

AD _____

Award Number: DAMD17-03-1-0122

TITLE: Regulated GDNF Delivery in Vivo Using Neural Stem Cells

PRINCIPAL INVESTIGATOR: Clive N. Svendsen, Ph.D.

CONTRACTING ORGANIZATION: University of Wisconsin
Madison, WI 53706-1490

REPORT DATE: April 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20050113 071

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE April 2004	3. REPORT TYPE AND DATES COVERED Annual (1 Apr 2003 - 31 Mar 2004)	
4. TITLE AND SUBTITLE Regulated GDNF Delivery in Vivo Using Neural Stem Cells			5. FUNDING NUMBERS DAMD17-03-1-0122	
6. AUTHOR(S) Clive N. Svendsen, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Wisconsin Madison, WI 53706-1490 E-Mail: svendsen@waisman.wisc.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) The major aim of this proposal was to establish lines of neural stem cells secreting GDNF under a regulatable promoter system which may be used for future transplant therapies for PD. The tasks of the first year have been met. We have changed from using rodent and monkey stem cells to using human stem cells (hNSC) due to better growth and infection profiles for human cells. We have shown that hNSC can be infected with lenti-GDNF. GDNF released from the cells is functional on dopamine neurons and can be switched on and off by doxycycline. We have enhanced infection rates such that over 85% of cells express GDNF. We have selected high expressing lines and shown that they can be expanded in culture for over 20 weeks without losing the ability to produce GDNF in a regulated fashion. We have performed 6-OHDA lesions in rats to induce PD and shown loss of TH expression, survival of hNSC transplanted into these animals and expression of GDNF for up to 8 weeks (this is ahead of our predicted time frame) but are currently starting in vivo regulation studies. PET analysis has shown that the lesion can be detected with imaging and we are currently optimizing this protocol for use in the more detailed studies next year. Our pilot monkey work has been delayed due to problems growing the monkey stem cells. However, we have now scheduled the first surgery using hNSC for May 2004.				
14. SUBJECT TERMS Neural stem cell; GDNF, toxicity; Parkinson's disease; viral deliver; inducible promoter			15. NUMBER OF PAGES 17	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments (Advances).....	4
Reportable Outcomes.....	
Conclusions.....	8
References.....	8
Appendices.....	10

This is the one year update for our DOD award including the periods between March 31st 2003 and April 1st 2004.

Major Changes to Original Proposal

We have followed the experimental design outlined in the grant but due to some of the first experimental data generated by this proposal we decided to change the types of cell used for these studies. In our original aims section we stated that we would assess the effects of transplanting rodent and monkey neural stem cells into the rat and monkey model of Parkinson's disease (PD). The rationale was that by using cells from the same species we would avoid having to use immune suppression. In our preliminary data we showed that monkey neural stem cells could be isolated and grown in culture for short periods. However, once the award was given we began more systematic studies of the monkey neural stem cells and have been surprised to find that they grow very slowly and are difficult to passage for long periods following infection with lentivirus. We had a similar problem with the rodent neurosphere cells. This is likely to be a species difference between human, rodent and monkey stem cells that we and others have noted previously. Despite various changes to the culture conditions we were unable to grow either efficiently enough to perform the long term experiments planned in the grant. As our preliminary data was using human cells we knew that long term growth was possible. Our original rationale for not using human cells was, again, that there would be no need for immune suppression of the animals. However, we have now established that Lewis rats with daily injections of cyclosporine can tolerate transplants of lentivirally infected human neural stem cells (see data to follow) and our collaborators have developed ways to immuno-suppress monkeys. Therefore, early in the granting period we decided to follow all of the protocols set out in the grant - but using human neural stem cells (hNSC) rather than monkey or rodent cells. This is actually far more relevant for diseases of the human brain such as Parkinson's disease.

The result of the delay in obtaining primate neural stem cells also led to a delay in our pilot sub-contract with Rush to perform monkey transplants. All of the protocols have now been updated to perform human neural stem cell grafts to monkeys and our first transplants are scheduled for May 2004.

Advances

During the last year our work with human neural stem cells has grown and we are now able to generate lines of hNSC that are non-transformed and for which we have a full genomic profile using gene array technology (Wright et al, 2003). These have been modified to release GDNF under an inducible vector system and have now been shown to survive transplantation into rodent models of PD based on the work in the update. Our cells are collected from the NIH funded Birth Disorders Laboratory in Washington and are covered under our local IRB. The primate work animal license has also been updated to reflect cyclosporine treatment and hNSC transplants.

Task 1. Optimize production of hNSC that secrete GDNF under an inducible promoter.

a. Assess and optimize GDNF release from hNSC using lentiviral vectors (Months 1-18)

In our original proposal, we had preliminary data demonstrating that neurospheres infected with an inducible lentivirus for GFP (^{ind}lentiGFP^{tet off}) expressed GFP that was turned off in the presence of doxycycline. We further reported that intact neurospheres

infected with an inducible lentivirus for GDNF (^{ind}lentiGDNF^{tet off}) expressed GDNF, which could be quantified using ELISAs.

