**­­­­­­­­­­­­­­­­UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2020/21**

***NB: This whole report will be posted on the Society’s website therefore authors should NOT include sensitive material or data that they do not want disclosed at this time.***

**Name of student:**

Oiher Serrano Asensio

**Name of supervisor(s):**

Lyndsay Murray

**Project Title: (no more than 220 characters)**

 Investigating masked pathology in selectively resistant muscles in a mouse model of SMA

**Project aims: (no more than 700 words)**

Spinal Muscular Atrophy is a childhood motor neuron disease with a genetic origin. In SMA, motor neurons are lost and pathology first evident at the neuromuscular junction. This include a loss of pre-syaptic terminals and a shrinkage and decrease in complexity of the motor endplate. Importantly, not all muscles are equally effected, with high levels of neuromuscular junction loss in some muscles while other remain comparatively unaffected. This selective vulnerability of motor endplates has not yet been characterised. In this project we seek to explore what happens to endplates when the pre-synaptic terminal is lost, whether there is intermuscular variability in the post-synaptic changes which are identified. Our specific aims are as follows (note that these have been altered slightly from the original proposal, due to covid related reasons):

1. **Compare total endplate number between SMA and WT mice in a selective vulnerable muscles.**

*Hypothesis: There will be a decrease in endplate number in selectively vulnerable muscles from a mouse model of SMA.*

* 1. Quantification of the percentage of fully occupied or vacant endplates is routine in the field of neuromuscular diseases. However, if endplates fade and are lost after denervation, this approach may be underestimating the levels of degeneration present. By quantifying total endplate number in a vulnerable muscle with high levels of pre-synaptic loss, we aim to determine whether endplates are lost following denervation
1. **Compare total endplate number between SMA and WT mice in a range of selective resistant muscles.**

*Hypothesis: There will be a decrease in endplate number in selectively resistant muscles from a mouse model of SMA.*

* 1. Here, we aimed to determine whether fading and loss of denervated endplates could be masking pre-synaptic degeneration in muscles designated as selectively resistant.
1. **Compare endplate pathology in differentially vulnerable muscles in a mouse model of SMA**

*Hypothesis: Endplate shrinkage and loss of complexity will be more severe in vulnerable muscles than in resistant muscles in a mouse model of SMA.*

* 1. Previous work has identified the atrophy and reduced complexity of endplates in muscles from mouse models of SMA. Here we aimed to explore whether the degree of post-synaptic pathology was related to the level of pre-synaptic pathology present.

**Project Outcomes and Experience Gained by the Student (no more than 700 words)**

1. **Compare total endplate number between SMA and WT mice in a selective vulnerable muscles.**

For this aim, fluorescent images of endplates stained with bungarotoxin and neurofilament were montaged in adobe photoshop. This analysis was done in the transversus abdominis muscle, a muscle which shows high levels of neuromuscular junction loss. Two consistent intercostal nerves were identified and all the endplates between the nerves were counted. Quantification of total endplate number between the two endplate bands revealed no difference between SMA and WT mice. This implies that, once denervated, endplates remain stable and are not lost.

1. **Compare total endplate number between SMA and WT mice in a range of selective resistant muscles**

For this aim, fluorescent images of endplates stained with bungarotoxin were montaged in adobe photoshop. Due to the comparatively larger size of the muscle, and high number of endplates, a quantification system was developed, which included image processing using Photoshop (making composites and highlighting desired shades of colour) and particle analysis using Fiji (ImageJ). Statistical analysis was performed in GraphPad Prism. Quantification of total endplate number revealed no significant difference between SMA and WT, which suggests that the fading or loss of endplates is not masking pathology in selectively resistant muscles.

