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14. ABSTRACT

The health benefits of regular exercise are documented in hundreds of studies. However, only recently have the potential benefits of exercise to alleviate symptoms of neurologically-based diseases come to light. In Parkinson's disease (PD), exercise can alleviate some of the locomotor impairments. However, not all PD patients are able to exercise due to other contraindications in health or lifestyle constraints which prevent them from exercising. For these reasons, it is vital to determine the neurobiological basis of how exercise improves locomotor function. Identification of a neurobiological mechanism that drives motor improvement following exercise can yield a target for a pharmacological or genetic approaches to improve motor function in PD patients unable to exercise. Using an established rodent model of PD, this proposal tests our hypothesis that exercise-related improvement in motor function is associated with increased expression of the dopamine-regulating protein tyrosine hydroxylase in the substantia nigra. Evidence to support this relationship will guide future research for genetic and pharmacological strategies to increase expression of tyrosine hydroxylase (TH) in the substantia nigra, which is severely decreased in PD, but still potentially salvageable in comparison to much greater TH loss in striatum.

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1. Introduction:

Parkinson's disease (PD) is a devastating neurodegenerative disease. With aging as the greatest risk factor for PD, coupled with a current acceleration in the number of individuals at or above retirement age, there is a major societal need to stall the development of motor symptoms and arrest further progression of motor impairment in PD. There has been a growing awareness that exercise for the PD patient can be a lifestyle practice to possibly decelerate the progressive nature of the disease. However, the mechanisms by which exercise imparts these beneficial effects are still yet to be fully elucidated. As motor impairment in PD is initiated by the loss of nigrostriatal neurons and resulting reduction in dopamine (DA) neurotransmission, much of exercise research has focused upon how DA may be restored in nigrostriatal terminals in the striatum. However, many studies conducted in PD rodent models indicate little or no change in DA neurotransmission markers in the striatum, despite an exercise-related reduction in motor impairment. Our research goal in this project is to rigorously evaluate the hypothesis that exercise impact on motor function is related to increased expression or function of tyrosine hydroxylase (TH), the rate-limiting enzyme of DA biosynthesis in the substantia nigra (SN). Using two models of PD, an established toxin model (6-OHDA) and a relatively new genetic rat model that models a familial form of PD that implicates defective mitophagy (Pink1 knockout), we will evaluate how an exercise regimen that can be practiced in humans in terms of frequency (three times per week), duration (40 min per exercise session), and intensity (10 m/min treadmill speed) can protect against further motor impairment with commensurate increases in TH expression, TH phosphorylation, and DA neurotransmission in the SN.

2. Keywords:

Parkinson's disease, exercise, glutamate transporter, tyrosine hydroxylase, dopamine, GDNF, GFR-alpha 1, Pink1, mitochondria

3. Accomplishments:

Major Goals (as described in updated and approved SOW, June 4, 2019) and current status

1) **Protocol submission and approval at institution and ACURO:** Completed by Dr. Michael F. Salvatore (PI) and Dr. Christopher Bishop (Co-I) in year 1.

2) **Experiments:** The experiments in year one had three primary objectives: **1)** Evaluating exercise impact on motor function and dopamine and allied neurochemistry in the 6-OHDA model, **2)** optimization of immunohistochemical approaches (with Richardson Lab), and, **3)** evaluate the differences in striatal and nigral extracellular DA and lesion impact vs sham-operated control group (with Bishop Lab).

Prior to launching the evaluation of exercise impact, we considered re-evaluating the rationale for the originally proposed timing of exercise intervention at 28 days post-lesion in the 6-OHDA model. Knowing that striatal and nigral loss of DA or TH was ~80-90% and 60-70%, respectively, in our previously published work and of others by day 7 post-lesion, we decided to conduct an investigation of the timeline of DA and TH loss (and changes in proposed related allied neurochemistry) at both day 7 and day 28 post-lesion. Furthermore, we included a sham-operated control for which to evaluate the impact of the unilateral (a.k.a. hemi-parkinson's model) 6-OHDA on nigral and striatal DA and TH expression and phosphorylation in the contralateral hemisphere. Accordingly, with this study we would have the results to draw much stronger conclusions on answering the following points to evaluate exercise impact;

a) *Does exercise restore DA or TH loss already occurring between day 7 and day 28 post-lesion?*

b) *Does exercise stall progressive loss of DA or TH occurring between 7 and day 28 post-lesion?*

c) *Does exercise affect DA or TH expression contralateral to the lesion during these two time periods post-lesion?*

In July 2020, the PI consulted with Co-investigators Dr. Bishop and Dr. Richardson with this plan and they both were in agreement that these results would provide maximal information for the rationale in timing of exercise intervention and increase our ability to draw conclusions on exercise impact. To this end, and thereby maximizing interpretation of exercise impact on lesion progression, both

laboratories agreed to conduct the analysis of extracellular DA in the SN and striatum (Bishop Lab) and cell loss vs. TH cell loss in the SN (Richardson Lab) in both lesioned and contralateral to lesion in the same day 7 and day 28 6-OHDA paradigm.

