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14. ABSTRACT

Recent studies in our labs have implicated a role for glial cells (astrocytes and microglia) in exercise- induced synaptogenesis and synaptic repair. The primary goal of this application is to investigate the role of astrocytes, microglia and peripheral monocytes in regulating exercise induced synaptogenesis and behavioral recovery in Parkinson's disease. Studies in this application will explore region specific metabolic changes mediatedby exercise, using imaging tools and molecular biology approaches to determine the mechanistic roles of glial cells,metabolism and immune processes associated with exercise induced neuroplasticity and potential disease modification. **Specific Aim 1** will test the hypothesis that exercise induced astrocytic activation and elevated lactatemetabolism regulates synaptogenesis and behavioral recovery in an animal model of PD. **Specific Aim 2** will test the hypothesis that activation of anti-inflammatory resident microglia and infiltrating peripheral mononuclear cells regulate synaptogenesis in the striatum and Prefrontal cortex of exercised 6-OHDA mice. We will further test the hypothesis that anti- vs. pro-inflammatory serum immune soluble factors (cytokines and BDNF) are associated withexercise benefits in PD.

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1. Introduction

Parkinson' disease (PD) is characterized by motor and non-motor (cognitive) impairments that lead to a progressive decline in quality of life (QoL) and increased morbidity. While there is no cure, research from our labs and others have demonstrated a role for exercise in improving motor/nonmotor features, QoL and repair of corticostriatal synaptic circuits). Recent studies in our labs have implicated a role for glial cells (astrocytes and microglia) in exercise-induced synaptogenesis and synaptic repair. It has long been known that activated glial cells accompany tissue damage in PD and contribute to an inflammatory cascade responsible for persistent injury that results in onset and progression of clinical disease. Astrocytes are essential for metabolic processes in the brain and are important in synaptic transmission. They couple multiple neurons and synapses into functional assemblies. Astrocytes also respond to increased synaptic neurotransmission via ability to sense extracellular concentrations of K^+ and glutamate. Neurons and astrocytes are metabolically coupled such that, even in the presence of cellular oxygen, astrocytes generate high levels of lactate through aerobic glycolysis and shuttle high-energy metabolites to neurons engaged in elevated synaptic activity. Astrocytes are also tightly associated with blood vessels and contribute to the blood brain barrier. In addition, synaptic loss and dysfunction, cell death, and neurite loss occurs in the presence of activated microglia, secretion of pro-inflammatory cytokines and chemokines, and recruitment of monocytes from the periphery. However, published studies and recent preliminary data from our lab have demonstrated that mechanisms capable of controlling pro-inflammatory activities in the CNS are induced by intensive exercise and involve production of anti-inflammatory cytokines (including the trophic factor BDNF), regional activation of microglia, and recruitment of monocytes with a "regulatory" phenotype to sites of synaptogenesis.

The primary goal of this application is to investigate the role of astrocytes, microglia and peripheral monocytes in regulating exercise induced synaptogenesis and behavioral recovery in PD. Studies in this application will explore region specific metabolic changes mediated by exercise, using imaging tools and molecular biology approaches to determine the mechanistic roles of glial cells, metabolism and immune processes associated with exercise induced neuroplasticity and potential disease modification.

Specific Aim 1 will test the hypothesis that exercise induced astrocytic activation and elevated lactate metabolism regulates synaptogenesis and behavioral recovery in an animal model of PD. This hypothesis is based on our preliminary data and recent reports demonstrating the importance of astrocytes and lactate metabolism in synaptic transmission, synaptogenesis and learning. We will also test whether exercise-induced changes in astrocytic activation and lactate metabolism is associated with increases in regional cerebral glucose uptake (rCGU) due to exercise. Studies will utilize a novel transgenic mouse model, established in our lab, which expresses astrocyte-specific red fluorescent protein tdTomato (ACT mouse) to isolate astrocytes. We have also developed a shRNA viral vector to selectively knock-down MCT-4, which we hypothesize to be critical for establishing the metabolic relationship between astrocytes and activated neuronal circuits through the ANLS.

Specific Aim 2 will test the hypothesis that activation of anti-inflammatory resident microglia and infiltrating peripheral mononuclear cells regulate synaptogenesis in the striatum and PFC of exercised 6-OHDA mice. We will further test the hypothesis that anti- vs. pro-inflammatory serum immune soluble factors (cytokines and BDNF) are associated with exercise benefits in PD. Our preliminary experiments in animals and pilot clinical study support this hypothesis. The CNS mononuclear cell population was more responsive to activation, which when cultured in the presence of stimuli secreted significantly higher amounts of IL-10, TGFβ, and BDNF suggesting enhancement of synaptic plasticity in these regions. processes are related to exercise benefits of cognitive function in physically (exercise) active PD subjects.

