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TITLE: Mechanisms of Enhanced Neuroregeneration Associated with the Common Human Single Nucleotide Polymorphism Val66Met

PRINCIPAL INVESTIGATOR: Margaret Caulfield, PhD

CONTRACTING ORGANIZATION: Michigan State University, East Lansing, MI

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14. ABSTRACT
Mechanisms underlying graft-induced dyskinesia (GID), a cell replacement side effect that can occur in individuals with Parkinson's disease (PD), remain unknown and controversial. As a potential genetic contribution to this therapeutic outcome, our lab focuses on a common single nucleotide polymorphism (SNP), rs6265, found in the gene for brain-derived neurotrophic factor (BDNF) resulting in decreased BDNF release. Using rs6265 knock-in rats, we previously demonstrated that homozygous rs6265 (Met/Met) parkinsonian rats engrafted with wild-type (WT; Val/Val) dopamine (DA) neurons uniquely developed GID. This behavioral phenotype was correlated with neurochemical signatures of immature DA-glutamate co-transmission normally seen during development. Based on the necessity of BDNF for DA neuron maturation, we hypothesize that decreased BDNF release in rs6265-carriers impairs maturation and synaptogenesis of grafted DA neurons, leading to GID, and that infusion of exogenous BDNF will allow for graft maturation, normalization of graft-derived synaptic innervation, and GID prevention. To test this hypothesis, male parkinsonian Met/Met rats were transplanted with intrastriatal embryonic WT DA neurons. Infusion cannulas were stereotaxically inserted 3µm dorsal to grafted cells at time of grafting and attached to 28-day Alzet™ osmotic minipumps containing BDNF or vehicle phosphate buffered saline (PBS). Pumps were removed after four weeks. Levodopa-induced dyskinesia (LID) and GID severity were evaluated over 10 weeks post-engraftment. DA-grafted animals infused with PBS or BDNF exhibited significant amelioration of LID compared to non-grafted animals, demonstrating successful engraftment. Contrary to our hypothesis, BDNF infusion in grafted animals increased amphetamine- and levodopa-mediated GID behavior in Met/Met hosts. BDNF infusion also increased contralateral amphetamine-induced rotational behavior, indicative of excess DA in the grafted striatum; GID in grafted PD patients has also been associated with excess striatal DA. Histological and molecular analyses of graft neurochemical phenotypes are ongoing. These data underscore the importance of tailoring therapeutics to improve clinical outcomes for individuals with PD.

15. SUBJECT TERMS
None listed.

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

   Parkinson's disease (PD) is the second most common neurodegenerative disorder with an estimated 1 million Americans currently living with the disease. Risk factors of PD include aging, traumatic brain injury, exposure to environmental toxicants like herbicides and pesticides including Agent Orange, and genetic susceptibility. Military environments can involve several of these factors thereby imposing significant risk to military personnel and aging veterans. There are several therapies for PD that are generally effective at alleviating symptoms in early- and mid-stage PD, however ultimately these therapies lose efficacy and side-effects develop as the disease progresses. There is growing consensus that PD is a complex and heterogeneous disease, with the molecular underpinnings, clinical presentation, and response to therapy varying greatly among individuals. The approach of replacing dopamine (DA) neurons that are lost in the disease that has had most clinical success has been primary ventral mesencephalic (VM) DA neuron engraftment (for review doi:10.1002/mds.27742). Unfortunately, the clinical experience with cell replacement therapy for PD is filled with conflicting results ranging from significant, sustained benefit to no effect, to a noteworthy incidence of graft-induced side effects known as graft-induced dyskinesias (GID) (for review doi:10.1002/mds.27742). The role of specific genetic variations in host response to grafting has remained entirely unexplored until a recent study undertaken by our group (doi:10.1016/j.nbd.2020.105175).The present study seeks to elucidate the role of a PD-relevant single nucleotide polymorphism (SNP), specifically rs6265 (aka:Val66Met) on the response of parkinsonian subjects to DA cell replacement therapy. The rs6265 gene variant occurs in the gene for the brain-derived neurotrophic factor (BDNF) and has a prevalence of ~20% in the general worldwide human population. Expression of this gene variant results in an activity-dependent decrease in available BDNF, a highly important neurotrophic factor. Our previous studies demonstrated that rs6265 transgenic rats grafted with wild-type (WT) DA neurons showed a dramatic enhancement of graft-derived neurite outgrowth and therapeutic efficacy compared to WT rats grafted with WT DA neurons (doi:10.1016/j.nbd.2020.105175). However, this rs6265 genotype was also uniquely associated with GID induction. The enigmatic finding of enhanced functional recovery in rs6265 Met allele carriers is corroborated by evidence in a rs6265 mouse stroke model and in people with traumatic brain injury, including in Vietnam veterans where the Met allele carriers showed enhanced functional recovery.

   The immediate goal of this study is to gain understanding into the mechanisms of how a single amino acid substitution (Met for Val) in the pro-domain of BDNF in rs6265 contributes to the enhanced graft-derived therapeutic efficacy and neurite outgrowth, and at the same time be uniquely associated with GID (doi:10.1016/j.nbd.2020.105175), the side-effect that has potential to impede the success of cell-based therapies for PD.

2. **KEYWORDS:** *Parkinson's disease, Neural grafting, Val66Met, Val68Met, rs6265, BDNF, BDNF pro-peptide, aging*

3. **ACCOMPLISHMENTS:**

   - **What were the major goals of the project?**

Specific Aim 1. Characterize the impact of the Met pro-peptide on neurite outgrowth in primary DA neuron cultures.	**Timeline Responsible Personnel**	**Progress and Accomplishments**
Major Task 1: Derivation, maintenance, and experimental treatment of VM DA neuron cultures from timed pregnant wild-type (WT) donors.		

