**University of Leicester**

**BBSRC MIBTP Studentship Project 2024-5 entry.**

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| **Project Reference** |  |

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**Section 2 – *Project Information***

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| **Project Title** | **Identifying the neurogenetic network underlying visually-driven sleep** |
| **Project Summary** | |
| **General background:** Sleep is a behavioural quiescence widely observed in the animal kingdom. During sleep, an animal’s motor activity, as well as their responsiveness to environmental stimuli, are largely reduced. The levels of sleep and wakefulness are maintained by homeostatic equilibrium: when we are awake for a period of time, the need to sleep (so-called sleep pressure) increases to trigger sleep, which in turn releases the sleep pressure so wakefulness resumes. Evidence from model organisms and humans indicates that daily light and visual stimuli contribute to this sleep pressure; our lab is interested in identifying the elusive molecular and neural basis of such vision/light-driven sleep pressure via several lines of investigation (see the related research topics in the lab website: <https://www.kofanchenlab.net/>).    We have identified *neurocalcin* (*nca*) as a gene with a key role in the regulation of light/vision-driven sleep in the fruit fly *Drosophila* *melanogaster* (*1*). Critically, circuit-based neurogenetic manipulation of *nca* indicates that *nca* is expressed within a novel neural pathway linking the visual system and the known sleep homeostatic centre in the fly brain (vision-sleep pathway). Moreover, we have used RNA-Seq to identify differentially expressed genes (DEGs) between the *nca* mutant and wildtype flies. We hypothesise *nca*-associated DEGs cooperate to facilitate vision-elicited sleep pressure in the novel vision-sleep pathway.    **Aim:** This PhD project, therefore, aims to combine the latest bioinformatic and bench techniques in single cell RNASeq (scRNA-Seq), connectomics and *Drosophila* sleep to map the neural population within the vision system that *nca*-associated DEGs act to facilitate visually driven sleep. In collaboration with the lab members and the wider research network, the student will conduct the project through the following three objectives.    **Objectives and Methods:**   1. **Identification of *nca*-associated DEGs enriched in the visual system:** Recent availability of single-cell RNA-Seq data of the fly brain and visual system (*2*) allow us to identify the spatial/temporal expression of the *nca*-associated DEGs within *Drosophila* visual system. Working with the Feuda lab this objective aims to map the DEGs into the scRNA-Seq data sets in order to isolate the neural clusters enriched for *nca*-associated DEGs which will be identified. Conversely, the mapping will also allow identification of a subset of *nca*-associated DEGs that express in the visual system. 2. **Identification of neural connection of vision-sleep pathway**: the preliminary data indicates that the vision-sleep pathway in *Drosophila* connects photoreceptors and a group of neurons just upstream to the sleep centre. Using the latest complete fly brain connectome (*3*), this objective aims to digitally construct a detailed neural connection of the vision-sleep pathway and examine overlaps with the *nca*-associated DEGs enriched neural clusters. The overlapped neuronal population will be confirmed experimentally by confocal brain imaging using a customised *nca*-associated DEGs-based enhancer trap reporter (*2*). 3. **Verification of the sleep modifying effect of *nca*-associated DEGs:** upon generation of the gene/neural cluster list, an automatic high throughput sleep monitor system will be used to test if reduction of these genes modifies the sleep phenotype in the *nca* mutant flies. The reduction will be implemented in all neurons as well as in the neural clusters identified.   Techniques that will be undertaken during the project  *Drosophila* Genetics, behaviour assay, *in silico* brain imaging, Bioinformatics on scRNA-Seq and Connectome, immuno-confocal brain imaging. | |
| **References** | |
| 1. Neurocalcin regulates nighttime sleep and arousal in *Drosophila*. *eLife*. 8 (2019), doi:10.7554/eLife.38114.  2. Using single-cell RNA sequencing to generate predictive cell-type-specific split-GAL4 reagents throughout development. *Proceedings of the National Academy of Sciences*. 120, e2307451120 (2023).  3. Neuronal wiring diagram of an adult brain. *bioRxiv.* (2023), 2023.06.27.546656 | |

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