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PRINCIPAL INVESTIGATOR: Su Guo

CONTRACTING ORGANIZATION: The Regents of the University of California, San Francisco
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14. ABSTRACT

Widespread neuroplasticity exists in the brain leading to adaptation or maladaptation. This project aims to understand how alteration of dopamine systems, which are profoundly affected in Parkinson's disease (PD), affects brain-wide plasticity at cellular resolution, employing the vertebrate model organism zebrafish. During the first year of this project, we have established transgenic animals that simultaneously express a reporter-tagged enzyme nitroreductase (NTR)(for chemogenetic ablation of dopamine neurons to mimic the loss of these neurons in PD)and GCAMP6s (a genetically encoded calcium indicator for imaging neuronal activity dynamics). We have found that, in freely behaving larval zebrafish, chemogenetic ablation of DA neurons significantly reduced locomotor activity measured by total distances travelled at the population level. However, at the level of individual animals, a clear correlation was not observed between the severity of total DA neuron loss and extent of locomotor impairment. Two possible interpretations of these findings are: 1) the lack of correlations between DA neuron loss and locomotor impairment at individual levels is due to the engagement of other neural systems in locomotor behavioral modulation. 2) The lack of linear correlations between DA neuron loss and locomotor impairment at individual levels is due to individual differences in the ability to activate a compensatory brain plasticity machinery.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The brain has a natural ability to reorganize itself by forming new neural pathways and connections. In the case of Parkinson's disease (PD), such dynamic adaptations might underlie the prolonged asymptomatic phase of the disease despite the loss of a majority of substantia nigra DA neurons. Likewise, maladaptation results in severe side effects such as dyskinesia when patients are on DA replacement therapies. The purpose of this research is to understand at cellular resolution brain-wide neuroplasticity upon DA alteration. We employ the vertebrate model organism larval zebrafish, which is saliently suited for this goal.

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

dopamine neurons, Parkinson's disease, brain-wide neuroplasticity, calcium imaging, locomotor behavior, zebrafish

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.

Specific Aim 1: induce DA neuronal loss in a quantitative manner and perform behavioral and brain-wide calcium imaging and data analysis at cellular resolution over time, at both resting state and upon stimulus delivery. Major Task 1: Determine the doses of MTZ to induce quantitative DA loss. Major Task 2: Brain-wide calcium imaging and data analysis upon DA neuronal loss.

Specific Aim 2: Over-activate DA signaling in control and DA neuron–degenerating individuals by administering L-dopa or DA agonists (mimicking DA replacement therapy), and carry out brain-wide functional connectivity and behavioral analyses. Major Task 3: Determine the doses of L-dopa or DA agonists for DA activation. Major Task 4: Brain-wide calcium imaging and data analysis upon DA over-activation.

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.

Major activity 1: determine the extent of dopamine neuron loss on the degree of locomotor impairment in larval zebrafish at both population and individual levels.

1.1. Approach: Five days post fertilization (dpf) transgenic larval zebrafish expressing NTR under the control of tyrosine hydroxylase (th) promoter and H2B-GCAMP6s pan-neuronally, as well as their Non-Tg siblings, were exposed to DMSO (vehicle control), 4mM or 8 mM MTZ (the pro-drug that ablates cells expressing NTR) for 24 hours, followed by washout of MTZ for 24 hrs. At 8 dpf, locomotor activity was tracked and DA neuron integrity was imaged.

1.2. Significant results and conclusions:

1.2.1. We observed that 8 mM MTZ treatment led to a significant loss of DA neurons and a significant impairment of locomotor activity assessed at the population level (**Fig.1**). This result suggests that the MTZ at 8 mM is an optimal dose for inducing DA neuronal loss with corresponding locomotor deficits.

1.2.2. When individual larval zebrafish's DA neuron pathology and locomotor scores were compared, no clear linear correlation was observed (**Fig. 2**). This result suggests that variables in addition to DA neuron integrity influence locomotor activity at the level of individuals. This may have to do with how individuals cope with DA neuron loss, either due to their genetic differences, or state-dependent variables influenced by environmental factors. One potential underlying mechanism that we wish to explore is differences in brain-wide plasticity.

Major activity 2: Brain-wide calcium imaging and data analysis pipeline.

2.1. Approach: Double transgenic larval zebrafish Tg[th-gal4;UAS-NTRmcherry][HuC-H2BGCAMP6s] were embedded in 1.2% low melt agarose and subjected to 2-photon imaging of neuronal activity brain-wide. Resting state activity was recorded. Both vehicle (DMSO) and D2R antagonist (sulpiride)-treated animals (50 uM for 1 hr). Calcium inaging data were computationally analyzed using softwares including CalMAn and CMTK.

