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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 (year 1 of 2) has applied methods of animal behavior, immunohistochemistry, molecular biology, functional brain mapping, voltammetry, 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

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1. INTRODUCTION:

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 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. Animal studies have been critical for providing evidence for exercise-induced neuroplasticity of corticostriatal circuits that are profoundly affected in PD. Work by our laboratory in a rat model of basal ganglia injury has provided evidence that SAE compared to simple aerobic exercise (AE) results in a differential enhancement of prefrontal cortex-mediated control of motor and possibly cognitive function. Using a rat model (striatal 6-hydroxydopamine model), the current study applies methods of animal behavior, immunohistochemistry, molecular biology, functional brain mapping, voltammetry, and micro-neuroanatomy, to the question of exercise-related restoration of cognitive function and the role of frontostriatal circuits. In the first year of this project, the following has been achieved (a) IACUC and ACURO approval; (b) Executive board meeting; (c) Equipment purchase and/or set up; (d) Establishment of standard operating procedures for all surgical, behavioral, molecular, imaging, electrophysiologic and neuroanatomic protocols; (e) Data collection and data analysis (behavioral, molecular, immunohistochemical, neuroanatomic). Analysis of data collected to date has shown that dorsomedial bilateral lesions of the striatum, while they do not alter general motor function or appetitive behavior, clearly impair learning of two separate matching-to-sample tasks (3-Choice serial reaction time task, T-maze task), with additional impairment noted during rule reversal. Further analysis of the operant serial reaction time task shows impairment in attention, processing speed, working memory, mental flexibility, as well as impulsivity – all components of executive function. Early data in a small number of animals, suggests that skilled exercise training results in a gradual and progressive, albeit small improvement in executive function. We are in the process of initiating a series of brain mapping studies to examine if the effects of lesioning and of exercise, respectively impair and improve engagement of the prefrontal-striatal circuit that underlies executive function during performance of the operant task. Results of the molecular analysis suggest a differential and dynamic effect of exercise across the striatal subsectors. Thus, while 4 weeks of exercise elicits significant changes in dopaminergic markers, plasticity markers, and metabolic markers in the dorsolateral striatum, changes in the dorsomedial striatum are largely in dopaminergic and synaptic markers. In the ongoing 2nd year of this project, we plan to (a) continue to add animals, (b) complete the functional brain mapping of the effects of lesions and exercise on the prefrontal-striatal circuit, (c) and to address the question of a possible differential effect of skilled versus simple aerobic exercise on cognitive, molecular and physiologic (including electrophysiologic) outcomes. 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.

2. KEYWORDS:

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

3. ACCOMPLISHMENTS:

What were the major goals of the project?

MAJOR GOALS PROJECT 2 (preclinical project from SOW months 0-12)

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?

Overview:

- Executive board meeting
- Establishing the lesion model
- Verifying the lesions: Tyrosine hydroxylase and Golgi staining
- Demonstrating absence of significant motor changes in lesioned animals
- Demonstrating absence of appetitive changes in lesioned animals
- Exercising of animals for behavioral studies
- Evaluation of cognitive function: T-test
- Evaluation of cognitive function: Operant testing
- Molecular studies and exercise paradigm

TASK 1: Executive board meeting: Completed 4/24/19

TASK 2: Given the lower priority of this task. Completion of tasks 3-6 is being prioritized, with plan to address task 2 at the end.

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

Dorsomedial striatal lesioning & lesion verification:

Methods:

Lesioning: The 6-OHDA basal ganglia injury model is a widely accepted model of dopaminergic deafferentation, and while not identical to PD, parallels the human disorder remarkably well [1]. To prevent any noradrenergic effects of the toxin, adult Wistar rats received desipramine before the start of surgery [2]. They were then placed under isoflurane anesthesia in a stereotaxic apparatus (David KOPF Instruments) and received injection of 6-OHDA.HCl (10 ug in 2µl, 1% ascorbic acid, Sigma-Aldrich Co.) at each of four injection sites targeting the dorsomedial striatum bilaterally (AP: +1.4, L: ±2.2, V: -5.2 mm, and AP: +0.2, L: ±2.8, V: -5.0 mm, relative to the bregma), which is the primary striatal sector targeted by the mPFC [3]. Such focal lesions result in minimal to modest motor changes, while demonstrating cognitive deficits characteristic of PD [4], and which have been proposed to offer a model for studying cognitive symptoms.

Immunostaining for tyrosine hydroxylase (TH), the rate limiting step in dopamine biosynthesis, was used to identify the site of the lesion and to determine its anatomical correlates to ensure specificity of the targeting. Rats were humanely anesthetized and subjected to transcardial perfusion and brain fixation. TH-immunostaining was performed in 25 µm thick coronal slices across the full extent of the striatum and substantia nigra using standard methods [5]. The volume of the striatum, substantia nigra compacta, substantia nigra reticulata, and cerebral hemisphere were defined bilaterally in the digitized, thresholded images of each rat by manual tracing using ImageJ 1.52k (Wayne Rasband, NIH) (striatum: AP+2.04 mm, +0.72 mm, 0.00 mm; substantia nigra: AP-4.68 mm, -5.76 mm, -6.48 mm).

