Unravelling Endocannabinoid Biology Using Omics Approaches in *Strongyloides* Parasites

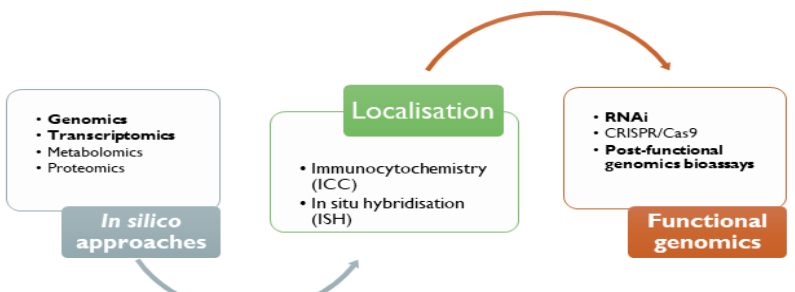
Luke Cadd, Nikki J. Marks, Aaron Maule, Angela Mousley, Louise E. Atkinson

Queen's University Belfast, UK

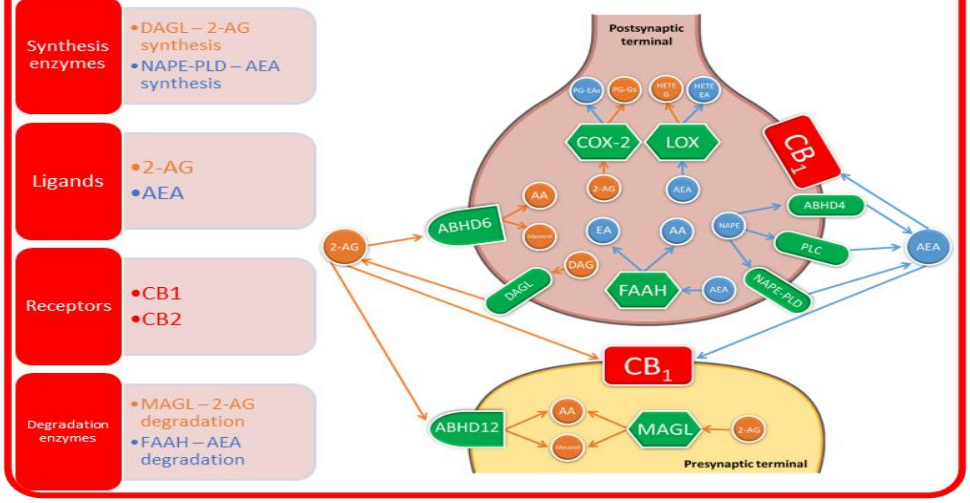
Parasitic nematodes cause neglected tropical diseases in 1.5 billion of the world's population and primarily affect the world's poorest communities[1]. Many of these parasites are soil-transmitted helminths (STHs) including *Strongyloides stercoralis*, *Ascaris lumbricoides*, whipworms *Trichuris trichiura* and hookworms (*Ancylostoma duodenale* and *Necator americanus*). Reliance on a limited range of anthelmintics and their sustained overuse is magnifying the risk of parasitic nematode drug resistance and intensifying the need for novel approaches to nematode control. **Endocannabinoid (EC) signalling** has received significant attention in human medicine due to its role in a variety of key biological processes, including movement, pain, reproduction and appetite[2]. Knowledge of EC-signalling in nematodes is restricted to the free-living model nematode *Caenorhabditis elegans*, where it is known to modulate cholesterol mobilisation, ageing, axon regeneration, locomotion, feeding and nociception[3-6]. The EC system in parasitic nematodes is largely uncharacterised, with current knowledge limited to data which suggest that EC-signalling may be involved in **host immune modulation** during hookworm infections[7]. This project aims to develop a platform for the identification and characterisation of novel drug targets using **in silico bioinformatics**, **in vitro bioassays** and **functional genomics** tools and will exploit this to explore EC signalling biology in ***Strongyloides* spp**. The data demonstrate that: (i) key elements of vertebrate signalling networks associated with EC biology are **conserved** in the genomes of 30 key parasitic nematodes representing 7 clades and free-living, animal-parasitic, entomopathogenic and plant-parasitic lifestyles and, (ii) both **novel** and **established** *Strongyloides*-specific bioassays have the potential to reveal fundamental information on parasite behaviour linked to EC-system function, including aspects of locomotion, sensory ability, development and parasitism. These fundamental data will aid the interrogation of EC-biology post-functional genomics (**RNAi**), and prioritise putative, novel, EC-targets for validation using **CRISPR/Cas9** approaches in *Strongyloides* species[8-9]. Knowledge of parasitic nematode EC-signalling systems has the potential to seed novel drug discovery pipelines for parasites of medical and agricultural importance.