Project 1. Rationale for timing of exercise intervention during nigrostriatal lesion progression. Exercise studies in rodent Parkinson's models can vary in experimental approach by virtue of the model being employed, the type of exercise being employed (voluntary or forced), and the timing of exercise initiation in relation to the induction of nigrostriatal lesion. Our goal is two-fold in this project. First, as stated in the proposal, we will apply exercise to rodent PD models such that the exercise regimen is one that reflects the intensity, duration, and frequency that is achievable in the PD patient. Second, as discussed above, the timing of exercise initiation will be such that the nigrostriatal lesion will be at a level in which the motor impairment is present, but progression of loss continues in the nigrostriatal pathway. This will permit evaluation of exercise impact on possible restoration of loss or prevention of further loss.

Results to date: We reported preliminary results from the 1st cohort of this project in the first semi-annual technical report. Thus far, the most significant finding was that TH protein loss showed a striking dissimilarity between the striatum and substantia nigra (SN) during the time course of 6-OHDA-induced nigrostriatal lesion. Loss of TH was >90% in the striatum by day 7 post-lesion (Fig. 1A), but ~60% in the SN (Fig. 1B). By day 28 post-lesion, loss of TH in striatum was not significantly different than that at day 7 (Fig. 1A) but was significantly greater, ~80%, in the SN (Fig. 1B).

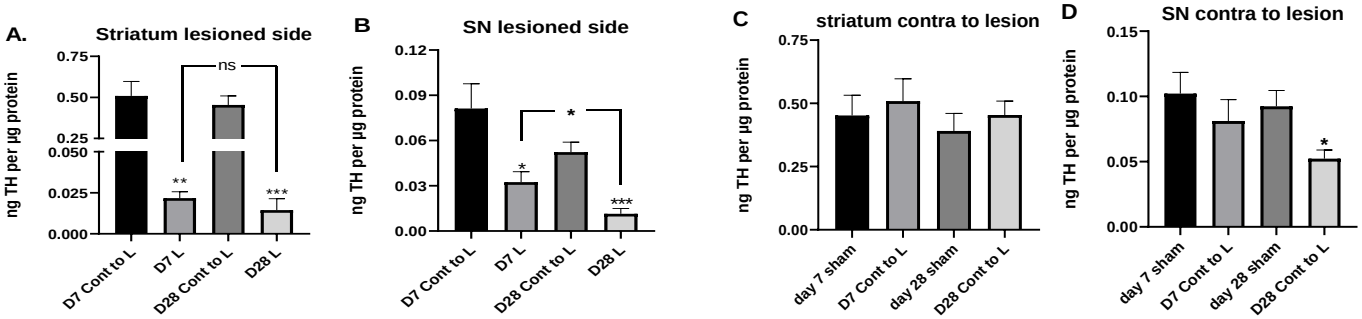


Figure 1. Timeline of TH expression (ng total TH per ug protein) in SN and striatum following nigrostriatal 6-OHDA lesion in lesioned hemisphere (A. striatum, B. SN) and hemisphere contralateral to lesion (C. striatum, D. SN). A. Striatum lesioned hemisphere. 6-OHDA reduced TH expression at day 7 (t=5.61, **p<0.01, df=4) and day 28 (t=7.30, ***p<0.001, df=5). There was no significant difference in TH expression between day 7 and day 28 (t=0.88, p=0.40, df=9). **B. SN lesioned hemisphere.** 6-OHDA reduced TH expression at day 7 (t=3.46, *p<0.05, df=4) and day 28 (t=7.15, ***p<0.001, df=5). TH protein was significantly less at day 28 vs. day 7 (t=2.85, p<0.05, df=9). **C. striatum contralateral to lesion.** TH expression in striatum contralateral to lesion was unaffected compared to sham-op on day 7 (t=0.48, p=0.64, df=9) or on day 28 (t=0.70, p=0.50, df=11). **D. SN hemisphere contralateral to lesion.** TH expression in SN contralateral to lesion was unaffected compared to sham-op on day 7 (t=0.90, p=0.40, df=9). At day 28, TH expression decreased in the contralateral SN post-lesion (t=2.75, p<0.05, df=11).

We also noted evidence of bilateral spread of the nigrostriatal lesion, but this effect was restricted to the SN, as there was no difference in striatum (Fig. 1C). As the time post-lesion increased, there was significant loss of TH in the SN by day 28 (Fig. 1D). In summary, these findings are highly relevant for the project for several reasons. **First**, there is progressive TH loss in the SN, but not striatum (which essentially hits the maximum degree of loss by day 7), in the lesioned nigrostriatal pathway. Notably, in human PD, this disparity of TH loss between striatum and SN is also observed, with major loss in striatum and loss of a lesser magnitude in the SN. Second, there is apparent spread of lesion impact contralateral to the lesion, but this is restricted to the SN. In human PD, motor symptoms begin unilaterally with eventual spread toward bilateral impairment.

