AWARD NUMBER: W81XWH-18-1-0666

TITLE: Investigating Exercise-Induced Neuroplasticity and Its Mechanisms in Parkinson's Disease: Targeting Executive Function and Brain Circuitry

PRINCIPAL INVESTIGATOR: Daniel P. Holschneider, MD,

CONTRACTING ORGANIZATION: University of Southern California
Dept. Contracts & Grants
1640 Marengo St., 7th floor, Site 701,
Los Angeles, CA, 90033-9263

REPORT DATE: OCTOBER 2022

TYPE OF REPORT: Final Report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JANUARY 2023	FINAL	15SEPT2018-14SEPT2022

4. TITLE AND SUBTITLE

Investigating Exercise-Induced Neuroplasticity and Its Mechanisms in Parkinson's Disease: Targeting Executive Function and Brain Circuitry

5a. CONTRACT NUMBER
W81XWH-18-1-0666

5b. GRANT NUMBER
W81XWH-18-1-0666

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Daniel P. Holschneider, MD

E-Mail: holschne@usc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Southern
California
Dept. Contracts & Grants
1640 Marengo St., 7th floor,
Site 701,
Los Angeles, CA, 90033-9263

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command

Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

An increasingly common problem in Parkinson's disease (PD) and its progression is cognitive impairment, yet it is rarely addressed with currently accepted therapeutics and is difficult to treat. Recent findings support the hypothesis that exercise, and particularly exercise that incorporates both skill and aerobic components (SAE), is a viable and effective treatment option for cognitive impairment in PD. Using a rat model of PD (striatal 6-hydroxydopamine model), the current project has applied methods of animal behavior, immunohistochemistry, molecular biology, functional brain mapping, and micro-neuroanatomy, to the question of exercise-related restoration of cognitive function and the role of frontostriatal circuits. Understanding the impact of exercise in the basal ganglia and its related circuitry may represent a new frontier in understanding mechanisms of neuroplasticity and repair and, thus lead to novel therapeutic targets for PD. It provides a framework for guiding future human trials aimed at optimizing specific, cost-effective rehabilitation strategies and reducing the burden of disease, not only for PD patients, but also for persons with a broad range of neurologic disabilities.

15. SUBJECT TERMS
Parkinson's Disease, exercise, skilled training, cognition, learning, executive function, dopamine, plasticity, metabolic, prefrontal, striatum, nigrostriatal, animal models, operant, brain mapping

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	28	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

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

   Parkinson's disease (PD) is the 2nd most frequent neurodegenerative disorder at old age and diminishes the quality of life in over 630,000 people in the USA, with numbers projected to double by the year 2040. An increasingly common problem in PD and its progression is cognitive impairment, yet it is rarely addressed with currently accepted therapeutics and is difficult to treat. Importantly, cognitive impairment leads to challenges in daily function, as well as significant social and psychological burdens. A wide range of exercise modalities have been examined in the motor rehabilitation of PD patients. However, investigation on the relationship between exercise and cognitive function in PD remains a major gap in knowledge. Recent preliminary findings support the hypothesis that exercise is a viable and effective treatment option for cognitive impairment in PD. Using a rat model (striatal 6-hydroxydopamine model), the current study applies methods of animal behavior, immunohistochemistry, molecular biology, functional brain mapping, and micro-neuroanatomy, to the question of exercise-related restoration of cognitive function and the role of basal ganglia-cortico-striatal circuits. Our results show that physical exercise results in a significant, gradual and progressive, improvement in executive function, with little significant difference in the type of exercise modality (skilled vs. unskilled vs. aerobic). Exercise also elicits differential neuroplastic and metabolic changes across the striatal subsectors. The return of functional connectivity with exercise appears to be most active in the dorsomedial (cognitive) and dorsolateral (motor) segments. Exercise elicited increased expression of dopamine receptors Drd1, Drd3, Drd4 and the synaptic protein PSD95 (mRNA transcript Dlg4) in the dorsomedial (cognitive) and dorsolateral striatum (motor).

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

   Parkinson's Disease, exercise, skilled training, cognition, learning, executive function, dopamine, plasticity, metabolic, prefrontal, striatum, nigrostriatal, animal models, operant, brain mapping

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

   What were the major goals of the project?
   List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

   MAJOR GOALS PROJECT 2 (preclinical)
   TASK 1: Executive Committee meeting

   TASK 2: Evaluate relationship between fitness and executive function

Subtask 1: Radiotelemetric recordings of heart rate and rotarod testing in response to skilled aerobic exercise or simple aerobic exercise or no exercise

TASK 3: Evaluate Effects of Skill-based v. Aerobic Exercise on Executive Function
Subtask 2: Performance of operant training (set-shifting task)
Subtask 3: Assessment of lesion size (TH staining)

TASK 4: Brain Imaging
Subtask 1: Perfusion autoradiography
Subtask 2: Assessment of lesion size (TH staining)

TASK 5: Bench Research
Subtask 1: Spine counts, dendritic branching
Subtask 2: Electrophysiology
Subtask 3: HPLC
Subtask 4: qRT-PCR
Subtask 5: Western Blots

TASK 6: Data Analysis
Subtask 1: Coordinate with Data Core for monitoring data

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

TASK 1: Executive board meeting: Completed

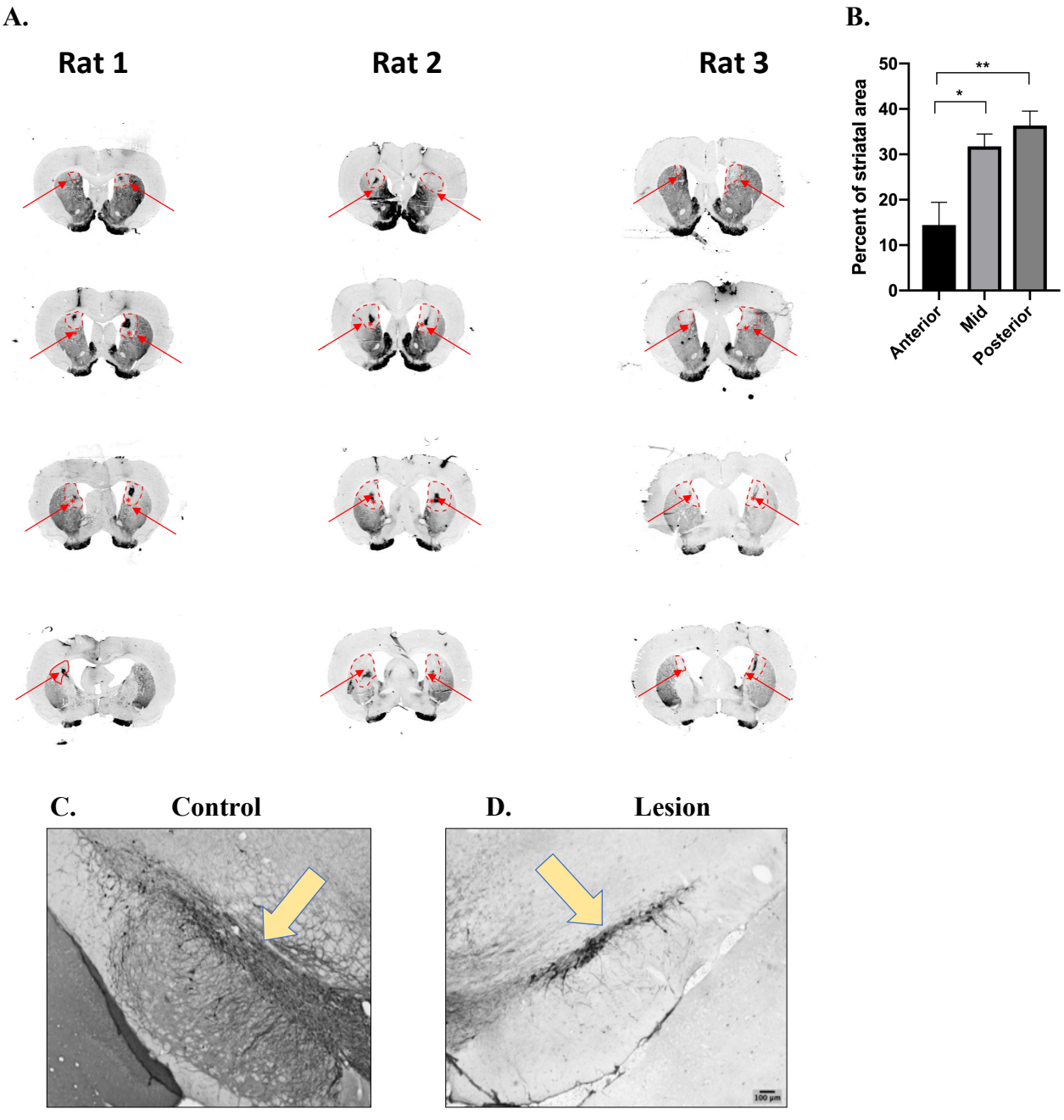
TASK 2: This task was not completed due to our findings that while exercise improves cognition, the type of exercise modality (skilled vs. nonskilled vs. aerobic) is not a significant factor.

