 rats were used for the study. The experiments were carried out in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 80–23, revised 1996). Approval was obtained from the Institutional Animal Ethics Committee before initiating the study. All efforts were made to minimize both the number and suffering of the animals used.

Lesioning of Ventral Subiculum

The rats from VSL group were anesthetized with ketamine (75 mg/kg, body weight, intraperitoneal [ip]) and xylazine (10 mg/kg body weight, ip). One percentage lignocaine was used as local anesthesia.

The animals were fixed on a stereotaxic instrument (David Kopf, Tujunga, CA, USA). The flat skull stereotaxic coordinates were taken from the Paxinos and Watson (1982) rat atlas. Ibotenic acid dissolved in phosphate buffered saline (pH = 7.4) was injected into the ventral subiculum (anteroposterior [AP] = −7.3 mm, mediolateral [ML] = 5.0 mm, and dorsoventral [DV] = 5.3 mm) bilaterally at a concentration of 0.25 μg/0.25 μl/site, at the rate of 0.25 μl/min with the help of a micro injector (Harvard Apparatus, Holliston, MA, USA) using a 5-μl Hamilton syringe. Following recovery from anesthesia, the rats were returned to their home cages and were provided postoperative care. The rats were given food and water ad libitum.

Transplantation of H3-GFP Hippocampal Cell Lines

The H3-GFP hippocampal cell lines were generated from the hippocampus (postnatal Day 1) of H-2K^b-ts A58 transgenic mice, which harbor a temperature-sensitive (TS) SV40 large T antigen

(Jat et al., 1991). The phenotypic characteristics of H3-GFP cells were studied extensively in vitro by our group (Sridhara Chakravarthy, 2001). The cell lines exhibit a neuronal phenotype (neurofilament-L [NF-L], microtubule associated protein-2 [MAP-2], nestin) and do not express any glial markers. In addition, the H3 cell line was genetically modified using a retrovirus encoding a nuclear-localized GFP to obtain H3-GFP to aid in the identification of cells in vivo. These cells are, in addition, resistant to G418 (neomycin) and zeocin. The cells have been maintained for several years in vitro and were found to remain stable in their expression pattern. Because H3-GFP cell lines contain a TS SV40 large T antigen, they proliferate at 33 °C (permissive temperature) but cease to divide at a nonpermissive temperature of 39 °C in low serum Dulbecco's modified Eagle's medium (DMEM), and only half of the initial number plated survived after 5 days in vitro. The cell lines were routinely maintained at 33 °C in 10% DMEM, with a doubling period of approximately 3 to 4 days. Before transplantation, the cells (80% confluence in the incubator at 37 °C) were washed with phosphate-buffered saline (PBS) and treated with 1× trypsin-EDTA for 2 min, resuspended in 5 ml of the same culture medium, centrifuged, and resuspended in 500 μl of supernatant media and incubated at 37 °C for 15 min prior to transplantation. Following incubation in DMEM medium prior to transplantation, the cell viability was found to be above 95% as assessed by trypan blue exclusion techniques.

Ten days following VSL, the VSL rats were subjected to transplantation of H3-GFP cell lines into the CA1 hippocampus bilaterally. The rats were anesthetized with ketamine (75 mg/kg body weight, ip) and xylazine (10 mg/kg body wt, ip) and fixed to a stereotaxic instrument. Four microliters of H3-GFP cell suspension (containing approximately 75,000 to 1,00,000 cells) was transplanted in to the CA1 hippocampus (stereotaxic coordinates, Bregma AP = 3 mm; ML = 2 mm; DV = 3 mm) bilaterally at a rate of 0.5 μl per minute with the help of a micro injector (Harvard Apparatus, MA) using 10 μl Hamilton syringe fixed with a 27-gauge needle. The needle was left in place for 5 min and was slowly withdrawn. Each rat received a total volume of 8 μl of H3-GFP cell suspension. The vehicle control group (VSL + VC) received an equal volume of DMEM (without serum) into the CA1 hippocampus. Healex was sprayed on the wound. Following recovery from anesthesia, the rats were returned to their home cages and postoperative care was taken. The rats were given food and water ad libitum. Two months following transplantation, the rats were assigned to various studies.

Immunofluorescence

Immunocytochemical studies were carried out 2 months following transplantation to assess brain-derived neurotrophic factor (BDNF) expression in the host hippocampus (n = 20, 5 rats per group, NC, VSL and VSL + VC and VSL + H3-GFP groups) and BDNF and basic fibroblast growth factor (bFGF) expression by the transplants (n = 6, VSL + H3-GFP group). Rats were deeply anesthetized and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. The brains were postfixed in the same fixative (4% paraformaldehyde [PFA]) for 48 hrs, followed by three washes in chilled 0.1 M phosphate buffer (pH = 7.4). The brains were then cryoprotected sequentially in 15%, 30%, and 40% sucrose and finally embedded in tissue freezing medium for cryosectioning. Thirty-micrometer thick coronal sections were taken at the level of the dorsal hippocampus using cryostat (Leica, Germany) and kept in deep well plates containing 0.1M-phosphate buffer (pH = 7.4). The sections were washed with phosphate-buffered saline containing 0.05% Triton X-100 (PBSTx); followed by incubation in 5% fat free skimmed milk solution for 4 hrs at room temperature to block nonspecific staining. Sections were processed for assessing the expression of trophic factors using appropriate antibodies.

Assessment of BDNF Expression in the Host Hippocampus

Fifteen sections per rat at the level of the dorsal hippocampus (between −2.3 mm to −3.8 mm with reference to Bregma using Paxinos and Watson (1982) rat atlas) were taken from each group (VSL, VSL + VC, NC, and VSL + H3-GFP, n = 5 rats per group) and were incubated with primary antibody (antirabbit BDNF immunoglobulin G [IgG] fraction, 1:500, Santa Cruz Biotechnology, CA, USA) for 72 hrs at 4 °C. Primary antibody was removed and sections were washed with PBSTx three times for 5 min each and then incubated in secondary antibody (1:200, antirabbit IgG fluorescein thiocyanate (FITC) conjugated; Sigma, St. Louis, MO) overnight at 4 °C. The sections were washed three times with PBSTx and mounted with 65% glycerol to avoid dehydration of the sections. The sections were cover slipped and sealed using nail enamel.

Microscopy and Quantification

Confocal imaging. The sections were viewed under laser scanning confocal microscope (Leica-TCS SL, Germany) for BDNF expression using a blue filter at an excitation of 488 nm under 20× magnification.

For Fluorescence intensity measurements, sections were viewed and images were captured using a Leica laser scanning confocal microscope. The FITC flourochrome bound to primary antibody against BDNF was excited by using an argon laser (488 nm) and detected by using standard FITC filters. All the images were captured at 20× Magnification at a constant PMT (Photo Multiplier Tube) voltage of 557 V and each image was averaged four times. The Image format was kept constant at 1024 × 1024 pixel resolution. Other features such as pinhole diameter, scan speed, and so forth were also maintained uniformly.

The eight-bit images captured on the confocal microscope were analyzed "offline" using Q-win plus software for image analysis (Bindu et al., 2007). Briefly, the captured images were converted into black and white images and the fluorescence intensity was measured on a scale of 0 to 255, where "0" refers to black (dark) and "255" refers to white (highly florescent/bright) pixels. Thereafter, using the "detect" command, the CA1 subregion of the hippocampus falling within the image frame of 500 × 500 μm was selected. The image was manually "threshold" between the values of 0 to 255, to select only the stained area for intensity measurement. The intensity values were then tabulated using the Excel program. A similar protocol was followed for CA3 area also. The images of both left and right hippocampi of each brain section were captured and values (of both right and left hippocampi) were pooled to obtain the mean value for each brain section and finally

the mean value for each rat was obtained by pooling the values from a total of 15 brain sections.

Assessment of BDNF and bFGF expression in transplants. The sections (5 rats from VSL + H3-GFP group; 15 sections for each trophic factor) were incubated separately with anti-BDNF (rabbit polyclonal, 1:200, Chemicon International, Billerica, MA, USA) or anti-bFGF (rabbit polyclonal, 1:200, Chemicon International) for 72 hrs at 4 °C. After three washes with PBSTx, sections were incubated overnight with antirabbit secondary tagged to Cy3 at room temperature in the dark. The sections were washed three times with PBSTx, mounted onto slides in mounting media (Sigma), and viewed under a laser scanning confocal microscope (Leica-TCS SL, Germany) at 488 nm for FITC/GFP and at 514 nm for Cy3 for the visualization of H3-GFP cells. The emission bandwidth of 495 to 540 nm for FITC/GFP and 550 to 625 nm for Cy3 was maintained to avoid any nonspecific overlap of emission frequencies as per our earlier study (Bindu et al., 2007).

In Vitro

H3-GFP cell lines were seeded onto glass cover slips ($n = 3$ cover slips for each antibody studied) precoated with 0.01% poly-D-lysine and 1 $\mu g/cm^2$ laminin and grown in DMEM/F-12 medium at 37 °C for 6 days and then processed for immunocytochemistry to study the expression of BDNF and bFGF.

On the sixth day, DMEM medium was removed, and cells (H3-GFP) were washed with phosphate buffered saline and fixed with ice-cold methanol for 4 min at −20 °C. The cells were incubated overnight in 0.1 M PBS (pH = 7.4), at 4 °C. Cells were washed three times with PBS (5 min each), followed by blocking with 3% bovine serum albumin for 30 min and incubated with primary antibody, either with anti-BDNF (rabbit polyclonal, 1:200, Chemicon International, Billerica, MA, USA) or antibFGF (rabbit polyclonal, 1:250, Chemicon International, Billerica, MA, USA) for 48 hrs at 4 °C. After three washes with PBS, cells were incubated with antimouse Cy3 (BDNF, bFGF) for 2 hrs at room temperature in the dark. The cells were rinsed three times with PBS and mounted with PVA-DABCO mounting media (Sigma). These cells were viewed under a laser scanning confocal microscope (Leica TCS SL, Germany) as in our earlier study (Shobha et al., 2007).

Behavioral Assessment of Spatial Learning

Behavioral studies were carried out following 2 months of transplantation using eight-arm radial maze and Morris water maze tasks in separate groups of rats.

Eight-Arm Radial Maze

The eight-arm radial maze is a computer-monitored plexiform maze (Columbus Instruments, Columbus, OH, USA) consisting of eight equally spaced arms radiating from an octagonal central platform. Each arm is 42 cm long, 11.4 cm high, and 11.4 cm wide. The entire maze was elevated 80 cm above the floor.

A total of 32 rats ($n = 8$ per group) were used for assessing the eight arm radial maze task. The groups studied were NC rats, VSL rats, ventral subicular lesion + DMEM as vehicle (VSL + VC), and ventral subicular lesioned rats with H3-GFP transplants (VSL + H3-GFP).