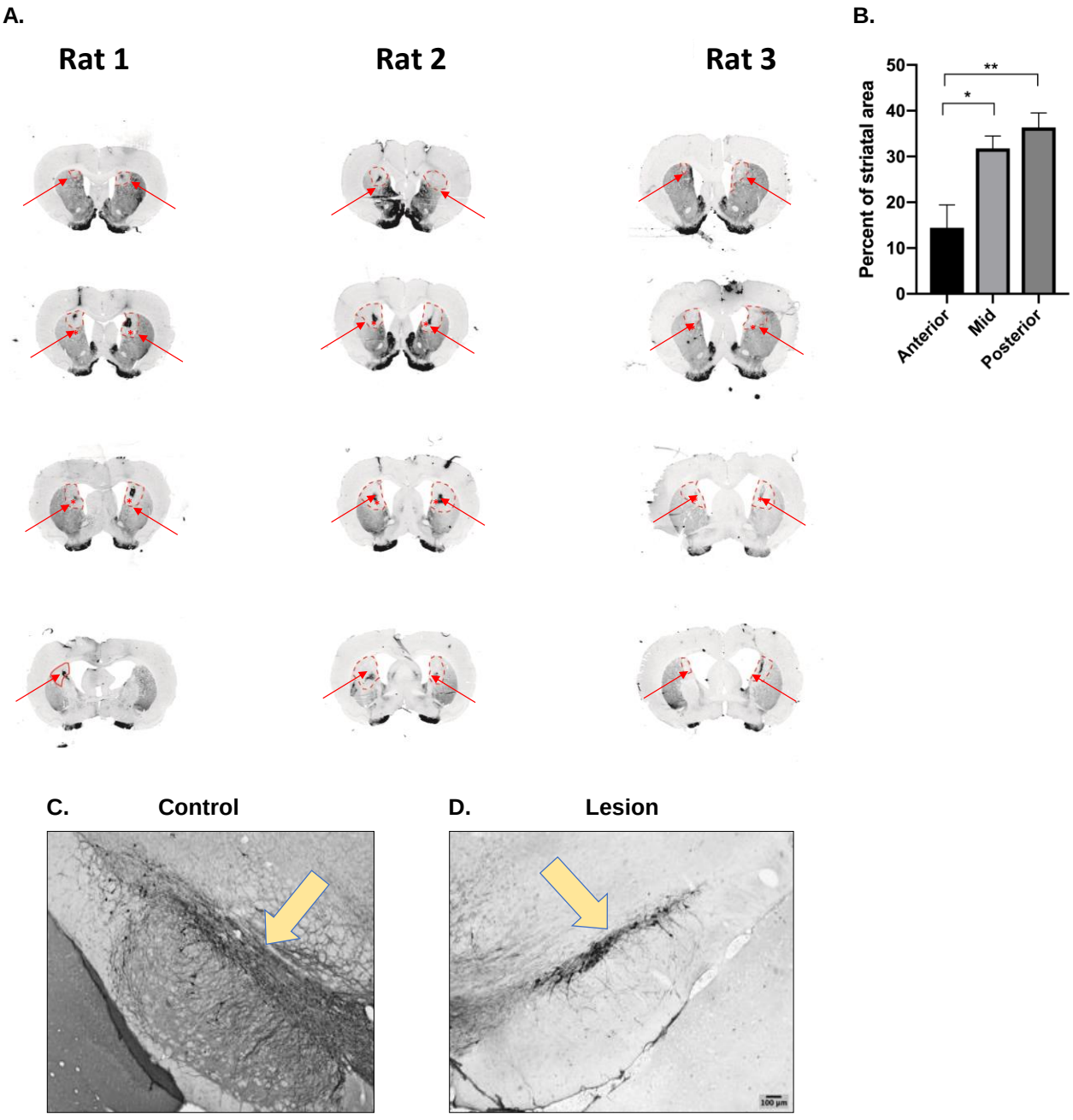
Golgi-Cox staining (PK401, FD NeuroTechnologies) was used to visualize neurons. Following the manufacturer's instructions, briefly, brains were collected fresh, impregnated for 2 weeks for processing, sectioned in the coronal orientation at 80µm thickness, and mounted on

gelatin-subbed slides. Sections were developed and dehydrated to process the contrast stain. Selection of cells for analysis include from both sham control of 6-OHDA rats subjected to skilled and aerobic exercise with studies focusing on the PFC, quadrants of the striatum, and ectorhinal cortex as a control region. Other areas for analysis will include the hippocampus. Analysis was carried out using the Bioquant Imaging system as we have previously published [6]. Changes in synaptic connectivity were evaluated based on both dendritic spine density and dendritic branching (Sholl analysis) of selective neurons.

Results:

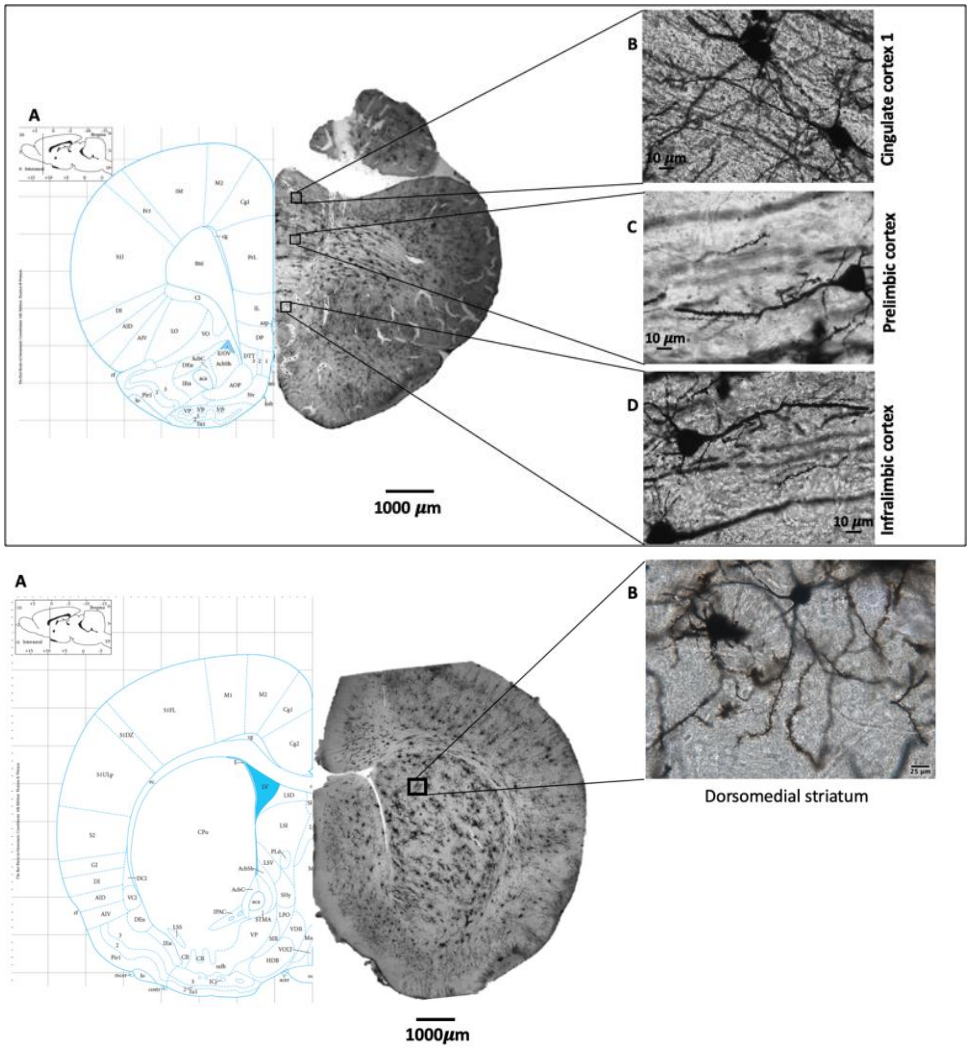
TH immunostaining. **Fig. 1A** shows the histological confirmation of lesion size after TH positive dopaminergic neurons in the anterior, mid, and posterior regions of the striatum. The loss of TH immunoreactivity was expressed as a percentage of total striatal area (**Fig.1B**). A one-way ANOVA revealed a statistically significant difference between the striatal regions ($F(2,12) = 9.401$, $p<0.01$). Post hoc analyses indicated that the mean lesion area of the anterior regions (14.42 ± 5.01/percent of striatal area ± SEM) differed significantly from that of both the lesion area in the mid (31.75 ± 2.74, $p<0.05$) and posterior (36.36 ± 3.18, $p<0.01$) regions of the striatum. **Fig. 1D** shows the extent of nigral TH+ cell loss induced by 6-OHDA of the dorsomedial striatum. Ongoing studies will use unbiased stereology to quantify degree of lesion at the anterior, mid and posterior levels of the substantia nigra.