Impact: Studies from this application will (i) elucidate the role of glia in mediating exercise-induced neuroplasticity in PD and its models, (ii) support that changes in neuronal metabolism in conjunction with activation of astrocytes and microglia support improved synaptogenesis, leading to improved motor andcognitive behavior, and (iii) demonstrate how small energetic molecules like glucose and lactate in conjunction with components of the peripheral immune system (monocytes) link region-specific neuronal activation in the brain with non-neuronal cells (glia) at sites of circuitry activation. Understanding the mechanisms of exercise- induced neuroplasticity are critical to the development of strategies to treat patients suffering from degenerative brain disorders such as PD. Indeed, our studies in both animal models of PD and in patients with PD have led to widespread adoption of exercise and physical therapy as standard of care. The immediate impact of the studies in this application will shed additional mechanistic light on the benefits of exercise to reveal region specificity, allowing refinement of neurological physical therapy approaches. The long-term clinical impact will provide a framework for future clinical studies to determine if exercise (i) can modify diseaseprogression, (ii) can impact immune components to promote synaptogenesis and restoration of motor and cognitive circuits, and (iii) whether benefits can be enhanced by including additional components of a healthy

lifestyle, including diet, mindfulness, and stress management. Finally, it is possible that the data will reveal potential for the addition of supplemental pharmacological interventions designed to target astrocytes, microglia and components of the peripheral immune system), and interventions that modify metabolism.

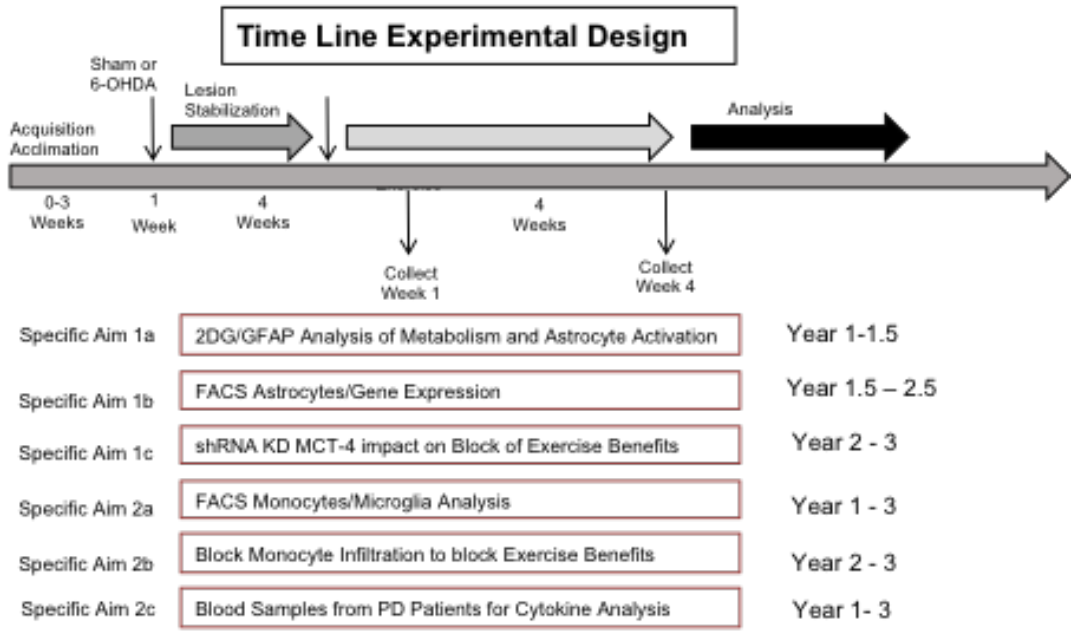
2. KEYWORDS

Parkinson's disease, exercise, metabolism, astrocytes, immune system, microglia, 6-OHDA, cognition, neuroplasticity, synaptogenesis.

3. ACCOMPLISHMENTS IN YEAR 1

3.1. Major Goals

The Following Section 3.1. outlines the major scientific goals achieved during Year 1 and 2. The Figure below highlights graphically the progress of the overall study. The Following Table lists the Statement of Word (SOW) with achievements in Year 1 and 2 highlighted in yellow. Following the Table are bullet points of major achievement and goals reached in Year 1 and Year 2.



