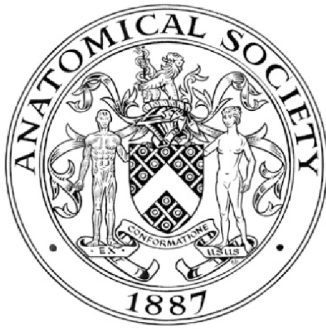




UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2025/26

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:

Eugenie Johanna Horisberger

Twitter Handle*:

(*optional)

Name of supervisor(s):

Dr Helena Chaytow & Professor Tom Gillingwater

Project Title: (no more than 220 characters)

Uncovering the Role of CCR2⁺ Macrophages in a Model of Motor Neuron Disease

Project aims: (no more than 700 words)

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterised by the progressive death of lower and upper motor neurons in the brain and spinal cord. ALS arises due to both genetic and environmental factors and is fatal, with patients having life expectancy of around 3 years following diagnosis (Masrori & Van Damme, 2020). ALS is characterised by the mislocalisation and aggregation of TDP43 protein within the cytoplasm, leading to the specific motor neuron cell death and neuroinflammation (Zhang et al., 2023). ALS progression leads to the denervation of neuromuscular junctions (NMJs) while the cell bodies remain intact (Dadon-Nachum, et al., 2011). This is known as the “die-back hypothesis”: early denervation of muscles causing weakness and wasting before neuronal death. It is now believed that proinflammatory factors, such as cytokines and infiltrating leukocytes, promote this early denervation of motor neurons in distant limbs (Dadon-Nachum et al., 2011). Significant CD68⁺ macrophages and CD45⁺ leukocytes have been observed around NMJs prior to denervation, leading to the hypothesis that early immune cell infiltration might be the cause of early denervation and promote the die-back hypothesis of motor neurons in ALS patients (Nógrádi et al., 2025).

In 2025, Nógrádi et al published a paper investigating the role of an important proinflammatory chemokine axis in NMJs denervation in ALS: the CCL2-CCR2 axis. CCL2 is a potent chemokine for monocyte infiltration in active inflamed tissue (Deshmane et al., 2009; Nógrádi et al., 2025). It attracts CCR2 positive cells, such as macrophages and leukocytes, into areas of active inflammation. Nógrádi et al. observed an increase in CD45⁺ leukocytes and CD68⁺ macrophages in ALS patient muscle as well as in ALS mouse models. Those infiltrated cells were hypothesised to contribute to NMJ denervation through the CCR2-CCL2 axis. Using CCL2 neutralising antibodies injected into the muscle, NMJ innervation was improved in correlation with an observed decrease of immune cell infiltration into hindlimb muscles (Nógrádi et al., 2025).

This project aimed to investigate the potential of CCR2 antagonists to prevent early denervation of motor neurons in gastrocnemius prior to early onset symptoms and neuronal death. Using a severe ALS model

(Thy1-hTDP-43) expressing hallmarks of ALS such as neuroinflammation, NMJ denervation, and motor neuron death, mice were treated with CCR2 antagonists.

The main aim of this project was to perform a quantitative morphological investigation at a published dose to determine whether systemic administration of a CCR2-targeting antagonists can prevent CCR2 +ve macrophage (CD68+ cells) infiltration into skeletal muscle and prevent NMJ denervation in an ALS mouse model..

Thus, this project had two main aims:

1. Determining the level of CCR2+ and CD68+ (macrophages) in skeletal muscle of the Thy1-hTDP43 mouse after treatment with a CCR2 antagonist.
2. Determining the effect of the CCR2 antagonist on NMJs denervation

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

Due to delays in colony breeding, CCR2-antagonist treated litters were only obtained towards the end of the summer project. Thus, morphological assessment on skeletal muscle samples was only possible on 2 mice – one wild type (WT) and one Thy1-hTDP43 mouse. Meanwhile, we optimised the protocol of CD68+ macrophages staining on mice treated with another immunomodulatory drug (MCC950). The CCR2-CCL2 images have been included in this report; however, the poster and final report will focus on the second immunomodulatory project.