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.



Golgi-Cox staining: We provide representative images of ongoing studies to demonstrate feasibility and progress. **Fig. 2** shows Golgi impregnation in representative coronal sections at the level of the PFC and mid-striatum. We have collected N = 6 brains from each group (Sham, Sham + aerobic exercise, Sham + skilled exercise, lesioned sedentary, lesion + aerobic exercise, and lesioned + skilled exercise). Brains have been processed for Golgi impregnation, mounted on slides, and are in the process of determination of dendritic spine density and dendritic arborization (Sholl analysis) in neurons at the level of the PFC and quadrants of the striatum. These studies are conducted in a blinded fashion. These findings of neuronal morphology will be correlated with additional molecular analysis of gene and protein expression, as well as changes in metabolism.

Figure 2: *Representative Golgi impregnations sections at the level of the prefrontal cortex (PFC) and mid-striatum.* The upper panel A shows a representative coronal section of the inclusive of the PFC (cingulate, prelimbic and infralimbic cortices) processed using the Golgi impregnation. Dark regions within the image represent individual stained neurons. The three regions of the PFC are outlined and are shown in higher magnification (B, C, D). The panels to the right show high magnification of individual neurons. The lower panel shows a similar stain but at the level of the mid-striatum. A high magnification shown in panel B demonstrates an individual neuron highlighting its dendritic arbor and visible dendritic spines.



No lesion effect on Rotarod performance: <u>*Methods*</u>: Rats were lesioned and after lesion maturation motor function was examined on the Rotarod. Rats were run using an acceleration paradigm (initial speed: 5 rpm = 1.15 m/min, acceleration rate: 6 rpm/min = 1.38 m/min^2, 2 trials/day, 30-min intertrial interval, x2 days) until they fell onto a padded surface or reached the 5 min cutoff time (maximum speed: 35 rpm = 8.02 m/min). The outcome variable was the latency to fall. <u>*Results:*</u> No significant difference was observed between lesioned and non-lesioned rats in the latency to fall from the Rotarod (**Fig. 3**). <u>*Conclusion*</u>: These results suggest that lesions do not affect motor strength, balance and coordination.

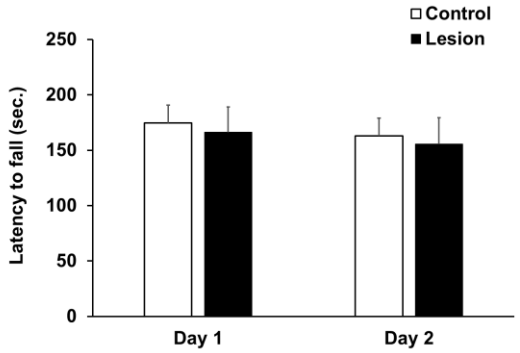


Figure 3*: Performance on the Rotarod:* Rats were exposed to the accelerating rotarod on sequential days. No significant difference in group mean latency to fall (+/- S.E.M.) was observed for animals (non-exercised) two weeks after striatal lesioning (n=8) and for controls (n=9).

No lesion effect on overnight home cage activity: *Methods*: Home cage activity was examined by infrared beam break overnight over 12 hours. Activity was examined at baseline and two weeks after bilateral lesioning of the dorsomedial striatum. Rats were run using an acceleration paradigm (initial speed: 5 rpm = 1.15 m/min, acceleration rate: 6 rpm/min = 1.38 m/min^2, 2 trials/day, 30-min intertrial interval, x2 days) until they fell onto a padded surface or reached the 5 min cutoff time (maximum speed: 35 rpm = 8.02 m/min). The outcome variable was the latency to fall. *Results:* Overnight activity counts in the animal's home cage did not differ from baseline when assessed two weeks after brain lesioning. No significant differences were noted for movements within the horizontal plane, ambulatory activity or rearing (**Fig. 4)**. There was no significant effect of lesioning on the group average maximum velocity during any 15-minute overnight interval (data not shown). *Conclusion*: These results suggest that lesions do not affect general motor activity.

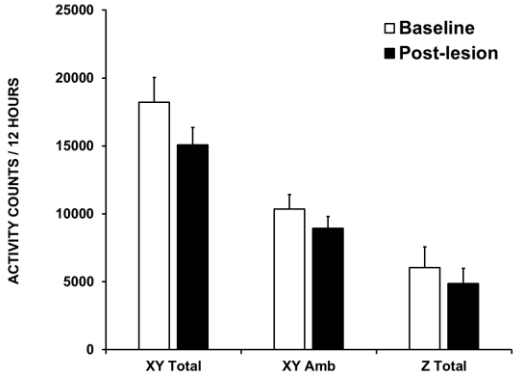


Figure 4*: Home cage overnight locomotor activity:* No significant group differences were observed in the number of overnight (6 a.m. – 6 p.m.) infrared beam break at baseline compared to those at two weeks following striatal lesioning. No difference was noted in the horizontal (xy) plane (total counts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

No lesion effect on sucrose preference: *Methods:* Sucrose preference was examined two weeks after bilateral lesioning of the dorsomedial striatum, as well as in control (non-lesioned) animals. Rats were water-deprived for 12 hours. Thereafter they were individually housed for 1 hour with access to 2 bottles, one containing 2% sucrose and the other with water, with location of each bottle randomized to the left or right side of the cage. Fluid consumption was measured. *Results:* see **Fig. 5.**

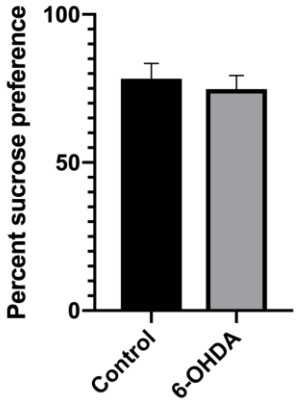


***Figure 5**: Sucrose Preference Test to evaluate anhedonia:* Shown is the percentage of the volume of sucrose intake over the total volume of fluid intake. There was no significant difference between the control (sham lesion) group and the 6-OHDA-lesion group ($t(12)=1.351$, $p=0.202$). Data are expressed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

Conclusion: These results suggest that there is no significant appetitive difference for sucrose consumption, which paves the way for the way for behavioral studies using sucrose pellet reward.