Acquisition of Spatial Task

The rats were maintained on a restricted food regimen during night prior to behavioral studies. During acclimatization, the rat was placed in the center of the maze and allowed to explore freely for 10 min. During training, all eight arms were baited with food pellets (Kellogg's chocolate wheat scoops), and the rats were trained to acquire the task of retrieving the food pellets from each arm. Reentry into the arm was considered as a working memory error. The trial was terminated when the animal obtained the reward from all arms or if 10 min had elapsed in the radial maze. Each animal was given two trials per day with an intertrial interval of 15 min. The performance was scored by calculating the percentage of correct responses (a correct response is when the rat enters an arm, not previously entered) divided by the total number of entries made by the rat. The average criterion for acquisition of the spatial task was attaining 7 out of 8 correct choices

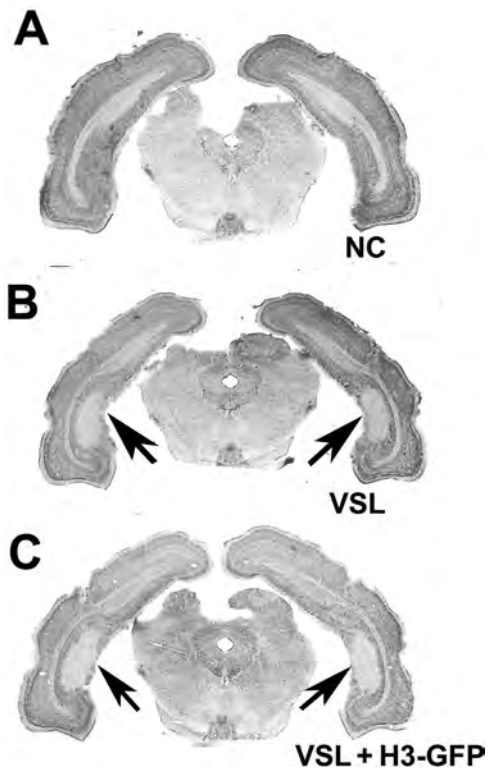

Figure 1. Photomicrographs of the cresyl violet-stained coronal rat brain sections at the level of ventral subiculum in normal control (NC) (A), ventral subicular lesioned (VSL) (B), and VSL lesioned rats transplanted with green fluorescent protein (GFP)-labeled hippocampal cell line (H3-GFP) (VSL + H3-GFP) (C) rats. The ventral subiculum was completely lesioned (indicated by arrows) in VSL and VSL + H3-GFP rats. Lesion size ranges between 1.5 and 2 mm^3 (measured with the help of stage micrometer using a Leitz [Germany] microscope under the same magnification). The stereotaxic coordinates correspond to anteroposterior (AP) = −7.3 mm, mediolateral (ML) = 5.0 mm, and dorsoventral (DV) = 5.3 mm using the Paxinos and Watson (1982) atlas. Adapted with permission.

(87%−100% correct choices). To avoid olfactory cues, the maze was wiped with 70% ethanol prior to each session (Devi et al., 2003).

Retention Test

A retention test was carried out 10 days following acquisition of the eight-arm baited task. In retention, same protocol of acquisition of the spatial task was followed. All eight arms were baited with food pellets (Kellogg's chocolate wheat scoops), and the rat was placed in the center of the maze. The performance was assessed in two trials and the mean average performance was taken. Finally, as mentioned above, the performance (percentage of correct choices) was scored by calculating the percentage of correct responses divided by the total number of entries made by the rat.

Water Maze Task

A separate set of 32 rats (n = 8 per group) was used for behavioral assessment of spatial navigation in Morris water maze. The groups studied were NC, VSL, VSL + VC, and VSL + H3-GFP.

The water maze was a circular pool (diameter = 168 cm, height = 60 cm) filled with water (temperature = 24 ± 1 °C) to a depth of 30 cm. An escape platform, 10 cm in diameter, submerged 1 cm below the water surface, was kept in the northeast quadrant of the tank. The water was made opaque by adding milk. The rats were given habituation in a single session of two trials during which they were allowed to swim for 90 s with no escape platform.

Acquisition of the Task

During training sessions, rats were released from two different positions of the pool (from the North West quadrant in the first trial and south west quadrant in the second trial keeping the face toward the wall), while the position of the hidden platform was kept constant (northeast quadrant). The rats were allowed to swim until they reach the escape platform or for a duration of 90 s. If the

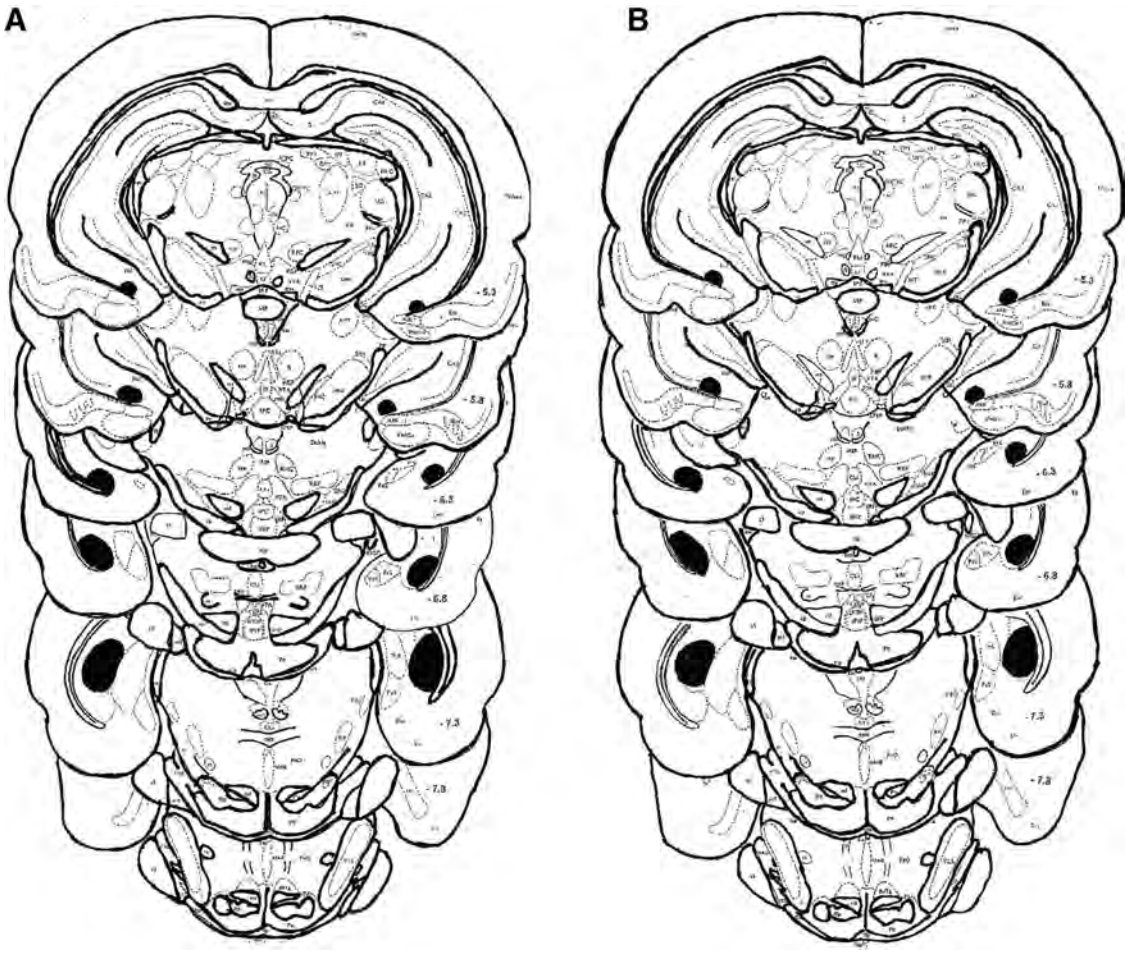

Figure 2. Schematic representation of the extent of ventral subicular lesion. Shaded areas represent the extent of damage (Bregma −5.8 to −7.8 mm) occurred following ibotenic acid lesioning of ventral subiculum using the Paxinos and Watson (1982) rat brain atlas from ventral subicular lesioned (VSL) (A) and VSL rats transplanted with green fluorescent protein (GFP)-labeled hippocampal cell line (H3-GFP) (VSL + H3-GFP) (B) rats. The ventral subiculum is completely lesioned by ibotenic acid infusion. Adapted with permission.

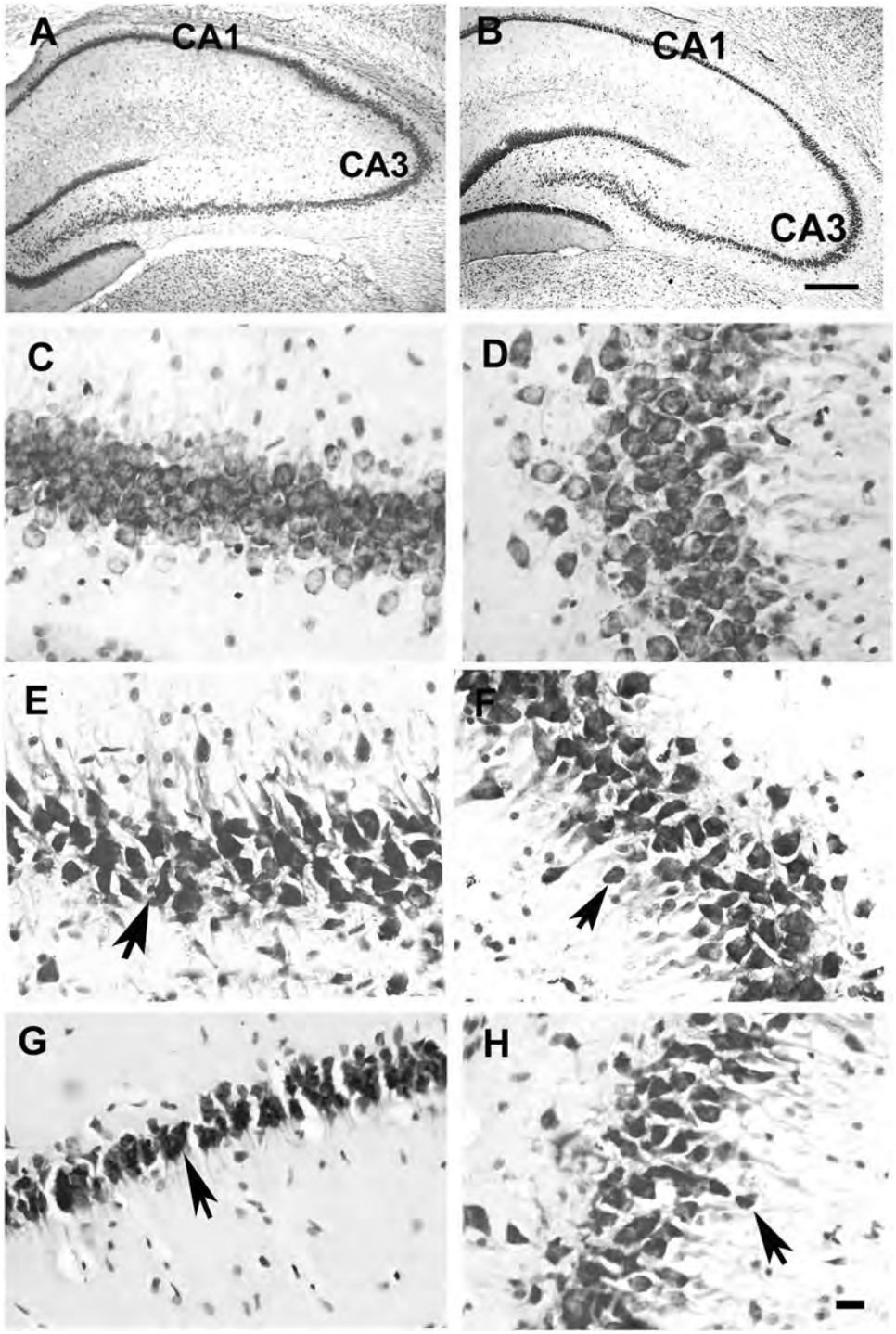

Figure 3. Photomicrographs of the cresyl violet-stained rat brain sections at the level of dorsal hippocampus in normal control (NC) (A) and ventral subicular lesioned (VSL) rats (B) at 4× magnification. (C) and (D) represent the magnified images of cornu ammonis 1 (CA1) and cornu ammonis 3 (CA3) from a NC rat to show the viable cells (large-sized, medium intensity stained cells with dark nucleoli). The ventral subicular lesion produced considerable degree of neurodegeneration in the hippocampus. The CA1 and CA3 areas of VSL (E and F) and VSL rats transplanted green fluorescent protein (GFP)-labeled hippocampal cell line (H3-GFP) (VSL + H3-GFP) rats (G and H) had mainly shrunken, darkly stained pyknotic cells indicating neurodegeneration. Scale bar (A and B) = 50 μm and (C–H) = 10 μm.

