Undergraduate Research Symposium
ADVANCING RESEARCH AND STEM FIELD ENGAGEMENT
PROJECT 46

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Major: **Biology**
Minor: **Chemistry**

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Kappa Opioid Receptor and its Role in Demyelination in a Multiple Sclerosis Model

Multiple sclerosis (MS) is a progressive autoimmune disorder that affects the central nervous system (CNS). MS is characterized by demyelination in the brain and spinal cord. In MS the myelin sheath is specifically targeted and damaged, leading to an impairment in nerve conduction. G-protein-coupled receptors (GPCRs) have been studied and are known to play an important role in the demyelination process. It has been documented that a specific receptor, Kappa Opioid Receptor (KOR), enhances the production and proliferation of myelin in the central nervous system. This study utilized mouse brain tissue from the cuprizone model of MS and investigated the role of KOR in demyelination. In order to assess the levels of myelin present in mice, myelin basic protein (MBP) and proteolipid (PLP) levels have been measured. These proteins are the most abundant protein components of myelin and play a major role in the structure and function of myelin. Mice have been generated and treated in a collaborator's lab (Rutgers University), however, the tissue was harvested and processed in our lab at the College of Saint Elizabeth. There were four groups of mice, WT (wild type) control, WT cuprizone, KOR KO (KOR knock out) control, and KOR KO cuprizone. The hypothesis is that the cuprizone treatment will lead to a decrease in MBP and PLP gene expression levels in KOR KO mice when compared to cuprizone treated WT mice; cuprizone treated WT mice will also show a decrease in MBP and PLP gene expression when compared to untreated WT mice. RNA has been extracted from the mouse corpus collosum tissue, cDNA has been synthesized, and RT-PCR has been performed to assess changes in MBP and PLP gene expression levels in the treatment groups described above.

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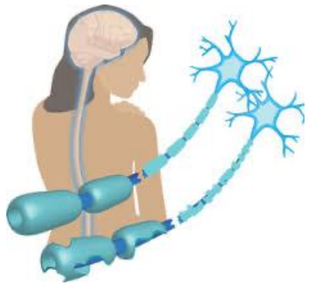
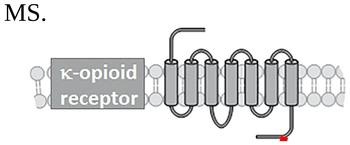
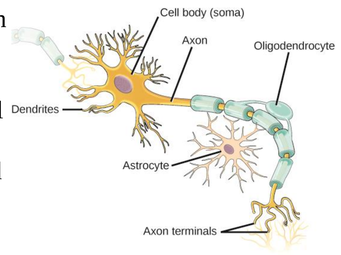
Background

- Multiple sclerosis (MS) is a debilitating autoimmune disease that causes destruction of the myelin sheath, the protective covering of the nerves. Myelin helps conduct electrical signals quickly and efficiently to and from the central nervous system (CNS). In MS, the myelin sheath is targeted and damaged, leading to an impairment in proper nerve conduction.
- G- protein coupled receptor's (GPCRs), such as the Kappa Opioid Receptor (KOR) have been studied and are believed to play an important role as targets for future therapeutic purposes in MS.





- KOR enhances the production and proliferation of myelin in the CNS. The enhanced production of myelin sheath is via oligodendrocytes. Oligodendrocytes are comprised of many proteins, however, the most common are myelin basic protein (MBP) and proteolipid protein (PLP).
- In order to study the demyelination process, mice are used, as MS can be mimicked in mice by administering cuprizone. Cuprizone is a copper chelating reagent, and it directly and indirectly causes oligodendrocyte death with subsequent demyelination.
- The purpose of this study is to assess the demyelination process and the amount of myelination proteins expressed in both the WT and KOR KO mice.



- **Hypothesis:** KOR knockout mice will exhibit altered gene expressions of myelin-related proteins like, MBP and PLP, in a cuprizone induced demyelination model.

Methods

C57BL/6 and 129S6 mice were fed with Cuprizone in their chow for about 100 days.