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Tasks in Specific Aim 1a	Timeline Month	Lab Group
Hiring and Training of Study Staff	All personnel are currently employed in labs	1, 2, 3

IACUC Approval for entire project (addendum to ongoing approved projects)	1	1, 2, 3

Milestone Achieved	2	1, 2, 3
6-OHDA lesioning of mice, stabilization of lesion, exercise regimen	2-6	1,2
2DG studies in Sham±exercise and 6-OHDA ± exercise mice	4-8	2
Milestone Achieved	8	1,2
GFAP analysis of astrocyte activation by IHC	4-8	1
Analysis of outcome data including correlation studies	8-9	1,2
Milestone Achieved	10	1,2
Analysis of data, completion of Aim 1b, report, publication, data storing	14	1,2
Tasks in Specific Aim 1b		
IACUC Approval for entire project (addendum to ongoing approved projects)	1	1,2
Milestone Achieved	2	1,2
6-OHDA lesioning of mice, stabilization of lesion, exercise regimen, breeding ACT mice	6-12	1,2
Construction and validation of ACT mouse	Completed	2
Milestone Achieved	Completed	2
FACS analysis of ACT mouse astrocytes	9-12 underway	2
Analysis of ACT astrocytes by qRT-PCR and RNAseq	10-14 underway	2
Milestone Achieved	14	2
Tasks in Specific Aim 1c		
Hiring and Training of Study Staff	All personnel are currently employed in labs	2
IACUC Approval for entire project (addendum to ongoing approved projects)	1	2
Milestone Achieved	2	2
6-OHDA lesioning of mice, stabilization of lesion, exercise regimen	14-18	2
Milestone Achieved	18	2
Construction of shRNA vector	Completed	2
Milestone Achieved	Completed	2
Validation of shRNA to KD MCT2 in astrocyte cultures	6	2
Milestone Achieved	8	2
Stereotaxic targeting of vector to mouse brain	14-18	2
Molecular and behavioral outcome measures	18-24	2
Analysis of data	20-24	2
Milestone Achieved	24-28	2
Tasks in Specific Aim 2a		
Hiring and Training of Study Staff	All personnel are currently employed in labs	3
IACUC Approval for entire project (addendum to ongoing approved projects)	1	3
Milestone Achieved	2	3
6-OHDA lesioning of mice, stabilization of lesion, exercise regimen	4-8	1, 3
FACS analysis microglia phenotype/function brain serum	8-18 underway	1, 3
Milestone Achieved	18	1, 3
Tasks in Specific Aim 2b		

Hiring and Training of Study Staff	All personnel are currently employed in labs	1, 3
IACUC Approval for entire project (addendum to ongoing approved projects)	1	1, 3
Milestone Achieved	2	1, 3
6-OHDA lesioning of mice, stabilization of lesion, exercise regimen	18-22	1
Blocking monocyte infiltration, behavioral molecular study of effects	24-28	1, 3

Milestone Achieved	30	1, 3
Tasks in Specific Aim 2c		
IRB approval of addendum to current protocol	1-2	3
Begin subject recruitment	Currently underway as part of ongoing IRB approved project	3
Collection of samples	6-24	3
Analysis of serum cytokines in PD patients at baseline, 9, 18 months	6-24	3
Milestone Achieved	24-30	3
Analysis of data, completion of Aim 2c, report, publication, data storing	36 underway	3
Tasks Overall		
Subtask 1: Coordinate with Data Core for monitoring data collection rates and data quality	Every 6 months	1, 2, 3
Perform all analyses according to specifications, share output and findings with all investigators	Every 6 months	1, 2, 3
Work with data core and dissemination of findings (abstracts, presentation, publications, DOD)		1, 2, 3
Completion of Studies	36	1, 2, 3

Major Goals Achieve in Year 1 and Year 2

Specific Aim 1a will test the hypothesis that exercise leads to increase regional Cerebral Glucose Uptake and astrocytic phenotypic activation. We will utilize [^{14}C]-2-deoxyglucose autoradiography mapping to measure glucose uptake in the brain and GFAP IHC as a phenotypic marker of astrocytic activation. This study is supported by our preliminary data showing exercise-related increase in astrocyte morphology consistent with activation. Adjacent brain slices will be used to examine for co-localization/correlation between increase in rCGU and astrocytic GFAP.

- Approval of IACUC for use of vertebrate animals. (Year 1)
- Conducting studies of [^{14}C]-2-deoxyglucose autoradiography mapping to measure glucose uptake in the brains of 6-OHDA mice subjected to exercise or that remain sedentary. All parameters for studies in Aim 1A are validated and currently underway. Brains have been harvested from non-lesioned mice with and without exercise and subjected to [^{14}C]-2-deoxyglucose autoradiography mapping. (Year 1)
- Significant number of 6-OHDA lesioned mice have been established to carryout studies in Aim 1. The site of the lesions in the basal ganglia have been verified with immunohistochemical staining for tyrosine hydroxylase. The impact of the 6-OHDA lesion in the dorsomedial stratum and its impact on motor and cognitive behaviors has been verified using rotarod, novel object recognition, open field, and pole test. (Year 1)
- **Year 2**: We have completed 2DG analysis of mice subjected to dopamine-depletion by 6-OHDA in the dorsal striatum and subjected to exercise training. This manuscript is now in preparation and will be submitted in early 2022.