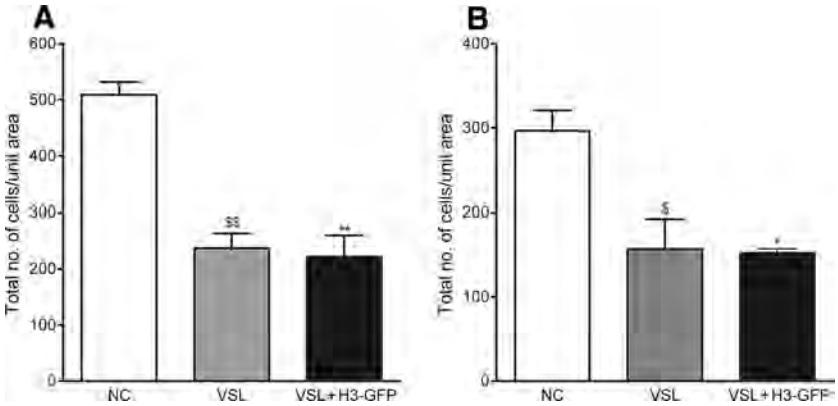

Figure 4. Quantitative assessment of cell density in the cornu ammonis 1 (CA1) and cornu ammonis 3 (CA3) areas of hippocampus in rats from different groups. The data represents mean ± *SEM*, $n = 6$ rats/group (15 sections per rat/group), normal control (NC), ventral subicular lesioned (VSL), and VSL rats transplanted with green fluorescent protein (GFP)-labeled hippocampal cell line (H3-GFP) (VSL + H3-GFP) groups. The cell density was significantly reduced following lesioning of subiculum, and no difference in the cell density was observed between the VSL and VSL + H3-GFP rats. The cell density was significantly reduced to 43% and 46% in the CA1 (A) and to 51% and 52% in the CA3 area (B) in VSL + H3-GFP and VSL groups, respectively. $\$ p < .01$, $\$\$ p < .001$ NC versus VSL, $^{*} p < .01$, $^{**} p < .001$ NC versus VSL + H3-GFP by one-way analysis of variance followed by Tukey's post hoc test.

rat failed to reach the platform within 90 s, it was gently guided to the platform and allowed to remain there for 30 s before the start of next trial. Escape latency and swim path were recorded using automated video tracking system (Columbus videomex-V). They were given two trials per day with an intertrial interval of 30 min. The training continued until the rat learned to reach the platform using the shortest route.

Probe Test

Twenty-four hours after acquisition of the water maze task, the platform was removed from the tank, and the rat was allowed to swim for 60 s. The amount of time spent in each target quadrant was measured.

Retention Test

Retention test was carried out 10 days following acquisition of the task. In retention, the same protocol of training (acquisition) was followed. Rats were released from the North West quadrant while the position of the hidden platform was kept constant at the North East quadrant. The rats were allowed to swim until they reach the escape platform or for a duration of 90 s. The performance was assessed with a single trial only because the first trial

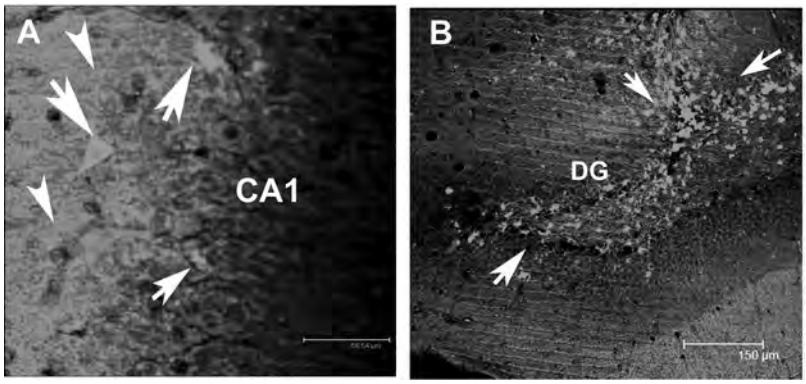

Figure 5. Confocal images of the green fluorescent protein (GFP)-labeled hippocampal cell line (H3-GFP) transplants in cornu ammonis 1 (CA1) hippocampus and dentate gyrus in the ventral subicular lesioned (VSL) + H3-GFP rats. Note that some transplant cells acquire the pyramidal shape with few processes (see arrowhead) among the native cells in CA1 hippocampus (A). Following 2 months of transplantation, most of the H3-GFP cells settled in the dentate gyrus (B). Scale bar A = 56.54 μm and B = 150 μm.

is not confounded by relearning and is therefore the cleanest measure of retention.

Histological Assessment of Lesion and Hippocampal Neurodegeneration

Following behavioral studies, all rats ($n = 8$ from different groups) were subjected to histological verification of lesion site of subiculum. In addition, the cell density assessment of CA1 and CA3 areas were carried out in 6 rats (randomly selected out of 8 rats used for behavioral studies) per group. The rats were perfused transcardially, with 0.9% saline followed by 10% formalin. The brains were subsequently removed and post fixed in 10% formalin for 48 hrs. 30-μm thick coronal sections were taken (Vibratome VT1000S, Leica Germany) at the level of ventral subiculum (to assess the lesion size) and also at the dorsal hippocampus [from –2.3 mm to −3.8 mm with reference to Bregma, using the Paxinos and Watson (1982) rat atlas], stained with cresyl violet and examined microscopically (Leitz, Germany). The data presented here are with lesion confined exclusively to ventral subiculum with a lesion size of 1.5–2 mm^3. To estimate the hippocampal damage following lesion, the cell density was assessed from CA1 and CA3 areas using the optical fractionator method. Thirty micrometer-thick vibratome (coronal) sections (every sixth sections, 15 sections/rat/group) were taken at the level of dorsal hippocampus. All the slides were coded to avoid experimenter's bias. Sections were stained with cresyl violet and viable cells were quantified under 40× magnification using the Stereo Investigator software (Stereo Investigator, MBF Bioscience, Microbrightfield Inc., Williston, VT, USA) using BX61 Olympus microscope.

The Stereo Investigator system, used for cell density quantification, consisted of a video camera (Optronix, Microfire, CA) attached to an Olympus BX61 microscope (Olympus Microscopes, Japan) and interfaced by the Stereo Investigator software (MBF Bioscience, Microbrightfield Inc., Williston, VT, USA).

The 30-μm thick sections, dehydrated and cleared following cresyl violet staining, were reduced to approximately 15 μm. Measurement of section thickness using the software revealed minimal variability (15 ± 0.5 μm) between sections. Briefly, every sixth section of the coded specimens was quantified, blind to the experimental manipulation involved. The contour of the area of interest was first delineated using a 4× objective, and the counting frame size (60 × 60 μm) and the systematic random sampling site frequency (150 × 100 μm, 0.01500 mm^2 area) were determined on the basis of preliminary population esti-

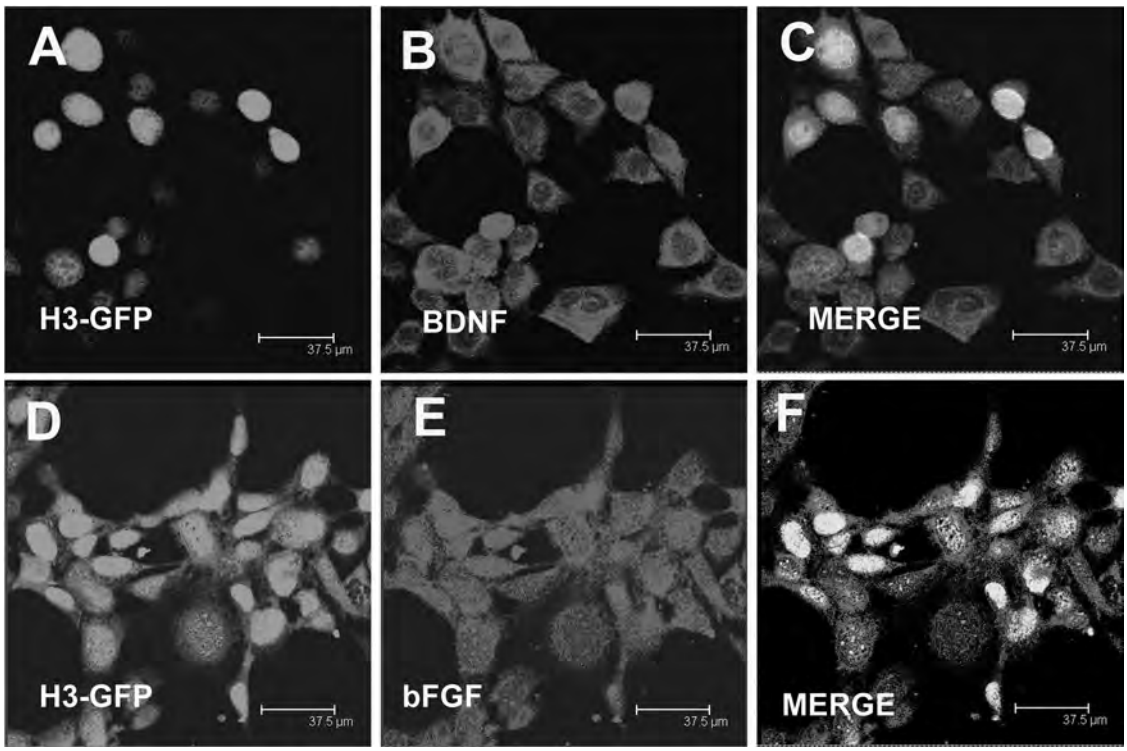

Figure 6. Confocal immunofluorescent images of green fluorescent protein (GFP)-labeled hippocampal cell line cells (H3-GFP) expressing brain-derived neurotrophic factor (BDNF) and basic fibroblast growth factor (bFGF) in vitro. (A) shows H3-GFP cells with nuclear localized GFP. (B) represents the H3-GFP cells expressing BDNF (cytoplasmic), and (C) highlights the merged images of (A) and (B). (D), (E), and (F) represent the H3-GFP cells expressing bFGF. In (D), the H3-GFP cells show both nuclear and cytoplasmic expression of GFP, (E) shows the H3-GFP cells express cytoplasmic bFGF, and (F) indicates the merged image of (D) and (E).