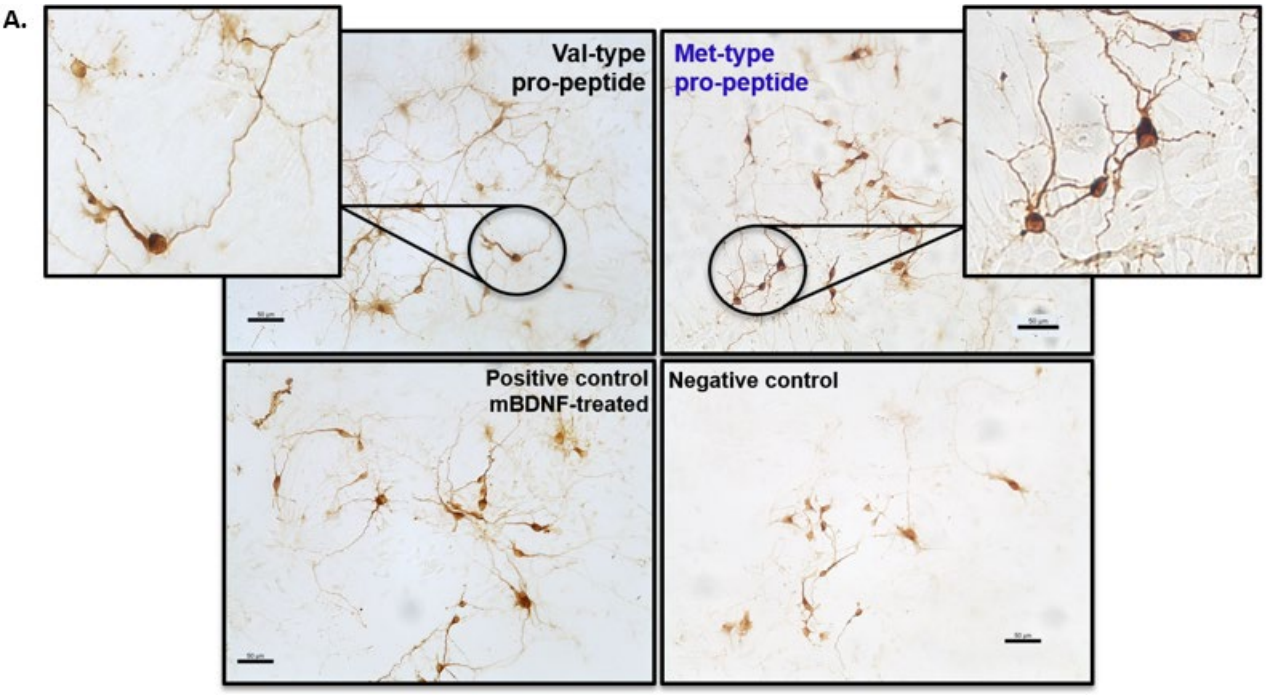
Subtask 1: Dissection and isolation of VM DA neurons (6 WT Sprague Dawley timed pregnant donors required) Subtask 2: Establishment and maintenance of VM DA neurons *in vitro* and BDNF pro-peptide treatments	Year 01 Month 1-5 Dr. Caulfield	Optimized protocols for cellular dissection, isolation, maintenance, treatment, and fixation of primary fetal VM DA cell cultures have been established. Pilot data from treatment of WT cells with BDNF and pro-peptides are provided below (Figure 1). Preparations for final experimentation are ongoing and are expected to be completed in year 02, months 1-6.
Major Task 2: Quantitate and compare neurite outgrowth in response to Met, Val, or Met+Val BDNF-pro-peptide treatment initiated at time of plating or following 7 days of *in vitro* maturation		
Subtask 1: Tyrosine hydroxylase (TH) fluorescent immunohistochemistry (IHC) and RNAscope in situ hybridization (ISH; *vglut2; bdnf, trkB, p75)*	Year 01 Month 1-5 Dr. Caulfield/ C. Szarowicz	TH+ IHC together with trkB and p75 RNAscope has been successfully piloted (Figure 2) and will be applied to upcoming pro-peptide treatment experiments. RNAscope® in situ hybridization for vglut2 and *bdnf* has not yet been attempted.
Subtask 2. Confocal imaging and Imaris software analysis utilizing neurite filament tracer, surface, and spots modules to quantitate receptor transcript numbers, neurite length, and neurite branching from individual TH+ cell bodies in 3-D	Year 01 Month 2-6 Dr. Caulfield/ C. Szarowicz	Pilot cultures were assessed via confocal imaging and Imaris® neurite filament tracer for preliminary workflow optimization and data (Figure 1). This technique will also be applied to upcoming in vitro experiments.
Major Task 3. Elucidate transcriptome profiles of VM cultures in response to Met BDNF-pro-peptide treatment.		
Subtask 1: Plate and process cells for Nano string® Whole Transcriptome Atlas analysis	Year 01 Month 6-12 Dr. Caulfield/ C. Szarowicz	Optimization of plating and morphology marker staining required for subsequent Nano string® WTA is underway. A few technical issues with the slides and stains have been encountered and are described below.
Milestone #1. *Manuscript/report preparation: 1) Confirm or refute that the Met pro-peptide enhances neurite outgrowth of VM DA neurons, and 2) Present of major transcriptome profiles altered by Met pre-peptide in VM DA neurons.*	Year 01-02 Month 12- 2 Dr. Caulfield/ Dr. Steece-Collier	Updated timeline, to be completed year 02 months 6-12.
Specific Aim 2. Characterize the impact of exogenous mBDNF on behavioral outcome, neurochemical phenotype, and graft-host circuitry in DA grafted Met/Met host rats.	**Timeline**	**Progress and Accomplishments**
Major Task 4: Intranigral lesioning of 24 adult male Met/Met Sprague Dawley rats and levodopa induced dyskinesia induction		
Subtask 1: Stereotaxic 6-OHDA lesion surgeries (50 male 6 mo Sprague Dawley rats required, 15 male Met/Met and 15 female Met/Met breeders will be paired to produce these experimental animals, approximately 50 female offspring from these litters will not be utilized in our experiments)	Year 02 Month 1 Dr. Caulfield/ Dr. Steece-Collier	Completed 1/10/2023 and 1/23/23
Subtask 2: Amphetamine-induced rotational behavior to set baseline	Year 02 Month 1 Dr. Caulfield	Completed 1/25/23 and 2/6/2023
Subtask 3: Daily levodopa priming injections for LID priming necessary for GID initiation	Year 02 Month 2 Dr. Caulfield	Completed 2/7/23- 3/7/23 and 2/23/23- 3/21/23