We have continued to optimize and assess GDNF release from hNSC infected with lentiviral constructs. Using a modified method of infection, which involves temporary neurosphere dissociation prior to infection, we can now achieve over 90% infection using a non-inducible lentivirus expressing GFP (Fig. 1). Similar methods were used with the dual vector inducible system - ^{ind}lentiGDNF^{tet off}. Dissociated cells were infected with both vectors and quickly reaggregated to form new spheres within 24 hours. Immunocytochemical staining revealed that over 70% of the cells within the sphere expressed GDNF (Fig. 2A). Using ELISAs, we determined that cells infected by our optimized methods produce GDNF and release 95.6 ± 18.1 ng GDNF/ml (SEM) into the media.

b. Establish doxycycline (DOX) regulation profile on GDNF secreted from hNSC infected with lentivirus (Months 6-24).

Having optimized and assessed GDNF production for task 1a, we next studied GDNF regulation using doxycycline (DOX). hNSC infected with ^{ind}lentiGDNF^{tet off} produce GDNF that is tightly regulated with DOX (Fig. 2B). Neurospheres infected with ^{ind}lenti-GFP did not release GDNF at levels high enough for measurement even with sensitive detection methods (Fig. 2B), showing that there is little or no constitutive production of GDNF from undifferentiated hNSC in our culture system. The range of GDNF levels released from individual neurospheres (Fig. 2B) suggested the future potential of selecting and propagating individual neurospheres with the highest gene expression (task 1c, months 18-36). Interestingly, the degree of GDNF regulation by DOX was similar amongst the neurospheres regardless of differing GDNF expression levels for each sphere. Following 2 days of DOX treatment, the range of decrease in GDNF levels was 56% to 68% compared to cells without DOX, with an average decrease of 64%. GDNF levels were reduced after 2 days of DOX treatment, but continued to decrease in a time-dependent fashion due to the long half-life of the GDNF protein. By 10 days of DOX treatment, there was an almost 90% decrease in GDNF levels compared to cells without DOX (Fig. 2C). Importantly, we demonstrated that the GDNF not only can be reduced by DOX administration but also can be reinitiated following DOX withdrawal (Fig. 4D).

We established that hNSC infected with ^{ind}lentiGDNF^{tet off} release high levels of GDNF. However, this may not be folded correctly or might not be biologically active. As discussed in the grant we next assessed the functional effects of this GDNF in a bioassay to determine whether it was functionally active. Primary rodent dopamine neurons that respond to GDNF were cultured in either basal media, supernatant from wildtype human neurospheres or supernatant from ^{ind}lentiGDNF^{tet off} infected neurospheres. The number of TH-positive cells significantly increased when cultures were grown in supernatant from wildtype human neurospheres or supernatant from ^{ind}lentiGDNF^{tet off} infected neurospheres compared to cultures grown in basal media ($p < .0001$) (Fig. 3A). This suggests an overall effect of conditioned media on dopamine neuron survival that is not further increased by GDNF. However, GDNF released by hNSC did have a significant effect on neurite length of cultured dopamine neurons (Fig. 3B; $P < .0001$). In addition, GDNF released from hNSC infected with ^{ind}lentiGDNF^{tet off} significantly increased the cell body size of cultured rodent dopamine neurons (Fig. 3C; $P < .0001$). The cell body size of neurons grown in wildtype supernatant was not increased compared to cultures in basal media,

suggesting no effect of conditioned media alone on this parameter of dopamine neuronal health. The fact that GDNF released from $\text{ind}_1\text{lentiGDNF}^{\text{tet off}}$ infected neurospheres has potent effects on cultured dopamine neurons demonstrates that these cells are releasing GDNF at physiologically relevant levels *in vitro*, and that the GDNF is biologically active.

c. Select and characterize lentiviral clones which express GDNF at high levels following differentiation (Months 18-36).

Though this is a task for later in the proposal, we have already shown that individual neurospheres can be assessed for GDNF levels and separated based on amounts produced (Fig. 2B). We have selected some of these high and low expressing neurospheres and grown them for extended periods of time in culture. Due to the integration of the virus being very stable, high expressing neurospheres continue to release high levels of GDNF for up to 20 weeks of continual expansion. Thus our system is proving very robust. We do not need to continually re-infect the neurospheres with new batches of lentivirus. Following differentiation there is little difference between high expressing GDNF spheres and low expressing GDNF spheres with regard to neuronal and glial production and so we are confident that modification of the hNSC is not affecting their long term characteristics *in vitro*.

d. Produce and optimize new vectors with combinations of GDNF/GFP (retrovirus/AAV)- assess which is most efficient at GDNF production in vitro and compare with lentivirus (Months 6-48).

We produced retroviruses to make GDNF/GFP under control of the tet inducible system and infected hNSCs with these constructs. After retroviral infection, cells were dissociated and stained for nestin, GFAP or beta III tubulin (markers for progenitors, astrocytes and neurons, respectively). Assessment of GFP expression in these cells showed only a small percentage was infected by retrovirus. This was consistent with the low infection by retrovirus that we reported for the constitutive construct. On the other hand, we achieve high levels of infection with our inducible lentivirus, consistent with what we reported for the constitutive lentiviral construct. By comparing retroviral and lentiviral infection, we have confirmed that hNSC are better infected by lentivirus and have therefore focused our efforts on *in vivo* characterization of this inducible vector.