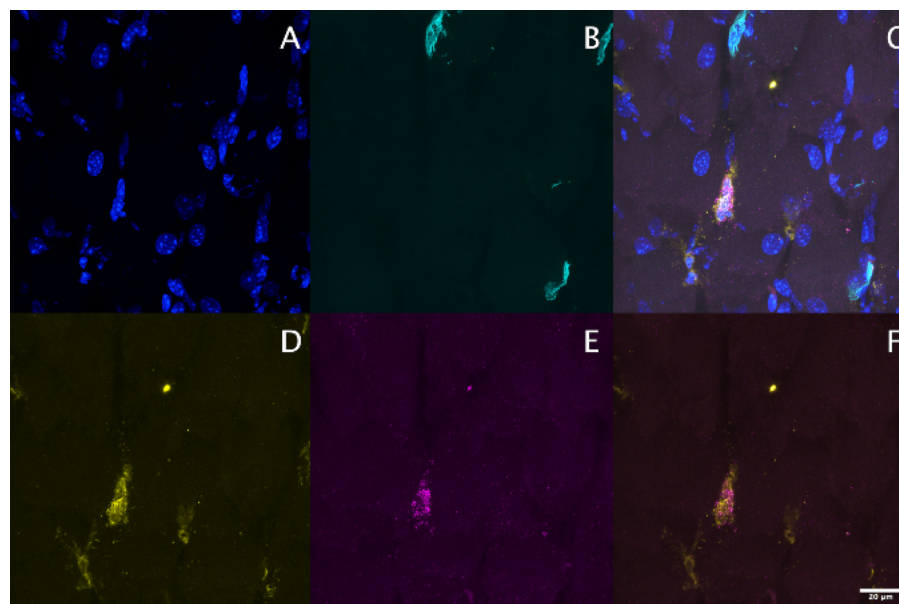


Figure 1: Successful optimization of immunohistochemical labelling of CCR2-CCL2 expression in hindlimb muscle from an FVBALS mouse model. (A) DAPI staining of cell nuclei. (B) α -bungarotoxin staining of acetylcholine receptors at NMJs. (C) Merge of all images. (D) CD45+ cells and (E) CCR2+ cells in hindlimb muscles. (F) Merge of CD45+ and CCR2+ cells in hindlimb muscles of FVBALS mouse model.

Our aim was to quantify CD68+ immune cell infiltration in hindlimb muscles in the Thy1-hTDP43 mice model after treatment. In order to do so, mouse pups were genotyped following birth using PCR and gel electrophoresis techniques. The mice were then assigned to treatment groups – e.g. drug or vehicle – and treated until they reached 19 days, where they show an ALS phenotype. The gastrocnemius muscle, as well as other hindlimb muscles (soleus, plantaris, flexor digitorum brevis, tibialis anterior, lumbricals, and extensor digitorum longus), was dissected and cryopreserved. Muscles were cryosectioned and mounted on slides for immunohistochemistry. Anti-CD68 antibody was used to stain CD68+ macrophages, as well as bungarotoxin for acetylcholine receptors in NMJs and DAPI for cell nuclei. Images were taken using an

epifluorescent microscope and analysed with ImageJ software. I learned and became independent in all stages of this process.

Bungarotoxin was used to label NMJs, which allowed us to take images over the innervation region of the GC muscle. Images were blinded for analysis to prevent subconscious bias. CD68+ cells were identified and showed an increased signal on the images, with adjustment of brightness and contrast used to optimize the identification of cellular overlaps. Following image analysis, across treatment groups, no significant differences in CD68+ macrophages were observed following MCC950 treatment. However, it should be noted that we also did not observe a difference in CD68+ macrophages numbers between wild type mice and Thy1-hTDP43, whereas previously published work has confirmed a strong immune cell infiltration into muscle across human patients and multiple mouse models of ALS (Nógrádi et al., 2025). These results could be due to technical issues in cell counting and image analysis, but also potential overcounts due to poor staining that included a lot of background noise. As such, we were not able to provide a definitive answer to the research question and further experiments will be required.