TASKS 3 / 6: Evaluate Effects of Skill-based v. Aerobic Exercise on Executive Function/Data Analysis

<u>Dorsomedial striatal lesioning & lesion verification:</u> Figs. 1 and 2 show the lesion location in the dorsomedial striatum, and related losses in tyrosine hydroxylase and its substrate dopamine.

Figure 1: Immunostaining for tyrosine hydroxylase to determine the degree of lesion and its anatomical site in the dorsomedial striatum. Images of coronal sections stained for TH at (A) anterior, mid, and posterior regions of the striatum in three representative 6-OHDA lesioned rats (left panel). The arrow identifies the site of loss of TH-immunoreactivity. (B) Quantification of

the loss of TH staining in lesioned animals is expressed as percent of striatal area at the three levels. (C, D) TH staining of the level of the midbrain showing the substantia nigra reticulata (SNR) and substantia nigra compacta (SNC) at -5.20 mm relative to bregma in control (panel C) and 6-OHDA lesion (Panel D). The arrow highlights the loss of immunostaining in the SNpc due to cell loss. Current studies are using unbiased stereological methods to quantitate the degree of lesion in rats from the first phase of these studies.



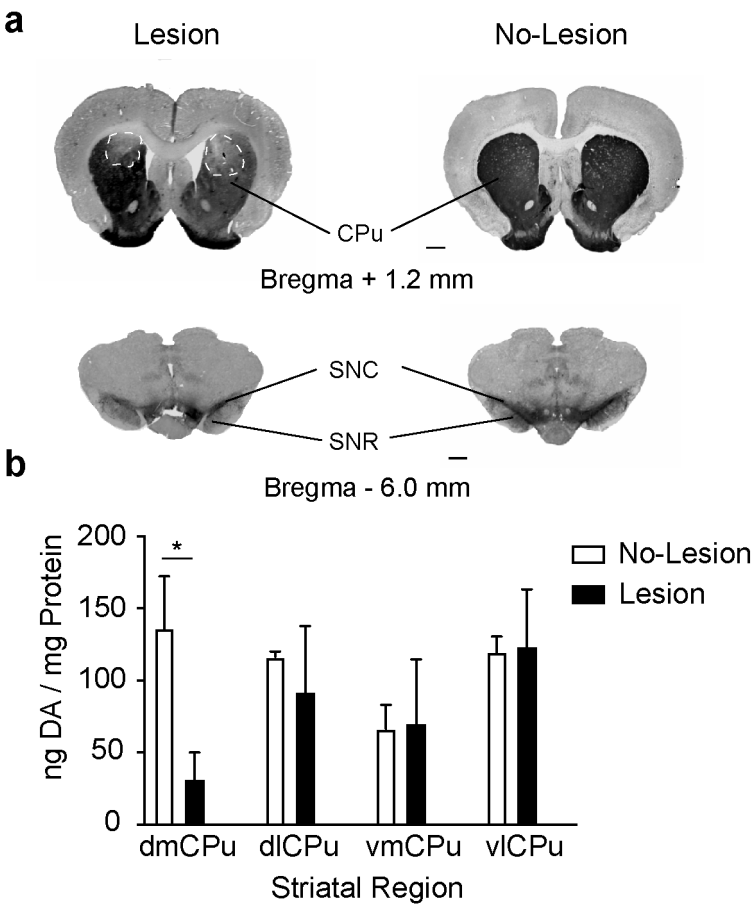


Figure 2. HPLC analysis of regional dopamine levels show that lesions reduced dopamine levels in the dorsomedial striatum (dmCPu), with no significant effects on dorsolateral, ventromedial and ventrolateral subregions (dlCPu, vmCPu, vlCPu).

(a) Representative images of coronal sections reveal bilateral loss in tyrosine hydroxylase immunoreactivity in the dorsomedial striatum (bregma AP +1.20 mm) and midbrain showing immunostaining of the substantia nigra pars compacta (bregma -6.0 mm). Scale bar = 0.5 mm.

(b) HPLC analysis of striatal dopamine from tissues collected from coronal slice (bregma AP +2.00 to 0.00 mm) from non-lesioned and lesioned rats in striatal tissue.

Lesions do not affect motor strength, balance and coordination as assessed with the Rotarod test. Results shown in **Figs. 3 and 4** suggests that motor impairment is not a factor that influences to any significant extent the results of our cognitive testing

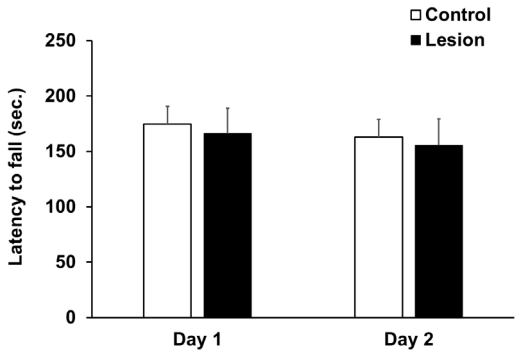


Figure 3**: **Performance on the Rotarod: Rats were exposed to the accelerating rotarod on sequential days. No significant difference in group mean latency to fall (+/- S.E.M.) was observed for animals (non-exercised) two weeks after striatal lesioning (n=8) and for controls (n=9).

Lesions do not affect general motor activity

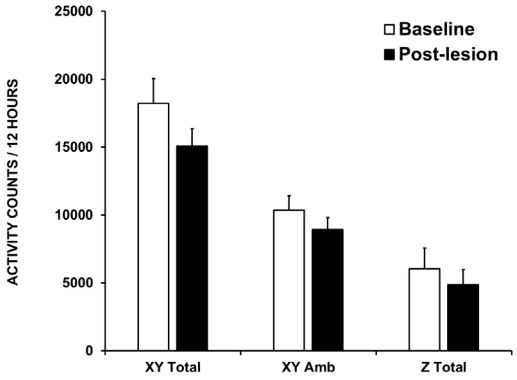


Figure 4**: **Home cage overnight locomotor activity: No significant group differences were observed in the number of overnight (6 a.m. – 6 p.m.) infrared beam break at baseline compared to those at two weeks following bilateral striatal lesioning. No difference was noted in the horizontal (xy) plane (total counts and ambulatory counts), and in the vertical (z) plane (rearing activity). n= 13 animals (non-exercised), group mean (+/- S.E.M.).

Lesions do not change appetitive preference for sucrose consumption

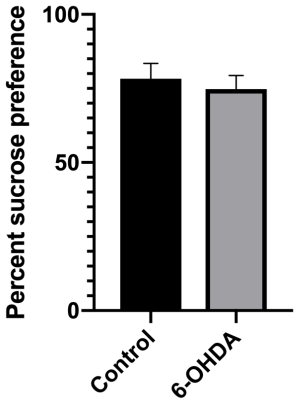


Figure 5**: **Sucrose Preference Test to evaluate anhedonia: Shown is the percentage of the volume of sucrose intake over the total volume of fluid intake. There was no significant difference between the control (sham lesion) group and the 6-OHDA-lesion group ($t(12)=1.351$, $p=0.202$). Data are expressed as mean +/- S.E.M. N = 14 per group.

