Inhibition of mTOR by Rapamycin Induces Neurodegeneration at the *Drosophila* Neuromuscular Junction

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***Abstract* — Rapamycin binds to and inhibits the mechanistic Target of Rapamycin (mTOR), but its neurological significance is not well documented. This study found that rapamycin reduced all measured indicators of neurological function, suggesting that rapamycin has an inhibitory role in neuronal function, long term potentiation (LTP), and impulse connections to physical development.**

I. Introduction

Rapamycin, an inhibitor of the mTOR pathway, has undergone extensive study and implementation as an immunosuppressant used to suppress the immune response that causes organ rejection in recipients. [1]

Rapamycin acts directly on the FKBP12 protein. The combined rapamycin-FKBP12 complex inhibits mTOR, which is a serine/threonine protein kinase that regulates cell growth, proliferation, survival, and protein synthesis. [2]

The purpose of this study was to quantify, both neurologically and developmentally, the role of mTOR at the neuromuscular junction. Based on the studies of Parsons et al. and Stoica et al., it was hypothesized that mTOR inhibition by Rapamycin would significantly decrease levels of cAMP, vesicle exocytosis, and calcium concentration at the neuromuscular junction, as well as locomotion and rate of development. [3][4]

II. Methodology

A GAL4 UAS crossed strain of *Drosophila melanogaster* was bred expressing a transgenic fluorescence resonance energy transfer (FRET) sensor for cyclic AMP (cAMP) at the motor neurons. These larvae were exposed to rapamycin concentrations 0 µM, 75 µM, 300 µM, and 600 µM. A locomotion assay and age stage cycle assay were performed, and larvae were dissected to analyze the neuromuscular junction (NMJ). A novel dissection chamber was created in order to be able to dissect and image without the use of a fixative. Three indicators of long term potentiation (LTP) and neuronal transmission were observed at 1000X oil-immersion magnification: the genetic FRET cAMP biosensor for the visualization of postsynaptic cAMP levels, FM 1-43 time staining used to measure presynaptic vesicle exocytosis, and FURA-2 staining to image Ca^{2+} concentrations. Pictures were taken through a Zeiss Axiovert fluorescent microscope and analyzed using ImageJ. Statistical analysis was conducted in IBM SPSS V20 with one-way ANOVA Post-Hoc Scheffe ($p<0.05$).

III. Results

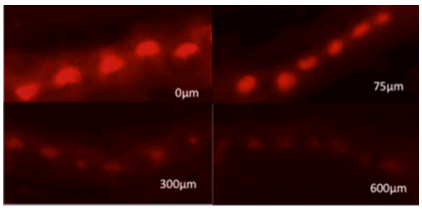

Figure 1. *Drosophila melanogaster* NMJ 1000x under a ZEISS Axiovert fluorescent microscope, using the FM 1-43 time stain.

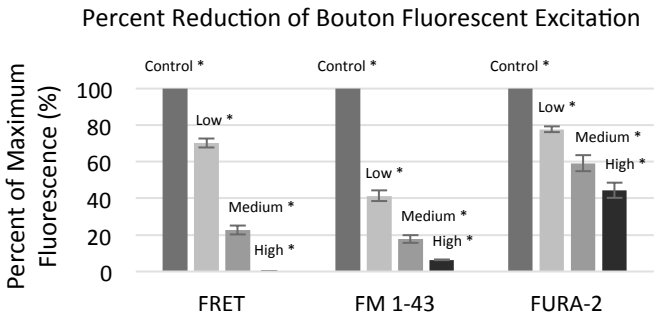

Figure 2. Average bouton fluorescence due to FRET CFP, FM 1-43 staining, and FURA-2 treatment, respectively, after treatment with rapamycin. Fluorescence is represented as a percent of the control fluorescence, recalculated for each sensor. * denotes significance $p<0.05$ between all concentrations
(Control: 0µM, Low: 75µM, Medium: 300µM, High: 600µM)

IV. Discussion & Conclusion

The results demonstrate that the inhibition of mTOR causes a statistically significant decrease in levels of cAMP (genetic FRET), the rate of presynaptic vesicle exocytosis (FM 1-43), and Ca^{2+} concentration at the *Drosophila* NMJ (FURA-2). Calcium was seen to decay linearly, indicating a near direct relationship with rapamycin. However, presynaptic vesicle exocytosis and levels of cAMP, which biologically are calcium-dependent, are shown to be thresholded values, decaying rapidly after a certain threshold is met. This threshold is the calcium level that marks the point at which LTP-dependent functions are triggered.

The loss of these functions suggests that long term depression has been triggered, associated with memory loss and neurological disease. Additionally, the age stage cycle assay and locomotion assay indicated slowed development rate and lack of mobility. This physical degradation, coupled with the neurological degeneration shown in processes essential to LTP indicates that mTOR has an integral role in neuronal function, LTP, and impulse connections to physical development.

Acknowledgments

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References

[1] Saunders et al."Rapamycin in Transplantation: A Review of the Evidence." Kidney International 59.1 (2001): 3-16. Web.

[2] Bjedov et al."Mechanisms of Life Span Extension by Rapamycin in the Fruit Fly Drosophila Melanogaster." Cell Metabolism 11.1 (2010): 35-46. Web.

[3] Parsons et al. "Translational Control via the Mammalian Target of Rapamycin Pathway Is Critical for the Formation and Stability of Long-Term Fear Memory in Amygdala Neurons." Journal of Neuroscience 26.50 (2006): 12977-2983. Web.

[4] Stoica, et al. "Selective Pharmacogenetic Inhibition of Mammalian Target of Rapamycin Complex I (mTORC1) Blocks Long-term Synaptic Plasticity and Memory Storage." Proceedings of the National Academy of Sciences 108.9 (2011): 3791-796. Web.

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