Exercise training in motorized running wheels (for behavioral studies): *Methods:* Rats with and without dorsomedial striatal lesions were trained on a complex wheel with irregularly spaced rungs (skilled aerobic exercise, SAE). These irregular wheels demand the constant adaptation of stride length. Animals were trained in a running wheel for 20 min/day (4 trials, 5 min/trial, 2-min inter-trial interval), 5 consecutive days/week for 4 weeks. No-exercise control animals were handled and left in a stationary running wheel for 30 min/day. *Results:* See figure below.

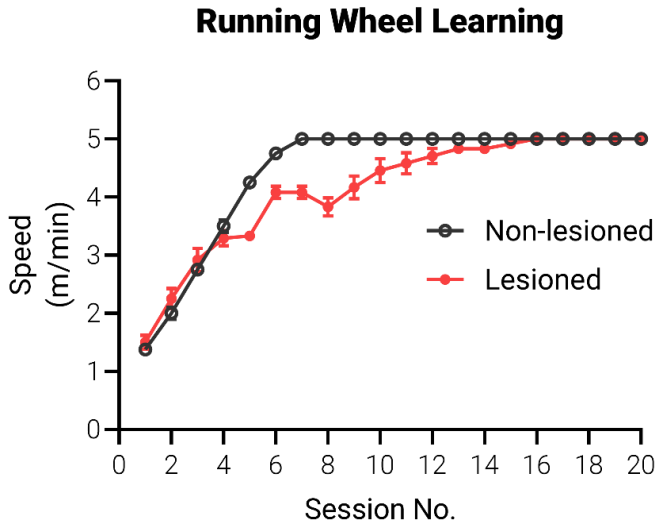


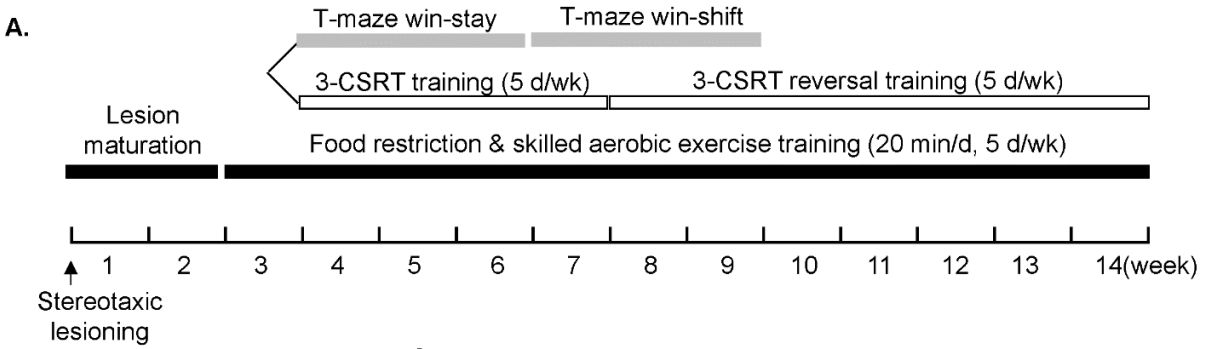
***Figure 6**: Acquisition of maximum running speed in the skilled running wheel exercise paradigm in non-lesioned and 6-OHDA lesioned rats.* Motor learning of the skilled motorized running wheel shown as average speed (in meters/min) over the initial four weeks of exercise (five sessions per week). Rats with a 6-OHDA induced lesion in the dmCP take longer to reach the plateau of running speed.

Experimental protocol for the 3-Choice continuous performance task (3C-CPT):

Methods: Rats were lesioned and 2 weeks after lesion maturation cognition was examined using the 3-choice continuous performance task (3C-CPT). Rats were food restricted to 85% of their free-feeding weight. Operant cages (MedAssociates) were comprised of a sound attenuating

cubicle with fan, modular test chamber with grid floor, house light, 3 bay nose poke wall, pellet dispenser pellet trough receptacle, receptacle light, head entry detector, smart controller, and infrared camera for real-time viewing of animals on a TV monitor. Cages were operated by MED-PC software using a personal computer. Pellet dispensers were loaded with dustless sucrose pellets. During the initial phase of 3-CSRT training, the animal was trained following a progressive schedule. Behavior was shaped in a fixed ratio 1 schedule response-reward task in which nose poke to a lit aperture resulted in a reward. During the reversal phase of 3-CSRT training (Fig. 1B, C), the stimulus was switched from a lit aperture among dark apertures to a dark aperture among lit apertures. The animal was trained progressively to learn to nose poke the dark aperture to receive reward. *Results:* see below

Figure 7*: Experimental protocol for operant training.* A. Timeline of experiment. B. 3-CSRT-reversal task. C. Progressive training schedule.