From the angle of motor impairment, lesion-related impairment of motor function is observed on the seventh day following lesion (Fig. 2). Therefore, the loss of TH in the striatum or SN in the lesioned

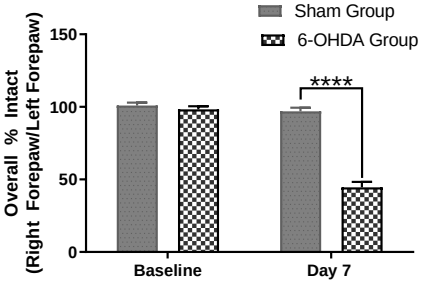


Figure 2. Right forepaw usage after unilateral 6-OHDA lesioning. A. There was a significant interaction between time after surgery and treatment ($F_{(1, 30)}$ = 99.49, p<0.0001) and a significant difference between the sham and 6-OHDA groups at day 7 (Uncorrected Fisher's LSD, p<0.0001).

side would be commensurate with the motor impairment detected by day 7. Therefore, we have the experimental evidence, both from the nigrostriatal TH loss assessment in striatum and SN and motor impairment associated with the nigrostriatal lesion, that the intervention with exercise on day 7 would enable us to evaluate whether the exercise could restore loss of motor impairment, and do so with either; **1)** a commensurate increase in TH expression in the SN, as hypothesized, or in the striatum, or **2)** prevent further loss of TH protein in the SN (Fig. 1B) by day 28. With these results, we have a strong experimentally-based rationale to begin the exercise intervention on day 8-10 following unilateral nigrostriatal lesion.

For this project to establish the experimental rationale for the timing of exercise intervention, we added an additional 10 rats to increase the number of rats for the day 7 cohort (n=4 successfully lesioned) and day 28 cohort (n=3 successfully lesioned) for the 6-OHDA lesioned group. We also have an additional day 7 sham-op group rats (n=2). This will increase the power of our report and help to establish the molecular link in terms of TH expression, DA tissue content and DA turnover (pending HPLC results). [Please note that the HPLC results for DA and DA turnover are delayed due to equipment issues experienced in Summer 2020 (see section 5 for further detail).] Once the results are obtained from this second cohort, we will collapse the data, and prepare our first publication, highlighting the dichotomous impact of 6-OHDA lesion in the striatum and SN in not only the lesioned side, but side contralateral to lesion in the SN.

Additional results; project update
From the PI (Salvatore) Lab:
-ser31 TH phosphorylation assessment in the lesion timeline: The phosphorylation of ser31 on TH is associated with DA tissue content levels in the rat and mouse CNS. There is also evidence that ser31 can affect TH activity from several studies from ours and other groups. Accordingly, we evaluated ser31 TH phosphorylation in the lesion timeline study and found yet more evidence of a dichotomous response to lesion between the striatum and SN (Fig. 3).

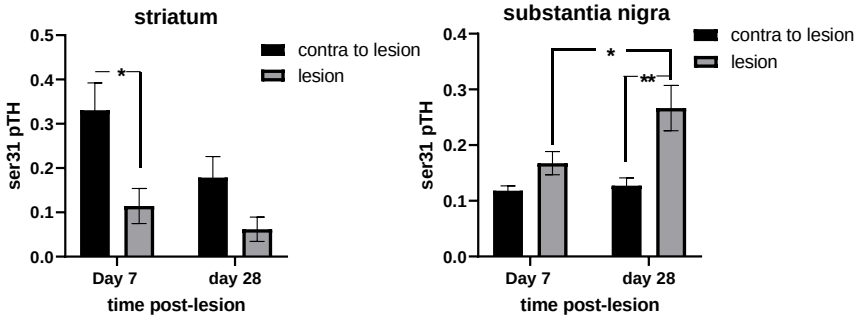


Figure 3. Ser31 TH phosphorylation in timeline following 6-OHDA lesion. In **striatum**, lesion produced a significant decrease in ser31 (Two way ANOVA repeated measure ($F_{(1,7)}$ =17.32, p=0.004), with decrease by day 7 (*p=0.01). In **SN,** lesion produced a significant increase in ser31 (Two way ANOVA repeated measure ($F_{(1,17)}$ =16.10, p<0.001), with significant increase at day 28 v day 7 (*p=0.02), and at day 28 vs contralateral SN (**p=0.001).

These results will also enable us to determine whether exercise intervention can affect ser31 TH phosphorylation in either region. With the lesion-related decrease in striattum, an exercise-related increase would suggest increased TH activity therein, whereas in the SN, an exercise-related increase would suggest increase a further increase in TH activity. The increase in ser31 TH phosphorylation from exercise is expected, given the literature reporting increased GDNF expression. GDNF has been reported by the PI to increase ser31 TH phosphorylation, particularly in the SN.

Assessment of Pink1 knockout rat neurochemistry (Salvatore Lab):

Pink1 knockout rats and aged-matched wild-type from Horizon Labs (now Envigo) (Fig. 4**)** were evaluated for DA and DA turnover in a 5-month old cohort. DA tissue content was significantly decreased 43% in the striatum of the Pink1-KO, whereas a 26% decrease in SN was not significant (Fig. 5**).** DA turnover, an index of major nigrostriatal neuron or TH loss, was significantly increased in both striatum and SN (Fig. 5, middle panel**).** Increased DA turnover was not seen in the ventral tegmental area ((VTA) t=0.80, ns, df=9, *data not shown*), signifying that loss of Pink1 function selectively increased DA turnover in the SN.