mate by optical fractionator probe of the stereo investigator software. The optical fractionator component was then activated to determine the number and location of counting frames and the counting depth for that section. The depth of top and bottom guard zones and the height of the optical dissector were maintained at 5 μm through out the quantification. Viable cells lying within the counting frame or touching the permissible zone of the frame were counted and those falling on the exclusion boundaries were excluded. The total number of viable cells was obtained from the "DAT" files computed by the optical fractionator probe. Both right and left hemispheres were counted separately and finally expressed together as a total

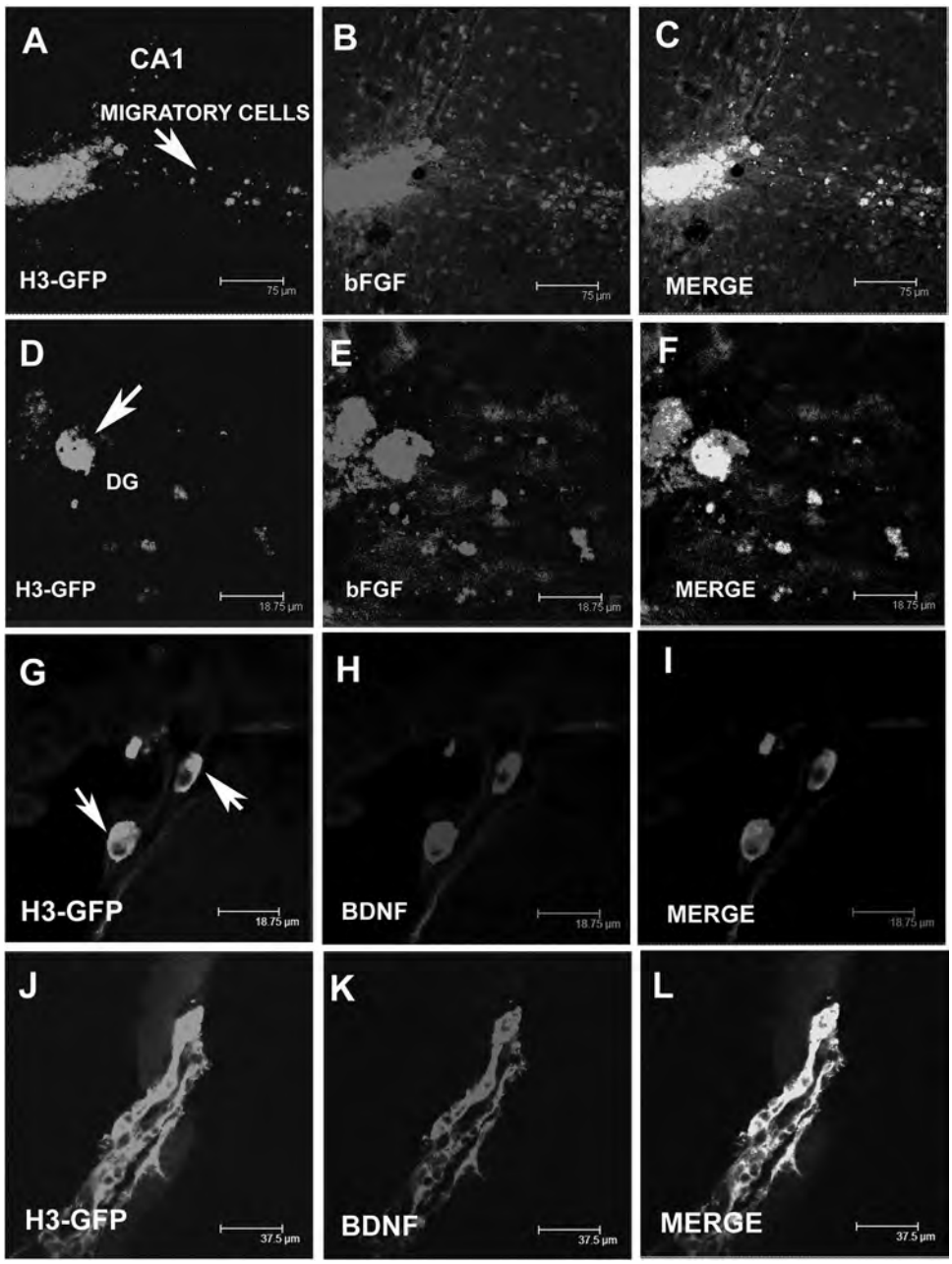

Figure 7. Confocal immunofluorescent images of green fluorescent protein (GFP)-labeled hippocampal cell line (H3-GFP) transplants expressing trophic factors after 2 months of transplantation. The H3-GFP transplants (A and D) express basic fibroblast growth factor (bFGF) in the cornu ammonis 1 (CA1) hippocampus (B) and dentate gyrus (E), and the merged images are shown in (C) and (F). The H3-GFP transplants with cytoplasmic GFP expression in the CA1 hippocampus (G) and dentate gyrus (J) express brain derived neurotrophic factor (BDNF) (cytoplasmic) as shown in (H) (CA1 area) and (K) (dentate gyrus), and the merged images of both GFP and BDNF are shown in Figure (I) and (L).

number of cells per unit area using the optical fractionator formula, $n = 1/ssf.1/asf.1/hsf$. EQ_(Dorph-Petersen, Nyengaard, & Gundersen, 2001), wherein ssf (section-sampling fraction) is 6 μm; asf (area-sampling fraction), which was calculated by dividing the area sampled with the total area, hsf (height sampling fraction), calculated by dividing the height sampled (i.e., 5 μm) with the section thickness at the time of analysis (i.e., 15 μm); EQ_ denotes the total count of particles sampled. The method was adopted from Rao, Hattiangady, and Shetty (2006).

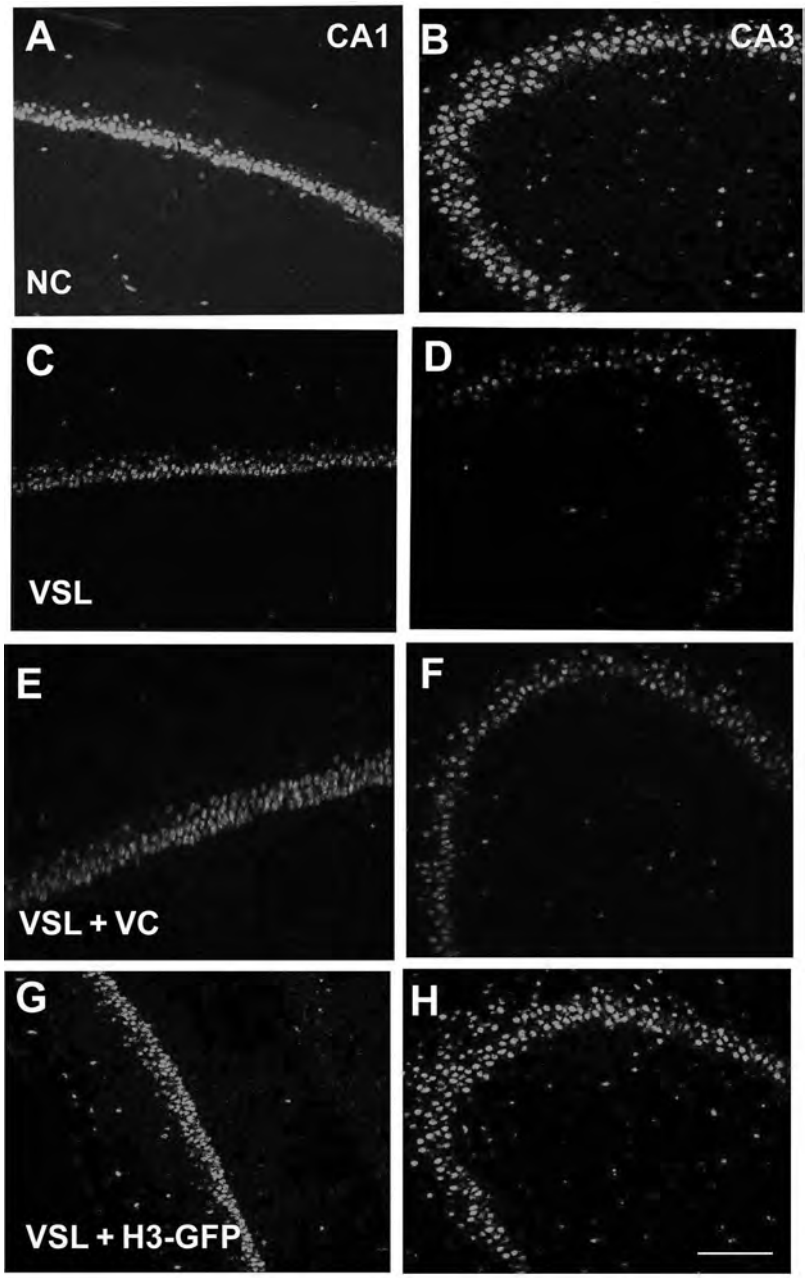

Figure 8. Confocal immunofluorescent images of brain derived neurotrophic factor (BDNF) expression in the cornu ammonis 1 (CA1) and cornu ammonis 3 (CA3) areas of the dorsal hippocampus in normal control (A and B), lesioned and transplanted groups (C–H). The images are taken under 20× magnification. Note the enhanced expression of BDNF (G and H) in the ventral subicular lesioned (VSL) + green fluorescent protein (GFP)-labeled Hippocampal cell line cells (H3-GFP) rats. The VSL (C and D) and VSL + VC rats (E and F) showed reduced expression of BDNF, indicating that lesioning of the ventral subiculum reduces the BDNF expression in the dorsal hippocampus. Scale bar = 150 μm.

Statistical Analysis

Two-way repeated measures (analysis of variance) ANOVA with days as within subjects factor and treatment as between subjects factor followed by trend analysis and comparison of the slopes of the learning curves were used to assess behavioral performance in radial arm maze and water maze tasks. Retention test, stereological assessment of cell quantification, and the Fluorescence intensity measurements of BDNF in the host cells were assessed using one-way ANOVA followed by Tukey's multiple comparison tests. All statistical analyses were carried out using SPSS Release 13 (SPSS Inc., IL, USA) and Prism Version 4 (Graphpad Software Inc., CA, USA).

Results

Histology

Ventral subiculum was lesioned completely (Figure 1B and 1C and 2A and 2B) following ibotenic acid infusion. The lesion was 1.5 to 2 mm^3 in size and was confined to the subiculum without any spread to other areas. Lesioning of ventral subiculum resulted in a significant decrease in cell density in CA1 and CA3 areas of the hippocampus (see Figure 3). Following lesioning, the cell density was reduced significantly to 43% and 46% in the CA1 area, $F(2, 15) = 29.59$, $p < .001$, and to 51 & 52% in the CA3 area, $F(2, 15) = 10.82$, $p < .001$, in VSL + H3-GFP and VSL rats, respectively (Figure 4B).

