CALIFORNIA STATE UNIVERSITY, NORTHRIDGE

HUMAN PLURIPOTENT STEM CELLS AMELIORATE NMDA-INDUCED
HIPPOCAMPAL DEGENERATION AND RELATED FUNCTIONAL DEFICITS

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By

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ABSTRACT

DO HUMAN PLURIPOTENT STEM CELLS AMELIORATE NMDA-INDUCED HIPPOCAMPAL DEGENERATION AND RELATED FUNCTIONAL DEFICITS?

By

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Masters of Science in Biology

Seizures, trauma and many neurologic diseases induce damage to the hippocampus, which can result in deficits in spatial navigation, memory consolidation and depressive-type behaviors. Current drug treatments have limited effectiveness in improving symptoms. Celavie Biosciences LLC has developed a human fetal brain-derived, pluripotent, nontumorigenic, hypoimmunogenic stem cell line with a normal karyotype (hfSC) that has previously shown an ability to migrate, differentiate and reduce structural and functional deficits in other neurodegenerative animal models. In this study, I examined if injected hfSCs into male Wistar rat CA3 hippocampal lesions will survive and possibly differentiate into mature pyramidal neurons, thereby diminishing neuronal damage. It has been established that the CA3 region of the hippocampus is involved in consolidating short-term memory to long-term memory and in spatial navigation. I stereotactically lesioned the CA3 regions at 50 days bilaterally with the neurotoxin NMDA (1 μ l containing 7.5 mg/ml; -3.5 mm AP; $\pm$ 2.0 L and -2.5 V). At 54 days of age, live hfSC (500,000 cells in 5 μ l cell suspension media) for our treatment group, frozen-killed hfSC (500,000 cells/ 5 μ l), HEK293T cells (500,000 cells/5 μ l), or cell suspension media (5 μ l) were bilaterally implanted directly into the NMDA damaged area. The rats were tested two weeks later (68 days) with various memory tests, including the water-maze task, and novel and place-object tests. My results showed that rats receiving live hfSC implantation performed significantly better in the water maze task than control groups. Novel and place object tests showed no significant differences among the treatment groups. Histology confirmed the survival of implanted hfSCs up to 28 days post-implantation. My study has shown that Celavie's human derived pluripotent stem cells were able to survive *in vivo* and improve hippocampal functionality, highlighting the potential promise for stem cell treatment of brain damage in neurodegenerative diseases.

INTRODUCTION

The hippocampus is the mammalian brain region that plays a crucial role in cognitive function including memory formations, spatial navigation and depressive behaviors (Amaral and Witter, 1995). Historically, hippocampal function has been analyzed using chemical lesioning studies; for example, subepileptic injections of excitotoxins like NMDA have been used to study the roles of specific regions on the formation of different types of memory. Using this approach, the CA3 region has been shown to be the area of the hippocampus involved in the consolidation of short-term memory into long-term memory, and spatial navigation (Kesner, 2007). While lesioning of the CA3 with a low dose of excitotoxins can show the link to memory formation, lesioning with high doses can lead to seizures and CA3 neurodegeneration (Maia et al., 2014). Besides neurotoxins, significant damage to the CA3 region can also occur from head injury, stress, hypoxia, and ischemia (Budde et al., 2013; Gao et al, 2014; Matsuoka et al., 1997; Olsson et al., 2003). Ultimately, hippocampal damage can be debilitating as current drug treatment and physical therapy have only limited effectiveness in reducing damage in patients with disorders like epilepsy and stroke.