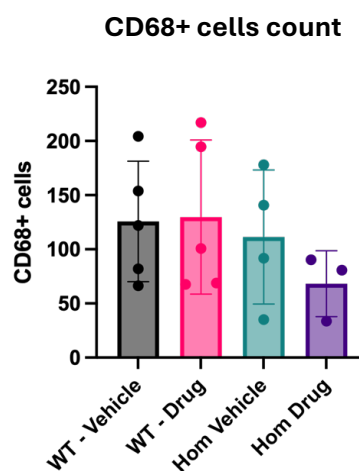


Figure 2: Quantification of CD68+ cells in Gastrocnemius muscle of the FVBALS Mice Model. The four treatment groups are wild type (WT) treated with a vehicle, WT treated with MCC950, Homozygous FVBALS mice treated with vehicle, and Homozygous FVBALS mice treated with the drug respectively. Data is presented as mean and S.E.M.

Throughout this project, we also assessed NMJ denervation status in hindlimb muscles of mice treated with CCR2 antagonists. Although no quantitative analysis could be performed due to small sample size, the first two obtained images confirmed successful SV2/2H3 immunolabelling for neurofilaments and synaptic vesicles, with α -bungarotoxin labelling acetylcholine receptors at motor endplates.

Throughout this 10 week summer project, I gained valuable experience in:

- Brain and spinal cord dissection and hindlimb microdissection, specifically for gastrocnemius, soleus, plantaris, flexor digitorum brevis, tibialis anterior, lumbricals, and extensor digitorum longus
- Embedding and cryosectioning hindlimbs, brain, and spinal cord tissues on slides for immunohistochemistry analysis
- Immunohistochemistry analysis assessing infiltrated CD68+ macrophages and CD45+ leukocytes, as well as CCR2+ macrophages, and NMJs using SV2/2H3 + α -bungarotoxin staining
- Image collection using Zeiss epifluorescence microscope to acquire high-quality images of both CCR2+, CD68+, and CD45+ with SV2/2H3 ICH labelling
- Morphological image analysis to process fluorescence images on ImageJ and perform simple cell counts on CD68+ macrophages and CD45+ leukocytes and denervation analysis on CCR2+
- Data handling learning how to consolidate the acquired data and present it in an informative manner such as lab group meeting.

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

AS Winter Meeting Trinity College Dublin, Ireland
TBD

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

Title: Immune Cell Infiltration in Skeletal Muscle of an ALS Mice Model After Immunomodulatory Treatment.

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.

The progressive denervation and ultimate death of motor neurons is the common pathological hallmark of amyotrophic lateral sclerosis (ALS). Due to excessive neuroinflammation seen in ALS patient and models, research is investigating whether motor neuron death might be exacerbated by proinflammatory factors such as cytokines and leukocytes. CD68⁺ macrophages infiltrate damaged muscles and are particularly found around neuromuscular junctions, the crucial nerve-muscle connection in ALS mouse models (Nógrádi et al., 2025). We therefore investigated whether immunomodulatory therapies could block this infiltration and protect the neuromuscular junction. We used immunohistochemistry to identify CD68⁺ cells in skeletal muscle surrounding neuromuscular junctions, labelled with bungarotoxin (Figure 1). While previous work had shown strong immune cell infiltration into the muscle of this mouse model, we saw wide variation between mice within each group. We also used immunohistochemistry to measure the denervation of neuromuscular junctions, where the motor neuron draws back from the muscle endplate in ALS (Figure 2).