1. **Compare endplate pathology in differentially vulnerable muscles in a mouse model of SMA**

Fluorescent images of endplates from 2 vulnerable (sterocleidomastoid and triceps) and 2 resistant (digastric posterior and hind limb lumbricals) muscles were used to quantify endplate size and endplate complexity. For endplate size, the outline of the motor endplate was traced and measured in image J, data was assembled in excel and analysed in graphpad prism. This revealed that, in resistant muscles there was a significant decrease in endplate size in both muscles. Interestingly, in vulnerable muscles, there was no significant difference in the average endplate size per muscle. Further analysis of individual endplate sizes revealed a sub-population of very small endplates and a small subpopulation of extremely large endplates. Further work will be required to determine whether these very small and very large endplate are innervated or denervated.

Endplate complexity changes during postnatal development, with endplates moving from an immature ‘plaque’ morphology to a mature ‘pretzel’ like morphology. The transition from plaque to pretzel has been previously described as delayed in mouse models of SMA. To explore how this relates to pre-synaptic vulnerability, endplates were designated as ‘plaque’, ‘intermediate’ or ‘pretzel’ in 2 vulnerable (sternocleidomastoid and triceps) and 2 resistant (digastric posterior and hind limb lumbricals) muscles. This analysis was done on fluorescent images with endplates labelled with bungarotoxin. In resistant muscles, there was a significant decrease in the number of ‘pretzel’ endplates and corresponding increase in ‘intermediate’ endplates. There were no ‘plaque’ like endplates. In the two vulnerable muscles, there was a significant increase in both ‘plaque’ and ‘intermediate’ endplates, with a corresponding decrease in ‘pretzel’ endplates. These results demonstrate that endplate maturation is delayed in both vulnerable and resistant muscles, but the delay is more severe in vulnerable muscles.

**Please state which Society Winter or Summer Meeting the student is intending to present his/her poster at:**

**Summer, 2022**

**Proposed Poster Submission Details (within 12 months of the completion of the project) for an AS Winter/ Summer Meeting – (no more than 300 words)**

The poster will display all the data gather from this project, as described in the preceding and proceding sections.

**Brief Resume of your Project’s outcomes**: **(no more than 200-250 words)**.

*The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.*

**Investigating motor endplate pathology of differentially vulnerable muscles in mouse models of Spinal Muscular Atrophy.**

Spinal Muscular Atrophy is a childhood motor neuron disease with a genetic origin. In SMA, motor neurons are lost and pathology is first evident at the neuromuscular junction. This include a loss of pre-synaptic terminals and a shrinkage and decrease in complexity of the motor endplate. Importantly, not all muscles are equally effected, with high levels of neuromuscular junction loss in some muscles while other remain comparatively unaffected. This selective vulnerability of motor endplates has not yet been characterised. In aim 1, we hypothesized that there would be a decrease in endplate number in selectively vulnerable muscles from a mouse model of SMA. Quantification of total endplate number in the vulnerable transversus abdominis muscle revealed no change in endplate number between wildtype and SMA mice. This suggests that denervated endplates do not fade following pre-synaptic loss. In aim 2, we hypothesized that there would be a decrease in endplate number in selectively resistant muscles. Quantification of total endplate number in the resistant diagastric posterior muscle revealed no difference between SMA and wildtype, which suggests that fading and loss of endplates does not mask degeneration in selectively resistant muscles. In the third aim, we hypothesised that endplate shrinkage and loss of complexity will be more severe in vulnerable muscles than in resistant muscles. Surprisingly, quantification of endplate size in two vulnerable and two resistant muscles revealed a significant decrease in endplate size in resistant, but not vulnerable muscles. Endplate complexity was decreased in both vulnerable and resistant muscles, but was more severe in vulnerable muscles. Further work is now required to investigate how loss of innervation affects endplate size and complexity.

**Other comments: (no more than 300 words)**

Oiher has been a great student – working really hard under very difficult circumstances. He has produced some really useful data which will trigger subsequent projects, and he has learned many project specific and general scientific skills during his time in the lab.

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*Signature of student.......................................................Date…28/09/2021…..*

*Signature of supervisor………………………………….............. Date…11th October 2021*

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