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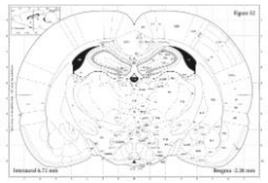
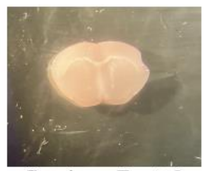
The medial portion of corpus callosum was viewed via a microscope and was dissected out

WT Control





Cuprizone Treated

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Homogenization and RNA Extraction

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Quantification of RNA concentration and absorption

Sample	Concentration (ug/ul)	A260/280
C57 WT	0.0211	0.528
C57 Cu WT	0.0218	0.545
C57 KO Control	0.0153	0.381
C57 Cu KO	0.0207	0.517
129 WT	0.0181	0.452
129 Cu WT	0.0207	0.517
129 KO Control	0.0129	0.323
129 Cu KO	0.0144	0.360

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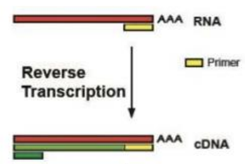
Rt- Reaction to obtain cDNA



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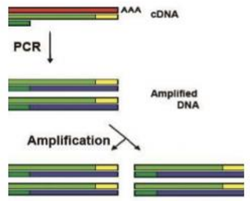
PCR Reaction for DNA amplification



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Data was obtained and analyzed

Results

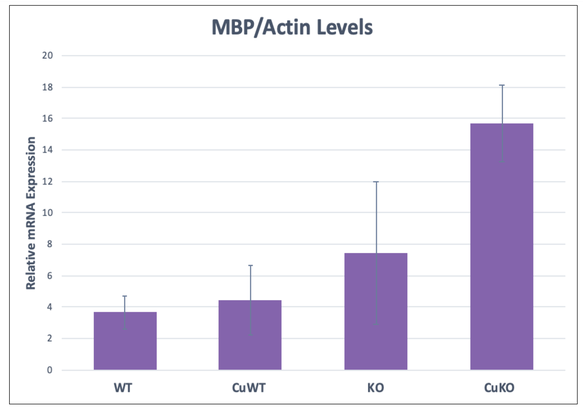


Figure 1: PCR data show levels of MBP gene expression in response Cuprizone treatment. CuWT has an increase in MBP expression compared to the WT group. CuKO also shows increased MBP expression compared to the KOR KO group. Overall there is an increase in MBP expression. Analysis of variance (ANOVA) determined that there are no significant differences between groups. MBP P = .48

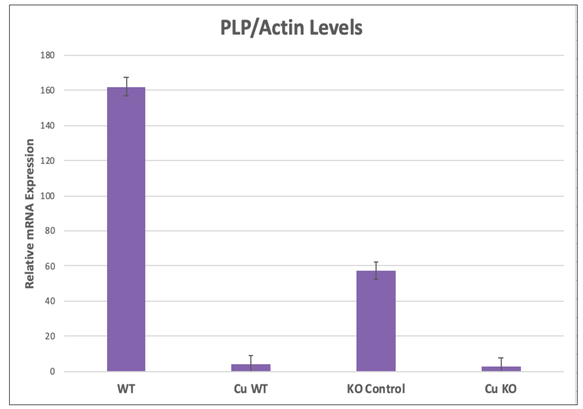
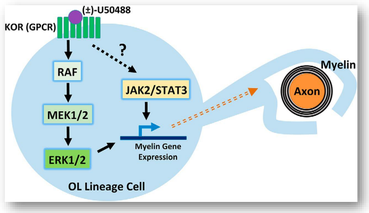


Figure 2: PCR data show levels of PLP gene expression in response to Cuprizone treated mice. CuWT has a decrease in PLP expression compared to the WT group. CuKO also shows a decrease in PLP expression compared to the KOR KO group. Overall, there is a decrease in PLP expression. Analysis of variance (ANOVA) determined that there are no significant differences between groups. PLP P= .51

Conclusions

- There is a trend for an increase in MBP expression in the Cu WT group compared to the WT control group. There is also an increase in MBP expression in the cuprizone KO treated group compared to the KOR KO group. In contrast to MBP, PLP expression decreased in both the Cu WT and Cu KO groups compared to the WT and KOR KO control group, respectively.
- MBP is a component of the myelin sheath and its increased expression indicates that remyelination is occurring.
- The significance of this study included understanding how the KOR functions not as an opioid receptor, but also a potential target in finding treatments for MS.



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