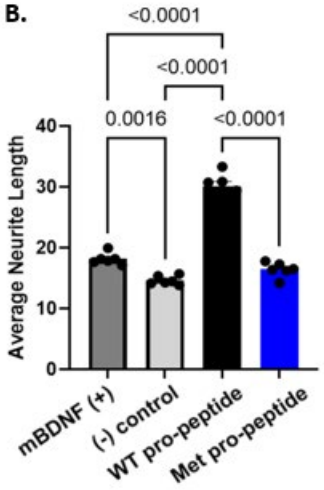
Major Task 5: Intrastriatal VM DA transplant and cannula surgeries of 24 adult male Met/Met Sprague Dawley rats, and instillation of Alzet minipumps		
Subtask 1: Stereotaxic graft and cannula implantation surgeries; subcutaneous implantation of Alzet minipumps (10 WT Sprague Dawley pregnant donors required; all pups to be utilized for obtaining embryonic ventral mesencephalic cell for transplantation)	Year 02 Month 2-3 Dr. Caulfield/ Dr. Steece-Collier/ C. Szarowicz	Completed 3/9/23 and 3/22/23
Major Task 6: LID induction, amphetamine- and levodopa-induced GID ratings		
Subtask 1: Post-graft daily levodopa injections	Year 02 Month 3-4 Dr. Caulfield	Completed 3/21/23 – 6/5/23
Subtask 2: Post-graft motor behavioral assessments (Amphetamine- and levodopa-induced GID)	Year 02 Month 3-4 Dr. Caulfield// Dr. Steece-Collier	Completed 3/21/23 – 6/5/23 (Figures 3 and 4)
Major Task 7: Necropsy and postmortem assessments of 24 adult male Sprague Dawley rats		
Subtask 1: Euthanasia/perfusions, sectioning, and triple label IHC (TH) and ISH (Vglut2, PSD95)	Year 02 Month 3-6 Dr. Caulfield, C. Szarowicz	Perfusions completed 5/19/23 and 6/5/23 Brain sectioning is currently underway, commenced 7/17/23 to be completed by 9/1/23. Staining will be completed year 02 months 3-6.
Subtask 2: Confocal Z-stack imaging and Imaris software analysis surface, and spots modules to quantitate receptor transcript numbers (*Vglut2, PSD95*) in TH+ cells	Year 02 Month 6-11 Dr. Caulfield	Imaging and analysis will be completed year 02 months 6-11.
Milestone #2: Manuscript/report preparation: Confirm or refute that mBDNF supplementation reverses immature graft phenotype and GID seen in Met hosts	Year 02 Month 12 Dr. Caulfield/ Dr. Steece-Collier	To be completed by the end of year 02.

What was accomplished under these goals?

Specific Aim 1. Characterize the impact of the Met pro-peptide on neurite outgrowth in primary DA neuron cultures. Primary embryonic WT DA neurons, the cell source used in our previous grafting studies, will be utilized to directly test the hypothesis that the Met pro-peptide acts as a novel bioactive molecule to promote neurite outgrowth.

Pilot results from Val and Met-type BDNF pro-peptide treatment of primary dopamine ~~precursor~~ cultures. Embryonic ventral mesencephalic DA neurons were plated and treated as described in Exp 1.1A (Figure 1A). We had originally hypothesized that the Met-type pro-peptide is sufficient to support increased neurite length and branching as compared to the Val-type based on previous in vivo results [1]. Our pilot experiment showed that the neurons exposed to the WT (Val) pro-peptide had greater neurite outgrowth but that the neurons exposed to the Met-type pro-peptide have a greater number of branches/complexity (Figure 1B, C). For these pilot experiments, the WT or Met pro-peptide was added to the culture wells via pipette twice daily for seven days. Considering the dynamic activity of cells in vitro together with the short BDNF half-life (<10mins), we have developed an approach to allow future experiments will utilize an osmotic pump to provide continuous administration of the peptides in culture to amplify the potential for significant and biologically relevant outcomes.





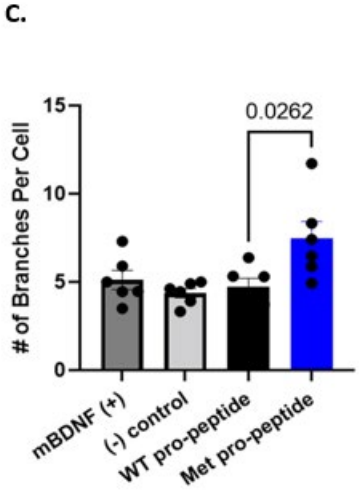


Figure 1. Successful derivation, treatment, and analysis of neurite growth for primary embryonic dopamine neurons. A. Tyrosine hydroxylase (TH) positive cells (brown, chromogenic DAB immunohistochemistry) isolated from E14 embryonic ventral mesencephalic brain. Circles and insets highlighting healthy cell bodies and neurites. B. Quantitation of average neurite length from 6 replicate wells of cells grown for 7 days showing greatest length in the wells treated with the WT (Val) pro-peptide. C. Quantitation of average number of neurite branches per cell from 6 replicate wells of cells grown for 7 days showing greatest number in the wells treated with the Met pro-peptide.

Optimization of TH+ immunohistochemistry (IHC) together with RNAscope® fluorescent in situ hybridization (FISH) for BDNF receptors trkB, p75, and binding partner sortilin. Significant progress has been made to optimize the culture and staining conditions to maintain untreated primary dopamine cell integrity and staining for TH protein together with transcript identification using RNAscope® for BDNF receptors and binding partners. This protocol will be applied to future invitro experimentation. Understanding when these receptors are developmentally present in our DA cultures is critical in understanding the timing of the application of the Met and WT pro-peptides, which act through these receptors.