H3–GFP Transplants and Expression of Trophic Factors

Two months following transplantation, H3-GFP transplants were homogenously distributed in the dentate gyrus (Figure 5B). A few H3-GFP transplants showed a pyramidal shape and extended a few processes (see arrow head, Figure 5A). We did not find any tumor formation following transplantation of the hippocampal cell lines in rats. H3-GFP cells expressed both basic FGF (bFGF) and BDNF in vitro and in vivo. H3-GFP cells showed nuclear localization of GFP between 1st to 4th day in vitro and by 6th day in vitro, the GFP expression was localized in the cytoplasm when grown on cover slips. H3-GFP cells in vitro show cytoplasmic expression of BDNF and bFGF (Figure 6B and E). Our study indicates that following transplantation, H3-GFP transplants survived mainly in the dentate gyrus and expressed both BDNF and bFGF (see Figure 7). In addition, the rats with H3-GFP transplants showed enhanced expression of BDNF in CA1, $F(3, 16) = 62.77$, $p < .001$, and CA3, $F(3, 16) = 69.26$, $p < .001$, areas, respectively, when compared with VSL groups (Figure 8 and Figure 9). Altogether, there was enhanced expression of trophic factors, both by the transplants and by the host hippocampus following transplantation.

Assessment of Spatial Learning and Memory Functions

Spatial learning in eight-arm radial maze. Figure 10 provides the details of task learning in the eight arm radial maze. In the eight-arm baited task, all arms were baited with food pellets (Kellogg's chocolate wheat scoops). The rats had to learn to visit each baited arm only once to obtain the food reward and reentry into an already visited arm was considered a working memory error. A two-way repeated measures ANOVA with days as within subjects factor and treatment groups as between subjects factor revealed a significant effect of days ($F = 53.27$, $df = 8$, $p < .001$), as well as significant group by day interaction effect ($F = 13.90$, $df = 24$, $p < .01$) on the percent correct choices suggesting group

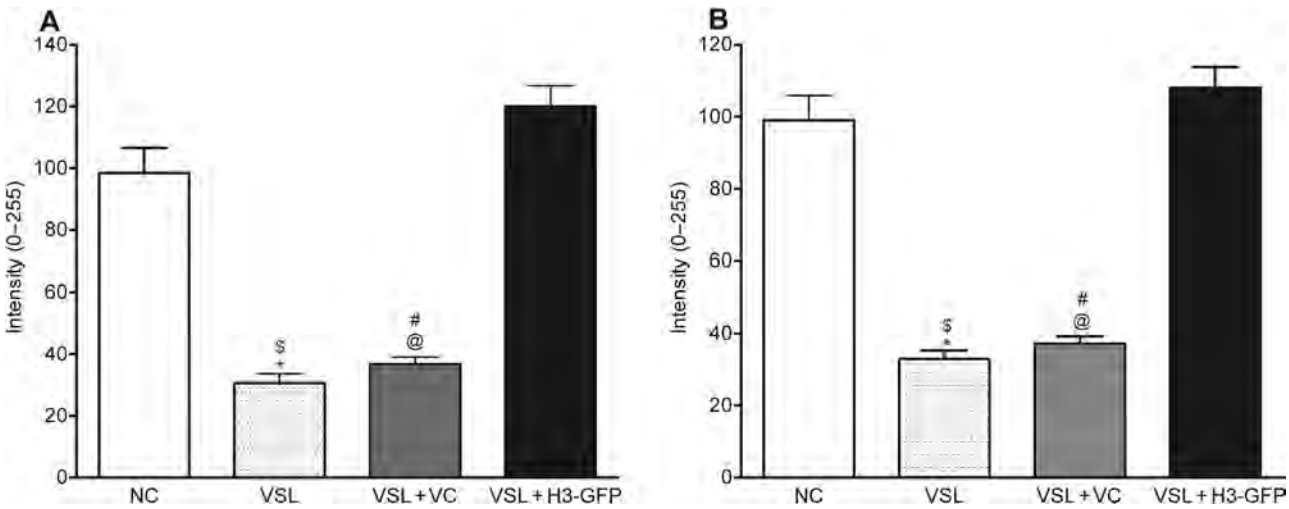

Figure 9. Intensity of brain derived neurotrophic factor (BDNF) expression in the cornu ammonis 1 (CA1) (A) and cornu ammonis 3 (CA3) areas of the dorsal hippocampus by means of Q-win plus software for image analysis. Each value represents the mean ± *SEM*, $n = 5$/group, 15 sections per group; normal control (NC), ventral subicular lesioned (VSL), VSL + vehicle control (VC) and VSL rats transplanted with H3-GFP cell lines (VSL + H3-GFP) groups. Note a significant decrease in BDNF expression in the CA1 (A) and CA3 (B) areas in the VSL and VSL + VC groups when compared with NC. The VSL + H3-GFP rats showed a marked increase in BDNF expression in the hippocampus when compared with VSL and VSL + VC groups. $\$ p < .001$, NC versus VSL, $@ p < .001$, NC versus VSL + VC, $^{*} p < .001$ VSL + H3-GFP versus VSL, $\# p < .001$ VSL + H3-GFP versus VSL + VC, by one-way analysis of variance followed by Tukey's post hoc test.

differences in the rate of learning. These learning differences were further explored by trend analysis over days. A significant interaction was found between the treatment groups and the linear ($F = 74.37$, $df = 3$, $p < .001$), quadratic ($F = 4.69$, $df = 3$, $p < .01$) and cubic ($F = 9.32$, $df = 3$, $p < .001$) trends of days. These results provide further evidence for group differences in the rate and temporal pattern of learning. Furthermore, the slopes of the learning curves for each of groups were calculated by performing linear regression over days and compared with one-way ANOVA which confirmed differences in learning rates, $F(3, 28) = 73.50$, $p < .001$). Post hoc Tukey's tests were used for pair wise group comparisons of the slopes which showed that both the NC group and the VSL + H3-GFP groups had significantly greater rates of learning compared to the VSL group (Table 1). Post hoc Bonferroni tests on the Day-9 performance showed that the VSL group had a significantly lower % correct choice compared to the NC

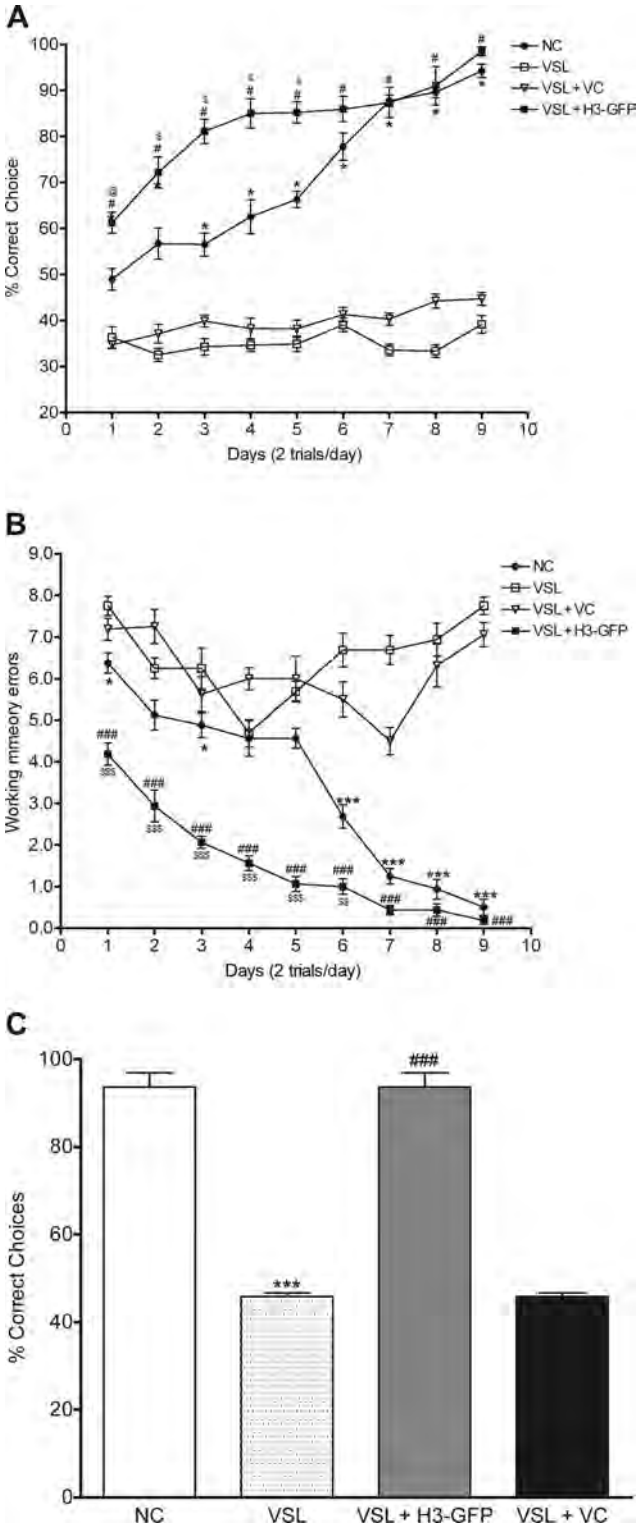

Figure 10. (A) Performance of rats in the eight-arm radial maze task. The graph depicts the performance as percentage of correct choices made by the rats as a function of time. Each value represents the mean ± *SEM*, $n = 8$ per group. The ventral subicular lesioned (VSL) and VSL + vehicle control (VC) rats showed impairment in task learning and working memory relative to normal control (NC) and VSL rats transplanted with H3-GFP cell lines (VSL + H3-GFP) rats. In addition, the performance of the VSL + H3-GFP group was better than the NC rats from first to fifth day of training and at a comparable level thereafter up to ninth day. Two-way repeated measures analyses of variance were followed by post hoc Bonferroni tests for group differences. $^{*}p < .001$, NC versus VSL; $\#p < .001$, VSL + H3-GFP versus VSL; $@p < .01$, $\$p < .001$, VSL + H3-GFP versus NC. (B) Working memory errors committed by rats during acquisition of the 8-arm radial maze task. Each value represents the mean ± *SEM*, $n = 8$ per group. During the initial days of training, rats from all groups made random reentries into arms already visited. As the training continued, the NC and VSL + H3-GFP rats showed progressive decrease in working memory errors as a measure of task learning. In addition, the VSL + H3-GFP rats made fewer working memory errors during the first 6 days when compared with NC rats. In contrast, the rats from the VSL and VSL + VC groups committed working memory errors throughout training when compared with NC and VSL + H3-GFP rats, indicating poor learning. Two-way repeated measures analysis of variance as followed by post hoc Bonferroni tests for group differences. $^{*}p < .05$, $^{***}p < .001$ NC versus VSL; $\#\#\#p < .001$, VSL + H3-GFP versus VSL; $\$\$p < .01$, $\$\$\$p < .001$, VSL + H3-GFP versus NC. (C) Retention of spatial performance in the eight-arm radial maze task. The retention of eight-arm radial maze task was assessed 10 days following acquisition of the spatial task. The retention scores (correct entries into baited arms) were assessed in two trials, the mean was taken for each rat, and finally the mean ± *SEM* was calculated for each group. In addition, the total number of entries made by the rats in each session was also noted. The performance as percentage of correct choices was assessed by calculating the percentage of correct response scores divided by the total number of entries made by the rat (including both correct response scores and working memory errors). The bar graph depicts the performance (percentage of correct choices) in NC, VSL, VSL + VC, and VSL + H3-GFP rats. Each value represents the mean ± *SEM*, $n = 8$ per group. The VSL + H3-GFP rats attained almost 100% of correct choices during the retention test. In contrast, the VSL and VSL + VC rats showed significant impairment in the retention of spatial task and made only 40% to 50% of correct choices indicating memory impairment. One-way ANOVA was followed by Tukey's post hoc test: $^{***}p < .001$, NC versus VSL; $\#\#\#p < .001$ VSL + H3-GFP versus VSL.