2.2. Significant results and conclusions:

2.2.1. Our preliminary data uncovered that treatment with D2R antagonist sulpiride significantly increased the activity of serotonin neurons in the hindbrain raphe (**Fig. 3**). This result suggests that D2R exerts an inhibitory effect on raphe 5-HT neurons.

2.2.2. Our preliminary data also uncovered that treatment with D2R antagonist sulpiride disrupts synchronized rhythmic activity in the brain (**Fig. 4**).

(Figures were presented in the appendices, due to difficulty in embedding them in this word file and space limitations).

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.

The project has provided training to postdoctoral fellows (Dr. Mariia Burdyniuk and Dr. Mahdi Zarei) and graduate students (Jeffrey Kim) in terms of research activities including one-on-one mentoring.
Professional development:
Jeffrey attended the Gordon Conference on Parkinson's disease.
Mariia attended the Woods Hole Neurobiology course.
Mahdi attended computational neuroscience workshops at Stanford and UC Berkeley.

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.

Nothing to report.

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

1. Brain-wide neuronal activity imaging and data analysis with simultaneous DA neuron and locomotor behavioral tracking: We are currently updating our 2-photon microscope that will enable us to image brain-wide neuronal activity with simultaneous tracking of DA neurons and locomotor behavior. We will perform this experiment in: 1) Control (vehicle); 2) MTZ-treated; 3) MTZ-treated plus L-dopa. 4) D1R or D2R agonist or antagonist-treated.
2. Brain-wide glial activity imaging and data analysis with simultaneous DA neuron and locomotor behavioral tracking: As the brain is made of not only neurons but also glial cells, we plan to perform brain-wide glial activity imaging in the same conditions as above.

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).

Dopamine neuron loss and/or upon administration of DA drugs (e.g. L-Dopa) lead to profound changes in neuroplasticity that remains poorly understood. Our work will uncover such mechanisms at systems level with cellular resolution employing larval zebrafish.

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.

Adaptive and maladaptive plasticity is thought to have functional consequences. For instance, PD patients are not presented with motor symptoms until ~60% of DA neurons undergo degeneration. What compensatory mechanisms occur in the brain resulting in such symptomatic delay? Our study will potentially shed new light on such mechanisms. This may potentially help aid with early diagnosis.

Upon prolonged treatment with L-dopa, debilitating side effects such as dyskinesia develop in PD patients. This significantly limit the utility of this important symptomatic management drug. Our study will potentially shed light on the mechanisms underlying L-dopa induced maladaptive plasticity. This may potentially help find new ways to circumvent such problem.

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.*

Nothing to report.

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.

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.

During the reporting period, the postdoctoral fellow Mariia Burdyniuk has decided to leave the lab due to personal reasons since August 2019. We have since filled the position and the new postdoctoral fellow is scheduled to start in December 2019.

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.

Nothing to report.

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

Not applicable.

Significant changes in use or care of vertebrate animals

None.

Significant changes in use of biohazards and/or select agents

None.

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).*

  Nothing to report.

  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.*

Nothing to report.

- **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.)*

Su Guo, Ph.D. PD/PI: 2.4 months, Direct the research, and provide advice to students/postdocs on how to perform experiments, interpret data, and troubleshoot problems.

Jeffrey Kim, graduate student, 12 months. Mr. Kim has performed work in the area of establishing transgenic lines.

Mariia Burdyniuk, Postdoc, 11 months. Dr. Burdyniuk has performed work to quantify DA neurons and locomotor behavior.

Mahendra Wagle, Associate Specialist, 4 months. Dr. Wagle assists Dr. Guo in personnel training and fish facility maintenance.

Amelia Dahlen, Lab Assistant, 4 months. Amelia assists with fish husbandry.

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:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (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://ers.amedd.army.mil*** *for each unique award.*

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

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.*

Figures:

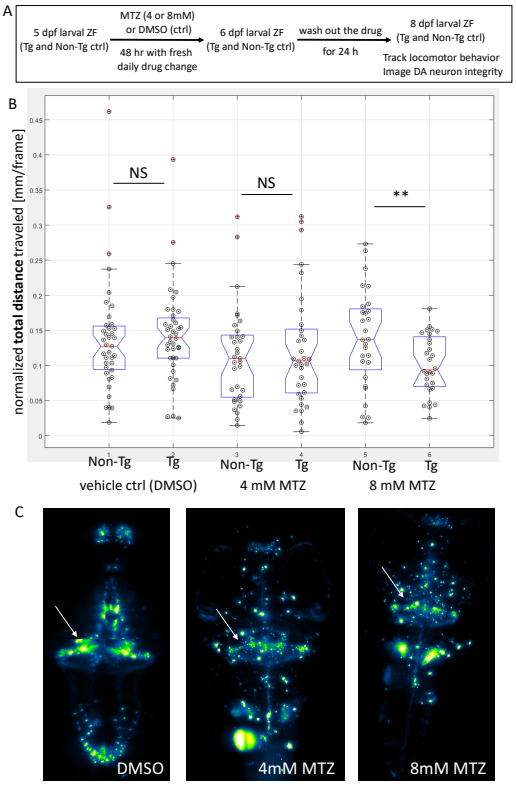


Figure 1. Chemo-genetic ablation of DA neurons is associated with significant locomotor impairment. (A) A schematic chart that depicts the work flow. (B) A scatter plot shows that 8 mM MTZ treatment significantly impairs locomotor activity in Tg compared to Non-Tgs. **, $p<0.01$. (C) Representative images show the loss of DA neuron fluorescence upon treatment with MTZ.