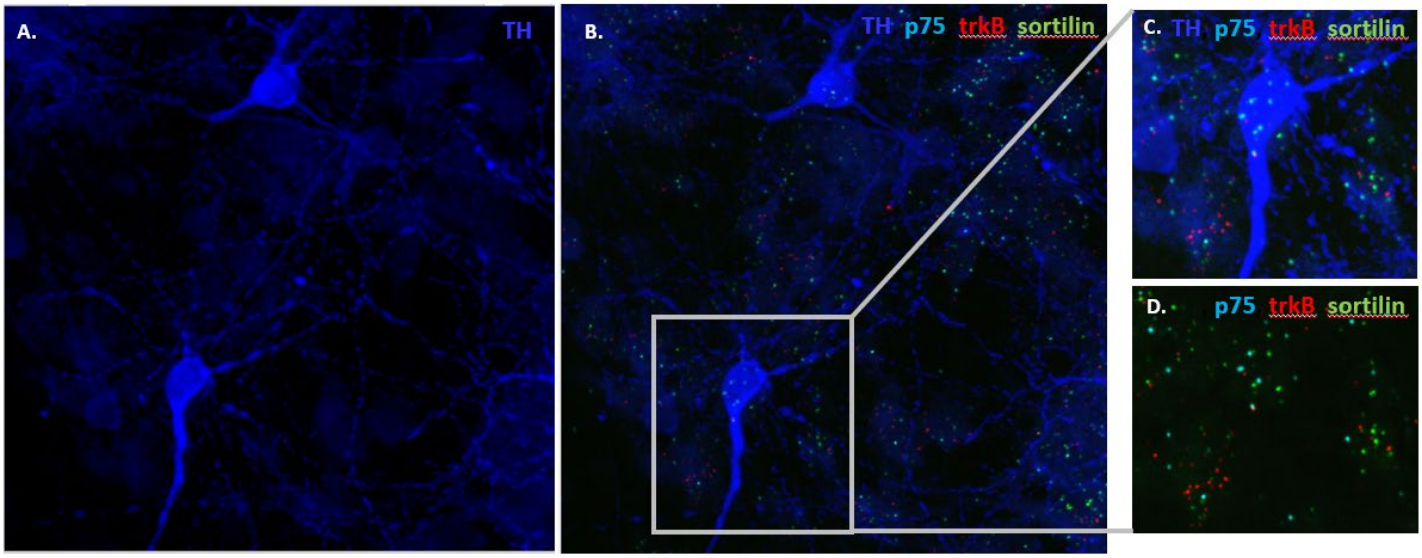


Figure 2. mRNA transcripts of TrkB, p75, and Sortilin are expressed by primary TH+ dopamine neurons. A. TH+ primary neurons (blue) with visible cell bodies and dendrites after 7 days in culture. B. TH+ dopamine neurons co-stained for p75 (cyan), trkB (red) and sortilin (green) mRNA (puncta). C. Enlarged image of single neuron. D. Enhanced image of same area as in C without the TH. Brightness and contrast increased 20% to increase visibility of neurites and puncta.

Specific Aim 2. Characterize the impact of exogenous mature (m)BDNF on behavioral outcome, neurochemical phenotype, and graft-host circuitry in DA grafted Met/Met host rats. Since mBDNF is crucially involved in neuronal and synaptic maturation, we will test the hypothesis that the diminished release of mBDNF in DA grafted Met/Met hosts results in the maintenance of immature DA neuron phenotype leading to GID expression, which can be prevented with mBDNF supplementation.

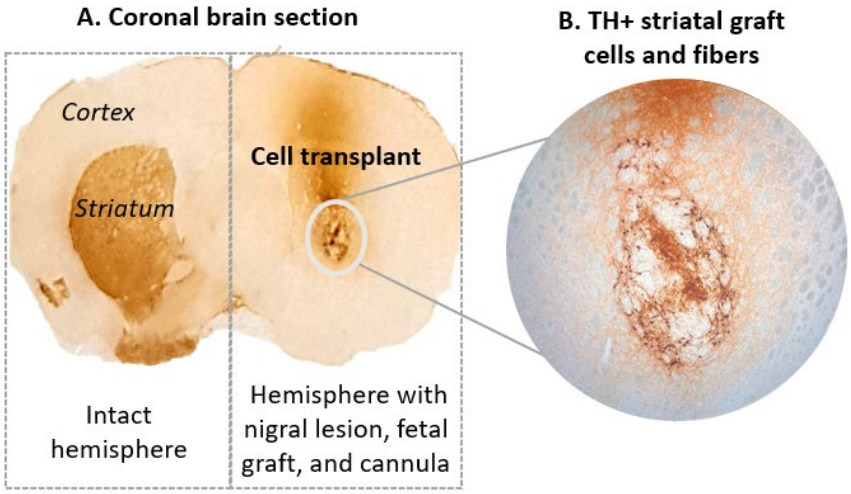


Figure 3. Successful nigral lesioning and fetal dopamine precursor cell transplant: individual example. A. Coronal brain section showing an non-lesioned left striatum (TH+, brown- resulting from intact nigral neuron processes extending into the striatum) and the contralateral lesioned striatum (parkinsonian) with embryonic dopamine cell transplant (TH+, brown: derived exclusively from grafted dopamine cells). B. Enlarged image of graft showing TH+ cell bodies (dark brown spots) and neurites (brown 'web').

Fetal dopamine neuron precursor grafts placed in the lesioned hemisphere striatum result in improvement in levodopa-induced dyskinesia (LID). As expected, the groups of animals with DA grafts had striking recovery from levodopa-induced dyskinesia (LID). LIDs are abnormal involuntary movement side-effects of levodopa therapy, and in the current studies, we used the validated rat model of LID because this complex behavioral malady can be ameliorated by DA neuron grafts in parkinsonian rats [2-5] and PD patients [6]. The two control groups that received either BDNF or the vehicle phosphate buffered saline (PBS) infusion and no grafted DA neurons maintained high

LID severity. Preliminarily, the grafted group with the BDNF infusion had an accelerated recovery at week 6 post graft (**p.g.**) timepoint as compared to the DA graft + PBS group that recedes by week 8 p.g. (Figure 4). It is possible that if the BDNF infusion was continued, instead of being disconnected at week 4, that this difference would have been maintained. Comprehensive statistical analysis of group data is ongoing.

