
**Eileen Cowan Studentship 2021**

**Project Title:** The role of sleep in dystonia pathogenesis.

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Department of Genetics and Genome Biology

**Project Description:**

**Project Reference EC21.4**

Applications are invited for a newly established **Eileen Cowan Studentship 2021** to be taken up within the College of Life Sciences at the University of Leicester

Application website:

https://le.ac.uk/study/research-degrees/funded-opportunities/cls-eileen-cowan-2021

Dystonia is a common movement disorder, characterised by involuntary movements or abnormal postures. Sleep disruption has been reported in dystonia despite the easing of motor symptoms during sleep[1], suggesting that the involuntary regulation of arousal/motor control is also perturbed in dystonia. Dystonia and sleep disturbances both correlate with changes in synaptic plasticity[2,3]. Yet it is unclear whether overlapping synaptic mechanisms cause sleep abnormalities and motor phenotypes in dystonia, and whether reduced sleep quality impacts the severity of dystonic movements. The induction of synaptic plasticity results in well-characterized changes in neuronal transcription[4]. In contrast, the transcriptional signatures of dystonia pathogenesis remain elusive, despite the identification of several dystonia-associated genes.

Loss-of-function mutations in the neuronal calcium sensor, Hippocalcin, are linked to autosomal recessive dystonia type 2 (DYT2) and increased excitability in cultured neurons[5]. However, it is unclear whether reduced Hippocalcin function perturbs neuronal transcription, and if so, how such alterations impact sleep and motor phenotypes in dystonia. Recently we successfully modelled DYT2 dystonia by generating knock-out (KO) and neuronal knock-down (KD) fly lines for the *Drosophila* Hippocalcin homologue, Neurocalcin (Nca). These flies show both sleep and locomotion phenotypes, associated with enhanced synaptic neurotransmitter release in the sensorimotor system[6].

This PhD project will use these fly models, combined with the versatile genetic toolkit, to investigate the potential role of sleep in dystonia pathogenesis by investigating the following objectives:

***1) Identifying pathogenic molecular pathways in DYT2 flies***

A recent study revealed that the differentially expressed transcriptome in a Huntington's disease mutant background contains pathogenic molecular pathways linked to disease phenotypes[7]. By applying the same strategy, we used RNA-Seq to define the differentially expressed transcriptome in NcaKO fly heads. Focusing on the role of these transcriptional targets in sleep control, we have performed an RNAi screen to knock down the top 50 differentially expressed genes in NcaKD flies. Among these genes, we have identified

candidate genes as sleep modifiers in the following cellular pathways: piRNA synthesis, telomere maintenance, and GPCR signalling. The PhD student will further verify these genetic interactions using CRISPR alleles of candidate genes. They will also investigate whether these candidate genes regulate Nca-mediated synaptic release using a fluorescent reporter (spH)[6].

***2) Identifying shared pathways underlying motor and sleep phenotype***

We previously found that NcaKO flies show reduced locomotion upon stimuli. Although this is an indicator of motor defects, the abnormal movement akin to dystonia has not been explored further in the fly models. *Drosophila* requires fine control over sensorimotor circuitry to perform the male's courtship song, which represents an ideal readout of precise motor control. The student will thus test for alterations in the courtship song of DYT2 models and whether the above identified candidate genes also modulate song and locomotion motor phenotypes in DYT2 flies.

***3) Testing whether sleep can ameliorate motor phenotypes***

The student will optogenetically activate known sleep-promoting circuits to increase sleep in DYT2 and four other previously established dystonia fly models (in collaboration with Jepson lab, UCL). They will then monitor subsequent changes in motor phenotypes. Conversely, constant vibration throughout the night will be applied to test whether sleep deprivation exacerbates motor phenotypes.

**References: 1.** Sleep Med. Rev. 26, 95-107 (2016). **2.** Neurobiol. Dis. 42, 162-170 (2011). **3.** Science 355.6324: 507-510. (2017**). 4.** Neuron 66, 337-351 (2010). **5.** Am. J. Hum. Genet. 96, 657-665 (2015). **6.** Elife 8, 38114 (2019). **7.** Cell Syst. 7(1), 28-40 (2018).

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