In recent years, stem cell transplantation has been shown to possess potential therapy for many neurodegenerative disorders (Hattiangady and Shetty, 2012), including hippocampus-based disorders like epilepsy (Waldau et al., 2010). Although, the current drug treatment and physical therapy have limited effectiveness in hippocampal-based neurodegeneration, stem cell transplantation studies in mammalian models have been proven as possible restorative therapy. In a recent study, scientists were able to functionally repair unilateral hippocampal lesions in rats, made by administrations of an

excitotoxin, by grafting neuronal stem cells (acquired from neonatal pups) into specific locations of hippocampal damage (Hattiangady and Shetty, 2012). These researchers found that the grafted neuronal stem cells were able to differentiate into all neuron types, and migrate into specific hippocampal areas. Rats that received the stem cell grafts showed a decrease in memory deficits and improved mood, suggesting effectiveness of the transplants. In another study, scientists used neural stem cells over-expressing choline acetyltransferase to restore cognitive function in an animal model of Alzheimer's disease (Park et al., 2012). In this study, researchers unilaterally damaged the CA3 region of rat hippocampi, then four weeks later implanted neural stem cells into the region of damage. They found that neural stem cells were able to differentiate into astrocytes and hippocampal neurons, and improve cognitive function in this disease model. In another study, researchers were able to protect against hippocampal degeneration and improve cognitive function and mood behavior in an animal model of temporal lobe epilepsy by implanting subventricular zone-derived (SZD) neural stem cell overexpressing IGF-1 grafts (Miltiadous et al., 2012). In this study, researchers injected a neurotoxin unilaterally into the hippocampi of mice to cause neurodegeneration and seizures. Four days following the hippocampal injury, mice received a unilateral implantation of SZD stem cells into the damaged area. Miltiadous et al. (2012) found that mice that received stem cell implants showed improved cognitive function when compared with control mice that received vehicle.

Celavie Biosciences LLC (Oxnard, CA) has developed a human fetal brain-derived, pluripotent, nontumorigenic, hypoinmunogenic neural stem cell line with a normal karyotype (hfSC). These stem cells have demonstrated their ability to migrate to the site of

brain lesions and differentiate according to the nature of the lesion (Kopyov, unpublished data). Ultimately, these cells have shown the ability to reduce structural and functional deficits in some animal models of neurodegenerative diseases. Recently, our lab has shown that Celavie's hfSCs implanted into the cerebellum of the ataxic Han-Wistar rat, with progressive degeneration in the hippocampus and cerebellum, significantly improved activity scores in animals compared with control animals that received dead hfSC implantation.

In my study, I worked in collaboration with Celavie and investigated whether implantation of hfSCs in NMDA-lesioned CA3 regions of rat hippocampi could alleviate and reverse the neurotoxin-caused memory deficits. The efficacy of Celavie's human fetal cells to repair the damaged CA3 region was tested through a series of cognitive tests conducted with these rats. The ability of Celavie's hfSC to ameliorate neuronal damage would suggest that these stem cells could be a possible effective treatment for people with hippocampal neurodegenerative disorders.

MATERIALS AND METHODS

Animals. A total of 50 male Han-Wistar rats were obtained from the California State University Northridge (CSUN) breeding colony. The animals were housed in standard rat cages, and provided with LabDiet 5001 rodent chow (Purina) and water *ad libitum*. The module room temperature was maintained at $23^{\circ}\pm 1^{\circ}\text{C}$ with a 12/12-hour light/dark cycle. Littermates were randomly divided into a live stem cell treatment group (n=21), a dead stem cell control group (n=17), a HEK293T cell group (n=7), and a cell media vehicle control group (n=5). Animals were assessed for their overall health by taking their weight once a week. This study was in compliance with prior approval of CSUN's IACUC committee.

Immunosuppression. At 45 days of age, all animals were implanted with an Alzet Osmotic Pump (Model# 2ML4) containing an immunosuppressive agent, cyclosporine (15 mg/kg per day for 28 days). Animals were anesthetized with 2.5% isoflurane, and a small subcutaneous pocket was prepared in the midscapular area. The pump was inserted, and the pocket was closed using wound clips.