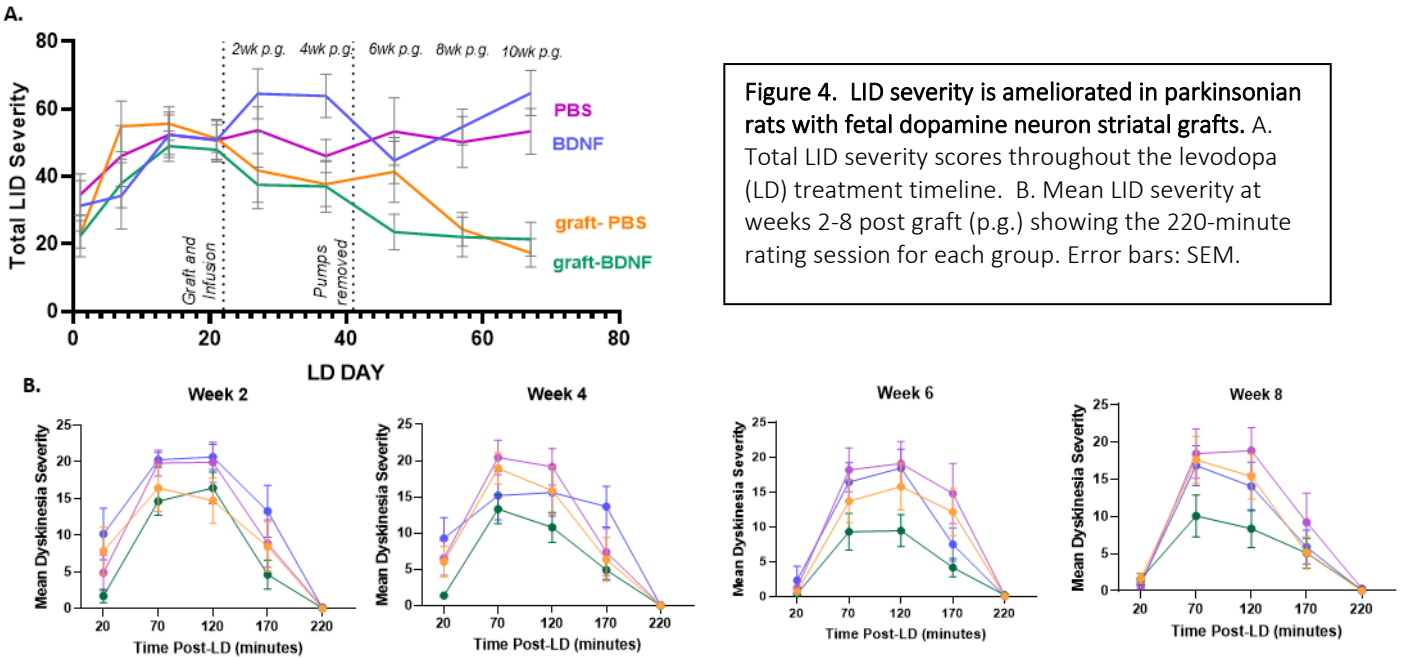


Figure 4. LID severity is ameliorated in parkinsonian rats with fetal dopamine neuron striatal grafts. A. Total LID severity scores throughout the levodopa (LD) treatment timeline. B. Mean LID severity at weeks 2-8 post graft (p.g.) showing the 220-minute rating session for each group. Error bars: SEM.

Counter to original hypothesis, BDNF infusion increased graft induced dyskinesias (GID) mediated by amphetamine as well as levodopa in Met host. In the SN, BDNF is a critical factor for the viability and function of DA neurons (e.g., [2-5], including the promotion of synapse development and maturation of nigral afferents within the striatum (e.g., [1, 7, 8]). We have previously demonstrated that the rs6265 genotype in the host rat is uniquely associated with development of GID [1]. Considering the decreased release of BDNF in Met allele expressing rats [1], we tested the hypothesis that infusion of mBDNF would allow for physiological graft maturation and normalization of grafted-derived synaptic reinnervation, with the consequence of preventing GID development. Preliminary behavioral analysis shows that grafted parkinsonian Met genotype rats with 4 weeks of BDNF striatal infusion have increased contralateral rotations (suggestive of too much DA release), amphetamine induced GID, and levodopa induced GID (Figure 5). GID in PD patients is suggested to be related to too much DA production (e.g., Freed et al., 2001, 10.1056/NEJM200103083441002; Shin et al., 10.1016/j.nbd.2012.03.038). Upcoming histological and molecular analysis of the graft maturation and neurochemical phenotype will be used to elucidate the mechanisms by which BDNF supplementation contributes to these maladies. It is reasonable to suggest that BDNF supplementation drives an increase in release of striatal dopamine in a non-physiological capacity. Even at this preliminary stage, our data highlight the need for careful tailoring of therapeutics to achieve the best possible clinical outcomes together with diminished negative side-effects. We posit that future studies focusing on titrating an optimized, lower dose, long-term BDNF infusion in Met hosts may allow for augmentation of graft function(i.e., LID amelioration) in grafted parkinsonian rats without side-effect development (i.e., GID induction).

