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

PRINCIPAL INVESTIGATOR: Su Guo

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

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13. SUPPLEMENTARY NOTES

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 second year of this project, we have employed the 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). Brain-wide calcium imaging data were collected using head-fixed and tail-free larval zebrafish preparation. The loss of tyrosine hydroxylase+ (TH^+) neurons was quantified in different locations throughout the brain that revealed a range from 20% to 100% neuronal loss in different brain locations. Computational tools for brain registration, neural activity and tail movement analysis were further refined. Together, these results poise us to uncover how the loss of TH^+ neurons in larval zebrafish affect brain-wide neural activity dynamics.

15. SUBJECT TERMS

NONE LISTED

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, tyrosine hydroxylase, 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: Collect brain-wide calcium imaging data.

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), or 5 mM MTZ (the pro-drug that ablates cells expressing NTR) for 48 hours, followed by washout of MTZ for 24 hrs. At 8 dpf, brain-wide calcium imaging was carried out using head-fixed tail-free preparation under the 3i 2-photon microscope.

1.2. Significant results and conclusions:
We observed that 5 mM MTZ treatment led to a significant loss of DA neurons (**Fig.1**). Further quantification uncovered that the severity of neuronal loss varies in different anatomical locations of the brain (**Fig. 2**).

Major activity 2: Refine computational tools for analyzing neural activity and tail movement data.

2.1. Approach: 1) we used ANTsPy (Advanced Normalization Tools) to register with reference 6-dpf brain (expressing Elavl3-H2B-RFP) from the Zbrain atlas, in which 294 regions have been identified. 2) For neural activity data analysis, we compared 4 software packages, CaImAn (Python), CNMFe (Matlab), Scalpel (R) & suite2p (Python) to determine their suitability for our data. 3) For tail movement tracking, we plotted the range of tail movement as a function of time, using custom written Matlab code.

2.2. Significant results and conclusions:

2.2.1. The ANYsPy enabled accurate registration of different brains (**Fig. 3**).

2.2.2. We decided to use suite2p based on two key criteria: 1) Minimal false negatives: In a single Z-plane from a 4D dataset, ~100 bright neurons were labelled manually from the standard-deviation projection. Ideally, all these neurons should be detected by the algorithm. 2) Minimal false positives: Since a single Z-plane contains thousands of neurons, it is not feasible to manually label all the neurons. Thus, a software-based method is used to estimate how many of the detected neurons do not correspond to a real neuron.

2.2.3. Four primary tail movement patterns were detected, which will be correlated with neural activity (**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. Krish Bose and Dr. Mahdi Zarei) and graduate students (Jeffrey Kim) in terms of research activities including one-on-one mentoring.
Professional development:
Jeffrey attended the UCSF Movement Disorder Retreat.
Krish and Mahdi attended the UCSF Neuroscience seminars and Retreat.

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 data analysis in control that will correlate DA neuron activity to the rest of the neurons in the brain, and to the different types of movement patterns.
2. Brain-wide neuronal activity data analysis comparing MTZ-treated with control that will determine how the loss of TH^+ neurons affect brain-wide neural activity and tail movement.
3. Collect Brain-wide neuronal activity data on MTZ- and L-dopa treated animals.

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, our laboratory was shut down due to the COVID-19 pandemic from March to June 2020. The laboratory was open with limited access capacity (25%) from June 2020 to September 2020. During these difficult times, remote research activities (e.g. computational tool refinement) were carried out, but no new experimental data were able to be collected.

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, 6 months. Mr. Kim has performed work in the area of transgenic lines.

Krish Bose, Postdoc, 7 months. Dr. Bose has performed work to collect neural activity and tail movement data.

Mahendra Wagle, Associate Specialist, 3 months. Dr. Wagle assists Dr. Guo in personnel training and fish facility maintenance.

Madhi Zarei, Postdoc, 4 months. Dr. Zarei has assisted with computational tool refinement.

Michael Munchua, Lab Tech, 1 months. Mr. Munchua does animal 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:
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://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:

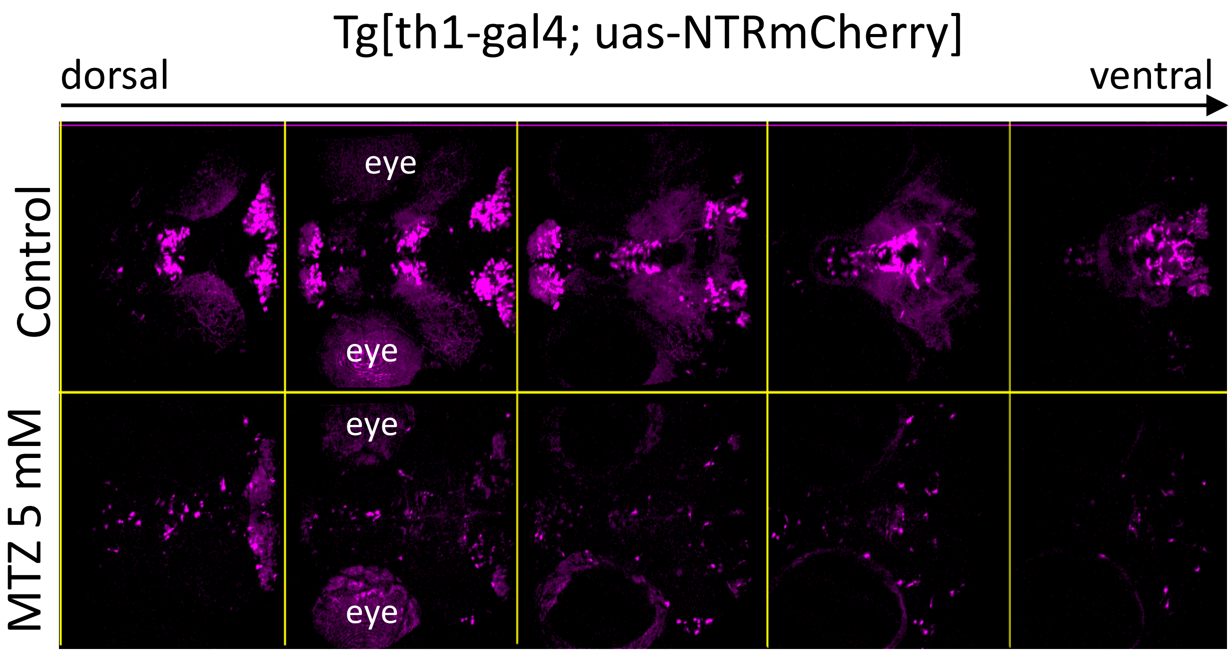

Figure 1. Chemo-genetic ablation of DA neurons visualized under the 2-photon microscope. Control is treated with DMSO; 5 mM MTZ was administered from 5-7 dpf, washed off for 1 day, and imaged on 8 dpf. Anterior is to the left in all images. Five representative optical sections were shown, arranged from dorsal to ventral positions in the animal. Significant loss of TH+ neurons were observed. For quantification, see Fig. 2.