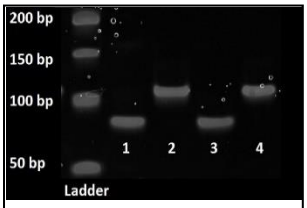


Figure 4. Verifying Pink1 KO 5% TBE electrophoresis of *PINK1* and wild-type PCR products of cerebellar extracts. Far left lane: DNA Ladder. Lanes 1, 3: Pink1 KO rats, Lanes 2, 4: WT rats. Gene fragment length is 109 bp in the WT and 83 bp in KO, reflecting deletion of 26 bp.

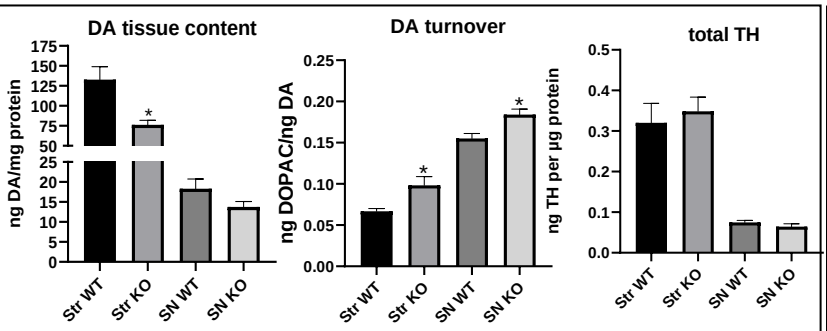


Figure 5. DA (left), and DA turnover (middle), and TH protein (right) in Pink1 KO rat vs wild-type (WT) Long-Evans at 5 months old. DA. Striatal DA was 46% less in Pink1 KO vs WT (t=3.32, **p*=0.01, df=8). DA decrease in SN was not significant (t=1.62, *p*=0.14, df=8). **DA turnover.** DA turnover increased in Pink1 KO in striatum (t=2.88, **p*=0.02, df=10) & SN (t=3.16, **p*=0.01, df=9). **Total TH.** No genotype difference in TH in striatum ((Str);t=0.46, ns, df=9) or SN (t=1.21, ns, df=8) was observed.

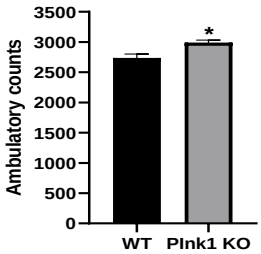


Figure 6. Locomotor activity in Pink 1 KO rat. Locomotor activity was evaluated in three 1 hr session. The mean activity was slightly increased in Pink1 KO (t=3.39, **p*=0.01 df=9).

Surprisingly, levels of TH protein did not decrease in Pink1 KO rat in striatum or SN (Fig.5, right), suggesting that decreased TH phosphorylation may be responsible for decreased DA. Despite the ~50% decrease in striatal DA and increase in DA turnover, locomotor activity did not decrease (Fig. 6). <u>These results are very relevant to our proposed experimental intervention with exercise and evaluate its impact over the course of 4 months.</u> Whereas there is no deficit in motor impairment in the open-field, there is evidence of decreased DA in the striatum and increased DA turnover in both the SN and striatum. In the coming year ahead, we will make the decision as to the timing of exercise intervention in the Pink1 knockout and age-matched wild-type. Current results from the literature indicate a motor deficit manifesting between 6-8 months of age. Thus, initiation of exercise is planned prior or within this time frame.

Results from Co-Investigators:

Bishop Lab: In the statement of work, item #4 detailed the microdialysis control experiments to investigate DA and DA turnover contemporaneously in striatal- and SN-targeted cannula implants in male and female Sprague-Dawley rats. Dr. Bishop and Salvatore consulted to plan this experiment in Fall of 2019 and his laboratory began the experiments in January 2020. In the original design of the proposal, we determined we would need a cohort for three different time periods during the course of exercise for evaluation of DA and DA turnover in striatum and SN (4,7, and 11 weeks post-lesion). However, in the interest (and possibility) that we could sample from these CNS areas *across* these time points (thereby increasing power for within subjects evaluation ***and*** reducing animal numbers possibly by one-third, we had two primary objectives in this pilot study;

1) Determine effects of nigrostriatal lesion on striatal and nigral basal and KCl-evoked DA, and DOPAC 28 days post lesion, with inclusion and analysis of a sham-op control group to ascertain contralateral to lesion effects.

2) Detemine if we can re-probe and measure DA, DOPAC in the striatum and SN across the time points of 4, 7, and 11 weeks.

3) Motor evaluation was done at 28 days post-lesion (the FAS test (as shown in figure 2 above)

A total of 20 rats were used in this pilot study, with equal division of 10 per gender and 5 lesioned and 5 sham-op within each gender group. Cannula were implanted in striatum at +1.0 AP, +/- 2.5 ML, and -3.5 DV and in the SN at -5.7 AP, +/- 2.5 ML and -7.0

At the time of collection, aCSF was infused for one hour and 3 samples were taken each 20 min. An additional 6 samples were collected to establish a baseline reading to compare against DA and DOPAC levels following KCl-infusion (beginning at 170 min) for 10 min. After another 10 min, KCl was replaced with aCSF and collection proceeded again for 20 min. 6 additional samples were collected at that time, thus a total of 15 samples per region (striatum or SN) were collected. Over the course of the study with the 20 rat cohort, 5 rats were removed from the study for either cannula failure or mistargeting (1 rat).