Neurotoxin Damage and Stem Cell Therapy. At 50 days of age, all animals were given bilaterally injections of the neurotoxin NMDA ($1\mu\text{l}$; 7.5mg/ml) at a rate of $0.25\mu\text{l}$ per minute in the CA3 region of the hippocampus (coordinates from Bregma: -3.3 anteroposterior, ± 1.8 mediolateral, and -2.6 dorsoventral). At 54 days of age, treatments began with $5\mu\text{l}$ of nutrient medium that contained 500,000 human fetal derived stem cells (hfSC), the same concentration of freeze-killed hfSC, the same concentration of HEK293T

cells, or an injection of cell media only. All injections took place in the same CA3 region of the hippocampus that received NMDA injections four days earlier.

Memory Testing. At 68 days of age, animals from all four groups were habituated to the empty field square arena (100cm by 100cm with 30.5cm walls) for three, 10 minute daily sessions, 24 hours apart. In the eight days after the final habituation, all animals were subjected to three different types of memory tests: novel-object recognition trial, place-object trial, and water maze trial. The novel-object recognition trials, and place-object recognition trials were conducted on alternating days during the first six days of memory testing, for a total of three trials apiece. The water maze trial was conducted during the last two days of the memory testing. All memory tests trials were recorded using a video camera. The videos were later analyzed for memory trial timings.

In the *novel-object recognition trial* (NORT), the animal was placed into the arena with two identical objects and allowed to explore for five minutes. The animal was then removed from the arena for a five minute retention period. After, the animal was placed back into the arena with one of the original objects and one novel object for a three minute trial; this trial was video recorded. Videos of the trials were analyzed with two separate hand-held timers to measured time (in seconds) spent at the original object versus novel object. An exploration ratio was generating by determining the time the animal's nose moved within 2 cm of the novel object divided by total time spent at both objects (see Figure 1).

In the *place-object trial* (POT), the animal was placed into the arena with two identical objects and allowed to explore for five minutes. Then, the animal was removed from the arena and after a five minute retention period, it was placed back into the arena for a three minute trial with one object moved to a new location. Videos of the trials were analyzed with two separate hand-held timers to measured time (in seconds) spent at the original object versus moved object. An exploration ratio was generating by taking the time the animal's nose moved the within 2 cm of the moved object divided by total time spent at both objects (see Figure 1).

The *water maze trial* (WMT) was conducted over two days, a training day and a trial day. The test was conducted in a 1.5 m diameter, 45 cm deep circular pool filled with water to a depth of 26.5 cm and made opaque with powdered milk. A circular platform, 25 cm high and 12 cm in diameter was placed into the tank at a fixed location in the center of one quadrant. For the training sessions, a red dart was affixed to the platform, making the platform location visible above the waterline. Each animal received three training sessions, which began with the animal positioned on the platform for a 10 second retention period. The animal was then released in one of the three empty quadrants not containing the platform and was given 90 seconds to find the platform. If the platform was not found within 90 seconds, the experimenter guided the animal to swim to the platform. This training was then repeated in the other two quadrants not containing the platform. On the next day ("trial" day), the red dart was removed from the platform. Each animal was given four test trials, in which the animal was released in the water at one of two locations equidistant from the platform. Each trial was video recorded. A rest period of 30 minutes was given between each test trial. The animal was allowed to swim for up to 90 seconds

to locate the platform, if it failed to locate the platform, escape was assisted. Trial videos were analyzed with a hand timer to measure latency time (in seconds) starting from initial entry into the water maze to when the rat found the hidden platform (see Figure 2).

Perfusion. Following the memory testing, all animals were sacrificed at 80 days. Animals were injected with a chloral hydrate (400mg/kg; IP), and then transcardially perfused with 0.9% saline solution followed by 4% paraformaldehyde (PFA) in 0.1M phosphate buffer solution (PBS). Brains were extracted, post-fixed in PFA for two days, and then soaked in 20% sucrose in 4% PFA/0.1M PBS for 24 hours prior to sectioning. Using a cryostat, the hippocampus was sliced on the coronal plane (35 μ m sections). The sections were placed on glass slides and examined for presence of human cells at the same stereologic level for all animals. Slides were visualized to identify the needle track and were stained within one week of slicing. All pumps were analyzed after perfusions to determine if cyclosporine was fully released.