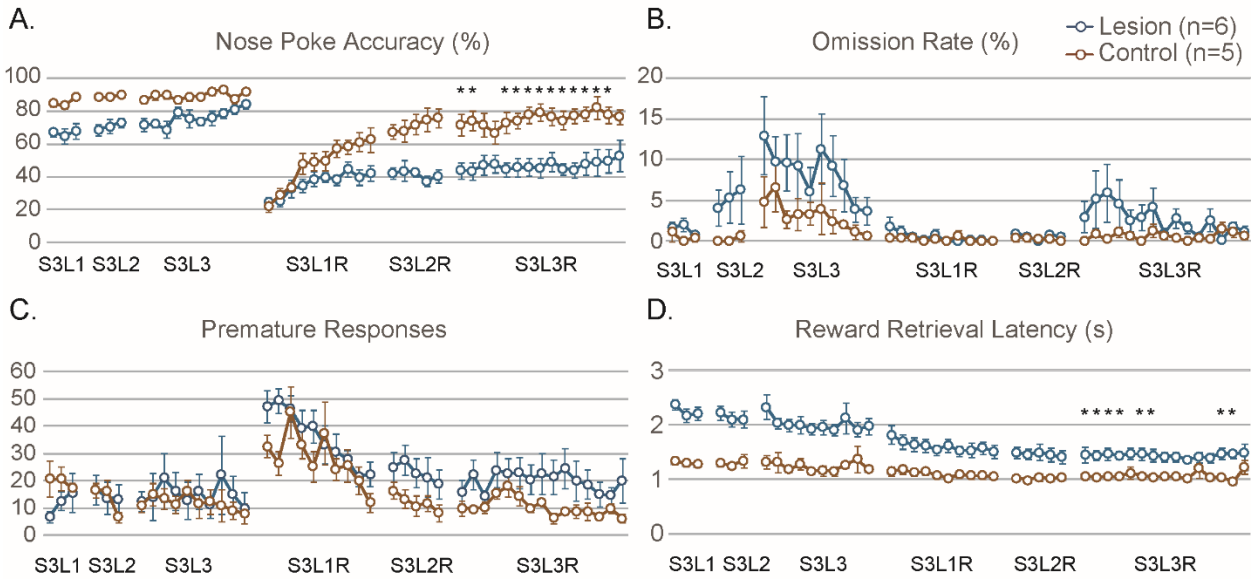


C.

3-CSRT	Stim Dur (s)	Limited Hold (s)	ITI (s)	TO (s)	Days
Stage 1a,b	n/a	n/a	n/a	n/a	1 (twice)
Stage 2	∞	∞	2	n/a	2
Stage 3 Level 1	30	30	2	2	3
Stage 3 Level 2	15	15	2	2	3
Stage 3 Level 3	5	10	2	2	10
3-CSRT Reversal					
Stage 1aR	n/a	n/a	n/a	n/a	1 (twice)
Stage 2R	∞	∞	2	n/a	5
Stage 3 Level 1R	30	30	2	2	10
Stage 3 Level 2R	15	15	2	2	5
Stage 3 Level 3R	5	10	2	2	15

Lesioned animals were able to learn regular 3-CSRT task while showing higher omission rate and reward retrieval latency compared to controls (**Fig. 8**, phase S3L1-S3L3). During the reversal phase (**Fig. 8**, S3L1R-S3L3R), lesioned animals showed statistically significant learning deficits in lower nose poke accuracy (**Fig. 8A**, 2-way ANOVA with repeated measure, P = 0.005) and higher reward retrieval latency (**Fig. 8D**, P = 0.007), as well as trend of higher omission rate (**Fig. 8B**, P = 0.2) and higher premature responses (**Fig. 8C**, P = 0.07).

Figure 8: *Deficits in 3-CSRT reversal learning in 6-OHDA lesioned animals (n = 6) compared to controls (n = 5)* in (A) Nose poke accuracy, (B) omission rate, (C) premature responses, and (D) reward retrieval latency. *: $P < 0.05$, Sidak post hoc test.



Ongoing experiments examined the effect of skilled aerobic exercise on reversal learning deficit in 6-OHDA lesioned animals. **Fig. 9** shows trend of increased nose poke accuracy and decreased premature responses in exercised compared to non-exercised lesioned animals in reversal learning phase. Sample size will be increased to achieve sufficient power.

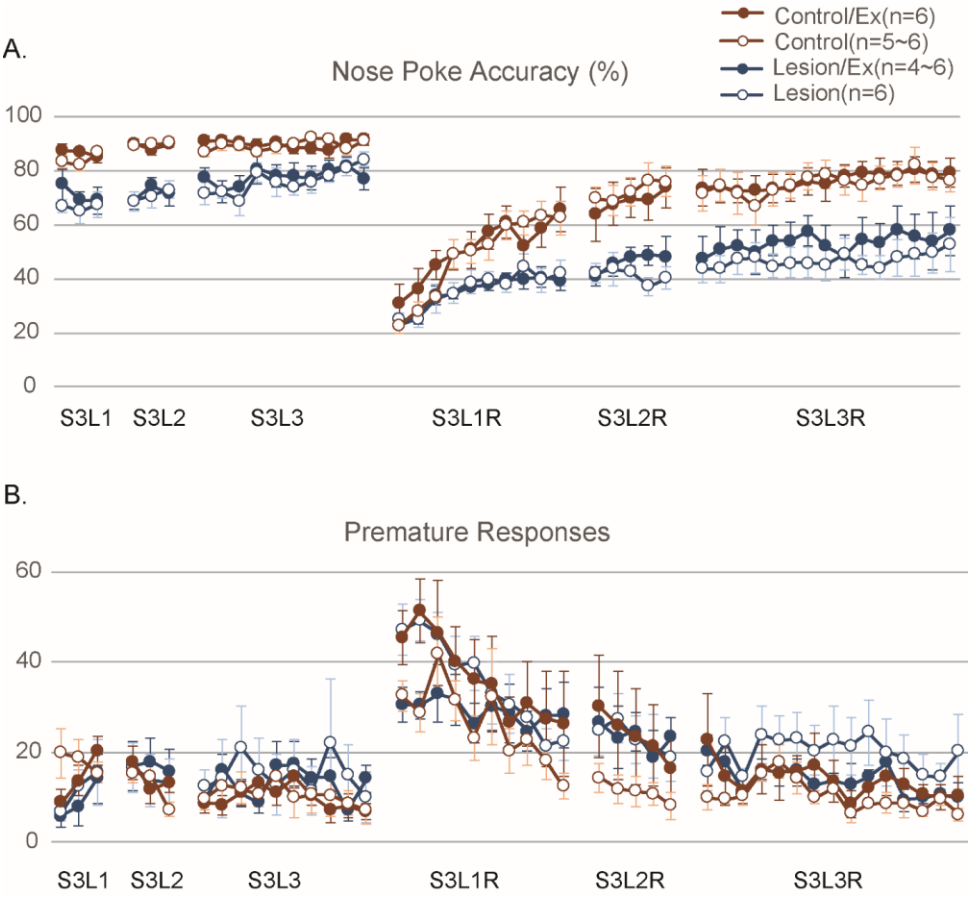
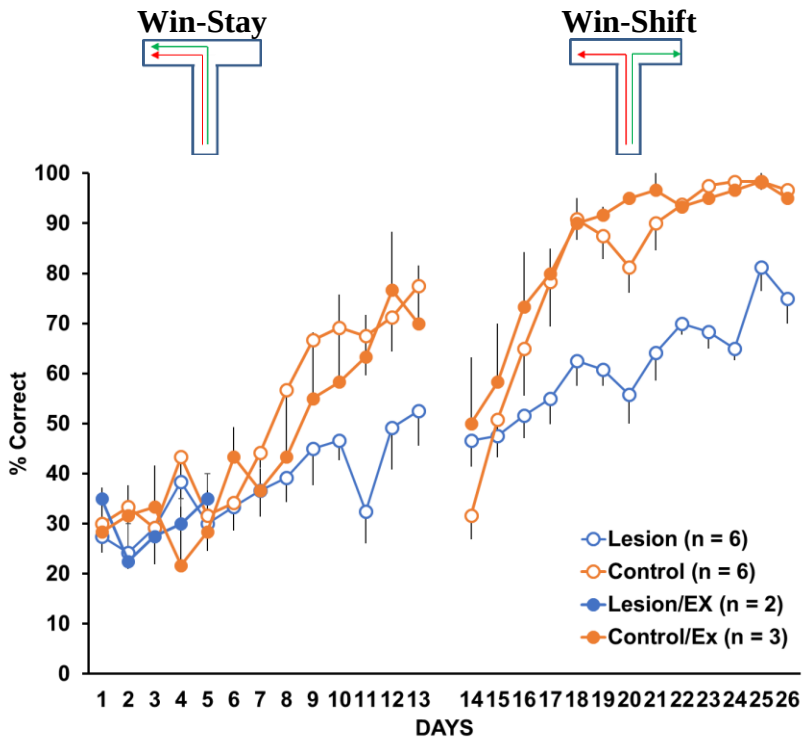