Specific Aim 1b will test the hypothesis that exercise leads to an increase of lactate metabolism of activated astrocytes in brain regions identified in SA1a and including PFC, dSTR, ERC, and vCB. Using our ACT transgenic mouse, FACS enrichment of astrocytes from these regions will be examined for expression of mRNA transcripts and proteins of interest including MCT-4 and LDHa and GLUT1 using qRT-PCR and WIB. We will also examine for astrocyte phenotype (S100A10, thrombospondin-1/2). (Year 1)

- Construction of the ART mouse expressing the red fluorescent protein tdTomato has been completed and validated using immunohistochemistry and microscopy. Currently these mice are continuing to be bred to achieve enough numbers to be used in studies as outlined in Year 2 of the research proposal. (Year 1)
- **Year 2**: Construction of the ART mouse has been achieved. Breeding continues to provide mice for subsequent Aims in Year 2 indicated below.

Specific Aim 1c will test the hypothesis that astrocyte mediated lactate transport is important in exercise-induced synaptogenesis. For these studies we have designed shRNA viral vector to selectively knock-down MCT-4 expression in astrocytes within the dSTR. Behavioral analysis will be conducted to examine recovery of

motor and cognition. Molecular analysis will be used to quantitate synaptogenesis using IHC and Golgi. We will also test whether knock-down of MCT-4 leads to decreased expression of GLUT-1, and LDHa using qRT-PCR and WIB in the PFC and dSTR.

- Construction of the shRNA vector to knockdown MCT4 expression has been constructed and validated in both in vitro (astrocyte cell cultures) and in vivo (injections into the mouse striatum). (Year 1)
- Studies have been carried out addressing Aim 1B where ACT mice have been administered L-lactate and the activation of genes of interest involve in metabolism and neurotrophic factor expression have been evaluated. These studies have been complemented with in vitro studies in astrocyte cultures. A manuscript describing these outcome measures is currently in review. (Year 1)
- **Year 2:** We have carried out 2 studies targeting the knock-down of the L-lactate transporter MCT4. In the first study we injected the lentiviral vector carrying shRNA to knock-down MCT4 to the M1 of the motor cortex. Overall findings indicated no impact on MCT1 expression, astrocyte cell death, or neuronal cell death. However, we did observe changes in neuronal dendritic spine density in M1, reduced uptake of the glucose transport ligand 2-deoxyglucose, and deficits in motor learning (but no deficit in motor performance). These findings are described in a manuscript (Lundquist et al, 2022) that is in review following its initial review in the journal Molecular Neurobiology. The second study utilized the approach described in the first study except the lentiviral vector was injected into the dorsolateral striatum. Studies have shown similar findings to the targeting studies in the motor cortex in terms of gene expression and cell viability. Interestingly, we found that knock-down of MCT4 results in enhanced sensitivity to the neurotoxin MPTP where a mild lesion (a single injection of MPTP) shows increased dopamine depletion, and increased loss of tyrosine hydroxylase expression, the hallmark of basal ganglia integrity, at the site of MCT4 knock-down. We have included these findings in a manuscript we will submit in 2022.

Specific Aim 2a will test the hypothesis that exercise leads to the activation of anti-inflammatory microglia in the STR and PFC. This Aim will utilize FACS analysis phenotypically identify and examine functional capacity as well as identifying infiltrating cells derived from peripheral blood.

- Mice to be used in this study have been constructed and validated. (Year 1)
- We have validated the FACS approach to be used in these studies. This Aim will be carried out in Year 2. (Year 1)
- **Year 2**: We have bred the ART mouse to now allow us to conduct FACS analysis on a subset of cell. We will compare total brain with samples of tissues from the striatum and cortex. We will examine know genes of interest using qRT-PCR, which requires less starting material, and then test FACS to determine the threshold of sensitivity for determination of changes in gene expression.

Specific Aim 2b will test the hypothesis that peripheral monocyte infiltration to the STR and PFC may regulate exercise-induced synaptogenesis and behavioral recovery. To test this hypothesis, we will block peripheral monocyte infiltration using Natalizumab, an inhibitor of integrin a4β1. We will use Golgi staining for dendritic spine density and IHC for markers of synaptogenesis (PSD95, synaptophysin) at the termination of the exercise intervention. Analysis will be conducted in the STR and PFC. We will conduct behavioral analysis of motor and cognitive function at completion of exercise.