Histology. Cresyl violet staining was used to visualize the nuclei of the cells within the hippocampus. Microscope slides were obtained from storage and dehydrated using series of ethanol dilutions (100%, 95%, and 70%) for 2 minutes each. This was followed by a 2 minute hydration rinse in water. The slides were then immersed in the cresyl violet stain (Sigma Aldrich, C5042-10g) for 30 seconds, followed by a 2 minute water rinse. Next, the slides were dehydrated in acetic formalin for 5 minutes, and rinsed again in water for 2 minutes. The slides were then dehydrated using three 2 minute ethanol rinse at 95%, 100% and 100% dilution. Finally ethanol was removed using a xylene, and slides were mounted with coverslips using Permount (Fisher Scientific, SP15-500).

Statistical Analysis. Statistical analysis of weight gain was analyzed using Systat with repeated measures ANOVA. For analysis on the memory tests, a one sample t-test (EXCEL) was used to determine significance. Significant levels were set at $p < 0.05$ for all analyses.

RESULTS

Animal Health. To determine if animal health was affected by immunosuppression, hippocampal lesioning, and cell implantation, all animals were weighed on a weekly basis. There were no significant differences (Figure 3; $F=0.435$, $p=0.729$) among treatment groups, cell media ($n=5$), HEK293t cells ($n=7$), live hfSC ($n=21$), and dead hfSC ($n=17$).

Novel Object Recognition and Place-Object Trials. Novel Object-Recognition and Place-Object trials were used to test object recognition and spatial memory in rats injected with cell media ($n=5$), HEK293t cells ($n=7$), live hfSC ($n=21$), or dead hfSC ($n=17$) into their NMDA-damaged hippocampi. The data show that stereotactic injection of live hfSCs did not improve Novel Object Recognition (Figure 4A; $F=0.855$, $p=0.471$) nor Place-Object (Figure 4B; $F=0.730$, $p=0.539$) in NMDA lesioned rats). All values are means $\pm$ SEM.

Morris Water Maze. Morris Water Maze trials were used to test spatial memory in rats injected with cell media ($n=5$), HEK293t cells ($n=7$), live hfSC ($n=21$), or dead hfSC ($n=17$) in NMDA-damaged hippocampi. Statistical differences were observed regardless of entry (Left Entry Point, $F=14.465$, $p<0.000$; Right Entry Point: $F=9.638$, $p<0.000$, Figure 5). Specifically, lesioned rats with live hfSC implantation performed significantly better than those implanted with cell media, HEK293T cells or dead hfSC in NMDA lesioned rats. All values are means $\pm$ SEM.

Alzet Pumps. All pumps were found to be completely devoid of cyclosporine, indicating there was no malfunction of Alzet Pumps.

Staining. Nuclear-staining (cresyl violet) was used to analyze survival of hfSC in hippocampal sections. All rats received 1ul of 1mg/ml NMDA injected directly into the CA3 region at day 50 of age, followed by implantation at day 55 of age. At 80 days of age, animals were perfused. Animal brains were sliced and processed within one week of perfusion. The HEK293T control group showed abnormal tumor-like growth in the hippocampus region (Figure 6a); this type of tumorigenic growth has been observed previously with HEK293T implantation (Shen et al., 2008). No tumors were observed in any other cell treatment, including live hfSC. The dead hfSC control group showed damage to the CA3 region and a clump of dead stem cells (Figure 6b). Finally, live hfSC were clearly visible using both a low magnification (4x) and a higher magnification (20x) (Figures 6c and d), indicating hfSCs had the ability to survive *in vivo* for at least 28 days. The higher magnification of the live hfSC treatment also shows many small, viable stem cells in the damaged CA3 region (Figures 6c, and d).