Our results in the sucrose preference test (**Fig. 5**) pave the way for the way for cognitive studies using sucrose pellet reward.

TASKS 3 & 6: Effects of exercise on cognition

Exercise improves cognitive flexibility, and impulsivity in the Parkinsonian rat model

Lesioned rats show a slower rate of learning and increases in response omissions, however, can reach levels of response accuracy equivalent to that attained in normal animals (3-CSRT). Lesion deficits in learning are unmasked during rule reversal (3-CSRT-R) where response accuracy compared to normal animals is unable to recover even after extended cognitive training (25 sessions).

Lesioned rats exposed to exercise showed a modest, yet significant improvement compared to sedentary lesioned rats in processing-related response accuracy in an operant 3-choice serial reaction time task (3-CSRT), as well as in the T-maze (**Figs. 6 & 7**). Exercise also elicited a significant improvement in lesioned rats in response accuracy and in cognitive flexibility as assessed by inhibitory aptitude during rule reversal in both the 3-CSRT-R and in the T-maze.

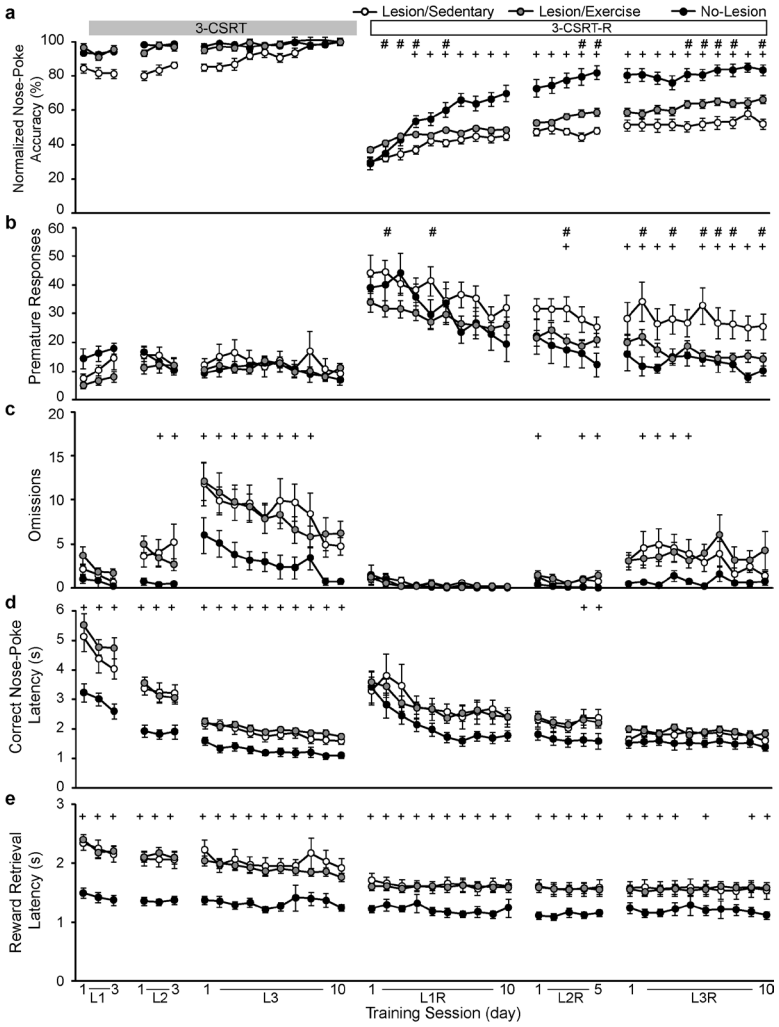


Figure 6. **Effects of exercise and lesion on the 3-choice serial reaction time task (3-CSRT) acquisition and reversal learning (3-CSRT-R).** Shown are group mean ± SEM of **(a)** normalized nose-poke accuracy, **(b)** premature responses, **(c)** omissions, **(d)** correct nose-poke latency, and **(e)** reward retrieval latency for No-Lesion (n = 12), Lesion/Sedentary (n = 12) and Lesion/Exercise group (n = 22). #: $p < 0.05$ Lesion/Exercise vs. Lesion/Sedentary; +: $p < 0.05$ Lesion/Sedentary vs. No-Lesion, Fisher's LSD multiple comparisons test. Data were also analyzed with two-way ANOVA with repeated measures (results not shown).

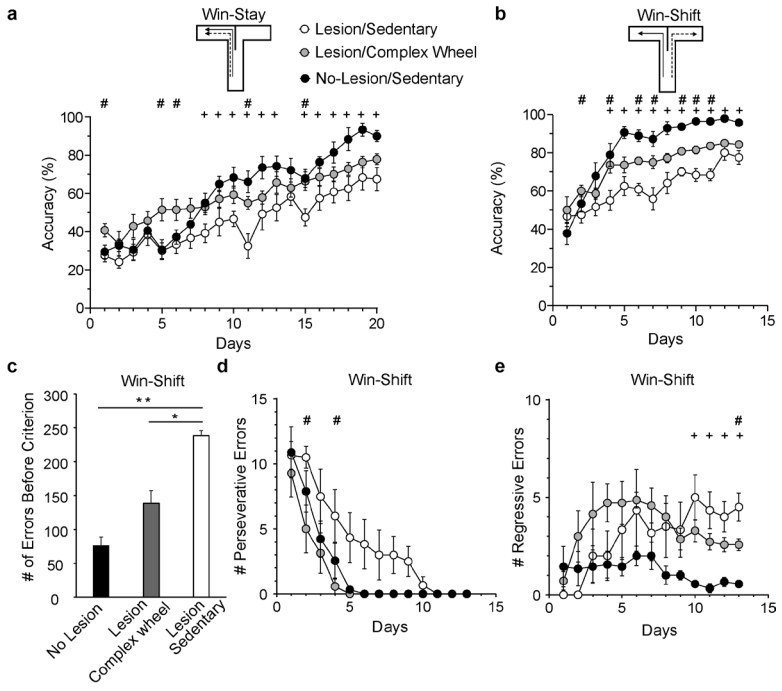


Figure. 7: Effects of lesion and exercise on T-maze learning of rewarded matching-to-sample (Win-Stay) followed by reversal (Win-Shift). (**a**) Rats were trained in a Win-Stay strategy (solid line arrow = sample trial; dashed line arrow = choice trial) for 20 days, followed by training in **(b)** Win-Shift strategy for an additional 13 days. Response accuracy (percentage of correct responses) is shown for No-Lesion/Sedentary (n = 9), Lesion/Complex wheel exercise (n = 7) and Lesion/Sedentary (n = 6). **(c)** Total number of incorrect trials performed until criterion (9 correct responses in 10 consecutive trials) was reached during the Win-Shift phase. **(d)** Perseverative errors during the Win-Shift phase. **(e)** Regressive errors during the Win-Shift phase. Mean ± SEM. #: $p < 0.05$ Lesion/Complex wheel vs. Lesion/Sedentary; +: $p < 0.05$ Lesion/Sedentary vs. No-Lesion/Sedentary, Fisher's LSD multiple comparisons test. *: $p < 0.0002$, **: $p < 0.001$ (Student's t-test).

No significant difference in cognitive outcomes in comparing different exercise modalities in lesioned rats.

Cognitive gains with exercise were observed with exercise in lesioned animals, independent of whether the exercise was simple (smooth running wheel), skilled (running in a complex wheel with irregular rung spacing) or pure aerobic (horizontal treadmill). This observation was valid even when exercise was undertaken for two different skill levels at comparable speeds and durations using the same exercise modality (complex versus smooth wheel running, **Fig. 8**).