Figure 9: *Effect of skilled aerobic exercise on reversal learning in 6-OHDA lesioned animals.* Preliminary data showed trend of increased nose poke accuracy (A) and decreased premature responses (B) in exercised compared to non-exercised lesioned animals.

T-maze with rewarded matching-to-sample and reversal was used to assess cognition. *Methods:* Rats were lesioned and 2 weeks after lesion maturation cognition was examined using the T-maze. Rats were food restricted to 85% of their free-feeding weight, thereafter they were habituated to the maze and then trained with for 3 days, 10 trials (5 sec. intertrial interval) in the morning and again in the afternoon using a sucrose pellet reward. Thereafter, the rat was trained in a 'Win-Win' paradigm (10 sample run→choice trials twice per day, 5 seconds intertrial interval, x 13days), in which animals had to choose the same arm during a choice trial (both arms open) that had previously been rewarded on the preceding sample trial (one arm closed). Sample trials were randomized across both arms. Thereafter, rats were exposed to a 'Win-Shift' strategy, in which the rat was only rewarded in the choice run if it entered the branch arm opposite the one chosen in the sample run (10 sample run→choice trials twice per day, 5 seconds intertrial interval, x 13 days). Number of correct entries into the bated choice arm were recorded for each trial. Performance was expressed as a percentage of correct choices made in each session. *Results*: T-maze detected a clear significant lesion effect ($P < 0.05$), both during the Win-Stay strategy and its reversal as Win-Shift. Data collection is ongoing to increase the number of animals in the Control-exercise and Lesion-exercise groups.

Figure 10: T-maze with rewarded matching-to-sample and reversal: Rats were trained in a Win-Stay strategy (red/green), followed after 13 days by a Win-Shift (red/green) strategy for an additional 13 days. Results are shown for non-exercised rats with and without lesions, and for lesioned rats exposed to exercise training. Shown are group means of the percent correct responses (standard error bars are bidirectional but for readability are shown only unidirectionally).



Conclusion: The T-maze is sensitive to detecting executive function deficits related to rule reversal. Data collection to detect exercise effects is ongoing. Early results suggest an absence of exercise effect in the control animals.

TASK 4: Brain Imaging

- An operant cage, controller and camera system dedicated to use with [^{14}C]-iodoantipyrine radioactivity has been set up outside the vivaria housing the other operant cages.
- Standard operating protocols have been set up for the novel imaging of tethered animals during performance of the operant task. Functionality of the tether system allowing relatively free animal movement has been tested in one rat.
- Two rats are being trained in the nonradioactive operant cages, and will be transitioned next week into the [^{14}C]-dedicated system to allow a pilot brain mapping study in the next 2-3 weeks.

TASKS 5 / 6: Bench Research / Data Analysis

Exercise training in motorized running wheels (for molecular studies): *Methods:* Normal, non-lesioned rats were trained, either on a simple running wheel (non-skilled aerobic exercise, AE) or on a complex wheel with irregularly spaced rungs (skilled aerobic exercise, SAE). These irregular wheels demand the constant adaptation of stride length. Animals were trained in a running wheel for 20 min/day (4 trials, 5 min/trial, 2-min inter-trial interval), 5 consecutive days/week for 4 weeks. No-EX animals were handled and left in a stationary running wheel for 30 min/day. *Results:* See figure below.

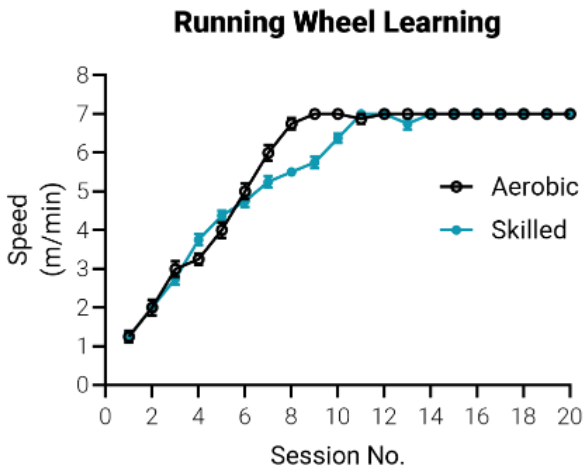


Figure 11: *Acquisition of maximum running speed in aerobic and skilled exercise groups.*
Motor learning of the aerobic or skilled automated running wheel shown as average speed (in meters/min) over the course of the four-week exercise period (five sessions per week). Rats engaged in the skilled exercise task take on average 2 extra sessions to reach the maximum running speed (7m/min) compared to rats in the aerobic exercise task.