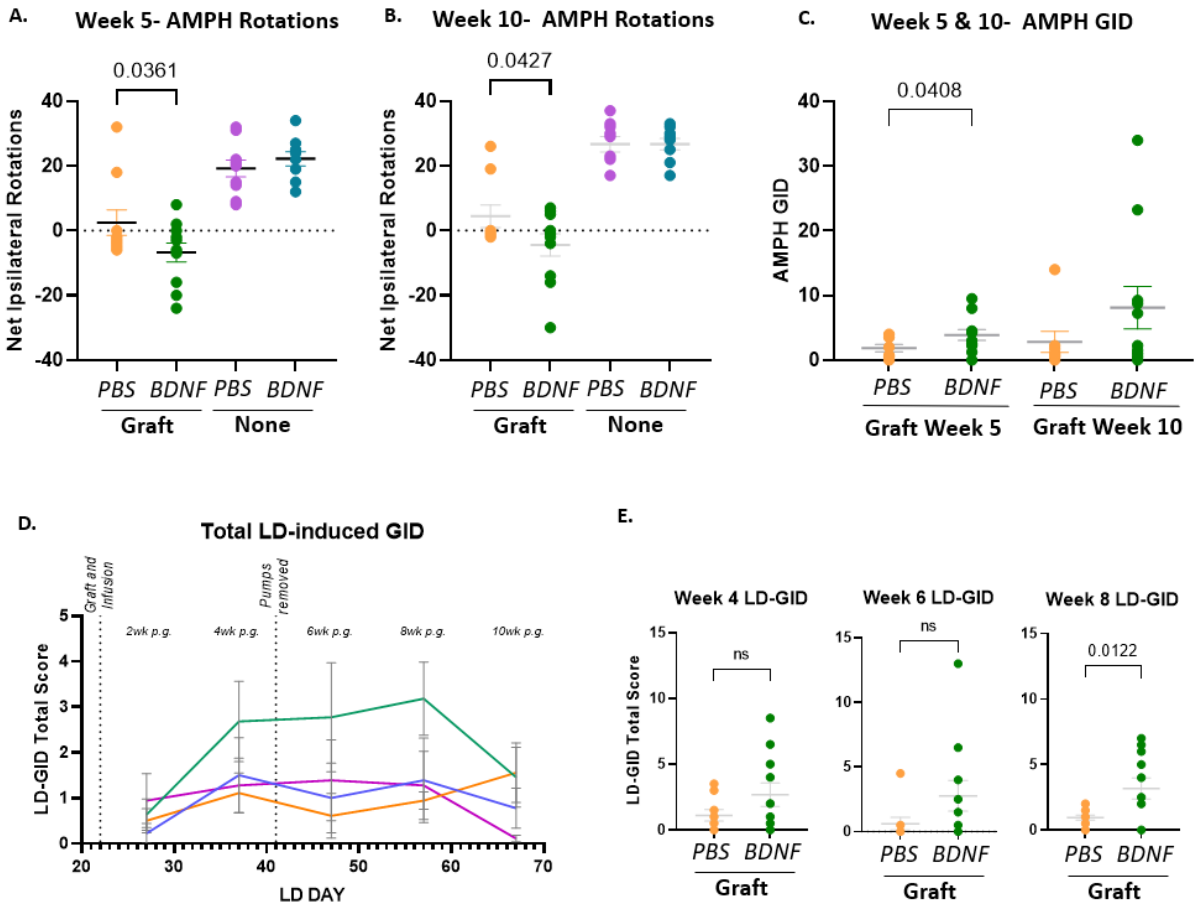


Figure 5. BDNF infusion into the grafted Met striatum results in increased contralateral rotations and graft-induced dyskinesia. A. & B. Amphetamine (AMPH) induced rotations were quantified one week after infusion cessation (week 5 post graft) and 6 weeks after (week 10 post graft). Dashed line indicated 'normalized' behavior. Data show that the sham grafted parkinsonian rats receiving PBS and BDNF rotate *ipsilateral* to the DA-depleted striatum as expected. DA grafted animals rotate *contralateral* to the lesioned side with the DA graft + BDNF group displaying significantly greater rotational rate compared to DA graft + PBS, indicating excess DA release in the grafted striatum. C. Total amphetamine-induced dyskinesias (i.e., amphet-GIDs) showed the greatest severity in DA grafted animals with BDNF infusion. D. Total levodopa induced GIDs (LD-GIDs) over the experimental time course. E. Total LD-GID scores for the DA grafted + PBS or BDNF infusion groups with the greatest GID severity in the BDNF+DA grafted animals. Error bars: SEM. Statistics: unpaired two-tailed Student's T-test.

- **What opportunities for training and professional development has the project provided?**
  - *This project provided critical support for the PI, Margaret Caulfield Ph.D., to be promoted from a post-doctoral fellow to a fixed-term Assistant Professor in the department of Translational Neuroscience at Michigan State University, effective 6/1/23.*
- **How were the results disseminated to communities of interest?**

Because these studies are either still ongoing or in development (in vitro) and because the in vivo grafting studies were just recently completed, there has been no formal dissemination of data to date. However, the rationale for the project and anticipated outcomes were presented as part of a university sponsored lecture series.

Margaret E. Caulfield, Kathy Steece-Collier. Parkinson's Disease in an Era of Personalized Medicine. Your Health Lecture Series. Sponsors: Calvin University, Spectrum Health and MSU College of Human Medicine. November 7, 2022.

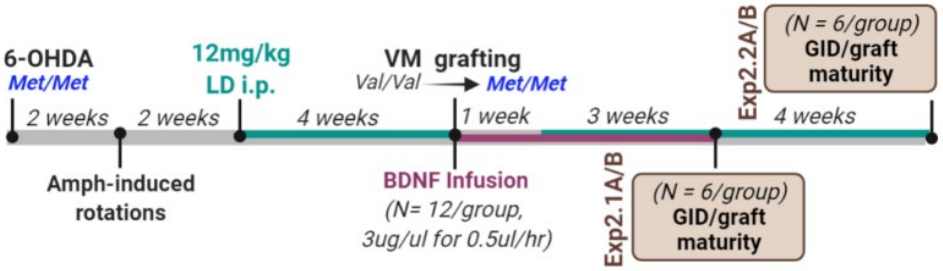
We will be presenting some of our new data at the Grand Challenges in Parkinson Disease meetings at the Van Andel Institute in Grand Rapids MI in September 2023.