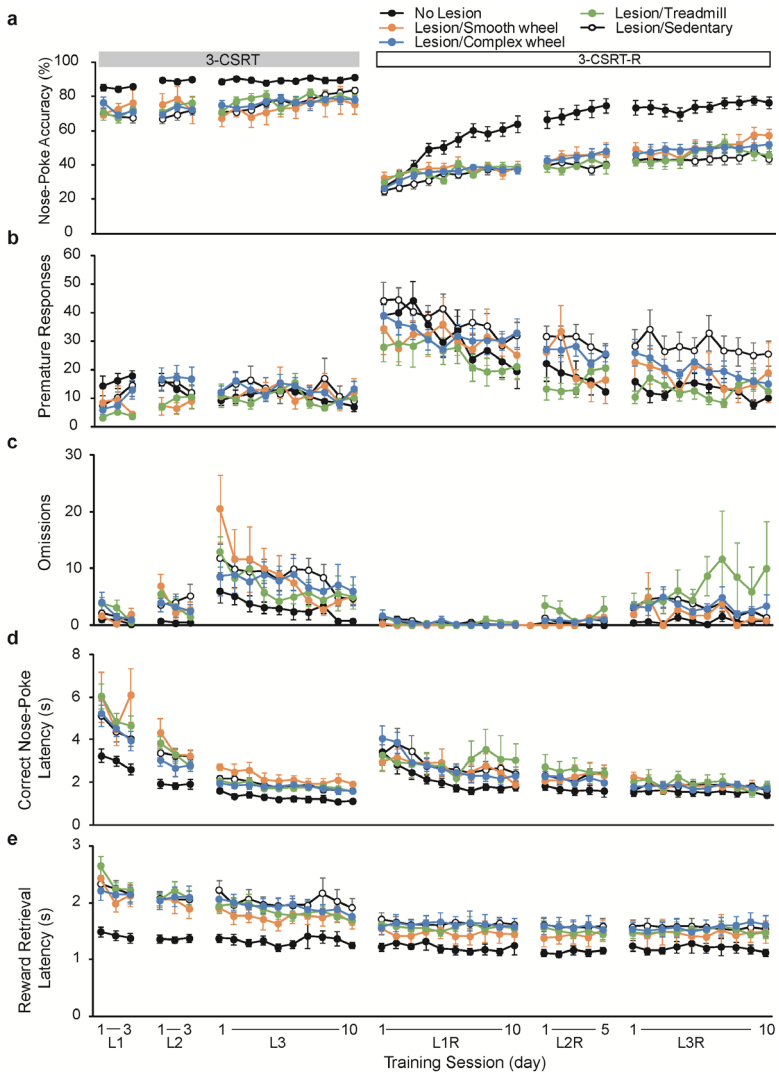


Figure 8. No difference of different exercise modalities on cognitive outcomes in the 3-Choice serial reaction time task with reversal learning (3-CSRT, 3-CSRT-R). Data are presented as mean ± S.E.M. Exercise effect was assessed using a two-way ANOVA with repeated measure among Lesion/Sedentary (n = 12), Lesion/Complex wheel running (n = 12), Lesion/Smooth wheel running (n = 4), and Lesion/Horizontal Treadmill (n = 6) groups. There was no statistically significant exercise effect in (**a**) nose-poke accuracy (p > 0.35), (**b**) premature responses (p > 0.077), (**c**) omissions (p > 0.13), (**d**) correct nose-poke latency (p > 0.39), and (**e**) reward retrieval latency (p > 0.73). Fisher's LSD multiple comparisons test showed significant difference in premature responses between the Lesion/Sedentary and Lesion/Treadmill group during the 3rd phase of reversal learning (L3R, p = 0.015). Due to institutional and local restrictions in response to the Covid-19 pandemic, we were not able to complete the Lesion/Smooth wheel and Lesion/Treadmill group, or to increase sample size.

TASKS 5 / 6: Bench Research / Data Analysis

Exercise in lesioned rats elicited increased expression of dopamine receptors Drd1, Drd3, Drd4 and two synaptic proteins (synaptophysin, Dlg4) in the dorsomedial (cognitive) and dorsolateral (motor) striatum (CPu).

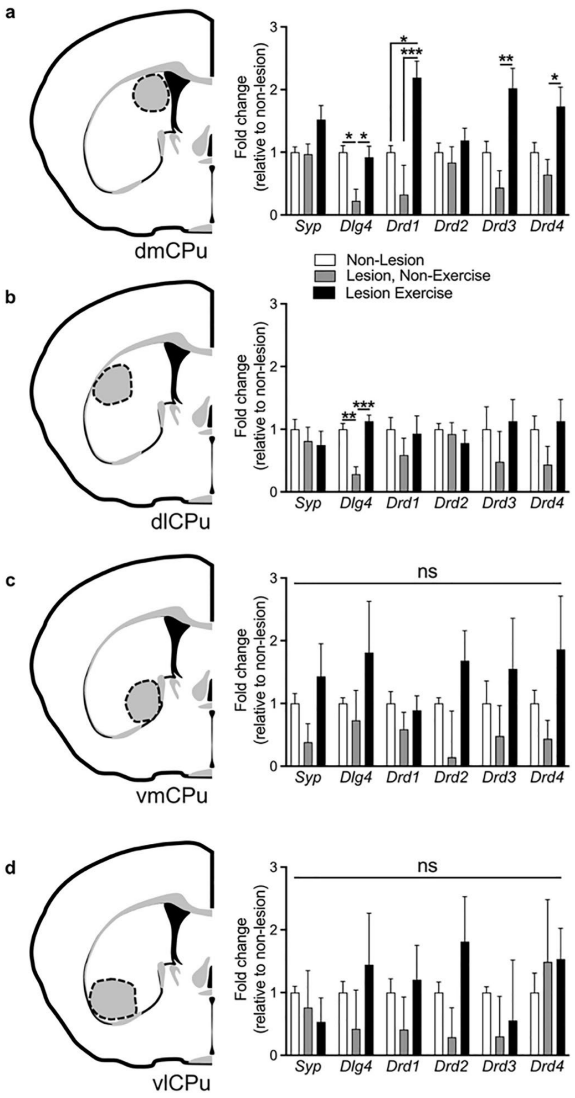
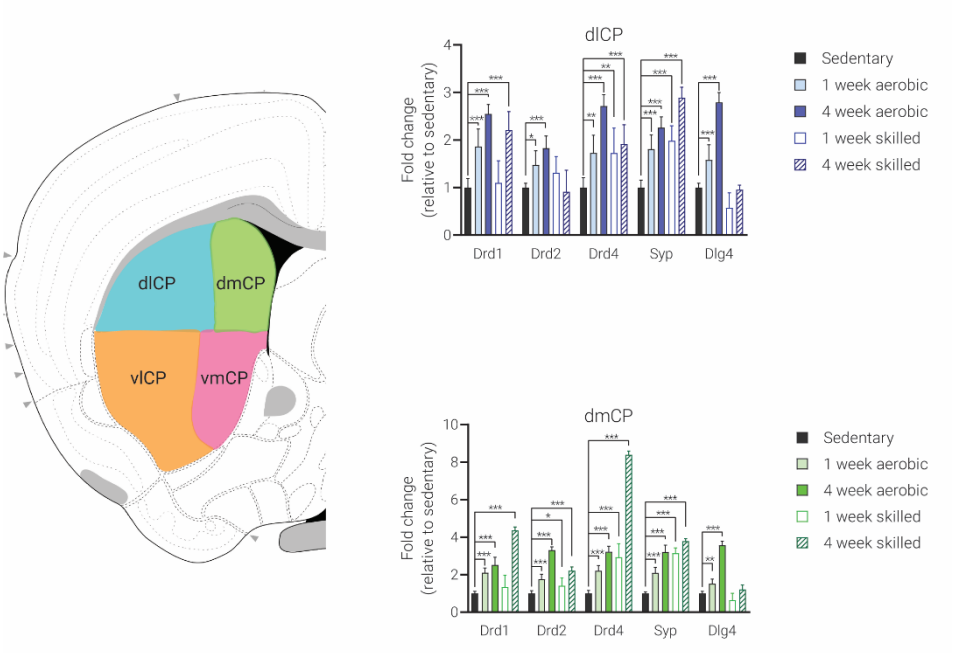


Figure 9. Lesioned rats: Dopaminergic signaling and synaptogenic gene expression changes across caudate putamen quadrants following exercise. Rat caudate putamen was divided into four quadrants for transcript analysis: **(a)** dorsomedial (dmCPu), **(b)** dorsolateral (dlCPu), **(c)** ventromedial (vmCPu), and **(d)** ventrolateral (vlCPu). Corresponding gene expression changes for four DA receptor (*Drd1, Drd2, Drd3, Drd4*) and two synaptic (*Syp, Dlg4*) genes in the exercise group (pooled complex and smooth wheel running, $n = 12$) compared to Non-Exercise controls ($n = 6$). Mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ relative to Non-Exercise, sedentary control (Student's *t*-test).