DISCUSSION

In my study, I examined at the ability of human fetal neuronal stem cells (hfSCs) to ameliorate bilateral NMDA lesioned CA3 region of the hippocampus. My results showed that rats receiving live hfSC implantation performed significantly better in the water maze task than control groups (Figure 5). This result highlights the potential ability of hfSCs to improve learning and spatial memory. Surprisingly, novel and place object tests showed no significant differences among the treatment groups (Figure 4), indicating that recognition memory was not improved by hfSC implantation. My histology results confirmed the survival of implanted hfSCs up to 28 days post-implantation (Figure 6c) with human stem cell growth observed within the CA3 region (Figure 6d). I also found that implantation of a different human embryonic cell type, HEK293T, did not improve performance in memory trials, and also showed atypical tumor growth in the area of implantation (Figure 6b), reiterating the fact that not any human embryonic cell type can be used to ameliorate neuronal damage in the brain. The improvement in spatial memory and learning highlights the potential ability of hfSCs to ameliorate CA3 hippocampal damage.

The CA3 region has extensive interconnections among its cells and receives inputs from the dentate gyrus as well as the medial and lateral entorhinal cortex (Amaral et al., 1995). It is the hippocampal region known to be involved in working spatial memory as well as memory consolidation (Gilbert et al., 2006). In a study using focal injection of a presynaptic zinc chelator, diethyldithiocarbamate, to temporarily inactivate the CA3 region during the consolidation phase of the Morris Water Maze, researchers found that mice showed significant deficits in working spatial memory performance when compared with

control animals (Florian et al., 2004), indicating a lack of memory consolidation in treatment animals. Other researchers have found similar results after looking at CA3 region inactivation also using diethyldithiocarbamate. They found that the CA3 region played a role in both the consolidation of spatial memory (also using the Morris Water Maze task), and contextual fear memory using sound memory tasks (Ceccom et al., 2007).

In my experiments, I was able to distinguish between short-term memory which has limited storage and rapidly decays over time, and long term spatial memory consolidation which is relatively permanent, using the object tests and the water maze test (Cowan, 2008). Short term memory was tested using the novel object and place object trials. In these trials, animals were given a short, five-minute familiarization period, in which they were placed into an arena with two identical objects; the animals were then moved to their cages for a five-minute retention period. The animals were then placed back in the arena with one object replaced (novel object trial), or one object moved (place object trials). These trials tested for short term recognition memory by the novel object and place object trials since the animals were only given a 5-minute retention period, and not enough time for memory consolidation. In the water maze test, the animals were given three training sessions on one day, and longer term memory trials were conducted on the following day. The repetitive water maze training sessions followed by the overnight retention period allowed for animals to consolidate spatial navigation information from short-term memory to long-term. As mentioned above, animals that received live hfSC treatment showed a significant improvement in water maze memory only when compared with all control groups, demonstrating the ability hfSCs to improve long-term, spatial memory. However, no significant differences between live hfSCs treatment and the

controls in the object tests, demonstrating no improvement in short term memory. These results corroborated the ability of hfSCs to improve lesioned CA3 function.

Hippocampal damage can occur from a number of incidences, including head trauma and epilepsies (Waldau et al., 2010). This damage can be debilitating as there are limited drug treatment and physical therapy treatments. The hippocampus and its role in learning and memory has long been studied using lesioning studies in which the whole hippocampus or a portion has been unilaterally lesioned and the effects on memory from this specific lesioning are studied (Mumby et al., 2002; Hattiangady and Shetty, 2012; Park et al., 2012; Miltiadous et al., 2012). Since the hippocampus is not normally involved in critical life functions it is possible to carry out these lesioning studies without critically injuring or killing the animal. Ultimately, the lesion effects can be tested through a series of cognitive tests.