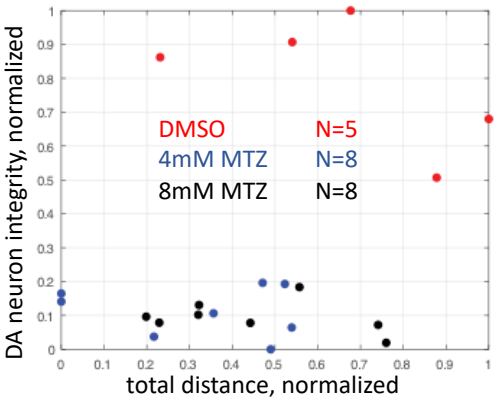


Figure 2. Lack of linear correlation between DA neuron integrity and locomotor scores. X-axis is the normalized total distances travelled, and Y-axis is the normalize DA integrity. No linear correlation can be observed at individual levels, suggesting the involvement of other mechanisms than total DA neuron contents in regulating locomotor behavior. Arrows point to the signal that represents DA neuronal subtypes homologous to human substantia nigra DA neurons.

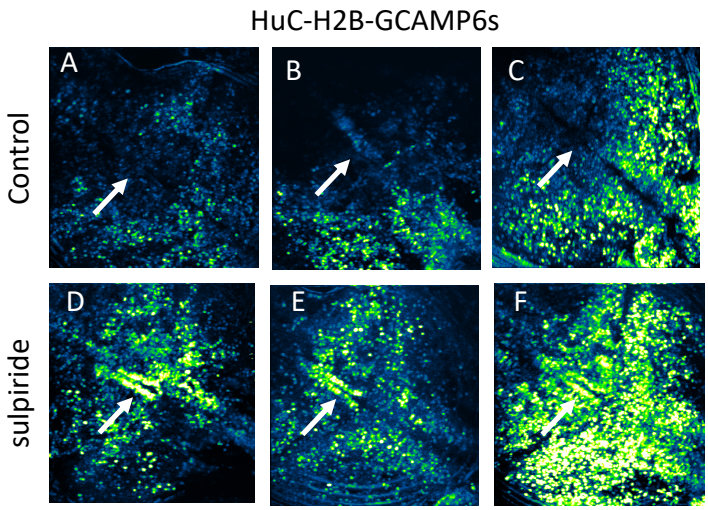


Figure 3. Brain-wide calcium imaging in control versus D2R antagonist-treated larval zebrafish shows an activation of hindbrain 5HT raphe neurons. Imaging was carried out in Tg[HuC-H2B-GCAMP6s]. (A-C) treated with vehicle (DMSO). (D-F) treated with the D2R antagonist sulpiride. Arrows point to the location of the hindbrain 5HT raphe neurons. Significant activation can be seen in sulpiride-treated individuals.

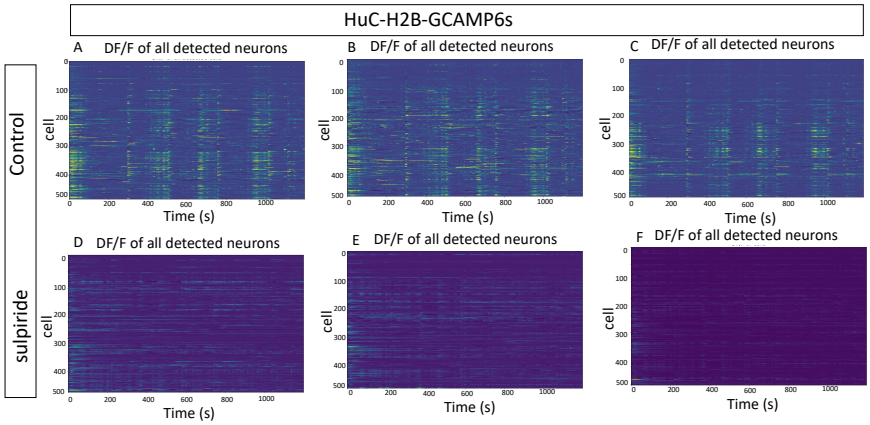


Figure 4. The D2R antagonist sulpiride disrupts brain-wide rhythmic activity. Raster plots show synchronized rhythmic activity brain-wide in control (DMSO-treated) larval zebrafish brain (A-C). Such synchronized rhythmic activity is largely absent in sulpiride-treated animals.

