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CONTRACTING ORGANIZATION: Emory University, Atlanta, GA 30322

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14. ABSTRACT

The causes of dystonia are not understood but abnormal signaling by the neurotransmitter dopamine occurs in many inherited forms of dystonia, including DYT1 dystonia. Abnormalities in dopamine signaling that are observed in patients with DYT1 are also observed in DYT1 knockin mice, suggesting a reduction in dopamine release. Further, our preliminary data suggest that trihexyphenidyl (THP), the most commonly used medication for the treatment of dystonia, corrects the dopaminergic defect. The overarching hypothesis is *the defect in DA transmission is caused by abnormal vesicular function or abnormal receptor-mediated regulation of release and rescued by THP.* The specific aims are: **1) To characterize presynaptic defects that mediate abnormal DA release in DYT1(ΔE) knockin mice** by assessing VMAT2 function, vesicle utilization, the ultrastructure of DA terminals, and D2 DA autoreceptor function nicotinic AChR (nAChR) heteroreceptors function. **2) To determine the mechanisms underlying the dopaminergic response to THP** using FSCV and microdialysis to to identify the role of nAChRs in the regulation of DA release by THP and to identify the specific mAChRs that mediate DA release.

15. SUBJECT TERMS

dystonia, dyt1, dopamine, knockin, mouse, fast scan cyclic voltammetry, microdialysis, electron microscopy, striatum, vesicle, muscarinic receptor

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1. INTRODUCTION

The causes of dystonia are not understood but abnormal signaling by the neurotransmitter dopamine occurs in many inherited forms of dystonia, including DYT1 dystonia. Abnormalities in dopamine signaling that are observed in patients with DYT1 are also observed in DYT1 knockin mice. Findings in both mice and humans point to a reduction in dopamine release but the mechanism underlying the abnormal release are unknown. Further, our preliminary data suggest that trihexyphenidyl (THP), the most commonly used medication for the treatment of dystonia, corrects the dopaminergic defect. The overarching hypothesis is *the defect in DA transmission is caused by abnormal vesicular function or abnormal receptor-mediated regulation of release and rescued by THP.* The specific aims are: **1) To characterize presynaptic defects that mediate abnormal DA release in DYT1(ΔE) knockin mice** by assessing VMAT2 function and vesicle utilization using fast scan cyclic voltammetry (FSCV) in slice, the ultrastructure of DA terminals, D2 DA autoreceptor function nicotinic AChR (nAChR) heteroreceptors function. **2) To determine the mechanisms underlying the dopaminergic response to THP** using FSCV and microdialysis to determine if the DYT1(ΔE) mutation differentially affects DA release in response to THP, to identify the role of nAChRs in the regulation of DA release by THP and to identify the specific mAChRs that mediate DA release.

2. KEYWORDS

dystonia, dyt1, dopamine, knockin, mouse, fast scan cyclic voltammetry, microdialysis, electron microscopy, striatum, vesicle, muscarinic receptor

3. ACCOMPLISHMENTS

What were the major goals of the project?

Goals	Timeline	%Completion
Major Task 1: ACURO approval for studies involving animals	Months 1-2	100%
Major Task 2: Specific Aim 1. To characterize presynaptic defects that mediate abnormal DA release in DYT1(ΔE) knockin mice	Month 3-36	25%
Major Task 3: Specific Aim 2. To determine the mechanisms underlying the dopaminergic response to THP	Months 13-36	20%

What was accomplished under these goals?

Major Task 1: ACURO approval for studies involving animals

Emory and ACURO approval was obtained for all animal studies. Shortly thereafter, the DYT1 knockin mouse colony was established to provide experimental mice for studies proposed in this grant.

Major Task 2: Specific Aim 1. To characterize presynaptic defects that mediate abnormal DA release in DYT1(ΔE) knockin mice.