- We have established and validated the 6-OHDA lesioning of mice for this aim. Studies are underway forYear 2 to determine infiltration of peripheral monocytes to the striatum and to determine if blocking the integrin receptor will block exercise-enhanced uptake to the striatum. (Year 1)
- **Year 2:** We pursued the analysis of the integrity of the BBB through several methods to complement the approach to examine the changes in BBB and the ability of cell to cross from the periphery to the brain. Using mice that have been lesioned with the dopamine -depleting neurotoxin MPTP and with the addition of exercise we have examined the patter of expression of proteins involved in tight junctions including occludin, and claudin. In addition, we have initiated studies to examine the integrity of the BBB in terms of its permeability to large molecules including Evans Blue and sodium fluorescein. Methods for these analyses are now in characterization and validation phases.

Specific Aim 2c will test the hypothesis that an increased level of serum anti-inflammatory (IL-10, TGFβ, and BDNF) vs. pro-inflammatory cytokines (TNFα, IL-1β, IL-6, and IL-2) is associated with higher levels of exercise intensity (average total METS/hr./week) and fitness levels (motor and/or cardiovascular) over an 18-month period in PD subjects. We will also examine whether increase level of serum anti-inflammatory vs. pro- inflammatory cytokines are associated with higher cognitive (executive function, EF) function at baseline, 9 and18 months of follow up.

- Addendum to IRB (PI: Dr. G. M. Petzinger, MD) to conduct blood collection and analysis of samples outlined in Specific Aim 2. (Year 1)
- Blood Samples are being analyzed for the presence of immune markers at the early time point (baseline) and we have completed the analysis of 46 samples (baseline) 20 samples (9 months) and 14 samples (18 months). Data are currently undergoing analyses and statistical validation. (Year 1)
- **Year 2**: Blood samples have been analyzed. A subset of these data are included in a manuscript in preparation Donahue et al 2022 (Listed below in Publications).
- **Year 2**: Overall we have examined 26 serum markers in patients with PD and conducted correlation and regression studies against cognition and fitness. We will publish these data as their own manuscript in 2022.

3.2. Opportunities for Training and Professional Development Provided by Project

This project has provided the following opportunities for training and development.

- Research electives for 5 undergraduate students
- Components of this project and data collection will be part of the doctoral thesis work of 3 USC doctoral students in the USC Neuroscience Graduate Program

3.3. Results Disseminated to Communities of Interest

Findings from this research study have been disseminated to the scientific research community through published manuscript (listed). In addition, findings on the impact of exercise eon brain health are used as a foundation for describing these studies and their translation to the medical and patient community at seminars for patients and support groups.

3.4. Plans During the Next Reporting Period

In Year 3 we will:

- The glucose uptake mapping in lesioned and non-lesioned mice with and without exerciseto identify regions of greatest activity and is now reported in a manuscript in preparation.
- Utilizer FACS to identify genes and proteins in astrocytes whose expression is altered with exercise and 6-OHDA-lesioning.
- Determination the impact of administration of L-lactate on gene and protein expression in our rodent modelof PD has been reported in a manuscript (Lundquist et al., 2021).
- Determine of blocking lactate transport leads to the attenuation of exercise-enhanced benefits in our mouse model of PD at both the motor cortex and striatum are reported in 2 manuscripts (Lundquist et al., 2022, in second review and Lundquist et al, 2022, in preparation).
- Continue to examine the alterations in infiltrating monocytes observed with exercise in the brain of our mouse model of PD.
- Analyzed the collect blood from patients with PD to conduct analysis of immune components and correlate them with disease and fitness levels. A subset of these data are reported in Donahue et al, 2022 and the complete analysis will be reported in a manuscript in 2022.

4. IMPACT

This application has immediate and long-term clinical and scientific impact. Studies from this application will (i) elucidate the role of glia in mediating exercise-induced neuroplasticity in PD and its models, (ii) support that changes in neuronal metabolism in conjunction with activation of astrocytes and microglia support improved synaptogenesis, leading to improved motor and cognitive behavior, and (iii) demonstrate how small energetic molecules like glucose and lactate in conjunction with components of the peripheral immune system (monocytes) link region-specific neuronal activation in the brain with non-neuronal cells (glia) at sites of circuitry activation. Understanding the mechanisms of exercise-induced neuroplasticity are critical to thedevelopment of strategies to treat patients suffering from degenerative brain disorders such as PD. Indeed, ourstudies in both