Task 2. To protect against toxic cell death in the brain by transplanting GDNF producing stem cells into rodent and primate models of PD.

a. Perform pilot monkey transplant study with GFP/GDNF retroviral construct using micro-PET and post mortem data to establish survival and possible function of cells (Months 6-18).

Although our pilot monkey work has been delayed, we have made great progress ahead of our predicted schedule in transplanting GDNF-producing hNSC into rodent models of PD. We have used lentiviral constructs for GFP and for GDNF rather than GFP/GDNF retroviral constructs due to the infection efficiencies detailed in task 1D. We first showed that hNSCs infected with $\text{ind}_1\text{lentiGFP}^{\text{tet off}}$ can survive transplantation into the rodent striatum and express GFP for 2 weeks (Fig. 4). Based on this successful survival and expression, we next wanted to transplant hNSC infected with $\text{ind}_1\text{lentiGDNF}^{\text{tet off}}$ into the 6-OHDA lesion model of Parkinson' disease. Before starting this work we needed to optimize several lesion parameters and behavioral tests, including amphetamine-

induced rotations, forelimb use and paw stepping, to confirm the 6-OHDA lesion. We established a robust system for generating partial parkinsonian lesions in Lewis rats with predictable behavioral changes (Fig. 5). Additionally, micro-PET was performed on two groups of animals (described in detail within the grant application) using the tracer 6^[18F] Fluoro-m-tyrosine (6-FMT) – a DOPA decarboxylase tracer. This technique was optimized to scan 4 rats simultaneously and was sensitive enough to detect the partial 6-OHDA lesion in these animals (Fig. 6). Finally, immunohistochemistry for tyrosine hydroxylase was used to verify the partial 6-OHDA lesion in the striatum and substantia nigra. Significant reductions were seen in localized regions of the striatum (Fig. 7A) which resulted in selective loss of TH positive neurons within the substantia nigra pars compacta (Fig. 7B,C).

Based on the successful survival and expression of hNSC infected with ^{ind}lentiGFP^{tetoff} and the characterization of this rodent model of PD, we transplanted hNSCs infected with ^{ind}lentiGDNF^{tetoff} into the partially lesioned striatum. Cells survived transplantation for two weeks within the region of the 6-OHDA lesion (Fig. 7D). In animals left for longer time periods we found continual survival of the grafts for up to 8 weeks (Fig. 8A) and strong expression levels of GDNF at this time that correlated directly with the human neural stem cells (Fig. 8B) suggesting continual release of GDNF. The level of GDNF produced by the hNSC was high enough to be detected both in the core of the transplant and a large surrounding area. We were also interested in using these cells in other models of striatal damage to assess how robust their survival was under different conditions. The rationale behind this is that in PD we are not sure exactly what the local environment will be for the cells following transplantation (our models of PD are very acute vs. the long term death of dopamine neurons in PD). To address this we also transplanted hNSC into the striatum of animals with induced Huntington's disease. This study was in collaboration with Jeff Kordower (who is going to perform the monkey studies with the hNSC). Our hNSC survived well in this rat model of Huntington's disease and produced functional recovery in some animals which will shortly be published in *Journal of Comparative Neurology* (McBride et al, 2004). This new paper serves as proof of concept that the cells we are generating can survive in different environments. Furthermore, two other papers have been published by the PI during the period of the grant using the non-modified hNSC transplanted into either a similar model of PD or a model of stroke. The cells survived, migrated and differentiated over extended periods of time (Burnstein et al, 2004; LaBelle et al, 2004) providing further proof of concept for the studies outlined in this proposal which are now well underway.

DOD supplement for scientific meetings related to this proposal

During the course of the grant we organized a meeting on behalf of the DOD for which we recently applied for a supplement. This supplement is to allow scientific meetings to be held over the period of the grant which will allow leaders in the field to come together and discuss various types of cell and gene therapy for Parkinson's Disease. This is an ideal opportunity to increase awareness of the role the DOD is playing in Parkinson's research through publications in high profile journals of meeting reports. We envisage small gatherings of leaders in the field (between 10-15) in addition to local stem cell experts from the University of Wisconsin. The format will be based around short presentations and much discussion of when and how stem cells may be used in the clinic for PD and possibly other diseases relevant to the DOD such as amyotrophic lateral sclerosis. The costs are mainly for travel and meal expenses for the speakers. By avoiding registration costs we can keep the meeting small and focused which

enhances discussion and ideas. This should also provide an ideal forum for the generation of new projects for the DOD to fund in the future. Key researchers in Parkinson's disease can find out how the DOD supports this work and will hopefully apply for grants in the future. The first of these meetings was held in November 2003 and was a great success leading to a publication of the conclusions in the premier medical journal *Nature Medicine* (Svendsen and Langston, 2004).