Subtask 1. Vesicular dopamine uptake assay. This subtask was designed to assess the functional integrity of synaptic vesicles by examining the integrity of VMAT2. In our initial experiments, we examined the expression of

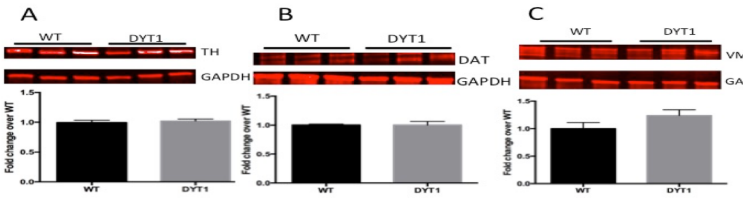

Figure 1. TH, DAT and VMAT2 protein expression, as assessed by western blot analysis in normal and DYT1 knockin mice. No significant differences were observed between genotypes for any protein (t-test p>0.5). Data represent means±SEM.

VMAT2 and other markers of presynaptic dopamine terminals including the dopamine transporter (DAT) and tyrosine hydroxylase (TH) by western blot analysis. These experiments were necessary to determine if presynaptic markers were increased or decreased, which could suggest a change in overall number of terminals, prior to performing functional assays of VMAT2 function. VMAT2, DAT and TH expression was comparable in normal and DYT1 knockin mice (Figure 1) suggesting that any difference observed in uptake assays can be attributable to changes in VMAT2 function rather than VMAT2 expression.

Subtask 2. Fast scan cyclic voltammetry (FSCV) for vesicle refilling. To determine if the vesicle refilling rate is affected in DYT1(ΔE) knockin mice, we used FSCV in dorsal striatum to assess dopamine release as the inter-stimulus interval of paired 1 pulse challenges was varied from 5 min to 0.3 sec with 3 min between paired pulses to allow DA release to recover. As the ISI decreased, both normal and DYT1 mice exhibit a reduction in DA release indicating a reduced ability to refill the readily releasable pool of vesicles as the ISI decreases, as expected. With a sample size of 4 for each genotype, the data do not suggest obvious differences. Our power calculations demonstrated that a sample size of 6/genotype is needed for this experiment, so 2 mice/genotype will be added to this experiment, which will be complete by the end of October.

Subtask 3. Ultrastructural analysis of dopaminergic terminals. DYT1 knockin and normal mice were perfusion-fixed with a mixture of paraformaldehyde (4%) and glutaraldehyde (0.1%). Their brains were post-fixed and cut in 60 um-thick coronal sections with a vibratome. From these animals, sections at the level of the striatum were processed for TH immunostaining which was localized with immunoperoxidase. After immunolabeling, some of these sections were shipped to Renovo Neural Inc for serial block face scanning EM (SBF/SEM) processing to collect series of images from ultrathin sections to be used for 3D EM reconstruction and morphometric analysis of TH-containing terminals between WT and DYT1 mice. At Renovo Neural, series of 200-300 blockface images are currently being captured (Zeiss Sigma VP scanning EM, Gatan 3 View in-chamber ultramicrotome). The images will be transferred back to us for analysis by investigators blind to the animal's genotype for 3D reconstruction to assess volume of the terminal, the number of synapses formed by single terminals, the surface of each synapse as well as the size and total number of synaptic vesicles/terminals. We are ahead of our anticipated timeline for this Subtask.