animal models of PD and in patients with PD have led to widespread adoption of exercise and physical therapy as standard of care. The immediate impact of the studies in this application will shedadditional mechanistic light on the benefits of exercise to reveal region specificity, allowing refinement of neurological physical therapy approaches. The long-term clinical impact will provide a framework for future clinical studies to determine if exercise (i) can modify disease progression, (ii) can impact immune componentsto promote synaptogenesis and restoration of motor and cognitive circuits, and (iii) whether benefits can be enhanced by including additional components of a healthy lifestyle, including diet, mindfulness, and stress management. Finally, it is possible that the data will reveal potential for the addition of supplemental pharmacological interventions designed to target astrocytes, microglia and components of the peripheral immune system), and interventions that modify metabolism.

Scientific Impact: Animal research over the past decade has shown that exercise and the way it is performed matters to neuro-rehabilitative outcomes, with changes in neuronal sprouting, restructuring of synapses and angiogenesis dependent. The current animal study provides a framework for understanding exercise-induced mechanisms of neuroplasticity at the regional level by examining synaptogenesis and metabolism and their influence on dopaminergic neurotransmission. An exciting aspect of this application is that it begins to investigate underlying mechanisms of exercise in animal models of PD and introduces astrocytes and microglia as important players in these processes including their impact on neuronal circuitry. Studies from thisapplication will (i) elucidate the role of glia in mediating exercise-induced neuroplasticity in PD and its models,

(ii) support that changes in neuronal metabolism in conjunction with activation of astrocytes and microglia support improved synaptogenesis leading to improved motor and cognitive behavior, and (iii) demonstrate how small energetic molecules like glucose and lactate in conjunction with components of the peripheral immune system (monocytes) link region-specific neuronal activation in the brain with non-neuronal cells (glia) at sites of circuitry activation. While the majority of the proposed studies are in young male animals, future studies can be designed to explore the impact of aging and sex in mediating the benefits of exercise in animal models. Also, benefits of exercise seen in our PD models may also show benefits in other models of disease and serve as an avenue to investigate these same phenomena in other human neurodegenerative disorders. Studies outlined in this application will begin to tease apart more specifically how non-neuronal components are impacted by exercise may be driving differential biological changes in brain circuitry, and in doing so also provides needed insights toward biomarkers that may be critical for future clinical studies to monitor exercise benefits.

Clinical Impact on Patients and Health Care Performance: The proposed application has the potential to lead to improvements in the efficacy of care for individuals with cognitive and motor impairment in PD by more specifically identifying mechanisms underlying and impacting the exercise prescription. This application examines exercise in a novel way by recognizing critical gaps that once addressed will greatly improve the efficiency of health care delivery such as (i) defining the patient characteristics (immune health, diet) that will be directly impacted by exercise; (ii) establishing the specific molecular and metabolic characteristics that are critical for improving and evaluating the impact and benefits of exercise in patients with PD, and (iv) may provide a biomarker for disease progression and its modification. Understanding the mechanisms of exercise- induced neuroplasticity is critical to further develop important therapeutic modalities for patients suffering from degenerative brain disorders such as PD. Studies from our labs over the past 16 years in both animal models of PD and in patients suffering from PD have led to the wide-spread application of exercise and physical therapy as a component of the current standard of care. Improved quality of life with improved cognitive performance will reduce falls, reduce the burden on healthcare providers, and certainly reduce the economic burden. Findings from this application can be leveraged to begin to apply exercise as a viable evidence-based
-treatment to modify other disorders of cognitive impairment and/or deficits in EF, including Alzheimer's disease, Huntington disease, schizophrenia, and traumatic brain injury. Studies in this application will provide important evidence-based data to allow neurologists and general internists to be able to prescribe a more precise form of exercise that targets cognition. Currently, physical therapy and exercise are not standard of care, and therefore not supported by most insurance policies. With information from this study, patients will be more empowered to take control of their personal treatment. The knowledge of a non-pharmacological and low-risk intervention falls within each individual's ability to control. Knowing they can truly impact their quality of life and disease progression, this will provide patients with a much-needed sense of self-empowerment and hope.

4.1. Impact on the Development of the Principal Disciplines of the Project

Studies in this research program are all underway. There are no technical or logistic issues to impede it progress. Findings from this program will demonstrate the important link between glia (astrocytes and microglia) and exercise-enhanced neuroplasticity in a rodent model of Parkinson's disease. We intend to demonstrate the important role played by astrocytes to regulate neuroplasticity especially through the transportof L-lactate that can act both as a metabolic substrate and a signaling molecular to strengthen synaptogenesis.We also aim to

link microglia and peripheral monocytes as mediators of the immune response in playing a role in enhancing synaptogenesis and repair of neuronal circuits damaged in Parkinson's disease. The outcome measures of synaptic repair are demonstrated by recovery of both cognitive and motor behaviors in our models.