- **What do you plan to do during the next reporting period to accomplish the goals?**
  - *During year 02, we anticipate completing the remaining objectives including tasks 1-3 working with the in vitro pro-peptide treatments of the primary dopamine neuron cell cultures. We have already made headway towards these tasks and do not anticipate issues with their completion. We similarly anticipate no issues with task 7 focused on analyzing the neurochemical phenotype of the grafted striatum. We anticipate compiling a manuscript from our results. Although our preliminary results are counter to our original hypothesis, this data provides valuable information about the role of BDNF in parkinsonian met allele carrier in graft dysfunction (i.e., GID) and function (i.e., LID).*

4. **IMPACT:**
   - **What was the impact on the development of the principal discipline(s) of the project?** *Nothing to Report*
   - **What was the impact on other disciplines?** *Nothing to Report*
   - **What was the impact on technology transfer?** *Nothing to Report*
   - **What was the impact on society beyond science and technology?** *Nothing to Report*
5. **CHANGES/PROBLEMS:**
   - **Changes in approach and reasons for change**
   - **Actual or anticipated problems or delays and actions or plans to resolve them**

     A change was made to our experiment groups on the day of grafting and mini-pump implantation. Originally, we proposed to have two experimental groups, one with PBS and one with BDNF infusion together with intrastriatal DA grafts as shown in the original timeline below. We were then proposing to euthanize one half of the animals at the cessation of infusion and the second cohort 4 weeks later. Due to technical problems associated with obtaining insufficient time-pregnant rats on the day of graft surgery, we adjusted our experimental plan based on the complex logistics of our studies.

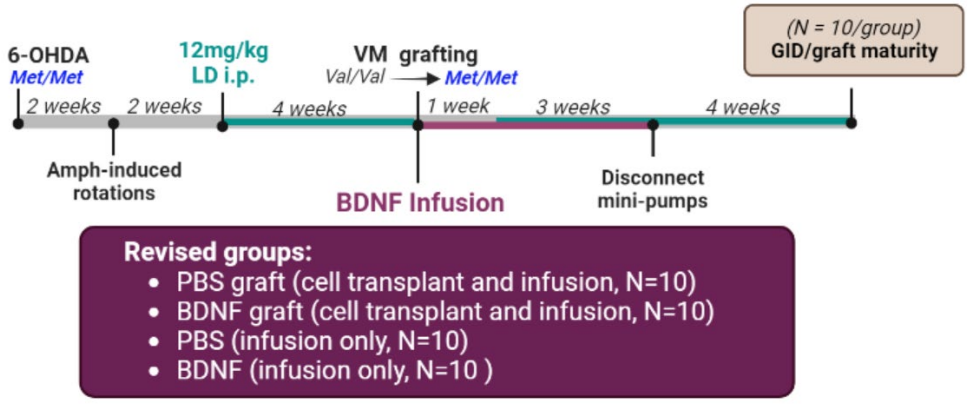
Original timeline SA2:



Specifically, to accomplish this surgical protocol, we required timed pregnant females purchased from Charles River to provide E14 pups for cell isolation. At the same time, the osmotic mini pumps required priming with BDNF or PBS for 48hrs prior to implantation. On the morning of the second fetal dissection day,

none of the 3 'pregnant' donors were pregnant, and therefore we were without cells for transplantation. We did, however, have many thousands of dollars of BDNF already primed in the osmotic pumps. To utilize this expensive reagent as well as rat hosts that had already been in study for 8 weeks, we decided the best course of action was to implant the remaining pumps into animals without grafts. This also meant that we would only sacrifice the experimental groups at one time point (see revised timeline below).

Revised groups and timeline for SA2:



While this change was a pivot from the proposed plan, IT DID NOT CHANGE OUR CORE QUESTION asking how BDNF infusion affects LID and GID outcomes in DA grafted parkinsonian Met allele hosts. In some respect, it was an improved design, allowing for control graft-free animals as well as an increase in final group numbers from 6 to 10 per group. This design allows us to examine additional inquiries about how BDNF itself affects the dyskinetic striatum in the absence of the graft. Finally, we were able to extend the experimental timeline to 10 weeks post-grafting to assess LIDs and GIDs at this later timepoint which has been shown previously to be when these behaviors peak [1].

- We have experienced some delays in the accomplishment of major task 3. For the proposed Nano string analysis of the transcriptome of the BDNF pro-peptide treated cultures in Experiment 1.2, we have experienced some supply issues. For example, we use poly-D-lysine-coated 8-well chamber slides and there have been major delays in supplier delivery of these slides. We also have encountered some technical issues with certain slide types being incompatible with apparatus used for imaging, as well as some markings on the slide interfering with the imaging. We have been working through these issues with the core staff who run the Nano string® platform as well as the Nano string® representative and have found a set up that will suffice moving forward and anticipate completing this task before the end of year 02.

- **Changes that had a significant impact on expenditures-** *Nothing to Report*
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents-** *Nothing to Report*
- **Significant changes in use or care of human subjects-** *Not Applicable*
- **Significant changes in use or care of vertebrate animals-** *Nothing to Report*
- **Significant changes in use of biohazards and/or select agents-** *Nothing to Report*

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  - **Journal publications.** *Nothing to Report*
  - **Books or other non-periodical, one-time publications.** *Nothing to Report*
  - **Other publications, conference papers, and presentations.**

    Margaret E. Caulfield, Kathy Steece-Collier. Parkinson's Disease in an Era of Personalized Medicine. Your Health Lecture Series. Sponsors: Calvin University, Spectrum Health and MSU College of Human Medicine. November 7, 2022.

- **Website(s) or other Internet site(s)**

  This work was chosen by the Neurotoxin Exposure Treatment Parkinson's (NETP)/Parkinson's Research Program (PRP) staff selected your research to be featured on the CDMRP PRP website.

  https://cdmrp.health.mil/prp/research_highlights/23Early_Investigators_highlight

- **Technologies or techniques**- *Nothing to Report*
- **Inventions, patent applications, and/or licenses-** *Nothing to Report*
- **Other Products**- *Nothing to Report*

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	*Margaret Caulfield*
Project Role:	*PI, Assistant Professor*
Researcher Identifier (e.g. ORCID ID):	0000-0002-9433-0881
Nearest person month worked:	*9*
Contribution to Project:	*Margaret has been a main contributor to the project's experimental design, animal surgeries, daily treatments, and testing, as well as data analysis and interpretation.*
Funding Support:	

Name:	*Carlye Szarowicz*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	0000-0002-3972-2161

Nearest person month worked:	*4*
Contribution to Project:	*Carlye has been a significant contributor to the piloting of in vitro studies, stereotactic surgeries, and animal behavioral ratings. Cell dissociation/dissections, Daily injections/animal husbandry, postmortem processing/analysis, etc.*
Funding Support:	*NINDS, Impact of Dysfunctional BDNF on Dopamine Terminal Remodeling in the Parkinsonian Striatum, R01NS105826*

