Publishable Summary

Project web site: https://www.southampton.ac.uk/biosci/research/projects/circadian-developmental-requirements.page?

Specific Objectives (SO):

1) Map the subsets of pupal clock neurons where CLK/CYC acts to control subsequent adult circadian locomotor behaviour. Genetic constructs and transgenic lines targeting CLK/CYC activity in relevant spatiotemporal patterns will be generated, validated, and used to generate relevant behavioural data.

2) Determine the impact of developmental inhibition of CLK/CYC on molecular circadian rhythms in adult clock neurons. Time course confocal immunofluorescence (IF) imaging will be conducted to determine molecular circadian phenotypes in different subsets of clock neurons.

3) Determine the neuro-anatomical phenotypes associated with loss of developmental CLK/CYC activity and their relevance to adult circadian behaviour. Neuronal morphology will be examined using confocal IF imaging of the circadian neuropeptide PIGMENT DISPERSING FACTOR (PDF) as well as a membrane-tethered fluorescent marker protein (CD8::GFP) expressed in clock neurons of interest.

4) Test the involvement of known CLK/CYC co-regulators and target genes in mediating the developmental requirement for CLK/CYC activity. Transgenic phenotypes will be created and tested for circadian behavioural phenotypes in an automated locomotor assay and for molecular phenotypes in quantitative Reverse Transcriptase PCR (qRT-PCR) and time course IF imaging experiments.

Work performed since the beginning of the project, main results:

SO1: Transgenic lines have been generated and behavioural genetics experiments conducted to identify the subset of clock neurons responsible for the developmental function of CLK/CYC. Two discrete subsets of clock neurons were found to be involved in pupal CLK/CYC function. Thus, the work for SO1/Work Package 1 (WP1) has been completed on schedule.

SO2: Confocal ImmunoFluorescence time course imaging was performed for several independent transgenic fly lines with conditional CLK/CYC function and molecular oscillator function was determined for neurons of the subsets identified for SO1. Significant changes in oscillator function were observed for select subsets of clock neurons. Therefore, results for the portion of WP2 associated with SO2 have been obtained in a timely fashion.

SO3: Both CD8::GFP and PDF IF imaging was performed, leading to the identification of neuro-anatomical defects associated with developmental and/or adult depletion of CLK/CYC function. Hence, progress on the portion of WP2 associated with SO3 has been made as anticipated and this work is now complete.

SO4: Work on SO4/WP3: The proposed genetic screen and its associated molecular characterization has been completed. Several candidate genes were found to be necessary for adult circadian behaviour. Two of these provided a link between developmental CLK/CYC activity and its control of normal clock neuron projection morphology. However, no single gene was found whose manipulation suppressed the behavioural or neuro-anatomical phenotypes of developmental CLK/CYC deficits.

Final results and their potential impact and use:

The successful completion of the project represents an important advance towards elucidating the poorly understood developmental mechanisms underlying circadian behaviour. This aspect of circadian biology has long been neglected, in part, due to the lack of suitable genetic tools.

Our work has potential relevance to the insect control community, which may profit from a better understanding of the mechanisms underlying clock development and function as they impact pest management. We anticipate that our research will also generate hypotheses regarding homologous aspects of clock development in mammals, which will be of interest to the medical and veterinary research communities. Finally, given the pervasive effects of circadian clocks on all types of organisms and the remarkable progress that has been made in deciphering the mechanisms underlying daily time keeping our work is also of general interest in educational outreach to the non-scientific community both as a source of factual information as well as an illustration of successful scientific strategies.