4.2. Impact on Other Disciplines

As pointed out in the Impact statements in this report the findings from this research program will impact our understanding of a wide spectrum of fields including neuroscience, physical therapy, disease progression in Parkinson's disease, and if such approaches can be implicated to alter disease progression. These studies will continue to implicate the importance of immunology in brain function as well as bringing together neuroscience, immunology, physical therapy, and clinical care.

4.3. Impact on Technology Transfer

Nothing to Report

4.4. Impact on Society Beyond Science and Technology

Findings in this research program will help guide our understanding of the role of exercise and physical therapy in treating neurodegenerative disorders including PD. For example, evidence medicine based on our outcome measures will provide additional support to guide clinicians, caregivers, and patients in the utility of exercise as an essential component of the first line of treatment and standard of care. It will also provide rationale for pharmaceutical and biotechnology companies to identify novel important therapeutic targets to enhance our findings and to find approaches for patients for which exercise may be challenging or difficult.

5. CHANGES / PROBLEMS

5.1. Changes in approach

Nothing to Report

5.2. Actual or Anticipated problems or delays and actions or plans to resolve

Due to COVID-19 outbreak the USC campus was closed to research personnel from March 13 to June 10. However, during this time all personnel were able to continue their responsibilities as outlined in the proposal. Breeding was allowed to continue such that there would be no loss of mice dedicated to these studies. However, some mice subjected to 6-OHDA lesioning just prior to the shutdown did not enter studies upon completion. We were able to utilize mice at 3 months post-lesion and match them to aged controls. The labwas allowed to reopen at 50% occupancy June 10^{th,} and this allowed proposed studies to commence. With careful planning and dedication of all researchers in this proposal we do not anticipate a significant delay other than possibly 3 or 4 months in the timeline. During this period, we were able to write and submit several manuscript supported by this application.

5.3. Changes that had a significant impact on Expenditures

Nothing to Report

5.4. Significant Changes in Use or Care of Human Subject, Vertebrate animal, Biohazards, or select Agents. (Outlined in the following sections)

5.4.1. Significant Changes in Use or Care of Human Subjects.

Nothing to Report

5.4.2. Significant Changes in Use or Care of Vertebrate animals.

Nothing to Report

5.4.3. Significant Changes in Biohazards.

Nothing to Report

5.4.4. Significant Changes in Select Agents.

Nothing to Report

6. PRODUCTS

6.1. Journal Publications

Lundquist, A.J., J. Parizher, G.M. Petzinger, and M.W. Jakowec, *Exercise induces region-specific remodeling of astrocyte morphology and reactive astrocyte gene expression patterns in male mice.* J Neurosci Res, 2019. **97**(9): p. 1081-1094.

Lundquist, A. J., T. J. Gallagher, G. M. Petzinger, and **M. W. Jakowec** (2021) Administration of L-Lactate to mice acts as a mimetic replicating exercise-enhanced changes in astrocytes. Submitted to Journal of Neuroscience Research. 99(5):1433-1447. doi: 10.1002/jnr.24804. Epub 2021 Feb 25. PMID: 33629362

Wang, Z., I. Flores, E. K. Donahue, A. J. Lundquist, Y. Guo, G. M. Petzinger, **M. W. Jakowec**, and D. P. Holschneider (2020) Cognitive Flexibility Deficits in Rats with Dorsomedial Striatal 6-OHDA Lesions Tested Using a 3-Choice Serial Reaction Time Task with Reversal Learning. NeuroReport PMID: 32881776.

Caldwell, C. C, G. M. Petzinger, **M. W. Jakowec**, and E. Cadenas (2019) Treadmill Exercise Rescues Mitochondrial Function and Motor Behavior in a CAG_{140} Knock-In Advanced Huntington's Disease Mouse

Model. Chem Biol Interact. 2019 Nov 26; 315:108907. doi: 10.1016/j.cbi.2019.108907. [Epub ahead ofprint] PMID: 31778667

Lundquist, A.J., G. N. Llewellyn, S. H. Kishi, N. A. Jakowec, P. M. Cannon, G. M. Petzinger, and **M. W. Jakowec** (2022) Knockdown of astrocytic monocarboxylate transporter 4 (MCT4) in the motor cortex leads to loss of dendritic spines and a deficit in motor learning. Molecular Neurobiology, In Resubmission.