Hattiangady and Shetty (2012) performed a study where they grafted neuronal stem cells acquired from neonatal rat pups into lesioned hippocampal areas. These researchers found that the grafted cells were able to migrate into specific hippocampal areas and differentiate into all neuron types. These rats also showed an improvement in cognitive function and improved mood, suggesting effectiveness of the stem cell transplants. Although Hattiangady and Shetty (2012) successfully showed the usefulness of stem cell implantation, their study was limited as they only used unilateral lesioning. Also, the acquisition of stem cells from neonatal pups may be unrealistic in real world applications due to the need to sacrifice neonates for a therapeutic source of stem cells. In another study, grafting human neural stem cells over-expressing choline acetyltransferase to restore cognitive function in an animal model of Alzheimer disease (Park et al., 2012), researchers

found that neural stem cells were able to differentiate into astrocytes and hippocampal neurons, and improve cognitive function in this disease model. This study also utilized unilateral damage. In a third study using unilateral hippocampal lesioning, researchers were able to protect against hippocampal degeneration and improve cognitive function and mood behavior in an animal model of temporal lobe epilepsy by implanting mouse subventricular zone-derived (SZD) neural stem cell overexpressing IGF-1 grafts (Miltiadous et al., 2012). These studies did show effectiveness of stem cell grafting, but used only unilateral damage models.

Although the technique of unilateral hippocampal lesioning has been extensively used, it may not account for the ability of the surviving contralateral hippocampus to compensate for damage. In a study comparing unilateral and bilateral hippocampal lesions, researchers found that unilaterally lesioned rats performed similar to sham rats in spatial memory tasks, whereas bilaterally lesioned rats performed significantly worse than sham controls (Li et al., 1999). In a similar study conducted by Zou et al., 1999, researchers discovered that unilateral hippocampal lesioned rats were able to recover working memory and spatial memory in the radial arm maze, and perform similar to sham control rats following 21 days of repeated training (Zou et al., 1999). In my study, the CA3 region was bilaterally damaged, thereby preventing the ability of contralateral hippocampal compensation, isolating the effects of stem cell implants.

Another form of the hippocampus' ability to compensate for neuronal loss is synaptic reorganization. The hippocampus has the ability to rewire itself following neuronal loss. Specifically, loss of CA3 neurons triggers granule cell axons (mossy fibers) to connect with surviving CA3 pyramidal neurons to maintain the hippocampal circuitry

(Cavazos et al., 2004). Although previous studies have shown that the hippocampus has the ability to self-repair through synaptic reorganization (Cheng, 2013), it would be hard to say that was the case in my experiments as there were significant differences between the live hfSC treatment group and control groups in the water maze trials. Had there been no difference among control treatment groups, then it could be implied that the hippocampus utilized another compensating mechanism.

In addition, synaptic reorganization in the hippocampus may not be beneficial for a lesioned rat. In a study looking at cognitive function immediately following traumatic brain injury, researchers found that it took up to 50 days for lesioned animals to perform similar to sham controls in Morris water maze tests (Sun et al., 2007). Although previous studies have shown that synaptic reorganization takes place following traumatic brain injury, it has been found to be inadequate in compensating for significant memory loss (Jorge et al., 2007; Potvin et al., 2006). It has also been determined that CA3 synaptic reorganization may lead to increased seizure activity, due to the formation of hyper-excitabile neurons (Hester et al., 2013). No seizures were observed in my study either before or after stem cell treatments.

One other form of compensation can occur in the hippocampus: neurogenesis. Following traumatic brain injury, endogenous hippocampal stem cells found in the dentate gyrus can migrate, differentiate and form synaptic reconnections (Shetty, 2014). The process of hippocampal neurogenesis takes approximately 60 days to mature from stem cell to fully functional neuron in the rat (Sun et al., 2007). Neurogenesis, however, did not affect my study since cognitive tests were conducted two weeks following implantation, which would not allow time for hippocampal neurogenesis (Sun et al., 2007). Despite the

ability at the hippocampus to compensate for neural damage via neurogenesis or synaptic reorganization, this compensation often falls short of full recovery of memory and mood deficits (Jorge et al, 2007).

