Expression of hTyr in *Drosophila* as a Novel Model of Parkinson’s Disease

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### Introduction

Recently, a main component of Parkinson’s disease development has been shown to be neuromelanin-induced neurodegeneration\textsuperscript{1-2}. Neuromelanin is an insoluble melanin created in dopaminergic neurons from dopaminergic precursors and byproducts, especially in the substantia nigra pars compacta (SNpc)\textsuperscript{3-4}. Its synthesis pathway, including the involvement of human tyrosinase (hTyr), is shown on the left, as well as its downstream consequences\textsuperscript{5}. hTyr was recently expressed in the SNpc and other dopaminergic neurons in rats and produced a PD-like phenotype\textsuperscript{6}. The goal of this thesis was to express hTyr for the first time in *Drosophila* melanogaster in order to create a naturalistic model of Parkinson’s disease for future research. In the creation of the model, we could provide evidence to determine whether hTyr expression is limited to mammalian systems. Additionally, *Drosophila* are easier to maintain, have faster turnover, have robust genetic tools and are overall more accessible for many labs\textsuperscript{7}.

Materials and Methods

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Figure 1: Synthesis and intracellular etiology of neuromelanin in dopaminergic cells. Modified from Mousavi et al. 2018 and Sulzer et al. 2018.

Figure 2: Cloning for the creation of the combined pUAST-hTyr-*Drosophila*-compatible plasmid.

Figure 3: Experimental design of hTyr + tissue-specific expressing driver lines. A. Review of the GAL4-UAS expression system. B. List of three main driver lines (WT control not shown) used and tissue-specific expression in the resulting mated hTyr flies.

Results

Figure 4: Conventional *Drosophila* activity monitor (DAM) protocol.

Figure 5: Raw 24-hour DAM activity plot, females (n = 76) and males (n = 67). Young flies (n = 79) were three weeks younger than old flies (n = 64). Young vs old was used to verify assay validity and old flies were a proxy for future changes in hTyr flies in the future. The novel cloning of a hTyr CDS into a pUAST plasmid and expression in live flies is nonetheless a critical step forward to making a neuromelanin-based *Drosophila* PD model.

Conclusion and Discussion

Climbing was shown to be an effective assay for quantifying negative geotaxis in flies in all sexes using old WT CantonS flies as a proxy. DAM could be effective for quantifying circadian rhythm periodicity and spontaneous activity, but more data is needed. Code written to quantify circadian rhythm periodicity is novel and can be used for future experimentation.

The homozygous hTyr line is still being mated, and thus the data in this thesis used old flies as a proxy while validating the assays that will be used to assess behavioral changes in hTyr flies in the future. The novel cloning of a hTyr CDS into a pUAST plasmid and expression in live flies is nonetheless a critical step forward to making a neuromelanin-based *Drosophila* PD model.

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References