Summary

In conclusion we have achieved all of the goals set out in the proposal for the first 12 months except transplantation of cells into the primate model which was delayed due to the poor growth of the primate neural stem cells. We have shown efficient infection of hNSC *in vitro*, regulation of GDNF release and biological effects on primary dopamine neurons. In addition, we have moved faster than we predicted with regard to transplantation and already have pilot data to show that hNSC can survive transplantation into the Parkinsonian rat model and continue to release GDNF over a period of 8 weeks. We can assess the lesions with behavioral tests and can follow the lesions dynamically with PET imaging as predicted in the grant. Our current goals (year 2) are to assess the functional effects of this GDNF release *in vivo*, establish DOX shut off of GDNF release and further optimize our culture methods through selection of high expressing neurospheres. We feel we are making rapid and solid progress over the first year of the grant.

Publications

We are currently putting together the first primary publication to arise from the current grant for which we have most of the data (Behrstock and Svendsen, In preparation). However, we have published a number of prominent reviews during the course of the grant which were in part funded by the DOD which supported part of Dr. Svendsen's salary during this period. Furthermore, other related papers published by our group during the period of the grant are also listed below to show constant productivity in this area of research which continually feeds into the current studies underway for the DOD. These are all listed below (**DOD funded).

Reviews

Behrstock, S. and Svendsen, C.N. (2003) Neural stem cells. *Nature Encyclopedia of the Human Genome*. 4: 298-302. Nature Publishing Group.

Tai Y.T., Svendsen CN. (2004). Stem cells as a potential treatment for neurological disorders *Current Opinions in Pharmacology*, 4; 98-104 **

Jakel RJ, Schneider BL, Svendsen CN. (2004). Modeling neurodegenerative diseases using human neural stem cells *Nature Reviews/Genetic*, 5; 1-8. **

Svendsen, C.N. and Langston, J. (2004) Stem cells for Parkinson's Disease and ALS: Replacement or protection? *Nature Medicine*, 10; 224-225 **

Behrstock, S. and Svendsen, C.N. (2004) Combining growth factors, stem cells, and gene therapy for the aging brain. *Annals of the New York Academy of Sciences*, 1019: In Press **

Papers

Gill S.S., Patel, N.K., Hotton, G.R., O'Sullivan, K., McCarter, R., Bunnage, M., Brooks, D.J., Svendsen, C.N. and Heywood, P. (2003). Direct brain infusion of glial cell line-derived neurotrophic factor (GDNF) in Parkinson's Disease. *Nat. Med.* **9**, 589-595.

Wright, L.S. Li, J., Caldwell, M.A., Wallace, K., Johnson, J. and Svendsen, C.N. (2003) Gene expression in human neural stem cells: effects of leukemia inhibitory factor. *Journal of Neurochemistry.* **86**:179-195.

Suzuki M, Wright LS, Marwah P, Lardy HA, Svendsen CN. (2004) Mitotic and neurogenic effects of dehydroepiandrosterone (DEHA) on human neural stem cell cultures derived from the fetal cortex. *PNAS* **101**: 3202-3207.

Burnstein RM, Foltynie T, He X, Menon DK, Svendsen CN, Caldwell MA. (2004). Differentiation and migration of long term expanded human neural progenitors in a partial lesion model of Parkinson's disease. *International J. of Biochem. and Cell Biology.* *In Press*

LeBelle JE, Caldwell MA, Svendsen CN. (2004). Improving the survival of human CNS precursor derived neurons following transplantation. *Journal of Neuroscience Research.* *In Press.*

McBride JL, Behrstock S, Chen EY, Jakel RJ, Seigel I, Svendsen CN, Kordower JH. (2004). Transplants of human progenitor cells improve motor function in a rat model of Huntington's Disease. *J. Comp. Neurol., In Press***

Ostenfeld, T. and Svendsen, C.N. Proliferative profile of EGF and FGF-2-responsive neural stem cells: obligatory requirement for neurogenesis to proceed through the division of progenitors emerging from differentiating neurospheres. *Stem Cells, In Press*

Patel, N.K, Bunnage, M., Heywood, P., Plaha, P., Hotton, G.R., Brooks, D.J., Svendsen, C.N. and Gill, S.S. Intraputaminal infusion of glial cell line derived neurotrophic factor in Parkinson's Disease: A two year clinical, cognitive and quality of life outcome study. *In revision.*

Hotton, G.R., Gill, S.S, Patel, N.K., Svendsen, C.N. Bailey, D, Asselin, M.C and Brooks, D.J. Assessment of glial derived neurotrophic factor in Parkinson's Disease: A F-dopa PET study. *In Revision .*

Behrstock, S. and Svendsen, C.N. Regulated release of GDNF from human neural stem cells in vitro and following transplantation into the parkinsonian rat. *In preparation**.*

Figure 1. Optimization of GFP lentiviral infection by dissociating neurospheres prior to exposure to virus. Following brief dissociation spheres were exposed to various titers of virus (A) and then viewed for GFP intensity (B). Maximum efficiency assessed by dissociating, acutely plating for 1 hour and counting number of GFP expressing cells using this method was over 90% (C). Expression levels and normal cell morphology following infection demonstrate that lentivirus does not affect cell health (D).