Table 1
Comparison of Slopes of the Percent Correct Choices (%CC) Over Days in Radial Arm Maze (RAM) Task

	NC	VSL	VSL + VC	VSL + H3-GFP
%CC in RAM	5.96 ± 0.33	0.28 ± 0.23	1.08 ± 0.21	3.65 ± 0.40
One-way analysis of variance, $F(3, 28) = 73.50$, $p < .001$				
	t	*p*		
Post hoc Tukey's tests				
NC vs VSL	18.86	<.001		
NC vs VSL + VC	16.19	<.001		
NC vs VSL + H3-GFP	7.67	<.001		
VSL vs VSL + VC	2.67	.05		
VSL vs VSL + H3-GFP	11.19	<.001		
VSL + VC vs VSL + H3-GFP	8.52	<.001		

Note. NC = normal control; VSL = ventral subicular lesioned; VC = vehicle control; VSL + H3-GFP = ventral subicular lesioned rats transplanted with H3-GFP cell lines. Values represent mean ± *SEM*, $n = 8$, for each group.

group ($t = 4.00$, $p < .001$). The transplanted group (VSL + H3-GFP) had significantly higher percent correct choices than the VSL group on Day 9 ($t = 18.79$, $p < .001$) and did not differ significantly from the NC group ($t = 1.35$, $p > .05$) (Figure 10A). The VSL + H3-GFP rats made about 60% of correct choices on first day and reached the criterion of 87% to 100% of correct choices by fifth day of training (Figure 10A). As the task learning progressed across days, the VSL + H3-GFP rats made fewer working memory errors when compared to VSL and VSL + VC rats. Working memory errors were also subjected to a two-way repeated measures ANOVA and trend analysis. A significant effect of days ($F = 37$, $df = 8$, $p < .001$) as well as a significant interaction effect between groups and days ($F = 14.40$, $df = 24$, $p < .001$) was observed. There was also a significant interaction between groups and the linear and quadratic trends over days. These results indicate that the rate of reduction of working memory errors during the course of learning was significantly different between groups. A comparison of the slopes of the working memory curves for the groups with a one-way ANOVA confirmed group differences in the rate of reductions in working memory errors, $F(3, 28) = 55.86$, $p < .001$). Post hoc Tukey's test showed a faster decline in working memory errors in the NC and VSL + H3-GFP group compared with the VSL group (Table 2). Post hoc Bonferroni test on the Day-9 working memory errors revealed significant impairment in the VSL group compared to the NC group ($t = 16.37$, $p < .001$). The transplanted group (VSL + H3-GFP) had significantly fewer working memory errors than the VSL group on Day 9 ($t = 17.08$, $p < .001$) and did not differ significantly from the NC group ($t = 0.71$, $p > .05$) (Figure 10B).

Retention Test

The rats were tested for retention of spatial task, 10 days following acquisition. The retention scores in terms number of correct performance (Mean ± *SEM*) were lower for VSL (5.41 ± 0.26) and VSL + VC (6.18 ± 0.38) rats when compared to NC (7.64 ± 0.28) and VSL + H3GFP (7.63 ± 0.17) rats. In addition, the working memory errors (Mean ± *SEM*) committed by the VSL (6.4 ± 0.32) and VSL + VC (6.62 ±

Table 2
Comparison of Slopes of the Working Memory Errors (WMEs) Over Days in Radial Arm Maze Task

	NC	VSL	VSL + VC	VSL + H3-GFP
	−0.75 ± 0.04	0.08 ± 0.06	−0.10 ± 0.06	−0.45 ± 0.03
One-way analysis of variance, $F(3, 28) = 55.86$, $p < .001$				
	t	*p*		
Post hoc Tukey's tests				
NC vs VSL	16.79	<.001		
NC vs VSL + VC	13.11	<.001		
NC vs VSL + H3-GFP	5.99	<.01		
VSL vs VSL + VC	3.68	>.05		
VSL vs VSL + H3-GFP	10.80	<.001		
VSL + VC vs VSL + H3-GFP	7.12	<.001		

Note. NC = normal control; VSL = ventral subicular lesioned; VC = vehicle control; VSL + H3-GFP = ventral subicular lesioned rats transplanted with H3-GFP cell lines. Values represent mean ± *SEM*, $n = 8$, for each group.

0.23) rats were greater when compared with VSL + H3-GFP (0.78 ± 0.17) and NC (0.9 ± 0.12) groups.

One-way ANOVA revealed significant group differences in the task performance as assessed by % of correct choices, $F(3, 31) = 104.20$, $p < .001$, and working memory errors, $F(3, 31) = 216$, $p < .001$. Post hoc Tukey's test showed that the VSL had significantly lower percent correct choices compared with the NC ($t = 18.73$, $p < .001$) group. The percent correct choices of the VSL + H3GFP group was comparable to that of the NC group and was significantly greater than that of the VSL group ($t = 17.64$, $p < .001$) (Figure 10C). Similarly, post hoc tests on working memory errors showed that the VSL group was significantly impaired compared with the NC group ($t = 18.73$, $p < .001$), whereas the VSL + H3GFP group showed significantly fewer working memory errors than the VSL group ($t = 16.57$, $p < .001$). These results indicate better memory retention among the transplanted group (VSL + H3-GFP) compared with the VSL rats.

Spatial Navigation in Morris Water Maze Task

The spatial navigational abilities in Morris water maze were assessed in terms of decreased escape latency to reach the hidden platform and by the velocity of swimming during training sessions. Differences in rate and temporal patterns of learning were analyzed using an approach similar to that adopted for the radial arm maze. A two-way repeated measures ANOVA on the dependent variable of escape latency was performed with days as within subjects factor and treatment groups as between subjects factor. While a significant effect of days was found ($F = 18.22$, $df = 8$, $p < .001$), the group by day interaction was not significant ($F = 1.20$, $df = 24$, $p = .247$). There was also no significant interaction of groups by linear ($F = 0.824$, $df = 3$, $p = .492$) or quadratic ($F = 1.12$, $df = 3$, $p = .358$) trend of days. However, a significant group interaction with cubic time trend was observed ($F = 3.04$, $df = 3$, $p = .046$). The slopes of the escape latency curves for the four groups were calculated and entered into one-way ANOVA which revealed that there was no significant differences between groups, $F(3, 28) = 1.23$, $p = .298$) (Table 3). These results suggest that the groups had similar rates of learning but differed in the cubic trend of task acquisition. Post hoc Bonferroni tests for Day 9 showed significant differences between VSL and VSL + H3-GFP groups ($t = 2.97$, $p < .05$) while no such differences were found between the NC and VSL + H3-GFP ($t = 0.22$, $p > .5$) (Figure 12A). The transplanted groups therefore performed better following 9 days of training compared with the VSL rats and their performance was comparable to the NC group. With regard to initial performance (Days 1–4), VSL rats took 65 to 75 s to reach the platform, later from sixth to ninth day of performance, took 35 to 50 s, suggesting a slow task learning. This is also evident in Figure 11, which shows that they took a circuitous route along the edge of the pool to reach the platform. The rats from all groups showed more or less same velocity of swimming and no significant difference was observed across various groups (Figure 12B).

Probe Trial

A two-way ANOVA was performed on the dependent variable of time spent in different quadrants during a 60-s swim trial with quadrants and treatment groups as the two factors. This revealed a significant effect of interaction between groups and quadrants, $F(9, 112) = 5.529$, $p < .001$. Post hoc Tukey's tests showed that rats from VSL + H3-GFP group showed a significant difference in target quadrant preference when compared to VSL ($t = 4.735$, $p < .001$) and VSL + VC ($t = 4.324$, $p < .001$) while there was no significant difference between NC and VSL + H3-GFP rats ($t = 0.7137$, $p > .05$). The VSL-H3-GFP rats spent about 38% of time in the target quadrant comparable to the 35% time spent by the NC rats. Whereas, the VSL and VSL + VC rats did not show a preference for target quadrant and spent almost equal time in all quadrants (see Table 4). These results indicate the establishment of spatial memory in the transplanted rats, and a poor learning by the VSL and VSL + VC rats.

Retention Test

Retention of the Morris water maze task was assessed 10 days after acquisition of task with a single trial. One-way ANOVA showed significant group differences in escape latency, $F(3, 28) = 8.78$, $p < .001$. Post hoc Tukey's tests revealed that the escape latency of the VSL + H3-GFP rats was significantly lesser than that of the VSL rats ($t = 6.24$, $p < .001$) and was comparable to that of the NC group (Figure 12C). Both NC and VSL + H3-GFP rats took less than 20 s to reach the hidden platform whereas; the VSL rats took more than 55 s to reach the platform. Since the swim speed (velocity) was comparable across the groups (graph not included), the poor performance of VSL rats indicates poor memory retention.

Discussion

The present study demonstrates the functional efficacy of H3-GFP transplants in restoring cognitive functions in VSL rats. The transplants settled mainly in the dentate gyrus and

Table 3
Comparison of Slopes of the Escape Latency Over Days in Water Maze Task

	NC	VSL	VSL + VC	VSL + H3-GFP
Escape latency	−4.44 ± 0.70	−6.066 ± 1.06	−3.992 ± 0.96	−3.951 ± 0.82
	One way analysis of variance, $F(3, 28) = 1.23$, $p = .298$			

Note. NC = normal control; VSL = ventral subicular lesioned; VC = vehicle control; VSL + H3-GFP = ventral subicular lesioned rats transplanted with H3-GFP cell lines. Values represent mean ± *SEM*, $n = 8$, for each group.