Impact of Dopamine Alteration on Brain-Wide Functional Connectivity at Cellular Resolution

PD170068

W81XWH-18-1-0176

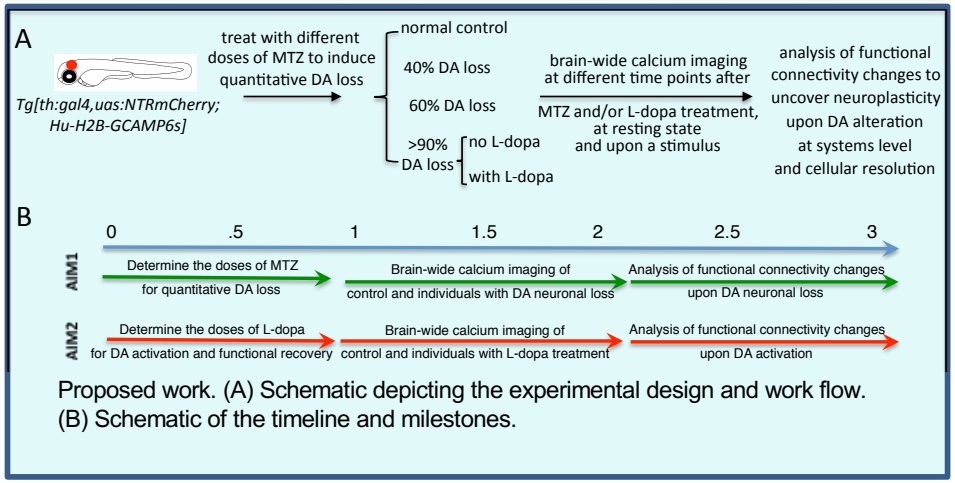
PI: Su Guo, Ph.D. **Org:** REGENTS OF THE UNIVERSITY OF CALIFORNIA, **Award Amount:** $988,211.00

Study/Product Aim(s)

- Induce quantitative DA neuronal loss and determine brain-wide functional connectivity changes at cellular resolution through brain-wide calcium imaging and data analysis.
- Over-activate DA signaling and determine brain-wide functional connectivity changes at cellular resolution.

Approach

Using a chemogenetic DA neuron degeneration model that we have established in our lab, we will perform brain-wide calcium imaging and carry out functional connectivity analysis at cellular resolution upon the induction of varying degrees of DA neuronal loss (mimicking DA deficits during PD progression). By administering L-dopa or DA agonists to this model, we aim to understand how DA over- activation might alter brain functional connectivity (similar to PD patients on dopamine replacement therapies). We anticipate that fundamental principles underlying brain adaptation might emerge, which could explain functional adaptation and maladaptation in disease progression and therapeutic outcomes.



Proposed work. (A) Schematic depicting the experimental design and work flow. (B) Schematic of the timeline and milestones.

Accomplishment: Established transgenic models for DA neuron loss and brain-wide imaging; Determined the doses of MTZ and DR drugs for altering the DA system; Collected preliminary data for brain-wide calcium imaging.

Timeline and Cost

Activities CY	18	19	20	21
Determine the doses of MTZ to induce quantitative DA loss				
Brain-wide calcium imaging and data analysis upon DA neuronal loss				
Determine the doses of L-dopa or DA agonists for DA activation.				
Brain-wide calcium imaging and data analysis upon DA over-activation				
Estimated Budget ($K)	**$108K**	**$330K**	**$330K**	**$220K**

Updated: (September 23, 2019)

Goals/Milestones

CY18 Goal – Establish models for DA neuron loss and brain-wide imaging

☑ Derivation of transgenic models through genetic breeding

CY19 Goals – Determine regimens for altering the DA system

☑ Identify doses of MTZ that induce significant DA neuron loss

☑ Identify doses of DR drugs to alter DA signaling

CY20 Goal – Elucidate brain-wide dynamics upon DA neuron loss/inhibition

☐ Uncover brain-wide activity linking to sensory/motor/DA neurons

☐ Uncover activity/connectivity changes upon DA neuron loss/inhibition

CY21 Goal – Elucidate brain-wide dynamics upon DA over-activation

☐ DA over-activation in normal brain

☐ DA over-activation in brains with DA neuron loss

Comments/Challenges/Issues/Concerns

- If timelines change, comment here. None
- If off by more than one quarter in spending, comment here. None

Budget Expenditure to Date

Projected Expenditure: $247,053 direct + 148,231 = $395,284

Actual Expenditure: $234,484.50 direct + 130,108.88 = $364,593.38