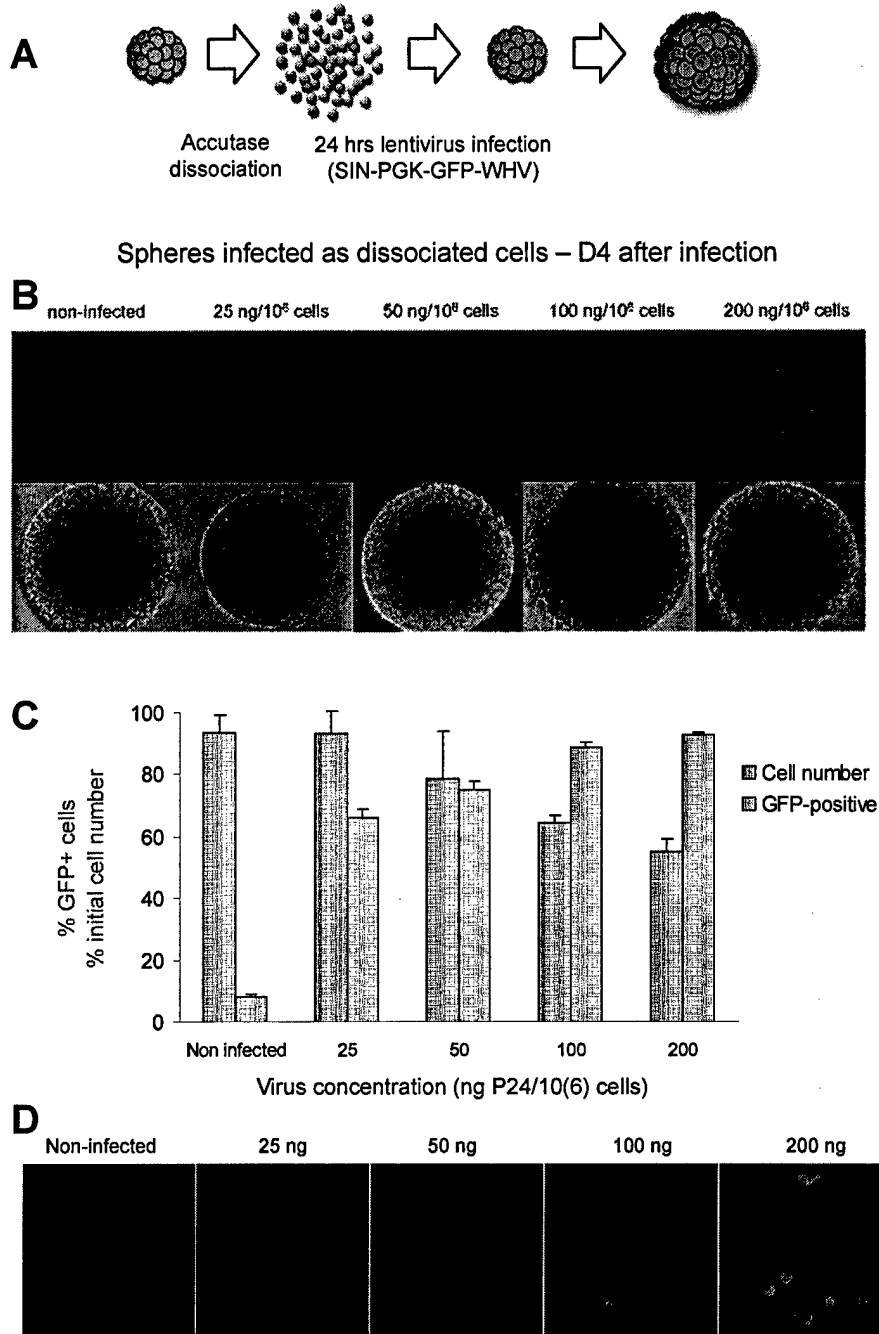
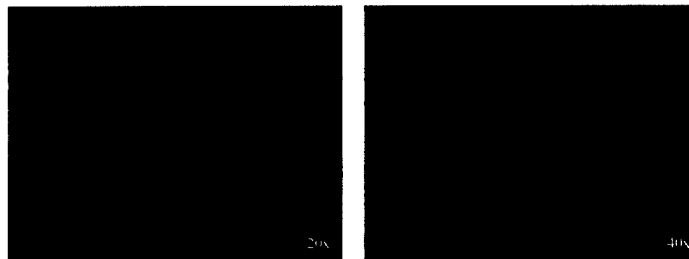
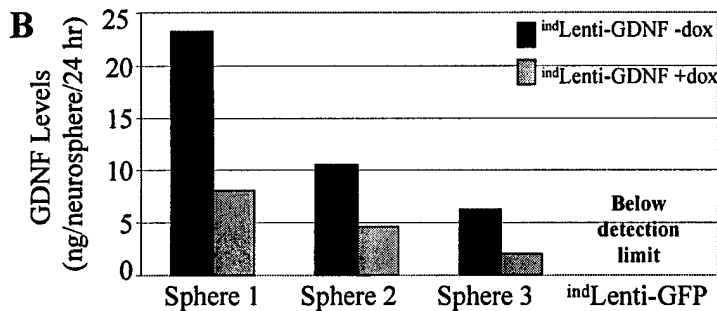


Figure 2. Human neural stem cells show regulated release of GDNF following infection with $\text{indLentiGDNF}^{\text{tet off}}$. Using the optimized methods established in Fig.1, hNSC were infected with the inducible GDNF constructs and approximately 70% of cells were shown to express GDNF (A). Individual spheres were isolated and levels of GDNF release assessed. Some spheres released more than others (B) but all showed down regulation of GDNF expression following DOX treatment for 2 days. Following longer DOX addition over 90% shutdown in GDNF production was established in hNSC (C). Importantly, this regulation was dynamic as when DOX was removed GDNF expression switched on again to levels similar to those seen before switch off (D).