At the four week time point, the Lab was able to collect samples from 15 rats. From these 15 rats, successful collection of sample was possible in 14 rats at the seven week time point. Unfortunately, the 11 week time point for collection was not possible due to COVID-related shutdown. The rats were euthanized at that time point.

Results: At this time (see section 5), an issue with our HPLC has prevented us from analyzing the samples that sent to us in July 2020. We expect to have this analysis done and results to report for the March 2021 report. The results will inform us of two outcomes to move the project forward: **1)** the impact of nigrostriatal lesion on the lesioned and contralateral to lesion (by between subjects comparison to the sham-op group) in the striatum and SN, and **2)** whether or not we can obtain stable measures (as indicated by comparing the baseline measures) of DA and DOPAC across the two time points of 4 and 7 weeks post-lesion or post-sham.

Assessment of lesion impact progression on extracellular DA in striatum and SN. Commensurate with the rationale for the timing of exercise intervention, the Bishop Lab has begun the work to assess extracellular DA and DA turnover (by evaluation of DOPAC) in the lesioned SN and striatum, and in these two regions contralateral to lesion at day 7 and day 28. This work is the logical extension of the work begun in the PI's lab, wherein differences in TH and TH phosphorylation were revealed in the striatum and SN during the time course of lesion progression, most notably in the SN (Fig. 1, 3).

At the time of this Annual Report, the lab has conducted studies in a total of 6 rats for the 7 day cohort (3 male and 3 female), and 7 rats for the 28 day cohort (2 male and 5 female). Within these numbers, the cannula were implanted to access the lesioned or contralateral to lesion side. <u>The experimental goal is to reach an n of 6 per side (contra and ipsilateral to lesion) for the lesion for each gender.</u>

Exercise pilot study: Dr. Bishop's group has purchased two treadmills and has reported success in exercising rats with compliance. Once I inform him of the motor outcome in our first exercise study, the lab is prepared to begin the proposed work to investigate impact of the proposed 3 time per week exercise regimen during lesion progression on extracellular DA dynamics.

Richardson Lab: The Richardson Lab will investigate lesion and exercise-related changes on TH+ cell counts against whole cell counts, astrocyte markers (GFAP, GLAST), and GFR-α1. In the SOW, item number 7 describes the need for optimizing our immunohistochemical (IHC) analysis of lesion and exercise impact (to be done in item number 9—identified in the SOW as exp. #4). In December 2019, the PI's Lab sent 14 different brain preparations to the Richardson Lab to begin the optimization in intact tissue (no sham or 6-OHDA in the rat) and also analyze (then) lesion impact 7-8 days post-6-OHDA.

In order to determine lesion severity in the SN vs the striatum, we reasoned the best approach would be for the Salvatore Lab to ascertain striatal TH loss by western blot and then compare that readout against TH+ cell numbers in the SN by IHC. The method to do this would be take the tissue without perfusion and then drop-fix the remaining midbrain after taking striatal sections. Four rats receiving the 6-OHDA lesion were dissected on day 7 or 8 post-lesion this way and the midbrain was drop-fixed. Two rats receiving sham op were dissected by the drop-fix method.

In intact source of tissue, the Richardson Lab began optimization to detect the following proteins; TH (Fig. 7), GFAP (Fig. 8), GFR-α1 (Fig. 9) and co-label of GFAP and GFR-α1 (Fig. 10) (as shown below). The remaining sham-op and 6-OHDA lesioned tissue sources have been cut and have been stored until the optimization is complete.

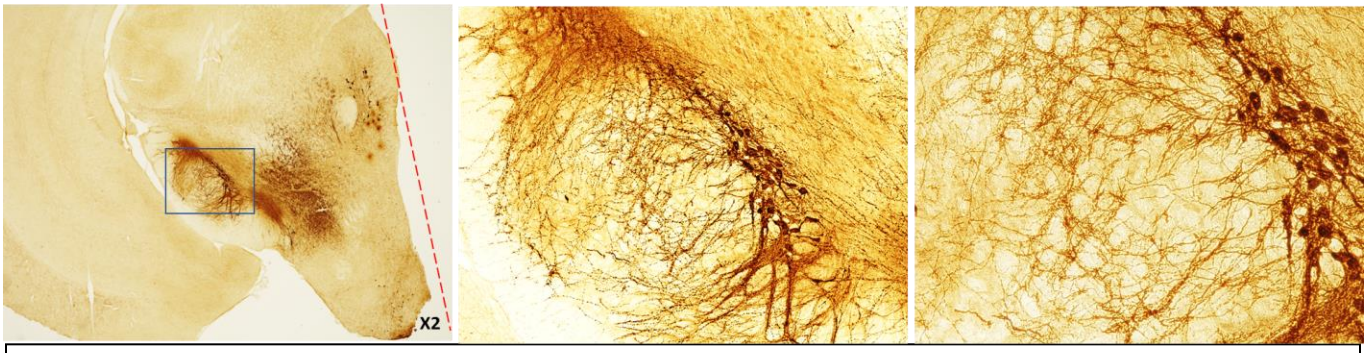


Figure 7. TH IHC staining in SN. midbrain from intact male Sprague-Dawley rat, stained with anti-TH (1:500) EDM Millipore AB152. Magnification 2X (left), 10X (middle), and 20X (right).