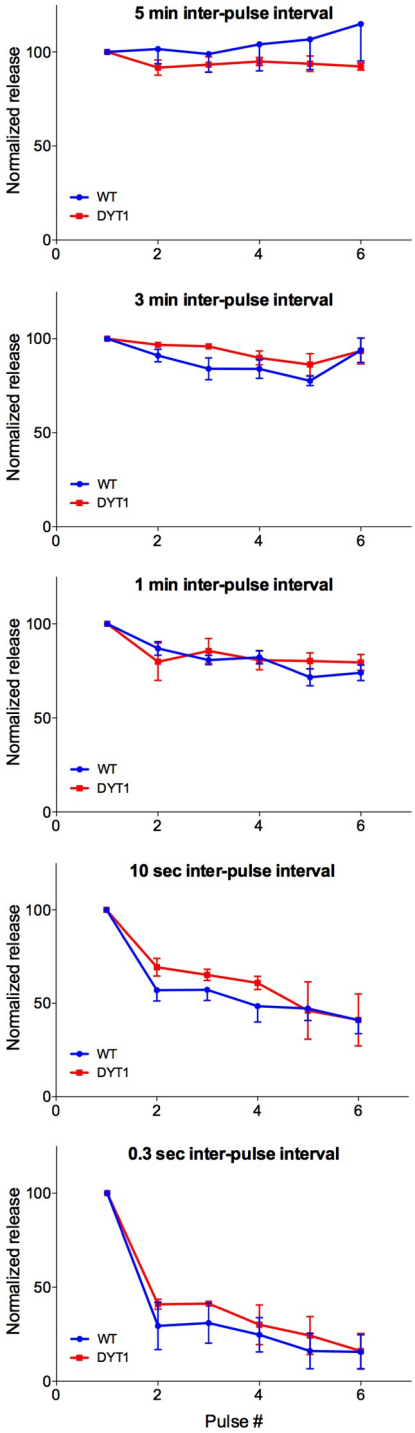

Figure 2. Rundown of DA release assessed by FSCV in normal and DYT1 knockin mice (n=4/genotype; n=6 for a fully powered experiment).

Major Task 3: Specific Aim 2. To determine the mechanisms underlying the dopaminergic response to THP. Because our access to a FSCV rig was somewhat limited (see Section 5 below), we initiated Major Task 3 earlier than anticipated.

Subtask 3. Reverse microdialysis for response to trihexyphenidyl (THP). We used microdialysis to measure changes in striatal extracellular DA in response to THP in awake behaving mice. We reverse dialyze THP (0.3 µM or 3 µM) to achieve striatal concentrations of 30 or 300 nM THP. 4 baseline samples were collected and then normal or DYT1(ΔE) knockin mice received infusions of THP directly into the striatum. Average extracellular DA was calculated from 4 baseline microdialysis samples and 6 samples during reverse dialysis of THP. For 0.3 µM infusion (n=7/genotype), there was an effect of genotype, as expected, but no effect of treatment. For 3 µM infusions, there was a significant effect of THP treatment (two-way repeated measures ANOVA, F=4.342, p = 0.05). As expected, there was a significant effect of genotype (F=38.02.8, p < 0.0001) whereby extracellular DA was significantly lower in DYT1 mice compared to controls. We are continuing to add THP concentrations to this experiment but have already performed a large amount of work on this labor-intensive subtask.

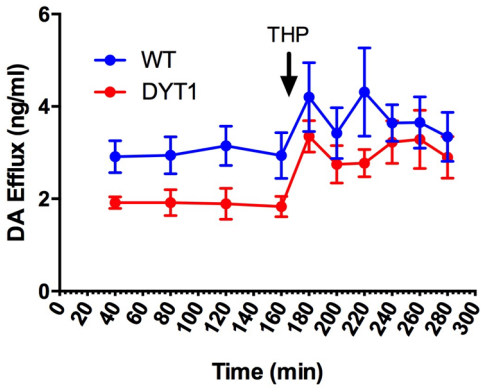

Figure 3. Effect of 3µM THP infusion on extracellular striatal DA concentrations. Data represent mean ± SEM of DA concentrations at each time point (n = 7-9/genotye)

What opportunities for training and professional development has the project provided?
Nothing to report
How were the results disseminated to communities of interest?
Nothing to report
What do you plan to do during the next reporting period to accomplish the goals?
We anticipate that the FSCV experiments described in Aim 1 will be complete within the first 6 months of Year 2. Additionally, we will be completing the reverse dialysis and microdialysis experiments for dopamine in response to trihexyphenidyl, which are described in Aim 2. These experiments are already well underway.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
Nothing to report
What was the impact on other disciplines?
Nothing to report
What was the impact on technology transfer?
Nothing to report
What was the impact on society beyond science and technology?
Nothing to report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change
Nothing to report
Actual or anticipated problems or delays and actions or plans to resolve them
The fast scan cyclic voltammetry (FSCV) experiments proposed in Aim 1 were delayed. In the first year of this grant we were planning to use Dr. Miller's FSCV rigs to perform experiments in Aim 1, as mentioned in our proposal. As Dr. Miller stated in the proposal, his rigs were used intensively so it was imperative for us to have our own rig, which we budgeted for in years 1 and 2; we anticipated having our own rig operational by the end of year 2. However, one of Dr. Miller's two rigs broke down during year 1 of this grant, so our access to a FSCV rig was more