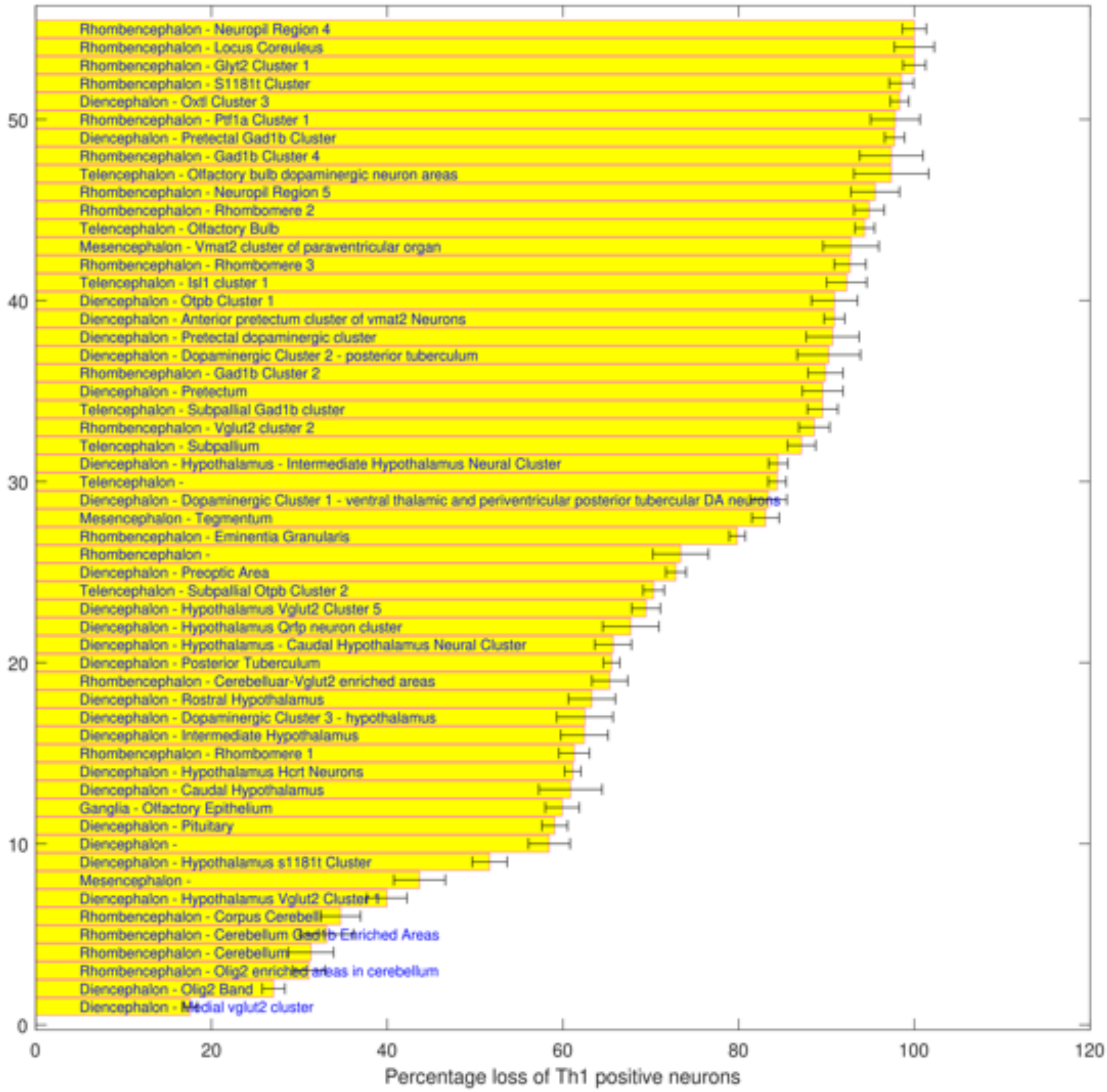

Figure 2. Quantification of DA neuronal loss in MTZ-treated animals. Bar graph depicting the percentage loss of TH positive neurons in different anatomical subgroups of DMSO-treated (n=12) and MTZ-treated (n=9) larvae. Relative errors in DMSO & MTZ groups were first calculated separately as standard deviation divided by mean; then they were added as: $[\Delta(MTZ)^2 + 2*\Delta(DMSO)^2]^{0.5}$. Differential rate of loss of TH+ neurons was observed in different anatomical regions.

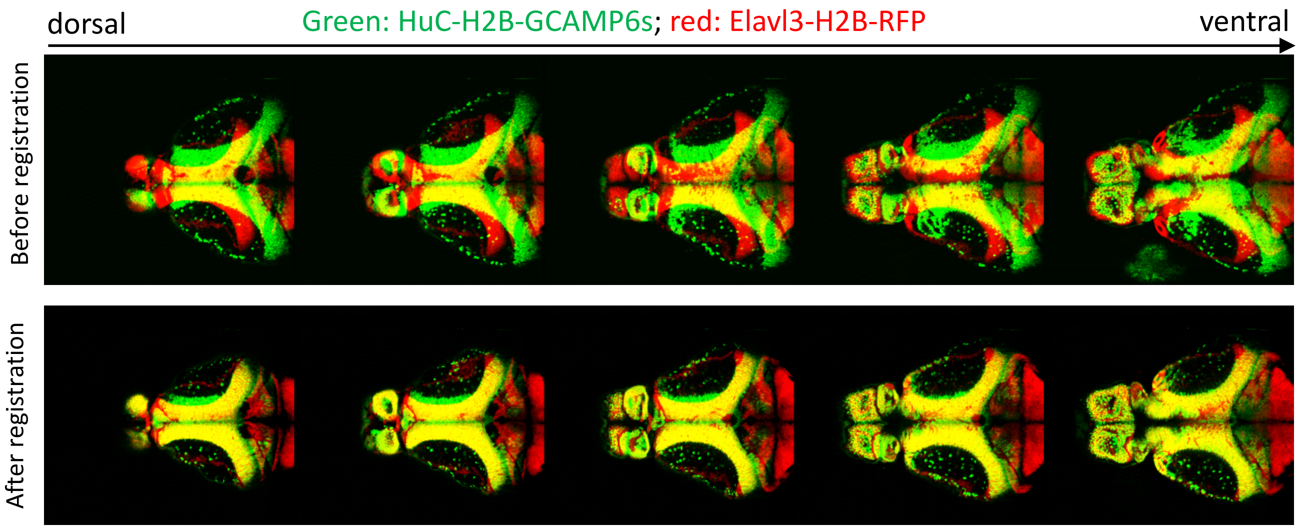

Figure 3. Brain resigration via the ANTs SynAggro transformation. Montage of representative optical brain slices at 10 µm Z-steps before (top) and after (bottom) registration with the Z-brain template (red). Our recorded Z-stack contained 151 slices while the Z-brain template contained 138 slices, so there is no direct Z-to-Z correspondence between green and red channels. Also, the image dimensions of green and red channels are different, so the green channel was resized to have same dimension as Z-brain template. 8 dpf larval brain (green) is bigger in volume compared to 6 dpf larval brain (red) of the Z-brain atlas. Both the registered Z-stack & Z-brain template contain 138 slices and have same image dimension.