<u>Exercise type and duration: Dopaminergic signaling and synaptogenic gene expression changes in normal, non-lesioned rats across dorsal caudate putamen.</u>

Fig. 10: Nonlesioned rats: Dopaminergic signaling and synaptogenic gene expression changes across dorsal caudate putamen across exercise duration and types.
(Left) Rat caudate putamen color coded into dorsomedial (dmCP), dorsolateral (dlCP), ventromedial (vmCP), and ventrolateral (vlCP) quadrants. (Right) Corresponding gene expression changes for three dopamine receptor (*Drd1, Drd2, Drd4*) and two synaptic genes, *Syp, and Dlg4 (also known as PSD95*) for the two dorsal quadrants. $n = 6$ rats per group; mean ± SEM. One-way ANOVA with Dunnett's multiple comparisons for each gene. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ relative to sedentary non-lesioned control.



While exercise changed dopamine receptor/synaptic markers and metabolic markers to the greatest degree in the dlCPu, robust changes, particularly in dopamine receptor/synaptic markers, were noted also in the dmCPu, with no significant changes noted in the vmCP. An 'exercise dose' effect (i.e. 4 weeks > 1 week) was noted for the dopamine receptor/synaptic markers and metabolic markers in the dlCPu, but only for select plasticity markers in the dmCPu.

<u>Summary:</u> We found that dopamine depletion in the dorsal CPu was associated with loss of PSD-95 mRNA transcripts (*Dlg4)* and that exercise significantly increased PSD-95 expression in 6-OHDA lesioned rats. This finding likely reflects the increased expression of this transcript in striatal medium spiny neurons, the cells within the striatum that express this synaptic gene and proteins at post-synaptic contacts. This increase in PSD-95 is consistent with synaptogenesis and

could be occurring in either or both glutamatergic and dopaminergic terminals at medium spiny neurons. We did not observe changes in synaptophysin mRNA transcripts in striatal tissues which differs in the patterns of change in protein we and others have reported. This may reflect the fact that this protein, expressed in pre-synaptic terminals is transcribes in cell bodies that reside outside of the striatum such as the cerebral cortex or thalamus. Increases after skilled training typically were slightly larger than those after aerobic training, with the largest significant increase noted in Drd4 expression within the dmCPU after 4 weeks of skilled exercise compared to the sedentary state (>8-fold increase).

TASKS 4 & 6: Brain mapping / Data Analysis:

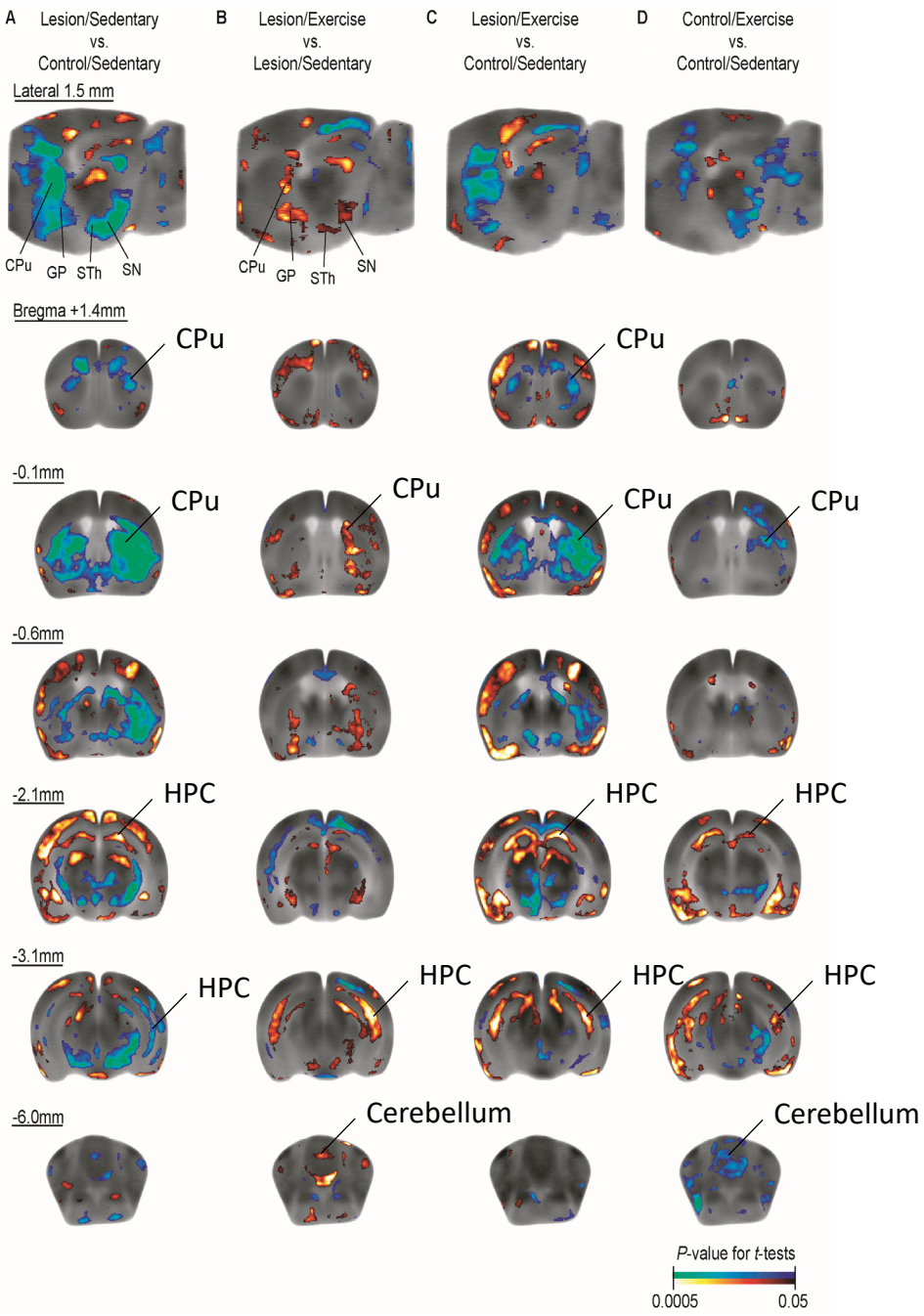
Mice were trained on a motorized treadmill for six weeks or remained sedentary (control), thereafter undergoing [^{14}C]-2-deoxyglucose metabolic brain mapping during wheel walking. Regional cerebral glucose uptake (rCGU) was analyzed in 3-dimensional brains reconstructed from autoradiographic brain sections using statistical parametric mapping. Functional connectivity was assessed by inter-regional correlation of rCGU.

To date we have focused on the analysis of the effects of exercise training on the basal ganglia-thalamo-cortical circuit (see below). The analysis is proceeding by first examining the effects of exercise in the normal animal, followed by exercise effects in the Parkinsonian animal. Analysis remains ongoing. Next, we will examine the effects of exercise on cognitive circuits.

Exercise reverses lesion-related hypometabolism in the striatum and in motor cortex

We have shown that the Parkinsonian animal (bilateral dorsomedial striatal 6-OHDA lesions) compared to normal controls shows a diminished glucose uptake in the basal ganglia-thalamo-cortical circuit (Lesion Effect). See **Fig. 11: (A)** striatum, CPu; globus pallidus, GP; substantia nigra, SN; subthalamic nucleus STh, lateral dorsal/posterior thalamus LD/LP; primary motor cortex, M1). This hypometabolism is dramatically reversed by long-term exercise training **(B)**. A similar pattern is observed in the posterior hippocampus (HPC in **Fig. 11,** bregma -3.1mm).