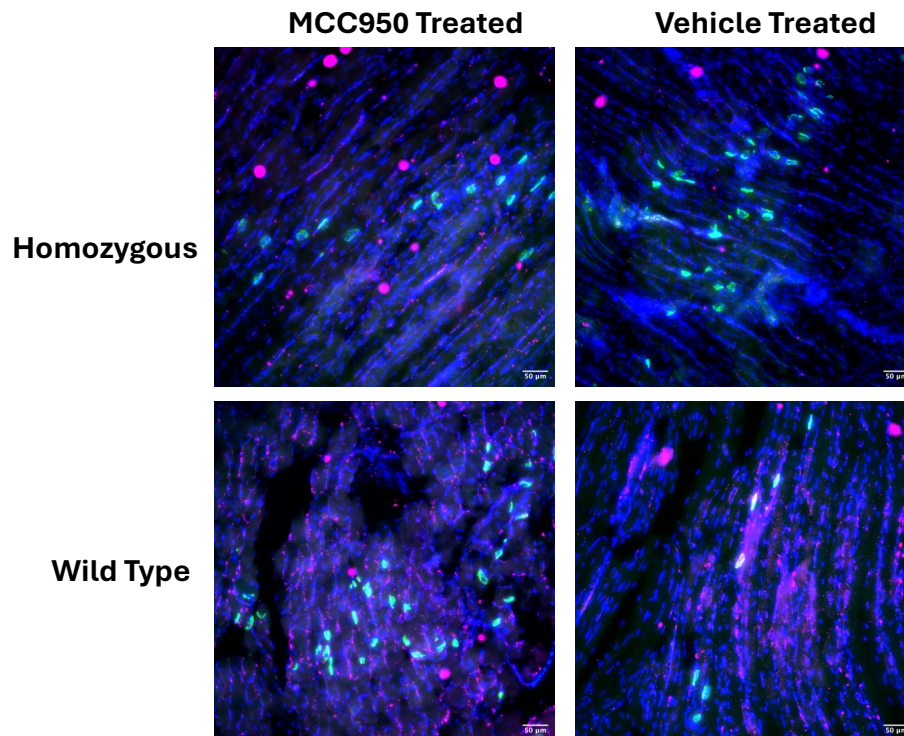


Figure 1: Immunohistochemistry of CD68+ Cells in Gastrocnemius of an FVBALS Mice Model. NMJs are stained with α -bungarotoxin in green, cell nuclei are stained with DAPI in blue, and CD68+ cells are stained with goat anti-CD68+ in magenta.

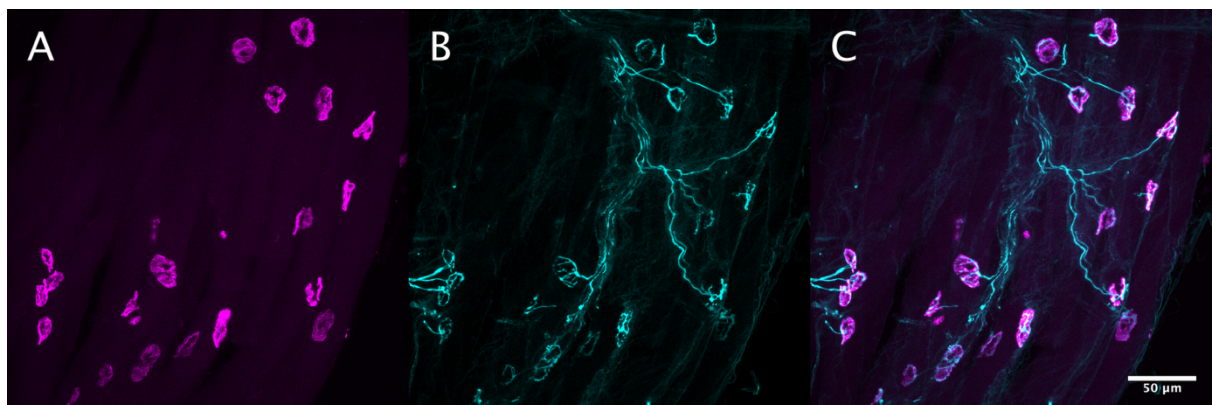


Figure 2: Neuromuscular junction innervation in the Gastrocnemius muscle of a Thy-hTDP mouse. (A) Acetylcholine receptors are stained with α -bungarotoxin (magenta). (B) Neurofilaments and pre-synaptic vesicles are stained with SV2/2H3 (cyan). (C) Merged images of endplates and presynaptic nerves in gastrocnemius muscle of a Thy-hTDP43 mouse model.

Other comments: (no more than 300 words)

Acknowledgments

I would like to thank the entire Gillingwater laboratory for making me feel so welcome during my internship. A special thanks to Professor Gillingwater for the opportunity to work on this project, and to my day-to-day supervisor, Dr. Chaytow, whose support was essential to its success. Finally, I am truly grateful to the Anatomical Society for the studentship that allowed me to pursue this project.

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Signature of student: Eugenie Johanna Horisberger Date: 17/09/2026

Signature of supervisor... ..Thomas Gillingwater ... Date...17/9/26.....

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