restricted than anticipated. In response, we accelerated the build of our own FSCV rig, which is now operational at the beginning of year 2 of this proposal, about 10 months earlier than expected (Figure 4). With our own rig, we are now able to assess 4-8 mice/week, in contrast to the 1-2 mice that we had anticipated using Dr. Miller's rig. As such, even using a conservative estimate of 4 mice/week for FSCV, we will easily accomplish the goals of this proposal, including FSCV data analysis by the end date of this proposal. Additionally, because our ability to perform FSCV experiments was limited, we initiated microdialysis experiments in year 1, which was earlier than planned, so our overall timeline and budget will not be impacted.

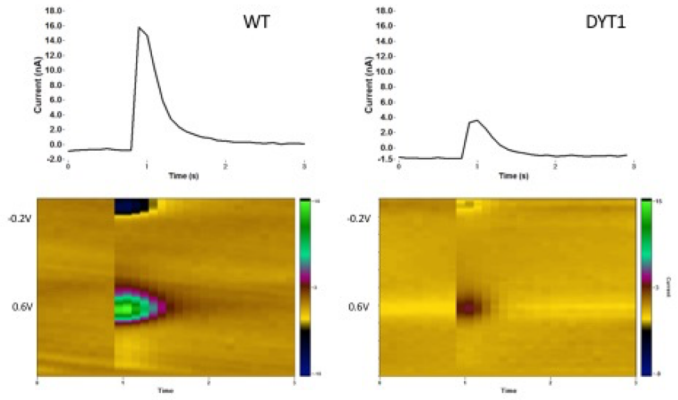

Figure 4. Sample fast scan cyclic voltammetry in dorsal striatal slice from wild type (WT) and DYT1knockin mice, illustrating results from our newly-constructed FSCV rig. Representative current traces and color plots are for a single pulse stimulation. Current traces are extracted at the peak oxidation voltage for dopamine. Oxidation (positive current) is shown in bright colors, and reduction (negative current) is shown in dark colors. As expected, dopamine release was reduced in DYT1 mice.

Changes that had a significant impact on expenditures

Nothing to report

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

Nothing to report

Significant changes in use or care of human subjects

Not applicable

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards, and/or select agents

Not applicable

6. PRODUCTS

Publications, conference papers, and presentations

Journal publications

Nothing to report

Books or other non-periodical, one-time publications

Nothing to report

Other publications, conference papers, and presentations

Nothing to report

Website(s) or other Internet site(s)

Nothing to report

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	Ellen Hess, PhD
Project Role	Principle Investigator
Researcher ID	ORCID ID #0000-0002-8546-8184
Nearest person month worked	5
Contribution to Project	She directs the progress of the research and supervises the efforts of the research team. She will also be responsible for the collection, interpretation and preparation of data for publication.
Funding Support	See attached active support page

Name	Gary Miller, PhD
Project Role	Co- Investigator
Researcher ID	none
Nearest person month worked	1
Contribution to Project	Dr. Miller has provided expertise and guidance for the construction of our FSCV rig. He has also helped with FSCV experimental design.
Funding Support	See attached active support page

Name	Rosa Villalba, PhD
Project Role	Postdoctoral Fellow
Researcher ID	None
Nearest person month worked	4
Contribution to the Project	Was involved in processing of mouse tissue and preparation of electron microscopic material. Involved in data collection, analysis and write-up of results from electron microscopic studies.
Funding support	NIH (in addition to DoD)