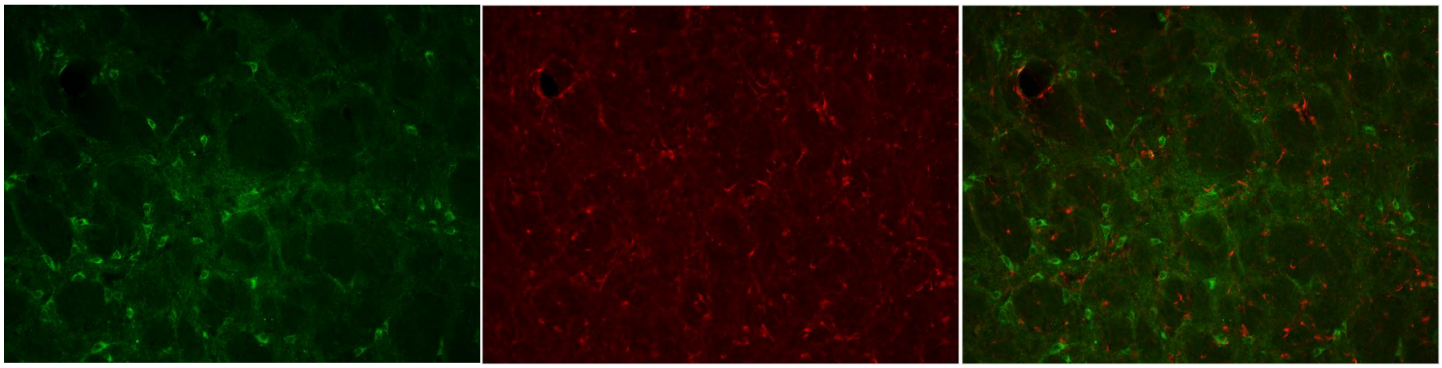


Figure 8 (left) GFR-α1 staining in striatum. Staining with 5 ug/ml goat anti-GFR (R&D Systems AF560), 20X. **Figure 9 (middle). GFAP staining in striatum.** Staining with chicken anti-GFAP (1:500 Invitrogen, PA1-10004) to identify astrocytes**. Figure 10 (right). Merge of GFR-α1 and GFAP in striatum.** 20X magnification.

Plans for the next reporting period:

Of note, given the agreement between the PI and Co-investigators on the rationale for timing of exercise intervention, as supported by the data obtained thus far in the PI's lab, an additional quantity of rats were obtained to acquire additional day 7 and a cohort of 28 day post lesion and sham rats (n=6 for all groups). These studies have been completed as of Sept 2020, and TH loss has been assessed in the striatum in the Salvatore Lab and reported to the Richardson Lab. The Richardson Lab will be coordinating with the Salvatore Lab to determine the priority of analysis, but it is expected that the order will be; 1) TH loss in lesioned vs. contralateral to lesion, TH counts in contralateral to lesion vs. sham-operated control group in the SN, and comparison against TH loss in striatum by western blot in Salvatore Lab, 2) GFR-α1 in same comparisons as for TH (Salvatore Lab will assess by western blot approach for striatal tissue), 3) GFAP, same approach as GFR-α1.

Also, as described in the section for Co-Investigator, Dr. Bishop, the lab will be completing the day 7 and day 28 studies and the Salvatore Lab will complete HPLC analysis.

The PIs lab will complete the analysis of TH, TH phosphorylation, DA, DA turnover, GFR-α1, glutamate transporters GLT-1 and GLAST, and including the results of the 10 rat second cohort and prepare for publication of the temporal aspects of expression and TH phosphorylation arising from nigrostriatal lesion and especially note the apparent effects on TH in the contralateral SN.

The PI will convene with Dr. Richardson and Bishop in January 2021 to devise the best strategies for publication of all results. Depending upon the timing, we may combine the results from all three labs into one major paper describing the unique lesion progression dichotomy between the SN and striatum and the unexpected effects on TH expression in the SN contralateral to lesion.

Finally, the PI will complete the assessment of TH expression, TH phosphorylation, and DA regulation in the Pink1 knockout rat (preliminary results in Figs. 4, 5) and submit for publication in February 2021.

Initiate first exercise experiment (item #5, Exp. 1): Scheduled and now in progress. There are 40 4-5 month old male Sprague-Dawley rats currently in the PIs animal colony. Accordingly, we are set to begin our planned exercise study, as stated in the approved SOW and in the proposal, in October 2020. Rats have been in our colony since August and have been regularly handled and will begin acclimation to the exercise regimen at this time. For this study, we will have four treatment groups; **1)** Sham-op, exercise, **2)** Sham-op non-exercise, **3)** 6-OHDA lesion, exercise, **4)** 6-OHDA lesion, non-exercise. In collaboration with Dr. Jason Richardson, the results of this study will be evaluated for loss of TH+ cells, as explicated in detail in experiment #4 in the proposal. Exercise will be implemented for 3 weeks on day 8-10 post 6-OHDA. Motor assessments are planned at the baseline (prior to lesion induction), day 7 (to confirm lesion and its extent on motor function by the FAS test), and then 3x per week on a treadmill at 10-11 m/min.