Overarching Aim: Characterisation of EC signalling systems in parasites using a *Strongyloides* drug target prioritisation pipeline.

Drug Target Prioritisation Pipeline



Mammalian Endocannabinoid System

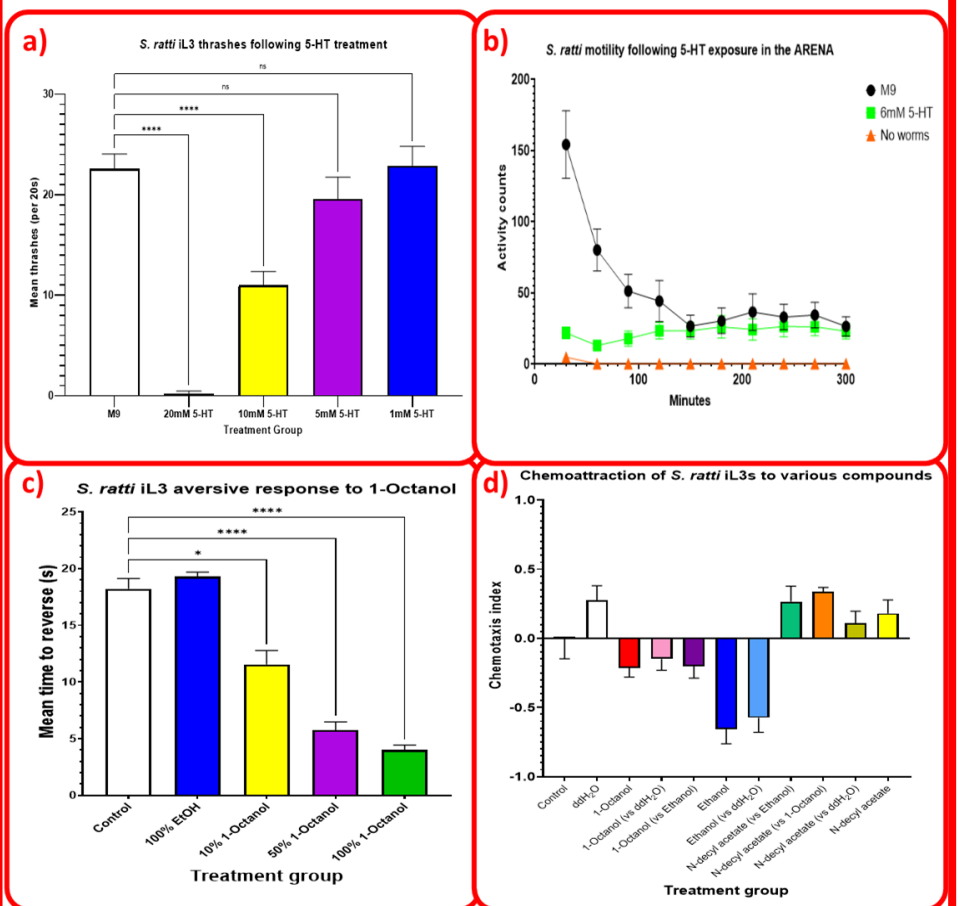


In silico characterisation of EC accessory receptors in Nematoda

- 30 nematode genomes; representing PN, FLN, EPN, PPN
- EC accessory receptors show varying levels of conservation
- Most conserved receptors will be used to explore **EC-pathway dynamics** post-functional genomics in *S. ratti*