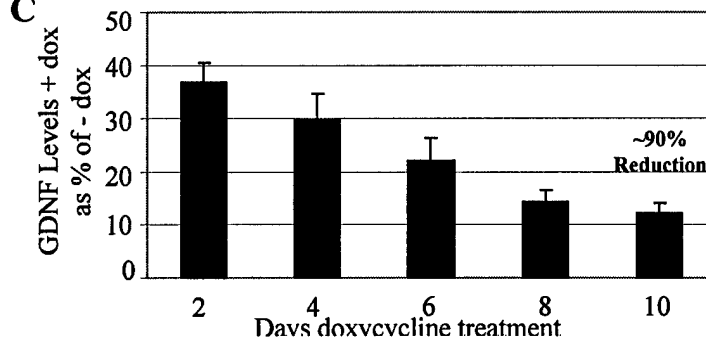
A



B



C



D

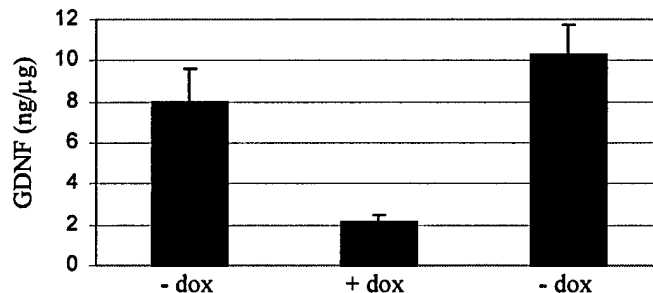


Figure 3. Functional effects of GDNF released from hNSC *in vitro*. Following plating of primary rat dopamine neurons overall survival was significantly enhanced with either conditioned media from wild type hNSC or conditioned media containing GDNF released from hNSC infected with lentivirus (A). However, when the fiber length (B,D,E) or cell body area (C,D,E) of dopamine neurons was assessed, significant increases were seen in response to GDNF released from the hNSC.

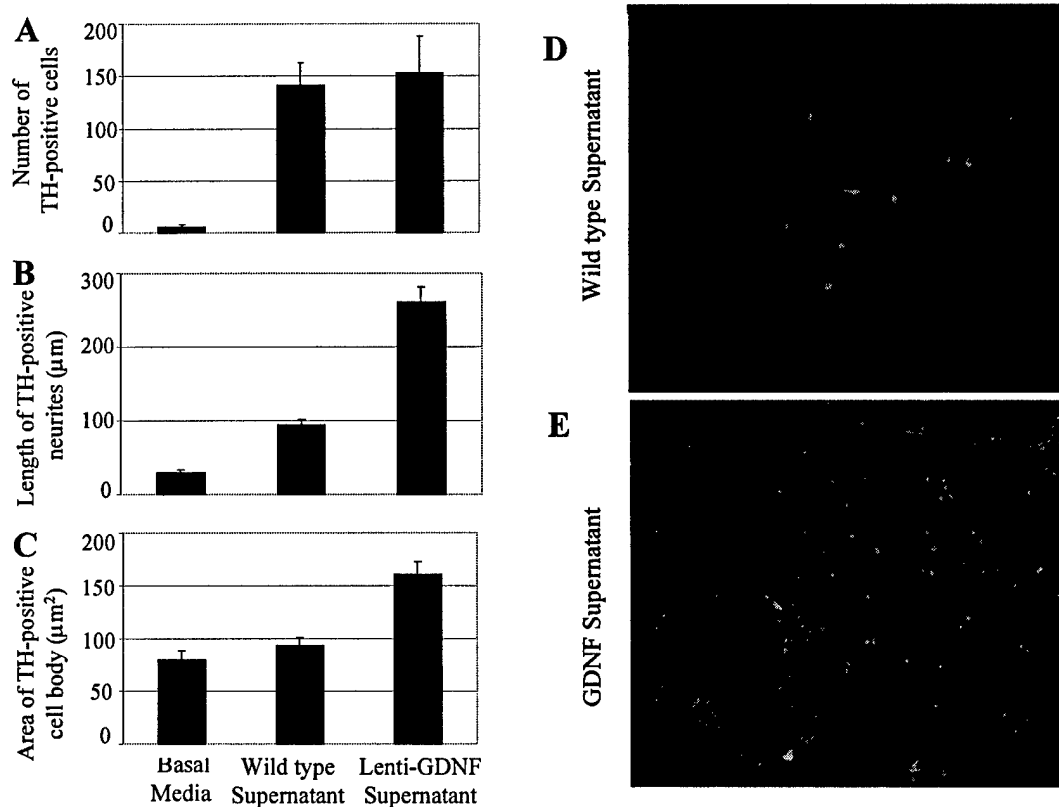


Figure 4. Human neural stem cells infected with the ^{ind}lentiGFP^{tetoff} construct survive transplantation into the rodent striatum for 2 weeks without down regulation of the transgene. The transplant is healthy and spans at least 300 μm (gap between B and D).

