The neuromuscular junction is the critical point of communication between the nervous system and skeletal muscle. Invertebrate model organisms like fruit flies and nematodes use both excitatory and inhibitory signals to control muscles. In contrast, the vertebrate neuromuscular junction, acetylcholine and nicotinic acetylcholine receptors are paired mediate current exclusively. Before innervation, embryonic muscle also expresses receptors for other neurotransmitters. When neurons targeting muscles release other neurotransmitters, the appropriate receptors are retained and upregulated, allowing non-cholinergic current. The goal of this project is to use the zebrafish as a genetically tractable system in which to explore the mechanisms of neurotransmitter-receptor matching in vivo. The central hypothesis of this proposal is that embryonic zebrafish skeletal muscle expresses non-cholinergic receptors that are eliminated upon targeting by cholinergic neurons, and can be re-expressed after loss of
neurotransmission. This hypothesis will be tested using gene and protein expression analysis, pharmacologic, and genetic tools to determine when, which, and by which mechanisms receptors for the neurotransmitters GABA, glycine, and glutamate are expressed in embryonic zebrafish muscle. Successful completion of these objectives will expand the greater understanding of signaling pathways that contribute to formation and maintenance of the vertebrate neuromuscular junction and extend this exciting form of neural plasticity and its underlying mechanisms to bony fish. These experiments use techniques and timetables that are appropriate for student researchers at a primarily undergraduate institution in a state that does not receive significant federal research support, yet trains many talented students for careers in science.

**Start date:** 09-01-2024

**End date:** 08-31-2027

**Last modified:** 11-13-2023

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Developmental receptor plasticity and its evolutionary implications at vertebrate neuromuscular junctions

Data and Materials Produced

Describe the types of data, physical samples or collections, software, curriculum materials, and other materials to be produced in the course of the project. (For collaborative proposals, the DMP must cover all the various data types being collected by each collaborator.)

This project will produce experimental data from the following methods: polymerase chain reaction, light and confocal microscopy, protein quantification, and video tracking behavior. Data will be collected from a minimum of three independent experiments consisting of wild-type, control vs. drug treated, or wild-type vs. mutants at ages 14-24 hours- and 7 days post-fertilization.

Standards, Formats and Metadata

Describe the standards to be used for all the data types anticipated, including data or file format and metadata. [Note: Where existing standards are absent or deemed inadequate, this should be documented along with any proposed solutions or remedies.]

We expect to generate the following data file types during this project: images (.CZI and .TIFF), tabular (.CSV and .XLS), and video (.MP4 and H.264 AVC). Imaging data will be made available in tif format, and video data will be made available in mp4 format and will not require the use of specialized tools to be accessed or manipulated.

Data will be analyzed to generate tabular files containing quantifications of protein levels, protein expression, and behavior to enable statistical analysis.

To facilitate interpretation of the data, protocol DOIs will be created and shared from protocols.io.

Roles and Responsibilities

Describe the roles and responsibilities of all parties with respect to the management of the data (including contingency plans for the departure of key personnel from the project).

Lead PI D.R. Weinberger, ORCID: 0000000299735685, will be responsible for the day-to-day oversight of lab/team data management activities and data sharing.
Dissemination Methods

Describe the dissemination methods that will be used to make data and metadata available to others during the period of the award, and any modifications or additional technical information regarding data access after the grant ends.

In this proposed project, the cleaned, item-level spreadsheet data for all variables will be shared openly, along with example quantifications and transformations from initial raw data. Final files used to generate specific analyses to answer the project goals and related results will also be shared. The rationale for sharing only cleaned data is to foster ease of data reuse. To facilitate interpretation of the data, protocol DOIs will be created and shared from protocols.io.

Data will be stored in common and open formats, such as csv. Information needed to make use of this data along with references to the sources of those standardized names and metadata items will be included wherever applicable.

Policies for Data Sharing and Public Access

Describe the PI’s policies for data sharing, public access and re-use, including re-distribution by others and the production of derivatives. Where appropriate, include provisions for protection of privacy, confidentiality, security, intellectual property rights and other rights.

Murray State University's Digital Commons provides searchable study-level metadata for dataset discovery. Repository assigns DOIs as persistent identifiers and has a robust preservation plan to ensure long-term access.

Data will be discoverable online through standard web search of the study-level metadata as well as the persistent pointer from the DOI to the dataset. We will include ORCID iDs for people, DOIs for outputs (e.g., datasets, protocols), and Research Resource IDentifiers (RRIDs) for resources as much as possible to make data identifiable and findable. Controlled access will not be used. The data that is shared will be shared by unrestricted download. There are no anticipated privacy concerns, factors, or limitations that will affect the access, distribution, or reuse of the scientific data generated by the proposal.

Archiving, Storage and Preservation

Where relevant, describe plans for archiving data, samples, software, and other research products, and for on-going access to these products through their lifecycle of usefulness to research and
data (or research products) will be deposited for long-term access and where. (What physical and/or cyber resources and facilities (including third party resources) will be used to store and preserve the data after the grant ends?)

Data described will be deposited into Murray State University's Digital Commons. All scientific data generated from this project will be made available as soon as possible, and no later than the time of publication or the end of the funding period, whichever comes first. The duration of preservation and sharing of the data will be a minimum of 10 years after the funding period.
Planned Research Outputs

**Data paper - "Receptor diversity in zebrafish neuromuscular junction"**

Short paper sharing results of PCR and IHC of developmental receptor patterns in muscle, spanning developmental innervation.

**Data paper - "Mechanisms of neurotransmitter-receptor matching in the zebrafish neuromuscular junction"**

Larger paper exploring the factors that promote retention or elimination of other receptor types, including pharmacology and genetic data.

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**Planned research output details**

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