Motor function is assessed at the end of each week of exercise and then at the end of the study at 28 days post-lesion. The tissues will be collected promptly after assessment of open-field activity and FAS within 24 hr of the final exercise session.

Training and professional development opportunities: The project has allowed for the doctoral training of Ashley Center at the Bishop Lab at Binghamton. Furthermore, Ella Kasanga, the graduate assistant in the PI's Lab, was awarded a Visiting Scholar Award from the Parkinson's Foundation in May 2020. She will visit the Richardson Lab and conduct imaging-based analysis on rat tissues collected in the first exercise experiment described above. Specifically, she will focus upon astrocytic and mitochondrial-based markers.

Dissemination of Results: Nothing to report at this time.

4. Impact

Potential impact on the Parkinson's disease research field:

The results thus far are proving to show evidence that the 6-OHDA model we have employed in our work, which target the toxin into the medial forebrain bundle at -1.8 AP, 2.0 ML, and 8.6 DV, may have some new translational relevance to the human PD condition. First, it is now well-established in human post-mortem assessment of TH and other dopamine markers that loss of DA markers in striatum is much greater in the striatal regions compared to the loss in the SN. We have seen the same outcome in this 6-OHDA paradigm, that striatal TH loss was already at its greatest extent by day 7 vs day 28, whereas nigral TH loss was significantly less at day 7 vs day 28. This would suggest that prevention of further TH loss in the SN between day 7 and day 28 may be possible by exercise. We are also in a position to ascertain if there is any possibility of rescue of striatal TH loss between these two time points.

Second, it is known that PD motor symptoms start unilaterally and progress to bilateral impairment. Given that we have new evidence that there is loss of TH in the SN, but not striatum, contralateral to lesion, this loss may also portend to contribute to motor deficits contralateral to the lesion over time. If this is the case, our hypothesis that decreased nigral TH expression or function and DA regulation are contributing to motor impairment would be supported by this finding. Furthermore, these data now allow for us to evaluate whether exercise does affect the contralateral SN by reducing or preventing loss that we observed at day 28 post-lesion (Fig. 1B). To our knowledge, this would be the first such report in the exercise field.

Impact on other disciplines: Nothing to report

Impact on technology transfer: Nothing to report

Impact on commercial technology, government, industry, start-up company, or adoption of new practices:

The dichotomous loss of TH between the striatum and SN in the 6-OHDA lesion model is expected to motivate other PD researchers to interrogate the SN with equal vigor as the striatum in approaches evaluating motor impairment and ways to improve it.

5. Changes/Problems:

Changes in approach: Nothing to report with regard to a change in objective or scope of work

Actual and anticipated problems:

The analysis of DA by HPLC: Our chromatogram for the quantitative analysis of DA was exhibiting a peak of unknown origin beginning in July 2020. Several contacts with the vendor to initiate a service call and attempt a number of trouble-shooting measures did not resolve the issue. During the final week of September 2020, the service technician has been on-site to work on resolution to eliminate this peak. The presence of this peak in study samples would be problematic for quantitation. Therefore, we are currently at a backlog for HPLC analyses to be completed for work in the PI's and Dr. Bishop's Lab. We are

currently expecting resolution of this matter the first part of October and there is a dedicated research assistant in the lab to run the hundreds of samples on backlog.

COVID-related delay in project 1 (rationale for timing of exercise intervention) The original additional cohort of 10 male Sprague-Dawley rats was set to be studied in March 2020, prior to the COVID shutdown. As three additional months passed until our re-entry in June 2020, we ordered another cohort of 10 to keep the age similar with the first cohort. The older cohort was nonetheless used in a 28 day lesion impact study. Those results will allow us to examine whether there are any changes in baseline neurochemistry that could affect lesion impact as would be reflected in the time course of an exercise study lasting up to three months.

Unanticipated expenditures:
This purchase of additional animals was an unexpected expense of roughly $1000 and the per diems to maintain the animals originally purchased totaled an approximate $1000.

Significant changes in care, approved protocols, or deviations in use of vertebrate animals:

Nothing to report from PI and nothing reported from Co-Investigator, Dr. Bishop

6. Products

Websites:

We will post publications and conference papers on our website: www.salvatorelab.net

Other products:

The comparative sparing of nigral, but not striatal, TH during lesion progression following 6-OHDA lesion induction to the nigrostriatal pathway represents an opportunity to potentially stave off further loss of the DA neuron phenotype. In this project, we will investigate exercise as one possible approach. However, given the evidence that nigral TH and related DA can influence motor function, the prevention of further TH loss in the SN at early stages of the disease would represent a top priority for the Parkinson's field to also determine other approaches to prevent further TH loss therein. The priority to focus only on striatal TH preservation or restoration may eventually become obsolete or unnecessary to improve certain aspects of motor function.