Lundquist, A.J., G. N. Llewellyn, S. H. Kishi, N. A. Jakowec, P. M. Cannon, G. M. Petzinger, and **M. W. Jakowec** (2022) Knockdown of astrocyte-specific monocarboxylate transporter-4 in the mouse striatum results in increased striatal dopamine levels and elevated sensitivity to the dopamine depleting neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. In Preparation.

Donahue, E.K., S. Venkadesh, V. Bui, D. Schiehser, R. Foreman, R. Wang, D. Haase, J. Duran, A. Petkus, B. Lund, D. Wing, M. Higgins, D. Holschneider, **M. W. Jakowec**, J. D. Van Horn, G. M. Petzinger (2022) Moderate to Vigorous Physical Activity is associated with better Memory, Executive Function, and Global Cognitive Function in Parkinson's Disease: A cross-sectional neuropsychological, immune, and neuroimaging study. In Preparation.

Wang, Z., A. J. Lundquist, E. Donahue, D. Phillips, G. M. Petzinger, M. W. Jakowec, and D. Holschneider (2022) Treadmill exercise enhances striatal metabolic connectivity and improves basal ganglia glucose hypometabolism in mice with intra-striatal 6-hydroxydopamine lesion. In Preparation.

6.2. Conferences and Presentations

Lundquist, A.J., G. N. Llewellyn, S. H. Kishi, N. A. Jakowec, P. M. Cannon, G. M. Petzinger, and M. W. Jakowec (2021) Knockdown of astrocytic monocarboxylate transporter 4 (MCT4) in the motor cortex leads to loss of dendritic spines and a deficit in motor learning. Gordon Research Conference, Astrocytes, April 2021.

6.3. Technologies or Techniques

Nothing to Report

6.4. Inventions, Patent Application, Licenses

Nothing to Report

6.5. Other Products

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7.1. Individuals that Worked on the

Project.

Name: Michael Jakowec, Ph.D.
Project Role: PI
Research
Identifier: N/A
Nearest person month worked: 2.4 Mo

Contribution to the project: No change. Project design, directing molecular, histology, and neuroanatomicstudies.

Name: Daniel P. Holschneider, MD Project Role: partnering co-I Research
Identifier: N/A
Nearest person month worked: 2.0 Mo
Contribution to the project: No change. Project design, project management, directing functional brain mapping studies, data analysis.

Name: Brett Lund, PhD Project Role: co-I Research Identifier: N/A
Nearest person month worked: 2.16 Mo
Contribution to the project: No change. Project design, project management, directing immune components,data analysis.

Name: Zhuo Wang, Ph.D. Project Role: co-I Research
Identifier: N/A
Nearest person month worked: 4.2 Mo
Contribution to the project: No change. Stereotaxic lesioning, directing operant studies, functional brain mapping, data analysis.

Name: Giselle M. Petzinger, MD
Project Role: co-I Research
Identifier: N/A

Nearest person month worked: 0.36 Mo
Contribution to the project: No change. Project design, data analysis, interpretations, alternative approaches.

Name: Yumei Guo, MS
Project Role: Staff
Research Identifier: N/A
Nearest person month worked: 7.0 Mo
Contribution to the project: No change. Skilled and nonskilled exercising of animals, immunohistochemical staining (tyrosine hydroxylase).

Name: Adam Lundquist, BS
Project Role: Graduate Student
Research Identifier:
Nearest person month worked: 5.0 Mo
Contribution to the project: Western blotting, qRT-PCR, brain dissection, data analysis.

Name: Erin Donahue, BS
Project Role: Graduate Student
Research Identifier:
Nearest person month worked: 5.0 Mo
Contribution to the project: Blood analysis, Animal behavior, Western blotting, qRT-PCR, brain dissection, data analysis

Name: Derek Phillips, BS
Project Role: Graduate student
Research Identifier:
Nearest person month worked: 1.5 Mo
Contribution to the project: immunohistochemistry, behavioral testing, brain dissection

Name: Enrique Cadenas, Ph.D.
Project Role: Collaborator
Research Identifier: N/A
Nearest person month worked: 0.12 Mo
Contribution to the project: No change. Advisement on interpretation of molecular biologic studies

Name: Wendy Gilmore, Ph.D.
Project Role: Collaborator
Research Identifier: N/A
Nearest person month worked: 0.12 Mo
Contribution to the project: No change. Advisement on interpretation of immune studies

7.2. Changes in Active Support for PI or Senior Key Personnel

Nothing to Report regarding Key Personnel.

Note: The Graduate Student Derek Phillips has been added to this study.

7.3. Other Organizations Involved as Partners

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

None

9. APPENDICES

No New manuscripts added to Annual Report 2.
Four manuscripts were included in Annual Report Year 1.
Three manuscripts in preparation are not included until submitted for review.