Fig. 11: Lesion and exercise effects on glucose uptake in the basal ganglia-thalamocortical circuit. Shown are statistically significant difference ($P<0.05$, >200 contiguous voxels) in the uptake of [^{14}C]-2-deoxyglucose during a motor learning task. Statistical parametric mapping of reconstructed whole brain autoradiograms compares **(A)** lesioned animals compared to normal controls show bilateral hypometabolism of the striatum, CPu; globus pallidus, GP; HPC hippocampus; substantia nigra, SN; subthalamic nucleus STh, lateral dorsal/posterior thalamus LD/LP; and primary motor cortex, M1, **(B)** lesioned animals after 6 weeks exercise training compared to their sedentary counterparts (n=10 per group) show a reversal of these changes in metabolism, with red/blue color indicating significant increased/decreased glucose uptake.



Exercise in the normal mouse enhances the functional connectivity in specific motor regions of the cortico-basal ganglia network.

We examined the effects of exercise on functional brain connectivity in the normal animal. Mesoscopic domain definitions were guided by the mouse brain connectome. Exercised compared to sedentary normal mice showed

(i) broad increases in intra- and inter-structural connectivity across the caudoputamen (CP), in particular the rostral and dorsal aspect, and in motor cortex,
(ii) reduced functional connectivity of the prefrontal cortex (PFC), and
(iii) newly emerged negative connectivity of the substantia nigra pars reticulata with the globus pallidus externus, and CP.

Figure 12: Connectivity degree changes in the *normal mouse* in subregions of the caudoputamen (CP) in response to exercise. **(A)** Connectivity degree of CP domains in the control (nonexercise) group is color-coded. Here 'degree' represents a measurement of the number of functional connections linking the node to other nodes in the network. **(B)** Connectivity degree of CP domains in the exercise group. **(C)** Connectivity degree changes in the exercise group compared to controls. CPr/CPi/CPc, rostral/intermediate/caudal caudoputament. Domain maps were drawn based on the mouse structural connectome [1].
1. Hintiryan, H., et al., The mouse cortico-striatal projectome. Nat Neurosci, 2016. 19(8): p. 1100-14.

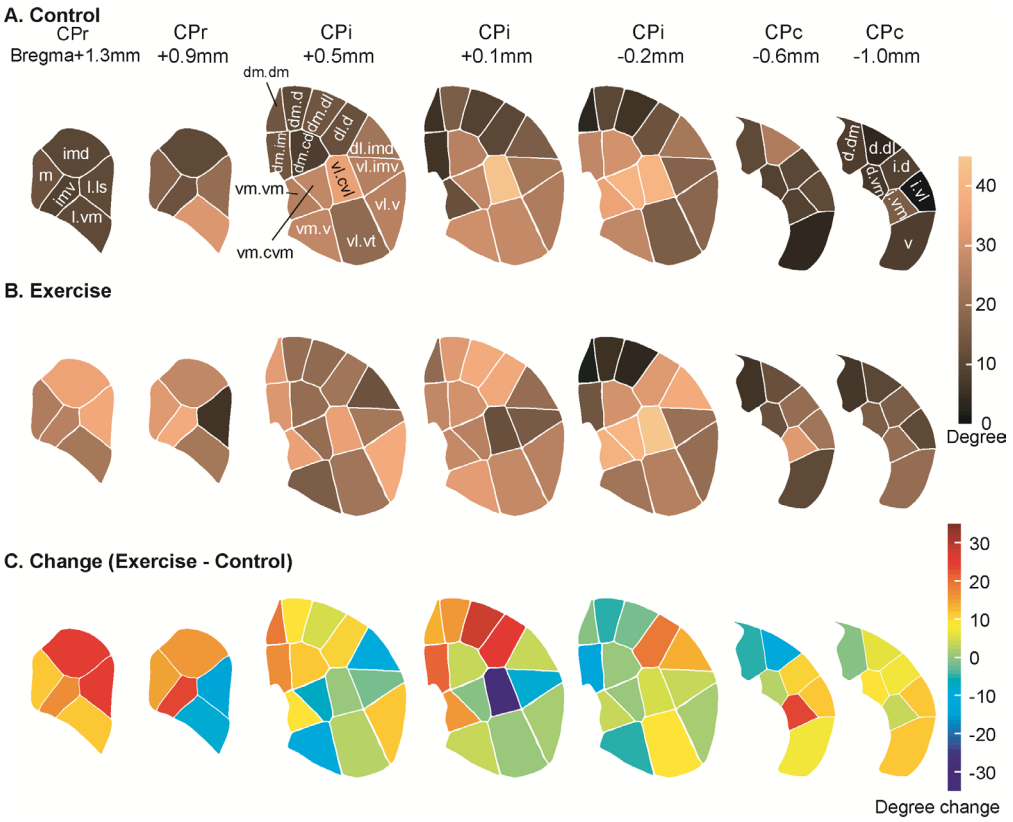
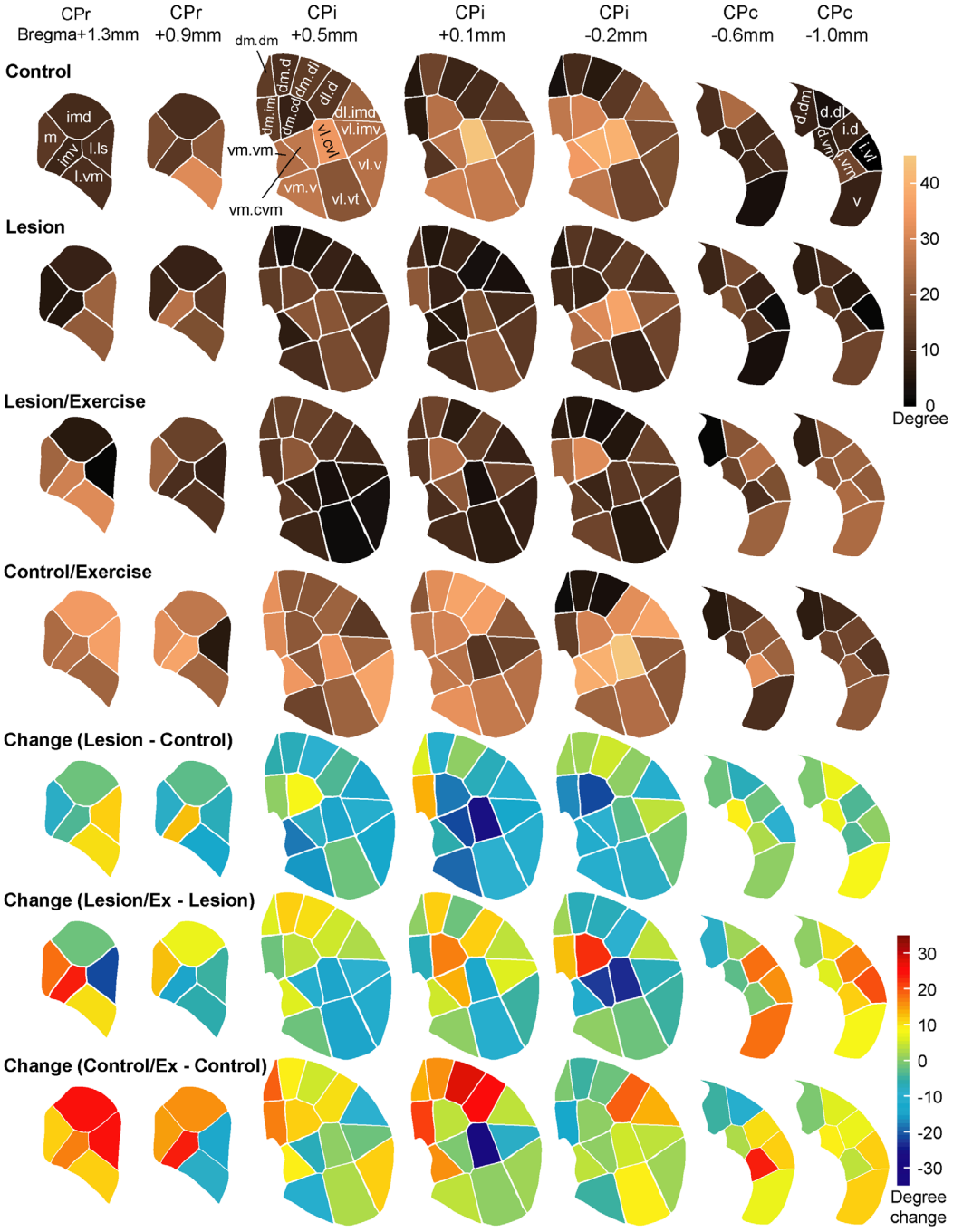