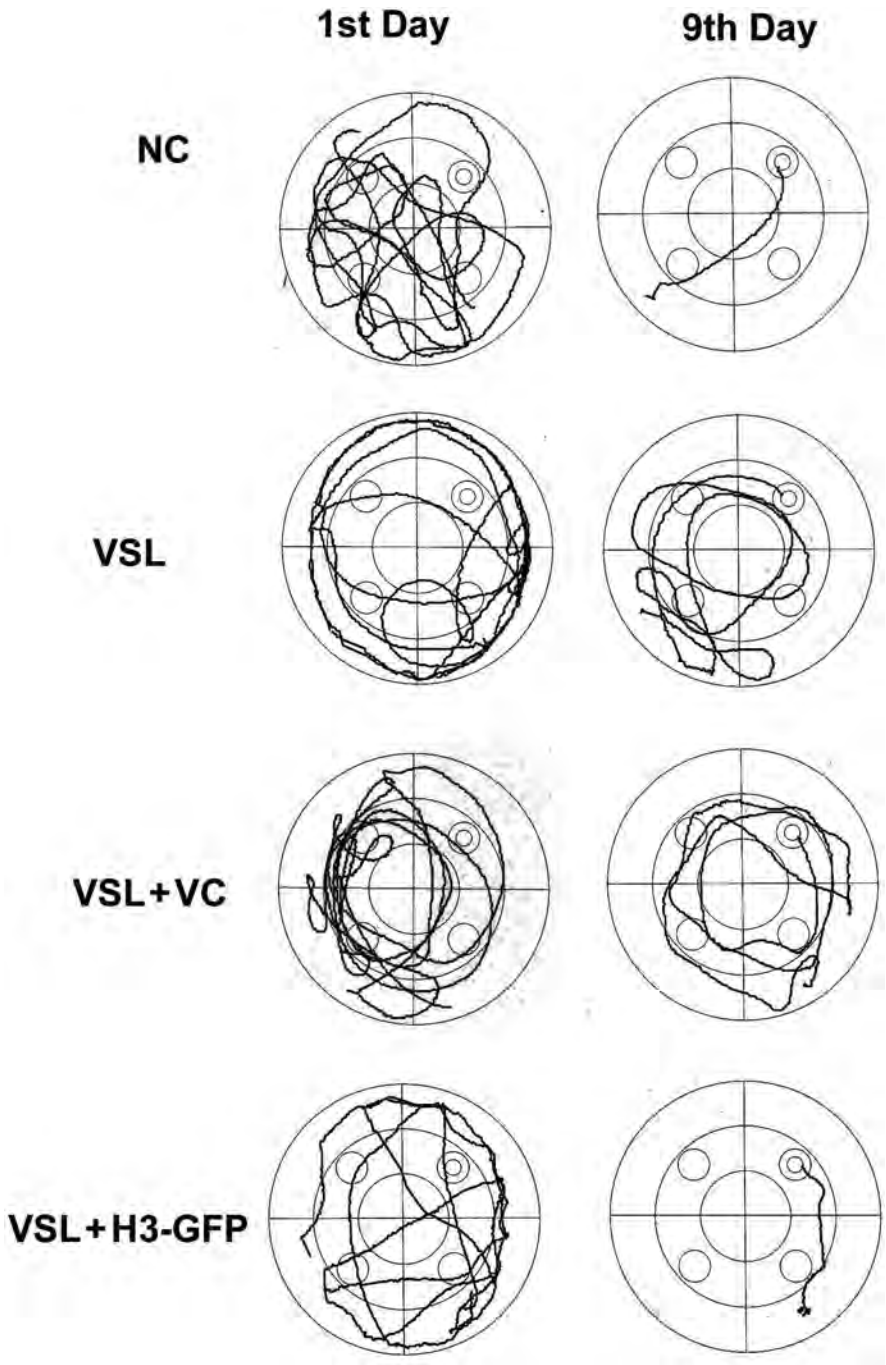

Figure 11. Representative examples of swim path during the water maze performance from different groups: the normal control (NC), ventral subicular lesioned (VSL), VSL + vehicle control (VC), and VSL rats transplanted with H3-GFP cell lines (VSL + H3-GFP). Note that the VSL + H3-GFP rats took the shortest route (as an index of learning) to reach the platform by ninth day of training similar to that of the NC rats. The VSL and VSL + VC rats took the more circuitous path even on the ninth day, suggesting impaired spatial abilities and thereby spatial learning.

expressed neurotrophic factors, BDNF and bFGF. The host hippocampal cells also showed enhanced BDNF expression following transplantation.

Ventral subicular lesion induced considerable degree of neurodegeneration in the hippocampus and significantly impaired the spatial learning in rats. Spatial learning impairment has been correlated with neurodegeneration and dendritic atrophy of hippocampal structures (Bindu et al., 2005; Devi et al., 2003; Jarrard, 1978, 1986; Jarrard et al., 1984). The anatomical connections between subiculum and other structures of hippocampal forma-

tion suggest the possibility of both retrograde and anterograde degeneration of hippocampus leading to behavioral impairment of spatial learning. The VSL rats showed spatial deficits in both eight arm radial maze and Morris water maze tasks. Hippocampal structures are essential to mediate various aspects of working memory and spatial localization components of water maze tasks (Cho & Jaffard, 1995; Floresco, Seamans, & Phillips, 1996; Galani, Jarrard, Will, & Kelche, 1997; Morris et al., 1982). Ventral subicular lesioning would have impaired these spatial abilities and thereby compromised the spatial map development (Galani et al., 1997; Redish & Touretzky, 1998; Riegert et al., 2004). Hippocampal place cells are essential in translating and storing of spatial information to construct a representation of the environment (O'Keefe & Nadel, 1978). Place cells are also found in the subiculum, and they fire in a direction and location specific manner to process the spatial information (Sharp, 1997, 1999). The subiculum, being a major output station of CA1 hippocampus, integrates and transfers the information to cortical structures (O'Mara, Commins, Anderson, & Gigg, 2001). Lesioning of the ventral subiculum alters the oscillating network events in CA1 hippocampus and entorhinal cortex (Laxmi, Meti, & Bindu, 2000). Subicular lesion is thought to interfere with the memory processing for spatial locations and does not alter the capabilities of rats to learn a spatial task when trials are repeated (Cho & Jaffard, 1995; Galani et al., 1997; Riegert et al., 2004). The VSL rats were able to acquire the task to a considerable extent by repeated training in the eight arm radial maze for a longer period. This may be achieved through the involvement of other structures like the dorsal subiculum, pre and para subiculum, because these are known to mediate spatial information (Cho & Jaffard, 1995; Riegert et al., 2004).

The H3-GFP transplanted rats showed better learning than lesion-only groups in both radial arm as well as in water maze

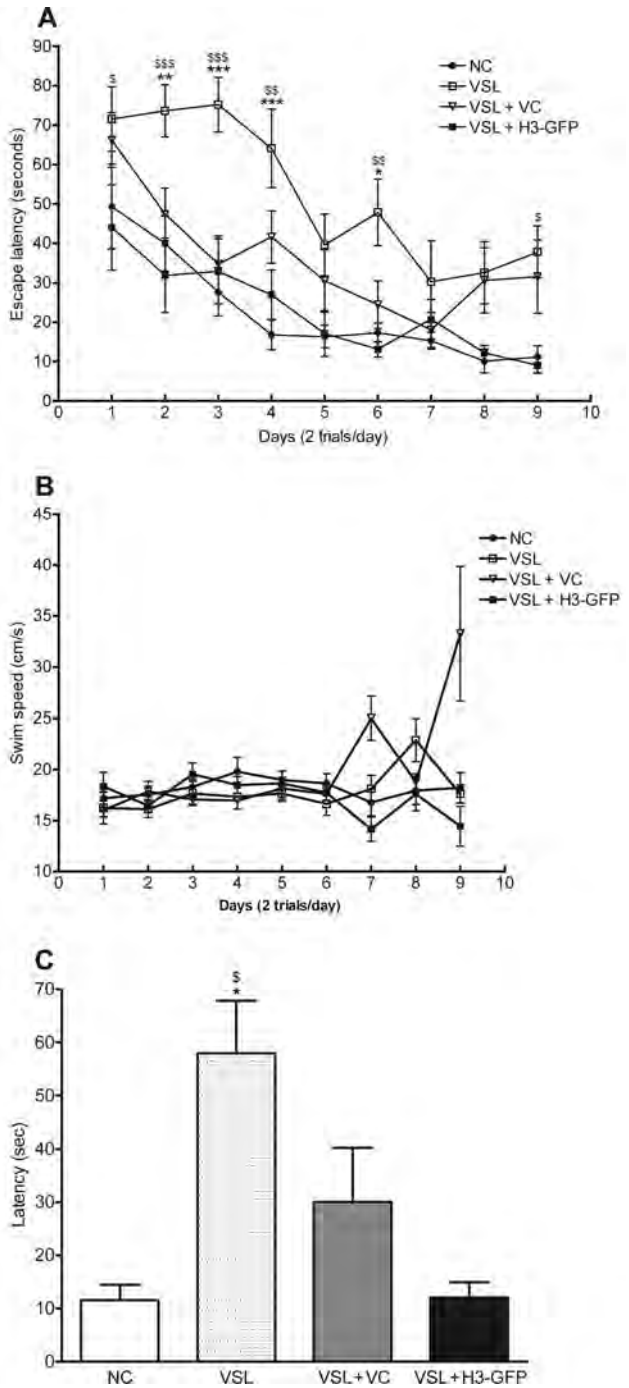

Figure 12. (A) Performance of rats in the Morris water maze task. The graph depicts the mean ± *SEM* of latency, $n = 8$ per group, normal control (NC), ventral subicular lesioned (VSL), VSL + vehicle control (VC), and VSL rats transplanted with H3-GFP cell lines (VSL + H3-GFP). As the training advanced in the water maze, the NC and VSL + H3-GFP rats showed a progressive decrease in the escape latency and learned to reach the hidden platform within a short time. The performance of VSL rats was impaired, and they took long time to reach the platform. Note that on Day 9, rats from the VSL + H3-GFP and NC groups took less than 10 s to reach the platform, whereas the rats from the VSL and VSL + VC groups took 35 to 40 s to reach the platform. Two-way repeated measures analysis of variance followed by post hoc Bonferroni tests for group differences: $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, NC versus VSL, $\$p < .05$, $\$\$p < .01$, $\$\$\$p < .001$, VSL + H3-GFP versus VSL. (B) Velocity of swimming in the Morris water maze. The graph depicts the velocity of swimming as an index of motor performance. The rats from different groups showed no difference in swim speed, and the velocity was between 15 and 20 cm/s, indicating no motor disability among the rats across groups. The data represent the mean ± *SEM* of the time taken to reach the hidden platform by the rats ($n = 8$ per group) from different groups (NC, VSL, VSL + VC, and VSL + H3-GFP). (C) Retention of Morris water maze performance in rats. The retention test was performed 10 days following acquisition of the water maze task in a single trial. The bar graph depicts the retention of Morris water maze task in terms of escape latency in rats from different groups. Data represent the mean ± *SEM* of escape latency, $n = 8$ per group, NC, VSL, VSL + VC, and VSL + H3-GFP. Both NC and VSL + H3-GFP rats showed better performance and took less than 20 s to reach the platform in the Morris water maze task. The VSL rats took more than 55 s to reach the platform, indicating a poor retention when compared with the NC and VSL + H3-GFP groups. One-way analysis of variance followed by Tukey's post hoc test $^{*}p < .001$, NC versus VSL and $\$p < .001$, VSL + H3-GFP versus VSL.