7. Participants & Other Collaborating Organizations

PI: Michael F. Salvatore, Ph.D., Professor, Dept. Pharmacology & Neuroscience, UNT Health Science Center-Fort Worth, 4 person months

Contribution to project: overall planning and scheduling of projects, direction of projects, coordination with lab staff and co-investigators, analysis and reporting of results

Graduate Assistant: Ella A. Kasanga. 9 person months
Contribution to project: study planning and execution, locomotor assessment, animal surgery, neurochemical analysis, analysis and reporting of results

Research Assistants: 12 person months each: Marla Shifflet, Christopher McElroy

Contributions to project: Shifflet: HPLC, RT-PCR, animal exercise and surgery, locomotor assessment, neurochemical analysis
McElroy: neuchemical analysis, animal exercise, locomotor assessment

Changes in active support of PI or key personnel: Nothing to report

Partner organizations:

Collaborators:
Binghamton University
Binghamton, New York

Dr. Christopher Bishop, Co-investigator, 1 month

Ashley Centner, Graduate Assistant, 12 months

Florida International University
Miami, Florida

Dr. Jason Richardson, Co-Investigator, 1 month
Yoonhee Han, postdoctoral fellow, 1 month

Increasing nigral tyrosine hydroxylase expression as a mechanism of exercise-mediated recovery: Evaluation in toxin and rat Parkinson's disease genetic models
Log number: PD180098
Award number: W81XWH1910757

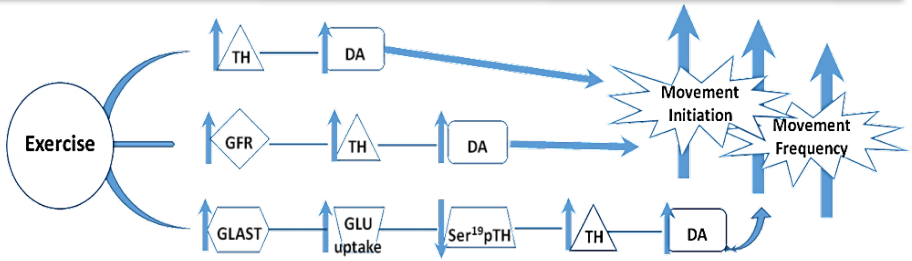
PI: Michael F. Salvatore **Org:** University of North Texas Health Science Center **Award Amount:** 1,491,860

Study/Product Aim(s)

- **Aim 1. Nigral TH expression affects exercise efficacy to attenuate or restore motor impairment.**
- **Aim 2. Increased GFR α1 increases TH expression and motor recovery in the SN following exercise.**
- **Aim 3. Increased GLAST expression following exercise prevents TH loss to increase exercise-related motor recovery.**

Approach

Completion of these studies will demonstrate that TH expression, specifically in the SN, affects DA neurotransmission locally therein to play a role in locomotor function in response to exercise. Such results would indicate that preservation of remaining TH in the SN in the PD patient could be targeted to improve motor function. Identification of GFR α1 and GLAST as upstream regulators of TH expression would provide new insight into understand how TH is regulated during nigrostriatal neuron loss, how exercise affects TH expression, and identify new targets for TH preservation or possible restoration in PD patients.



Accomplishment: Using two different rat models of Parkinson's disease (PD) (the 6-OHDA and Pink1 knockout), we are establishing the state of nigrostriatal neuron loss , phenotype, and relationship to motor impairment at the early stages. Our work indicates tyrosine hydroxylase (TH) loss in the substantia nigra (SN) is progressive in the lesion timeline, whereas striatal TH loss is much greater and reaches its peak very early. Furthermore, TH loss in the SN is progressive in both hemispheres, whereas loss in striatum is not. These differences will allow determination of whether treadmill exercise will restore striatal TH loss, or prevent further nigral TH loss, in relation to expected increase in motor function.

Timeline and Cost

Activities CY	19	20	21	22
Nigral TH targeting and DA analysis of exercise impact in 6-OHDA rat				
Nigral TH targeting and DA analysis of exercise impact in Pink 1 KO rat				
Targeting GFR role in exercise impact				
Targeting GLAST role in exercise impact)				
Estimated Budget ($K)	**$445K**	**$455**	**$455**	

Updated: October 1, 2020

CY19-20 Milestones – Establish exercise impact using human Parkinson's patient exercise frequency (3 times/week) as metric.

1) begin collaborative exercise studies with Bishop and Richardson Labs to reliably measure extracellular DA contemporaneously in SN and striatum and assess TH cell loss vs astrocytic markers

2) establish rationale for timing of exercise intervention following experimental outcomes using the 6-OHDA and Pink1 knockout models

3) established Pink1 knockout rat does exercise with compliance

CY20-21 Goals – continue collaborative exercise studies with Bishop and Richardson Labs. Evaluate impact of exercise on lesioned and contralateral to lesion SN and striatum on TH cell loss, astrocytic markers, and extracellular DA and DA turnover

- start mechanistic studies to target substantia nigra as center of motor impact

CY21-22 Goals –continue GFR and GLAST impact on exercise outcome studies

Comments/Challenges/Issues/Concerns: Lab shutdown for 3 months due to COVID.

Budget Expenditure to Date: August 31, 2020
Projected Expenditure: 538,000
Actual Expenditure: 309,000