Conclusion: Lesioned rats are able to run vigorously in the skilled and simple (aerobic) running wheels. For the molecular studies, running speeds for the aerobic wheel were normed to speeds achievable by lesioned rats in the skilled wheel.

qRT-PCR: Regions of interest (mPFC, dm/dl/vm/vlCP), were rapidly and unilaterally microdissected and submerged in an RNA stabilization solution (pH 5.2) at 4°C, Gene expression changes were measured with quantitative RT-PCR (qRT-PCR) as previously described [7, 8]. Briefly, qRT-PCR was run with 2 µl of cDNA and qPCRBIO SyGreen master mix on an Eppendorf Mastercycler Ep Realplex using a program of 15 min at 95°C, followed by 40 cycles of 15 seconds at 94°C, 30 seconds at 55°C, and 30 seconds at 72°C. Data was collected and normalized on Eppendorf Realplex *ep* software. Gene expression changes were examined for four metabolism-related genes (*Hif1a, Vegfa, Slc2a1, Ldha*), as well as three dopamine receptor (*Drd1, Drd2, Drd4*) and two synaptic (*Syp, Dlg4*) genes. Standard ΔΔCT analysis [9] was used to quantify fold changes in gene expression in experimental groups normalized to controls, with *Actb* serving as a housekeeping gene.

Results: see figure below.

Figure 12: *Metabolic gene expression differentially changes across caudate putamen quadrants, exercise duration, and exercise type.*
(Left) Rat caudate putamen color coded into dorsomedial (dmCP), dorsolateral (dlCP), ventromedial (vmCP), and ventrolateral (vlCP) quadrants. (Right) Corresponding gene expression changes for four metabolism-related genes (*Hif1a, Vegfa, Slc2a1, Ldha*) in each of the CP 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 control.

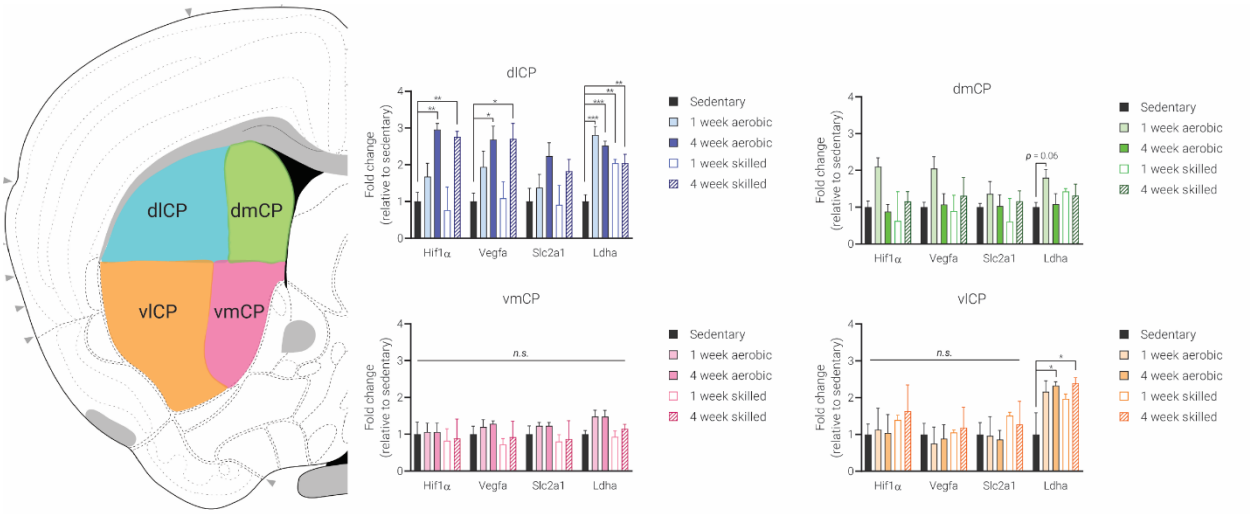
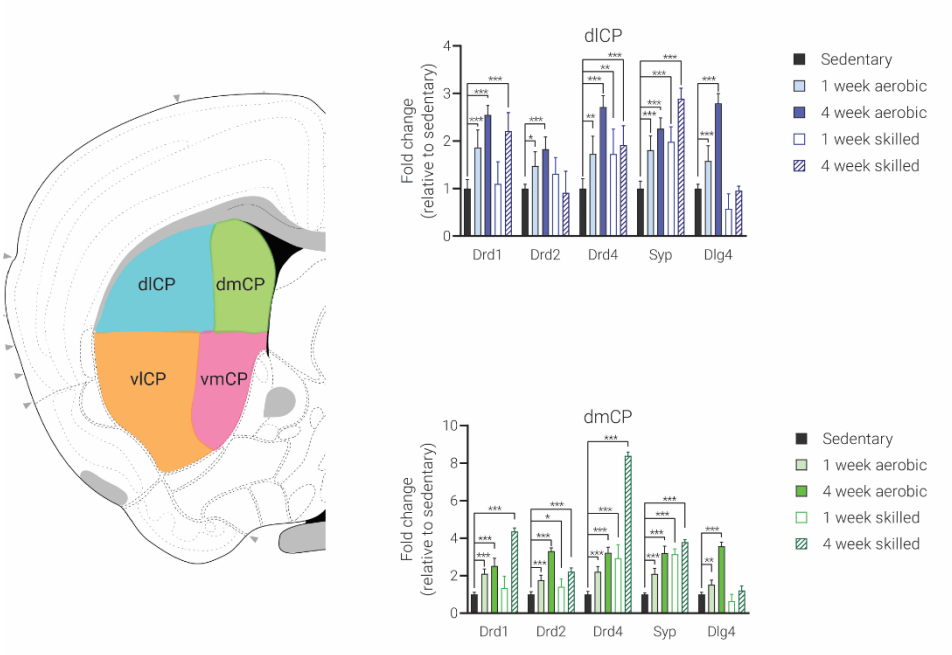


Figure 13: *Dopaminergic signaling and synaptogenic gene expression changes across dorsal caudate putamen across exercise duration and types.*
(Left) Rat caudate putamen color coded into dorsomedial (dmCP), dorsolateral (dlCP), ventromedial (vmCP), and ventrolateral (vlCP) quadrants. (Right) Corresponding gene expression changes for three dopamine receptor (*Drd1, Drd2, Drd4*) and two synaptic (*Syp,*

Dlg4) genes 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 control.



Conclusion: Overall these results suggest a differential effect of exercise across the striatal subsectors. 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, and only Lhda showing significant exercise effects in the vlCP. 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. 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). Thus, while exercise elicits in the dlCPu both significant plasticity and metabolic changes, in the dmCPu the changes are largely in dopamine receptor/synaptic markers. These results are broadly consistent with findings that acute treadmill walking in the rat, both in the skilled and simple wheels [10], as well as on the rotarod [11] shows greatest changes in rCBF in the dlCPu, though functional connectivity to medial prefrontal cortex increases particularly in the dorsomedial striatum [10].