Glutamate receptor
Acetylcholine receptor
Opioid receptor
Adenosine receptor
CatSper-related receptor
Ca^{2+} channel
Serotonin receptor
Dopamine receptor
TRP channel
Orphan GPCR
Thrombin receptor

Strongyloides Bioassay Toolkit



Strongyloides bioassay toolkit. a) Serotonin as a positive control for observing *Strongyloides* locomotion in liquid. b) The Wormtracker ARENA as an automated method for exploring locomotion in *Strongyloides* – appropriate for up to 2 hours. c) Using 1-octanol to elicit a nociceptive response in *Strongyloides*. d) *Strongyloides ratti* chemoattraction to different compounds.

Conclusions & Next Steps

1) Varied conservation of **EC accessory receptors** – explore further with transcriptomics and investigate other accessory components

2) ***Strongyloides* bioassay toolkit** can be used to explore phenotypes post-functional genomics

3) RNAi to explore roles of **core EC components** (e.g. npr-19) – explore **EC-pathway dynamics** utilising accessory components & direct targets for **CRISPR-Cas9**

References

1. Hotez, P.J., Bethony, J., Bottazzi, M.E., Brooker, S., Diemert, D. and Loukas, A., 2006. New technologies for the control of human hookworm infection. *Trends in parasitology*, *22*(7), pp.327-331.
2. Oddi, S., Scipioni, L. and Maccarrone, M., 2020. Endocannabinoid system and adult neurogenesis: a focused review. *Current opinion in pharmacology*, *50*, pp.25-32.
3. Lucanic, M., Held, J.M., Vantipalli, M.C., Klang, I.M., Graham, J.B., Gibson, B.W., Lithgow, G.J. and Gill, M.S., 2011. N-acylethanolamine signalling mediates the effect of diet on lifespan in *Caenorhabditis elegans*. *Nature*, *473*(7346), pp.226-229.
4. Pastuhov, S.I., Matsumoto, K. and Hisamoto, N., 2016. Endocannabinoid signaling regulates regenerative axon navigation in *Caenorhabditis elegans* via the GPCRs NPR-19 and NPR-32. *Genes to Cells*, *21*(7), pp.696-705.
5. Oakes, M.D., Law, W.J., Clark, T., Bamber, B.A. and Komuniecki, R., 2017. Cannabinoids activate monoaminergic signaling to modulate key *C. elegans* behaviors. *Journal of Neuroscience*, *37*(11), pp.2859-2869.
6. Galles, C., Prez, G.M., Penkov, S., Boland, S., Porta, E.O., Altabe, S.G., Labadie, G.R., Schmidt, U., Knölker, H.J., Kurzchalia, T.V. and de Mendoza, D., 2018. Endocannabinoids in *Caenorhabditis elegans* are essential for the mobilization of cholesterol from internal reserves. *Scientific reports*, *8*(1), pp.1-12.
7. Batugedara, H.M., Argueta, D., Jang, J.C., Lu, D., Macchietto, M., Kaur, J., Ge, S., Dillman, A.R., DiPatrizio, N.V. and Nair, M.G., 2018. Host-and helminth-derived endocannabinoids that have effects on host immunity are generated during infection. *Infection and immunity*, *86*(11), pp.e00441-18.
8. Lok, J.B., 2019. CRISPR/Cas9 mutagenesis and expression of dominant mutant transgenes as functional genomic approaches in parasitic nematodes. *Frontiers in genetics*, *10*, p.656.
9. Dulovic, A. and Streit, A., 2019. RNAi-mediated knockdown of daf-12 in the model parasitic nematode *Strongyloides ratti*. *PLoS pathogens*, *15*(3), p.e1007705.