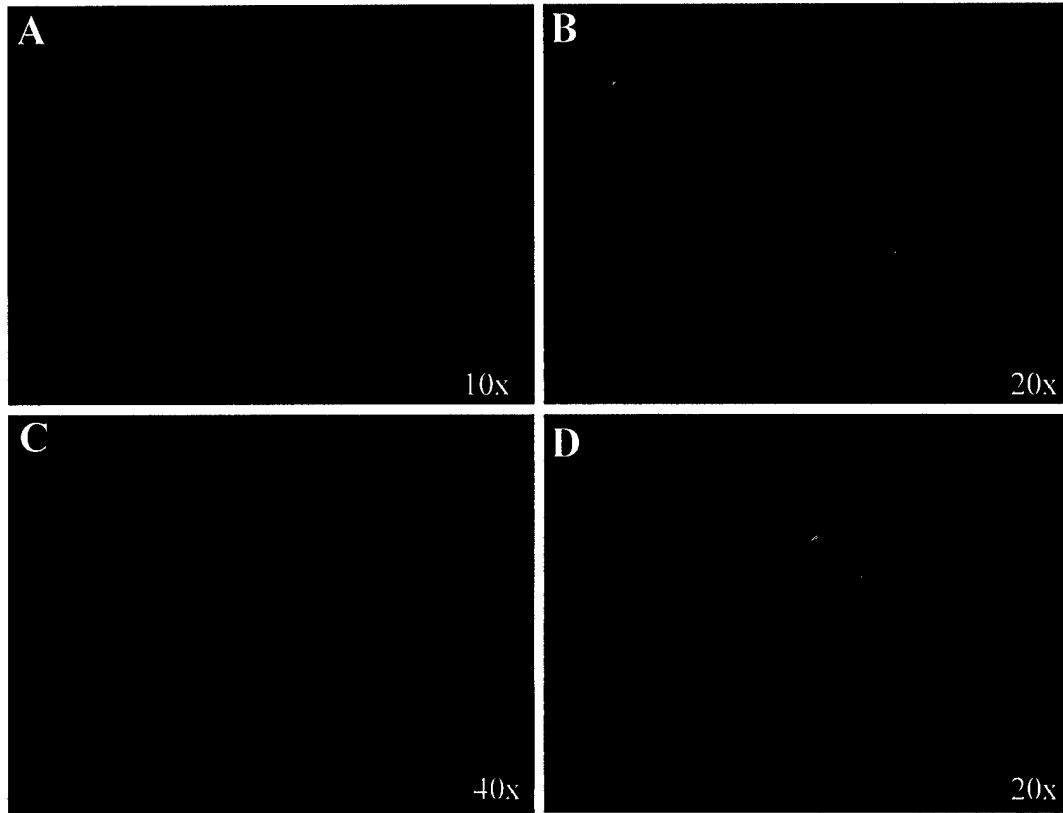


Figure 5. The partial 6-OHDA lesion leads to predictable changes in forelimb stepping, forelimb use and amphetamine-induced rotation.