In previous studies, implanted human neural embryonic stem cells have been shown to differentiate into neuronal cells and migrate to areas of damage (Kelly, 2004; Chang et al., 2013; Hurelbrink et al., 2002). Celavie's human fetal derived stem cells have also demonstrated their ability to migrate to the site of brain lesions and differentiate according to the nature of the lesion (unpublished data). My immunohistochemistry results show small hfSCs growing within the lesion site, CA3 region, which appear to reduce the behavioral damage caused by NMDA (Figure 4d). In my study, I did not determine if these cells had matured into either neurons or glia.

My finding that the implantation of hfSCs improve spatial memory and learning bilaterally CA3 region lesioned rats is astounding since there is currently limited treatments to repair fully hippocampal damage. It is also important to note that the stem cell implantation did not have an effect on health, as animals that received treatment resulted in normal weight gain. My study has shown that Celavie's human derived pluripotent stem cells were able to survive *in vivo* and improve hippocampal functionality, highlighting the potential promise for stem cell treatment of brain damage in neurodegenerative diseases.

FIGURES

Figure 1. Memory Tests Schematics. (a) Novel-Object Trial. In the novel-object tests, the objects remained in the same location, but one object was substituted for the test. (b) Place-Object Trial. For the place-object test, one object was moved prior to the test.

Figure 2. Morris Water Maze Schematic. In this cognitive test, the rat used visual cues to find the hidden platform. Rats were tested from two start locations equidistant from the platform: right start and left start.

Figure 3. Mean weight gain for rats whose hippocampi were lesioned with NMDA and subsequently injected with cell media (n= 5), HEK293t cells (n=7), live hfSC (n=21), or dead hfSC (n=17). All treatment groups had similar weight gain over the course of the experiment, suggesting that hfSC implantation did not affect rat health as measured by weight gain ($F=0.435$, $p=0.729$). All values are means $\pm$ SEM.

Figure 4. Novel Object-Recognition (A) and Place-Object (B) assays were used to test object recognition and spatial memory in rats injected with cell media (n=5), HEK293t cells (n=7), live hfSC (n=21), or dead hfSC (n=17) into their NMDA-damaged hippocampi. The data show that stereotactic injection of live hfSCs did not improve object recognition or spatial memory in NMDA lesioned rats compared to the other treatment groups (ANOVA, Novel-Object: $F=0.855$, $p=0.471$; Place-Object: $F=0.730$, $p=0.539$). All values are means $\pm$ SEM.

Figure 5. Morris Water Maze trials were used to test spatial memory in rats injected with cell media (n=5), HEK293t cells (n=7), live hfSC (n=21), or dead hfSC (n=17) in NMDA-damaged hippocampi. Rats with live hfSC implantation performed significantly better (ANOVA, Left Entry Point: $p < 0.001$; Right Entry Point: $p = 0.005$) than those implanted with cell media, HEK293T cells or dead hfSC in NMDA lesioned rats. All values are means $\pm$ SEM.

Figure 6. Photomicrographs of cresyl violet stained hippocampi of rats that received 1 μ l of 1mg/ml NMDA injected into the CA3 region at day 50 of age, followed by injection of HEK293t (Fig. 6a), dead hfSC (Fig. 6b), or live hfSC (Fig. 6c and 6d) implantation at day 55 of age. Fig. 6a shows the typical tumor growth (arrow) caused by HEK cell implantation. Fig. 6b shows the damaged CA3 region and a clump of dead stem cells (arrow). Fig. 6c. (hfSC, low magnification) shows many small stem cells growing within the damaged CA3 region (arrow); while Fig. 6d is a higher magnification image of this area. Arrow shows location of surviving hfSC.

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