Name:	*Kathy Steece-Collier*
Project Role:	*Co-Investigator, mentor*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Kathy participated in animal surgeries, dissections, and behavioral ratings.*
Funding Support:	*NINDS, Impact of Dysfunctional BDNF on Dopamine Terminal Remodeling in the Parkinsonian Striatum, R01NS105826, Genetic Silencing of Striatal CaV1.3 Calcium Channels as a Potent Antidyskinetic Therapy for PD. R01NS110398*

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** *Nothing to Report*
- **What other organizations were involved as partners?** *Nothing to Report*

8. **SPECIAL REPORTING REQUIREMENTS-** *Nothing to Report*
9. **APPENDICES:** *References*

1. Mercado, N.M., et al., *The BDNF Val66Met polymorphism (rs6265) enhances dopamine neuron graft efficacy and side-effect liability in rs6265 knock-in rats.* Neurobiol Dis, 2021. **148**: p. 105175.
2. Soderstrom, K.E., et al., *Impact of dendritic spine preservation in medium spiny neurons on dopamine graft efficacy and the expression of dyskinesias in parkinsonian rats.* Eur J Neurosci, 2010. **31**(3): p. 478-90.
3. Maries, E., et al., *Focal not widespread grafts induce novel dyskinetic behavior in parkinsonian rats.* Neurobiol Dis, 2006. **21**(1): p. 165-80.
4. Lee, C.S., et al., *Embryonic ventral mesencephalic grafts improve levodopa-induced dyskinesia in a rat model of Parkinson's disease.* Brain, 2000. **123 (Pt 7)**: p. 1365-79.
5. Lane, E.L., et al., *The impact of graft size on the development of dyskinesia following intrastriatal grafting of embryonic dopamine neurons in the rat.* Neurobiol Dis, 2006. **22**(2): p. 334-45.
6. Hagell, P., et al., *Dyskinesias following neural transplantation in Parkinson's disease.* Nat Neurosci, 2002. **5**(7): p. 627-8.
7. Lai, K.O. and N.Y. Ip, *Structural plasticity of dendritic spines: the underlying mechanisms and its dysregulation in brain disorders.* Biochim Biophys Acta, 2013. **1832**(12): p. 2257-63.
8. Zagrebelsky, M. and M. Korte, *Form follows function: BDNF and its involvement in sculpting the function and structure of synapses.* Neuropharmacology, 2014. **76 Pt C**: p. 628-38.

Mechanisms of Enhanced Neuroregeneration Associated with the Common Human Single Nucleotide Polymorphism Val66Met

Annual Report Y1

W81XWH-22-1-0804, PD210014

PI: Margaret Caulfield, PhD **Org:** Michigan State University **Award Amount:** $399,632.00

Study/Product Aim(s)

Specific Aim 1. Characterize the impact of the BDNF Met SNP pro-peptide on neurite outgrowth in primary dopamine neuron cultures.

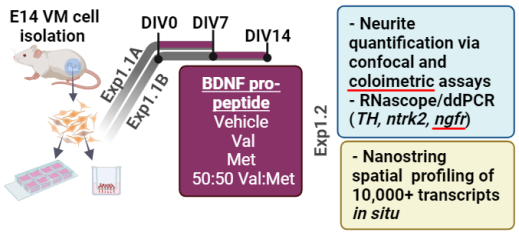
Specific Aim 2. Characterize the impact of exogenous mBDNF on behavioral outcome, neurochemical phenotype, and graft-host circuitry in dopamine grafted Met/Met host rats.

Approach

SA1. Derive, maintain, and treat primary dopamine neuron cultures from timed pregnant wild-type (WT) donors with Met, Val, or Met+Val BDNF-pro-peptide. Then quantitate neurite outgrowth and transcriptome profiles.

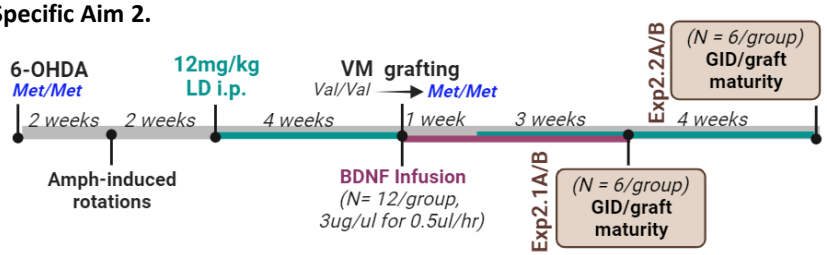
SA2. Stereotaxic nigral lesioning, levodopa induced dyskinesia induction, striatal dopamine cell transplant, BDNF/PBS cannula and mini pump insertion, dyskinesia ratings, necropsy and postmortem tissue processing to determine graft survival and maturity.

Specific Aim 1.



Accomplishment: The majority of SA2 was completed in YR1 of this award. Results are to be presented in poster format at the 2023 Van Andel Institute Grand Challenges, and 2024 AD/PD annual meeting.

Specific Aim 2.



Timeline and Cost

Activities / Year	1 (SA2)	2 (SA1)
Primary cell culture and treatment		
Neurite quantitation and transcriptome analysis		
Animal model induction and dyskinesia ratings		
Postmortem analysis of grafts and animal behavior		
Estimated Supplies Budget ($K)	**$39.956.00**	**$40,574.70**

Updated: (7/31/2023)

Goals/Milestones

SA1 Goals

- ✓ Primary dopamine cell culture optimization
- ❑ Quantitate neurite outgrowth between treatment groups
- ❑ Transcriptome analysis

SA2 Goals

- ✓ Animal model induction- lesioning, grafting, cannula placement
- ✓ Levodopa induced dyskinesia and graft induced dyskinesia ratings
- ✓ Necropsy and postmortem tissue sectioning
- ❑ Determination of graft survival and maturity

Comments/Challenges/Issues/Concerns

A change was made to our experiment groups on the day of grafting and mini-pump implantation due to lack of properly pregnant females received from Charles River.

Budget Expenditure to Date

Projected Expenditure: $199,816

Actual Expenditure: $172, 200.31