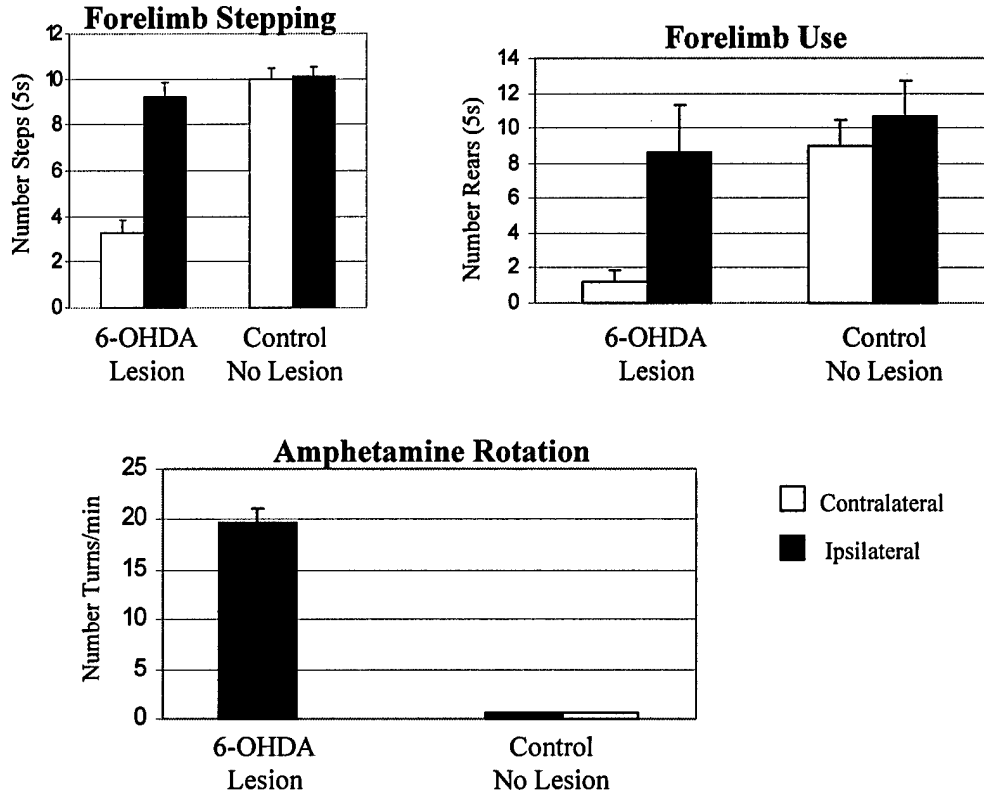


Figure 6. PET imaging (see grant for details) reveals significant differences in dopamine storage following a parital 6-OHDA lesion. Coronal sections through the whole rat head. Animals prior to the lesion have even distribution of dopamine uptake on both sides of the striatum (A; cross represents midline of striatum). Following 6-OHDA lesions there is a significant reduction in signal on the lesioned side (B; arrow)

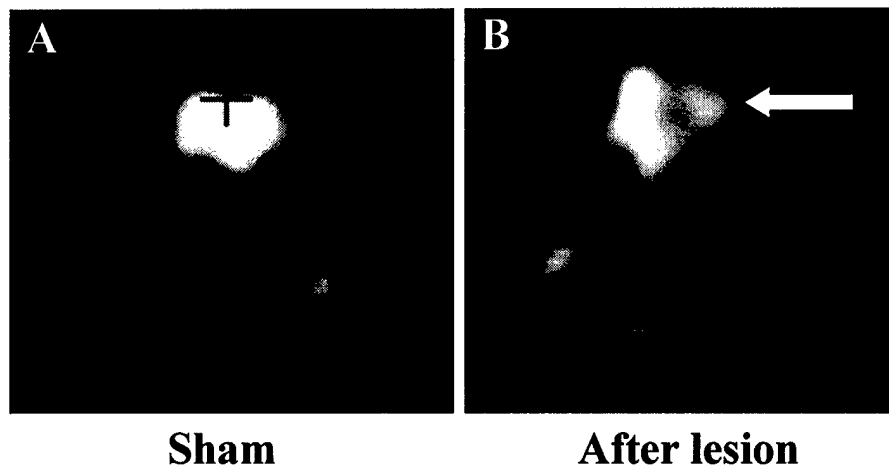


Figure 7. 6-OHDA leads to reduction in tyrosine hydroxylase (TH) staining in the striatum (A) which in turn results in significant loss of dopamine neurons in the substantia nigra pars compacta (C) when compared to the non lesioned side (B). Transplanted human neural stem cells survive well in the lesioned striatum (D) stained here with the specific human nuclear marker which does not recognize rodent cells.

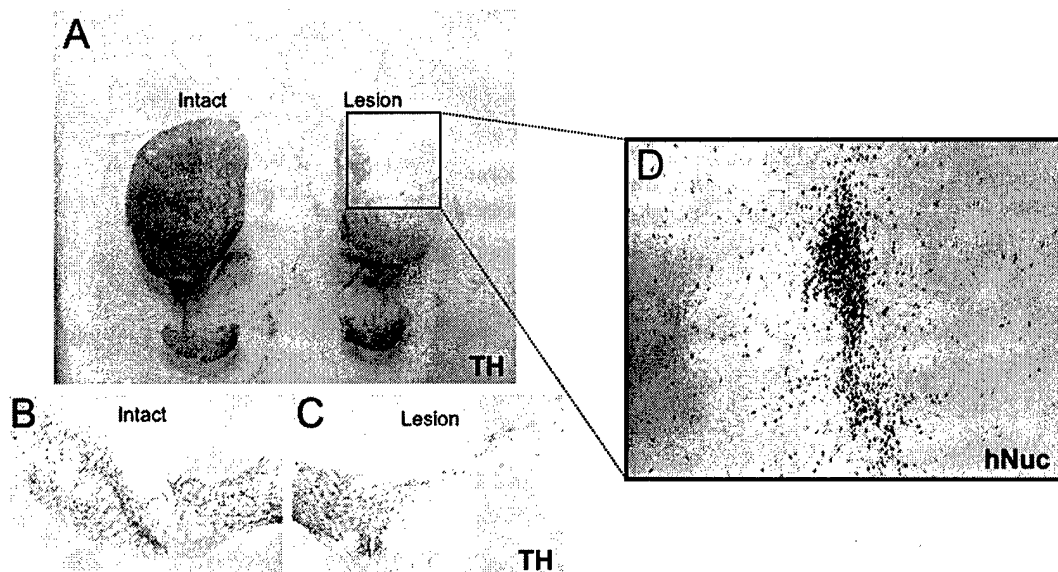


Figure 8. Human nuclear and GDNF staining co-localize in the striatum and GDNF produced by the hNSC is robust enough for detection both within and around the graft core. Human nuclei staining revealed many cells within the region of the transplant that had begun migrating out into the striatum at 8 weeks as shown here (A). Staining adjacent sections with an antibody to GDNF revealed a 100% overlap with human cells and GDNF release (arrow shows overlap of same blood vessel) (B). GDNF release was robust enough to spread from the graft core into the surrounding striatum.