Figure 13 (below): Connectivity degree changes in the *lesioned mouse* in subregions of the caudoputamen (CP) in response to exercise. Connectivity degree of CP domains in the control (nonexercise) group is color-coded. Here 'degree' represents a measurement of the number of functional connections linking the node to other nodes in the network. 6-hydroxydopamine lesions were initiated in the bilateral CP (AP: + 1.5 mm, ML: ± 2.2 mm, DV: 5.2 mm, and AP: +

0.3 mm, ML: ± 2.8 mm, DV: 5.0 mm, relative to bregma). While lesion by itself decreases degree connectivity broadly across the CP (both dorsal and ventral regions), the return of connectivity with exercise appears to be most active in the dorsal segment. This suggests a regional specificity of the exercise effect. In addition, we saw an increased recruitment with exercise of the anteriormost and posteriormost regions of the CPu (outside the epicenter of the lesion). This suggests an overal reorganizing of the functional brain map, as well as making 'healthy brain cells work harder". Domain maps were drawn based on the mouse structural connectome as in **Fig. 12**.



NOTE: Similar maps have been generated for the external globus pallidus and substantia nigra (data not shown). Analysis of these regions and the hippocampus is ongoing.

Summary of Figs. 12 & 13 above:
Exercise alters functional connectivity with subregional specificity. While lesion by itself decreases degree connectivity broadly across the CP (both dorsal and ventral regions), the return of connectivity with exercise appears to be most active in the dorsomedial (cognitive) and dorsolateral (motor) segments. This suggests a regional specificity of the exercise effect. In addition, we saw an increased recruitment with exercise of the anteriormost and posteriormost regions of the CP, striatal regions that exhibit high integration among cortical afferents, across different cortical subnetworks, suggesting cross modality integration.

Our findings highlight exercise-induced enhancement of functional integration in specific motor circuit of the cortico-basal ganglia network, with a similar pattern in normal and Parkinsonian animals. Increased functional connectivity in the motor circuit in the absence of increases in rCGU strongly suggests greater network efficiency, which is also supported by the reduced involvement of prefrontal cortex-mediated cognitive control during the performance of a new motor task (data not shown). Our study provides a framework for understanding the effects of exercise on brain functional reorganization and motor learning, and a roadmap for further region- and pathway-specific, mechanistic investigation.

6 weeks of treadmill training increases functional connectivity between dorsal striatum and primary motor cortex (M1) in the lesioned Parkinsonian mouse.

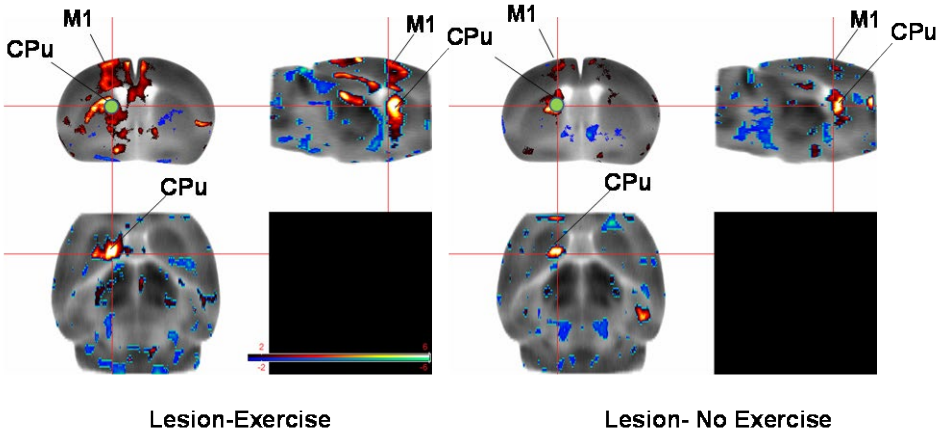


Fig. 14: 6 weeks of treadmill training increases functional metabolic connectivity between dorsal striatum (CPu) and primary motor cortex (M1) in the lesioned Parkinsonian mouse. Shown are coronal, sagittal, and transverse views of functional connectivity of a single seed region (green circle) placed into the left striatum of lesioned mice. Red/blue colors denote significant positive/negative correlations between metabolic activity of these brain regions and the seed ($P<0.05$, >200 contiguous voxels).

As noted above, we continue our analysis of the effects of exercise on the functional connectivity in the Parkinsonian animal, as well as in cognitive circuits.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

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

- Research electives for 4 undergraduate students
- Components of this project and data collection will be part of the doctoral thesis work of 4 USC doctoral students in the USC Neuroscience graduate program (A. Lundquist, I. Flores, E. Donahue, D. Phillips)

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Work from this project provided the impetus for Drs. Jakowec and Petzinger to apply and receiving university funds for an annual conference entitled "Metaplasticity and Megaplasticity: Changing the Brain from Synapse to Community" which was held at Lake Arrowhead, CA 12/6-8/2019 and in 11/18-20/2022. Participants included faculty, doctoral and undergraduate students, from USC, other California universities, as well as the East Coast.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Data analysis of the imaging data is ongoing.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Results from this project are likely to provide evidence for the benefits of exercise in the cognitive neurorehabilitation of Parkinson's patients.

Work from this project provided the impetus for Drs. Holschneider, Jakowec and Petzinger to apply 9/2022 for a grant from the Dept. of Defense for FY22 Parkinson's Research Program - Synergistic Idea Award entitled "Impact of Diet and Exercise on Cognition in Parkinson's Disease."

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Our study selected basal ganglia functional regions-of-interest (ROI) based on novel, mesoscopic domain definitions in the mouse brain structural connectome (Figs. 12 & 13). This sampling method, informed by state-of-the-art structural connectomic data, reflects a first effort to delineate functional units within subregions of the brain. This manner of a systematic selection of functional ROIs has not previously been undertaken. Mesoscopic sampling provides sufficient data simplification, while avoiding the risk of losing relevance to the interpretation of behavior through an exhaustive reductionist analysis. It represents a necessary 'next step' in moving beyond the structural connectome and into the challenge of understanding the functional significance of subregional structure. While databases such as the Allen Mouse Brain Atlas are beginning to describe regional gene expression within brain subregions, our work begins to implement this approach for an application examining changes in regional brain metabolism and brain activation. This approach is likely to make an impact into the broader fields of functional brain mapping in animal models.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Community lectures by our extended Parkinson's Research group is raising awareness of the benefits of daily exercise training in the management and treatment of, not only the motor deficits, but also cognitive impairment characteristic of Parkinson's Disease.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to report

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Impact of COVID-19: During the height of the pandemic the laboratory was shut-down as per the university's mandate. All animal colonies were mandated by the university to be euthanized to minimize need for vivarium staff to care for them. This included animals in the pipeline for our long-term, ongoing studies to evaluate the behavioral, molecular, imaging, electrophysiologic outcomes of chronic exercise using both skilled and nonskilled training. While a portion of brain tissue was harvested according to our prescribed experimental protocols, other had to be prematurely harvested, or could not be used (e.g. rats intended for functional brain mapping or awaiting lesioning). Labs were permitted to reopen at 50% capacity on 8/31/2020, and at 100% occupancy by 3/29/21. The delay resulting from the pandemic initiated the request for the 1st no-cost extension (9/12/20-9/14/21), as well as the 2nd no-cost extension (9/15/21 – 9/14/22).