Table 4
Comparison of Performance in Morris Water Maze Task During Probe Trial of Retention Testing

	NC		VSL		VSL + VC		VSL + H3-GFP	
Groups	Time spent (s)	% of time spent	Time spent (s)$$*	% of time spent$$*	Time spent@*	% of time spent@*	Time spent	% of time spent
Northeast (target quadrant)	22.03 ± 1.93	35.38 ± 3.37	13.40 ± 1.84	22.51 ± 2.41	14.28 ± 0.78	23.96 ± 1.34	23.56 ± 2.30	38.46 ± 3.06
Northwest	10.54 ± 1.89	18.34 ± 2.58	14.75 ± 0.96	24.77 ± 1.01	16.17 ± 1.35	27.10 ± 2.23	13.34 ± 0.92	21.81 ± 1.30
Southwest	11.81 ± 1.67	18.43 ± 2.19	13.40 ± 1.60	22.55 ± 1.97	13.30 ± 1.02	22.28 ± 1.67	9.75 ± 1.61	16.02 ± 2.08
Southeast	17.55 ± 1.38	27.83 ± 2.01	17.86 ± 1.21	30.15 ± 2.14	15.88 ± 1.29	26.65 ± 2.22	14.38 ± 1.54	23.69 ± 2.25

Note. NC = normal control; VSL = ventral subicular lesioned; VC = vehicle control; VSL + H3-GFP = ventral subicular lesioned rats transplanted with H3-GFP cell lines. Values represent mean ± *SEM*, $n = 8$, for each group. The VSL and VSL + VC rats did not show any preference for target quadrant and distributed their search evenly across all quadrants at chance level (close to 15 s in a 60-s trial), while the NC and VSL + H3-GFP rats spent significantly longer than chance level in the target quadrant.
One-way analysis of variance followed by Tukey's post hoc test: $$ $p < .001$, NC versus VSL, @ $p < .01$, NC vs. VSL + VC, * $p < .001$, VSL + H3-GFP vs. VSL, VSL + VC.

tasks. Although the underlying mechanisms of functional recovery are not clearly understood, studies suggest various possibilities of host regeneration induced by the grafts. The bulk trophic support provided by the transplants may help to reestablish the damaged host neural connections either by forming new networks or by reconstructing the old pathways through host regeneration. Sinden et al. (1997) showed restoration of water maze performance in ischemic rats following transplantation of MHP36 cell lines. The MHP36 cells were mainly found in the damaged CA1 field and showed both neural and glial differentiation. It is suggested that these different phenotypes can promote repair and restoration of functions. Toda et al. (2001) also demonstrated the graft-mediated repair of the damaged CA1 field and the behavioral recovery. Though these studies demonstrate the importance of graft meditated repair of neural network, additional role of cytokines and neurotrophins has been emphasized in promoting sustained functional recovery. The present study clearly demonstrates the H3-GFP transplants meditated recovery of spatial and cognitive performance in VSL rats. We attribute the functional recovery to the enhanced expression of BDNF in the transplanted rats. Enhanced expression of trophic factors suggests the possibility of enhanced neuroprotection (Bonde et al., 2000; Kim et al., 2001). Many studies support the graft-mediated host regeneration through trophic support as the basis of improved cognitive functions (Brodhun, Bauer, & Patt, 2004; Chu et al., 2003; Kerr et al., 2003; Shear et al., 2004; Sinson, Voddi, & Mclntosh, 1996; Watson et al., 2003). Borlongan, Hadman, Sanberg, and Sanberg (2004) reported the trophic factor mediated reduction of cerebral infarct following injection of human umbilical cord blood cells. Kerr et al. (2003) have reported the capacity of transplanted EBD cells (embryoid body derived human pluripotent cell) to restore neurologic functions in animals with diffuse motor neuron disease. EBD cells are thought to halt motor neuron degeneration and facilitate the reconstitution of synaptic contacts on surviving host motor neurons. It is shown that EBD cells help neurite out growth in culture by secreting various factors such as TGF-α and BDNF and these trophic factors are known to support the survival of motor neurons and axon regrowth after axotomy (Barde, Davies, Johnson, Lindsay, & Thoenen, 1987; Herzog & Otto, 2002; Lindsay & Peters, 1984; Tobias et al., 2001; Yuan et al., 2000). Similarly, Shear et al. (2004) demonstrated the versatility of neural progenitor cells in promoting both motor as well as the behavioral recovery in a mouse TBI model. Because they did not see any specific neuronal or glial differentiation, they suggested the possibility of trophic factor mediated mechanisms for repair. Gao et al. (2006) also reported the recovery of cognitive functions in rats with acute traumatic brain injury soon (10 days) after transplantation of primed hNSC grafts. This acute functional recovery is attributed to the enhanced expression and release of GDNF by the hNSCs grafts. The neurotrophic factors appear to protect the host brain cells from secondary brain damage and would promote host self repair and cognitive recovery. Other studies have also provided supporting evidence of trophic factor mediated cognitive recovery in CNS insults. Rao et al. (2006) showed better graft survival following transplantation of pretreated embryonic hippocampal tissue suspension with trophic factors such as bFGF and BDNF and a poor survival when transplanted without pretreatment (Zaman & Shetty, 2003). Furthermore, Hicks et al. (2007) found that a combination of neural stem cell transplants together with enriched housing conditions and voluntary wheel running facilitated the behavioral recovery within 7 days of transplantation. Such combinations probably help to mediate a significant degree of synaptic plasticity in terms of enhanced expression of growth factors in promoting the behavioral recovery. We have also demonstrated the possibilities of enhanced neuronal plasticity and host regeneration in ventral VSL rats following exposure to enriched environment (Anandh et al., 2007; Bindu et al., 2007).

The present study clearly demonstrated the H3-GFP transplant mediated recovery of cognitive functions following subicular lesion. The Dentate gyrus may have facilitated the migration and differentiation of grafted cells probably due to its capacity to secrete various growth factors together with its ability to continue neurogenesis (Daniel & Lynn, 1996). The trophic factors released by the graft cells probably are potent survival factors for hippocampal neurons (Bonde et al., 2000) and in our study the VSL + H3-GFP rats showed enhanced BDNF expression in the hippocampus. In addition, we have got

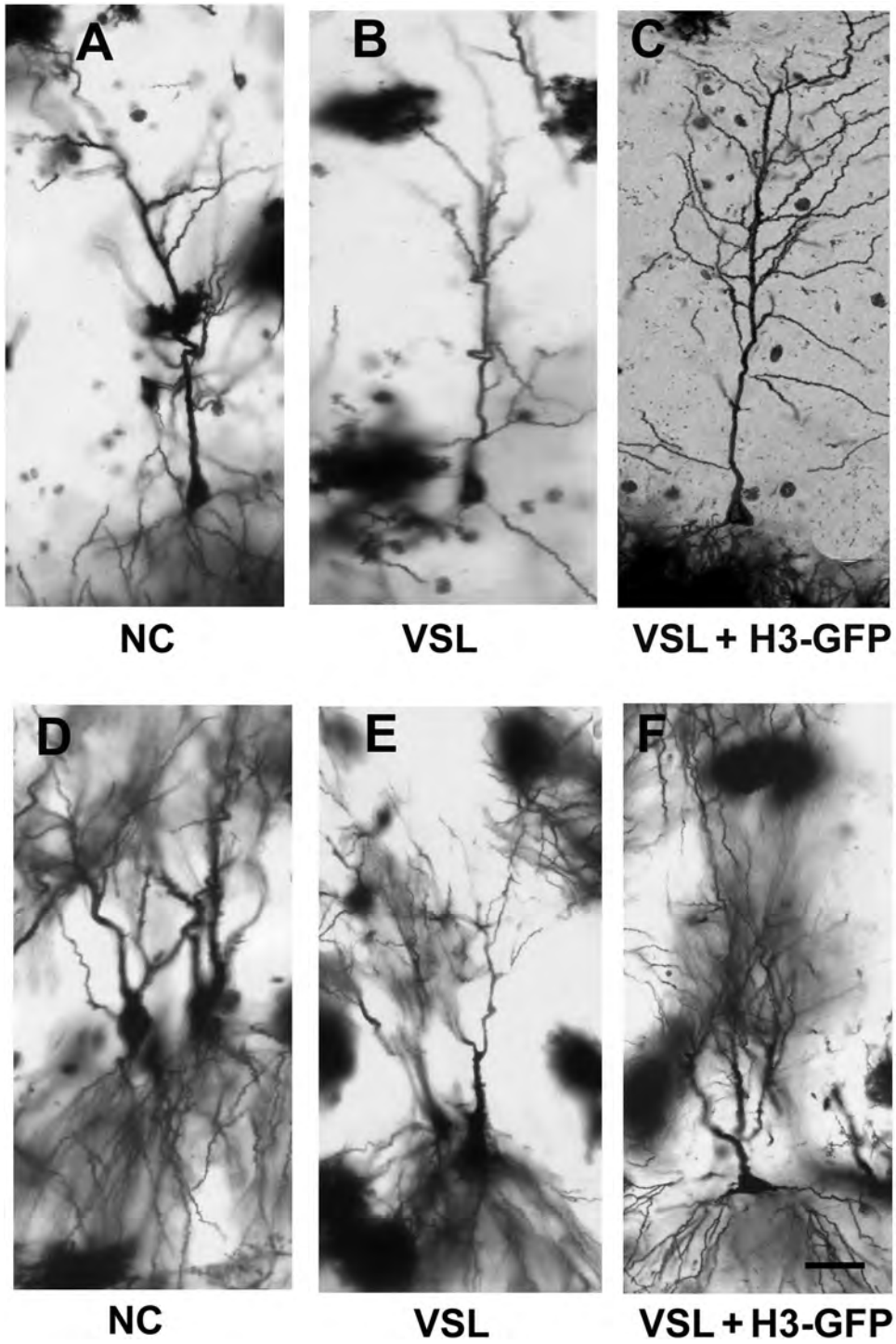

Figure 13. Representative examples of Golgi stained pyramidal neurons of hippocampus in rats from different groups. (A–C) and (D–F) represent the pyramidal neurons from cornu ammonis 1 (CA1) and cornu ammonis 3 (CA3) areas, respectively, among the normal control (NC), ventral subicular lesioned (VSL), and VSL lesioned rats transplanted with H3-GFP cell lines (VSL + H3-GFP) rats. The dendritic morphology of pyramidal neurons appears different in VSL rats. The VSL rats showed reduced dendritic arbors (qualitative observation) when compared with the NC and VSL + H3-GFP groups. Scale bar = 25 μm.

supporting evidence (from a preliminary study conducted) of host regeneration in terms of enhanced dendritic branching of hippocampal neurons in transplanted rats (see Figure 13) though this needs to be elucidated further in future studies. Moreover, the contribution of other trophic factors cannot also be ruled out in mediating host regeneration since the grafts settled mainly in the dentate gyrus. This notion of graft meditated host regeneration and functional recovery through trophic support opens up various possibilities of functional recovery through pharmacological intervention, genetically modified cells, glial or other non-neural transplants and so forth in CNS injury. In addition, the H3-GFP transplants could have also promoted various nonspatial search strategies that primarily depend upon the sensory, motor, or motivational aspects leading to enhanced behavioral performance. On the whole, transplantation of H3-GFP cells promoted the spatial and cognitive functions in VSL rats.

In conclusion, our study demonstrates the functional recovery in VSL rats following transplantation of H3-GFP cells. Although various studies have demonstrated the graft survival, integration and functional recovery in animal models of CNS lesion and damage, exact physiological functions of these transplants remain to be investigated. Our study, however, supports further investigation into the role of trophic mechanisms in graft-mediated behavioral recovery.

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