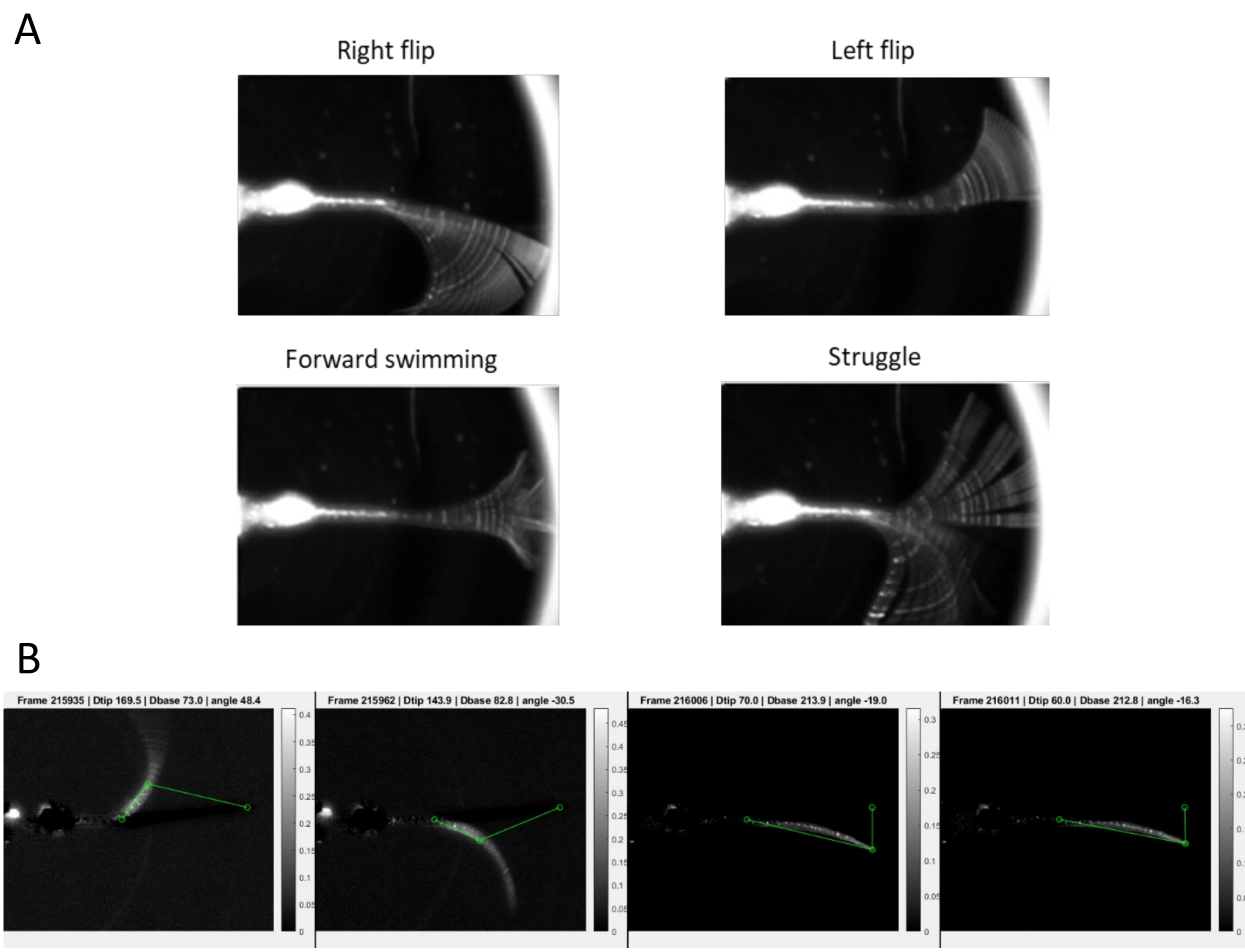

Figure 4. Characterization of locomotor behavior in head-fixed animals based on tail movement patterns. (A) Four primary tail movement patterns are observed to be correlated with neural activity. (B) Tail tracking using the custom written Matlab code. The program generates a movie containing the frames in which tail motion was detected. This video was used for manual verification of the angles printed in a .csv file. Here, 4 such frames from 1 movie are shown.