Electrophysiology Analysis of Neuroplasticity

In the second year of this proposal, studies focused on analysis of changes in neuroplasticity using electrophysiological methods focused in excitatory postsynaptic currents (EPSCs) are to be conducted. One of the graduate students in this proposal is currently working with colleagues at UCLA (labs of Nigel Maidment and Carlos Cepeda) to conduct these experiments. We have

constructed an electrophysiological rig here in our labs to be used in these studies, assisted by our collaborators. These studies will complement analysis of genes and protein involved in neuroplasticity as part of the molecular studies in this proposal.

What opportunities for training and professional development has the project provided?

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

- Research electives for 3 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
- Work from this project provided the impetus for Drs. Jakowec and Petzinger to apply and receiving university funds for a conference entitled "Metaplasticity and Megaplasticity: Changing the Brain from Synapse to Community" USC Wrigley Center, Catalina Island", to be held 12/6-8/2019. Participants include faculty, doctoral and undergraduate students, from USC, other California universities, as well as the East Coast.

How were the results disseminated to communities of interest?

- Results are being presented at the above-mentioned meeting.

What do you plan to do during the next reporting period to accomplish the goals?

In the ongoing 2nd year of this project, we plan to (a) continue to add animals, (b) complete the functional brain mapping of the effects of lesions and exercise on the prefrontal-striatal circuit, (c) and to address the question of a possible differential effect of skilled versus simple aerobic exercise on cognitive, molecular and physiologic (including electrophysiologic) outcomes.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Results from this project are likely to provide evidence for the benefits of exercise in the cognitive neurorehabilitation of Parkinson's patients. Work by our extended Parkinson's Research group is aiding through community lectures to raise 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:

Changes in approach and reasons for change

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.

Delays:
(1) Work by Dr. Jakowec, Dr. Chow and the graduate student Adam Lundquist is ongoing for the calibration of the hardware to initiate the electrophysiologic studies. This has been slowed availability of Dr. Chow. We are currently working with Dr. Nigel Maidment (UCLA, no % effort requested) whose expertise in in-vivo voltammetry is accelerating this work.
(2) Hardware problems with the microscope and motorized stage have delayed the counting of dendritic spines. Funds from the Neurology department (Dr. Jakowec) have allowed replacement with stereologic studies to resume in November.
(3) Addition of percent effort by two additional USC doctoral students (Erin Donahue, Ilse Flores) will aid in the timely execution of planned behavioral/imaging studies.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Not applicable

Significant changes in use or care of vertebrate animals

Not applicable.

Significant changes in use of biohazards and/or select agents

Not applicable.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

- Manuscripts in preparation:
  - "Functional remodeling of corticostriatal function with exercise"
  - 'Skilled exercise training differentially regulates gene expression in subsectors of the rat striatum"
- 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

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

- 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.
- Halliday, M.R., D. Abeydeera, A.J. Lundquist, G.M. Petzinger, and M.W. Jakowec, Intensive treadmill exercise increases expression of hypoxia-inducible factor 1alpha and its downstream transcript targets: a potential role in neuroplasticity. Neuroreport, 2019. 30(9): p. 619-627.

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

Not applicable.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: 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: 2.0 mo
Contribution to the project: No change. Project design, project management, directing functional brain mapping studies, data analysis.

Name: Michael Jakowec, Ph.D.
Project Role: co-I
Research Identifier: N/A
Nearest person month worked: 1.0 mo
Contribution to the project: No change. Project design, directing molecular, electrophysiologic, and neuroanatomic studies.

Name: Zhuo Wang, Ph.D.
Project Role: co-I
Research Identifier: N/A
Nearest person month worked: 4.0 mo
Contribution to the project: No change. Stereotaxic lesioning, directing operant studies, functional brain mapping, data analysis.

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: Enrique Cadenas, Ph.D.
Project Role: Collaborator
Research Identifier: N/A
Nearest person month worked: 0.24 mo
Contribution to the project: No change. Advisement on interpretation of molecular biologic studies

Name: Robert Chow, Ph.D.
Project Role: Collaborator
Research Identifier: N/A
Nearest person month worked: 0.19 mo
Contribution to the project: No change. Advisement on electrophysiology experiments

Name: Adam Lundquist, BS (replaces former doctoral student Matthew Halliday)
Project Role: Graduate Student
Research Identifier: N/A
Nearest person month worked: 5.0 mo
Contribution to the project: Western blotting, qRT-PCR, brain dissection, data analysis

Name: Ilse Flores, BS
Project Role: Graduate student
Research Identifier: N/A
Nearest person month worked: 0.3 mo
Contribution to the project: immunohistochemistry, Golgi staining, sucrose preference testing, brain dissection

Name: Erin Donahue, BS
Project Role: Graduate student
Research Identifier: N/A
Nearest person month worked: 0.2 mo
Contribution to the project: operant behavior, 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?

Updates to the active support of members of the research team (Holschneider, Jakowec, Wang) are outlined below.

1. 5RO1 DK118402 (NIDDK), direct cost $1,000,000 07/1/18-06/30/22
   PI: Kanoski, Co-investigators: D. Holschneider, Z. Wang
   "Control of feeding behavior by melanin-concentrating hormone"
   Major Goal: Evaluation of neural circuits underlying feeding behavior

2. GRANT # W81XH19/ PD180100 (Department of Defense), direct cost $900,000
   9/1/19- 8/31/22
   PI: Jakowec, Co-Investigators: D. Holschneider, Z. Wang
   "The Role of Astrocytes and Microglia in Exercise-Induced Neuroplasticity in Parkinson's Disease"
   Major Goals: To investigate the role of the immune system in regulating exercise induced synaptogenesis and behavioral recovery in rodent models of PD using imaging and molecular biology approaches.

3. 1K01DK118000, NIDDK, direct cost $725,900 3/26/19 -12/31/23
   PI: Noble, Role: D. Holschneider is a co-Mentor with Dr. Kanoski
   "Melanin-Concentrating Hormone and the Neural Regulation of Feeding"
   Major Goals: To study the neural systems that lead to excessive feeding behavior and food impulsivity. To identify the mechanisms through which the neuropeptide, melanin-concentrating hormone, promotes excessive food intake.

What other organizations were involved as partners?

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://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *See attached*

9. APPENDICES: *N/A*

10. BIBLIOGRAPHY

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7. Halliday, M.R., D. Abeydeera, A.J. Lundquist, G.M. Petzinger, and M.W. Jakowec, *Intensive treadmill exercise increases expression of hypoxia-inducible factor 1alpha and its downstream transcript targets: a potential role in neuroplasticity.* Neuroreport, 2019. **30**(9): p. 619-627.
8. 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.
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