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

N/A

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:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

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

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title;*

journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

- Holschneider DP, Wang, Z, Guo Y, Sanford MT, Yeh JC, Mao JJ, Zhang R, Rodriguez LV "Exercise modulates neuronal activation in the micturition circuit of chronically stressed rats: A multidisciplinary approach to the study of urologic chronic pelvic pain syndrome (MAPP) research network study", Physiology & Behavior, 2019, Dec 27;215:112796. doi: 10.1016/j.physbeh.2019.112796. *Acknowledgement of federal support (yes).*
- Sanford MT, Yeh JC, Mao JJ, Guo Y, Wang Z, Zhang R, Holschneider DP, Rodriguez LV "Voluntary exercise improves voiding function and bladder hyperalgesia in an animal model of stress-induced visceral hypersensitivity: a multidisciplinary approach to the study of urologic chronic pelvic pain syndrome (MAPP) research network study", *Neurourology & Urodynamics, 2020, Jan 13. doi: 10.1002/nau.24270. Acknowledgement of federal support (no).*
- Caldwell CC, Petzinger GM, Jakowec MW, Cadenas E, "Treadmill exercise rescues mitochondrial function and motor behavior in the CAG140 knock-in mouse model of Huntington's disease", *Chemico-Biological* Interactions, 315 108907, 2020, doi: 10.1016/j.cbi.2019.108907. *Acknowledgement of federal support (yes).*
- Wang Z, Flores I, Donahue E, Lundquist A, Guo Y, Jakowec MW, Holschneider DP, "Cognitive Flexibility Deficits in Rats with Dorsomedial Striatal 6-OHDA Lesions Tested Using a 3-Choice Serial Reaction Time Task with Reversal Learning", *NeuroReport, 31(15):1055-1064, 2020. Acknowledgement of federal support (yes).*
- Petkus AJ, Jarrahi B, Holschneider DP, Gomez ME, Filoteo V, Schiehser DM, Fisher BE, van Horn JD, Jakowec MW, McEwen S, Petzinger GM
  "Thalamic volume mediates links between cardiorespiratory fitness (VO2max) and cognition in Parkinson's Disease", *Parkinsonism and Related Disorders*, 2021 Mar 29;86:19-26. doi: 10.1016/j.parkreldis.2021.03.019. *Acknowledgement of federal support (yes).*
- Jarrahi B, McEwen S, Holschneider DP, Schiehser D, Petkus A, van Horne JD, Filoteo V, Jakowec MW, Petzinger GM
  "The Effects of Cardiovascular and Motor Skill Fitness on Intrinsic Functional Connectivity of Neural Networks in Patients with Parkinson's disease", *Brain Plasticity*, 2021 Oct 19;7(2):77-95. doi: 10.3233/BPL-200115. *Acknowledgement of federal support (yes).*
- Wang Z, Lundquist AJ, Donahue E, Guo Y, Phillips D, Petzinger GM, Jakowec MW, Holschneider DP
  "A mind in motion: Exercise improves cognitive flexibility, impulsivity and alters dopamine receptor gene expression in a Parkinsonian rat model", Current Research in Neurobiology, 3, 100039, 2022. doi.org/10.1016/j.crneur.2022.100039. *Acknowledgement of federal support (yes).*
- Donahue EK, Venkadesh S, Bui V, Tuazon AC, Foreman R, Wang R, Haase D, Foreman RP, Duran J, Petkus A, Wing D, Higgins M, Holschneider DP, Bayram E, Litvan I, Jakowec MW, Van Horn JD, Schiehser D, Petzinger GM.

"Physical activity intensity is associated with cognition and functional connectivity in Parkinson's disease", Parkinsonism and Related Disorders, 104, 7-14, 2022. *Acknowledgement of federal support (yes).*

- Wang Z, Donahue E, Guo Y, Rentel M, Petzinger GM, Jakowec MW, Holschneider DP, "Exercise alters cortico-basal ganglia network functional connectivity: A mesoscopic level functional analysis informed by anatomic parcellation defined in the mouse brain connectome", in review. *Acknowledgement of federal support (yes).*

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

- Lundquist, A.J., Gallagher, T.G., Petzinger, G.M., Jakowec, M.W., "Lactate administration recapitulates the astrocyte-specific neuroplastic effects of exercise", Abstract #204.12; Annual meeting of the Society for Neuroscience, Chicago, IL, 10/19/2019
- Rodriguez LV, Stephens A, Kutch J, Appelby DH, Newcomb C, Landis R, Afari N, Holschneider DP. "The Association of Physical Activity and Lower Urinary and Pain Symptoms and Improved QoL in Individuals with Urologic Chronic Pelvic Pain Syndrome is Mediated by Depression, Nociceptive and a Peripheral Phenotype: Findings from Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network", Annual meeting, American Urologic Association, Washington DC, 5/15-19/2020
- Rodriguez LV, Stephens-Shields A, Rude T, Kutch J, Appelby DH, Newcomb C, Afari N, Holschneider DP, "The Association of Physical Activity, Urinary and Pain Sumptoms and QoL in individuals with Urologic Chronic Pelvic Pain Syndrome (UCPPS) is Moderated by Depression, Nociceptive and a Peripheral Phenotype: Findings from Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network", Annual meeting, American Urologic Association, Las Vegas, NV 9/10-13/2021

- *Sanford MT, Yeh JC, Mao JJ, Zhang R, Wang Z, Holschneider DP, Rodriguez LV, "The effect of exercise on an animal model of stress-induced bladder hyperalgesia", AUA, Chicago, IL, 5/2019.
- *Sanford MT, Yeh JC, Mao JJ, Zhang R, Wang Z, Holschneider DP, Rodriguez LV, "The effect of exercise on an animal model of stress-induced bladder hyperalgesia", Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction (SUFU), Miami, FL, 3/2019. 3rd place award for best basic science abstract.
- *Sanford M, Yeh JC, Mao J, Zhao R, Wang Z, Holschneider DP, Rodriguez LV. "Voluntary exercise improves voiding function and bladder hyperalgesia in an animal model of stress-induced visceral hypersensitivity." Journal of Urology, Vol. 201, No. 4S, Supplement:e887. 4April 2019. https://doi.org/10.1097/01.JU.0000556826.86596.39

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*

- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

For the period 9/15/2021 – 9/14/2022 (2nd no-cost extension)

Name: Daniel P. Holschneider, MD
Project Role: partnering PI (with Dr. Giselle Petzinger, award # W81XWH-18-1-0665)
Research Identifier: N/A
Nearest person month worked: 0.6 cal. month (DoD funds), 0.4 mo (nonDoD funds)
Contribution to the project: No change. Project design. Currently engaged in drafting of manuscripts.

Name: Michael Jakowec, Ph.D.
Project Role: co-I
Research Identifier: N/A
Nearest person month worked: 1.0 mo (non-DoD funds)

Contribution to the project: No change. Project design, aiding with the Western blotting, qRT-PCR and HPLC.

Name: Zhuo Wang, Ph.D.
Project Role: co-I
Research Identifier: N/A
Nearest person month worked: 4 cal. months
Contribution to the project: No change. Functional brain mapping, animal behavior, data analysis, manuscript writing.

Name: Adam Lundquist, BS
Project Role: Graduate Student
Research Identifier: N/A
Nearest person month worked: 0.5 mo (non-DoD funds)
Contribution to the project: Western blotting, qRT-PCR, brain dissection

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)

Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

See attached

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

- Wang Z, Flores I, Donahue E, Lundquist A, Guo Y, Jakowec MW, Holschneider DP, "Cognitive Flexibility Deficits in Rats with Dorsomedial Striatal 6-OHDA Lesions Tested Using a 3-Choice Serial Reaction Time Task with Reversal Learning", *NeuroReport, 31(15):1055-1064, 2020*
- Wang Z, Lundquist AJ, Donahue E, Guo Y, Phillips D, Petzinger GM, Jakowec MW, Holschneider DP
  "A mind in motion: Exercise improves cognitive flexibility, impulsivity and alters dopamine receptor gene expression in a Parkinsonian rat model", Current Research in Neurobiology, 3, 100039, 2022. doi.org/10.1016/j.crneur.2022.100039.
- Wang Z, Donahue E, Guo Y, Rentel M, Petzinger GM, Jakowec MW, Holschneider DP, "Exercise alters cortico-basal ganglia network functional connectivity: A mesoscopic level functional analysis informed by anatomic parcellation defined in the mouse brain connectome", in review.