Name	Susan Jenkins
Project Role	Research Specialist
Researcher Identifier	None
Person-month work	4
Contribution to the Project	Perform tissue sectioning, immunohistochemical reactions and preparation of tissue to be used in the EM studies
Funding support	NIH (in addition to DoD)

Name	Jean-Francois Pare
Project Role	EM Lab supervisor
Researcher Identifier	None
Person-month work	1

Contribution to the Project	Involved in the sectioning of tissue for EM. Maintain EM-related equipment. Help with EM data collection.
Funding support	NIH (in addition to DoD)

Name	Yoland Smith, PhD
Project Role	Co-investigator
Researcher Identifier	None
Person-month work	1
Contribution to the Project	Supervise EM-related work of this grant. Helps with design and execution of EM experiments. Involved in data analysis. Communicates frequently with PI and other members of the team about progress of the work.
Funding support	See attached active support page

Name	Xueliang Fan, PhD
Project Role	Postdoctoral Fellow
Researcher Identifier	None
Person-month work	12
Contribution to the Project	Performs all surgeries associated with microdialysis, makes microdialysis probes, runs microdialysis experiments
Funding support	

Name	Christine Donsante
Project Role	Research Specialist
Researcher Identifier	None
Person-month work	12
Contribution to the Project	Maintenance of the mouse colony, including the attendant paperwork, genotyping, vet consults etc. Supplies collaborating laboratories with mutant and control mice. Performs all ordering, lab maintenance and assists postdoc when needed.
Funding support	

Name	Rong Fu, PhD
Project Role	Postdoctoral Fellow
Researcher Identifier	None
Person-month work	4
Contribution to the Project	Maintenance and day-to-day operations of our HPLCs. Performw the HPLC associated with in vivo microdialysis; she also performs the initial analyses of the chromatographs from all HPLC samples.
Funding support	NIH

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Ellen Hess (Principle investigator)
No longer active
Research Grant Ataxion Pharmaceuticals

New active award
1 R21 NS093550-01 (Chin) 07/01/15-06/30/17 0.6 calendar
NIH/NINDS $125,000
Molecular Analysis of TorsinA Function and Dysfunction
The goal of this project is to study the role of the torsinA-printor complex in normal physiology and dystonia pathogenesis.

Dr. Yoland Smith (co-I)
No longer active
Extrastriatal Functions of Dopamine (NIH)
Parkinson's Disease Foundation
MJ Fox Therapeutic Development Initiative (Michael J Fox Foundation)
BP-ENDURE-Atlanta: Engaging undergraduates in neuroscience research (NIH)

New active awards
Research grant (Simpson, PI; Smith, Emory PI) 10/01/2016-09/30/201 1.2 months
Weston Brain Institute $500,000
Human MiniPromoters for Brain Gene Therapy; Focused on Parkinson Disease
The goal of this project is to develop new viral vector tools for brain gene therapy that allow specific cell-type and network transfection in rodent and nonhuman primate CNS

P51OD011132 (Caughman, PI) 05/01/16-04/30/17 0.6 months
NIH/ORIP $9 000
Salary support for Yerkes Core Faculty
These funds support part of the salary of Dr Smith as core faculty at the Yerkes Primate Center

Dr. Gary Miller (co-I)
No longer active
- Udall Parkinson's Disease Center at Emory University: Circuitry to Therapy (NIH)
- Pathogenic Mechanism of Environmental Toxicants in Parkinson's Disease (NIH)

New active award
Title: National Exposure Assessment Laboratory at Emory
Time Commitment: 1.80 calendar months
Supporting Agency: National Institutes of Health (NIEHS)
Name and address of the Funding Agency's Procuring Contracting/Grants Officer:
George Tucker, National Institute of Environmental Health Sciences, 111 T.W. Alexander Drive, Research Triangle Park, NC USA 27709
Performance Period: 09/01/15-08/31/19
Level of Funding: $1,404,657 annual direct costs
The goal of this project is to provide the children's health research community with access to state-of-the-art analysis of environmental factors related to health and disease.

What other organizations were involved as partners?
Nothing to report

8. SPECIAL REPORTING REQUIREMENTS
Not applicable

9. Appendices
None