**BBSRC MIBTP Studentship Project**

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| **Project Title** | Unravelling the interplay between circadian clock and stress response using neurogenetics  |
| **Project Summary**  |
| Molecular clock also interacts with a key kinase-signalling network called the integrated stress response (ISR) (*1*). Cellular stressors such as protein aggregates and reactive oxidative stress trigger ISR and modulate downstream gene transcription. Furthermore, altered ISR levels in fly neurons modify circadian rhythm, suggesting that the ISR is a cellular mechanism linking cellular stress to sleep-wake cycle. ISR can be triggered by misfolded protein aggregates that are associated with aged-related neurodegeneration. Initially ISR in the affected neurons eliminate the protein aggregates and the related cellular stresses, yet, prolonged cellular stress leads to neuronal dysfunction and cell death (1). Typically, the protein aggregates and the related ISR first affect specific brain areas. This selectivity is a widely observed phenomenon termed, selective vulnerability. However, the molecular mechanisms underlying selective vulnerability remain unclear.   The neuronal toxicity directed by aggregate-prone proteins including Aβ, SCNA, HTT and TDP43 has been studied by expressing misfolded protein aggregate in *Drosophila*. Recently we found that the 150 so-called “clock neurons” that express the molecular clock in the fly brain are resistant to Aβ-related ISR stress and toxicity (3). Intriguingly others found that clock neurons are more vulnerable to HTT-mediated toxicity (3).   Taken together, these findings suggest that the molecular clock regulates ISR stress to modify neuronal vulnerability to specific protein aggregates.  **Aim:** Using the versatile genetic and imaging tools available in the fruit fly, this PhD project aims to investigate the role of the ISR in shaping selectivity of the circadian clock against protein aggregates.  **Objectives and Methods:** the project will investigate the following question-oriented research objectives: **1.** ***Which* *aggregates selectively cause ISR in clock neurons?*** Fly strains have been generated to express the aforementioned protein aggregates and the transgenic fluorescent reporters for ISR (4). The project will follow a protocol using techniques including brain dissection, immunostaining, confocal microscopy and image analysis. This protocol allows investigation of the fluorescent signals that indicates the extent of ER stress in the fly brains. Clock neurons will be immuno-labelled in the same brains in order to compare the levels of ER stress between clock and non-clock neurons. To further evaluate the direct effect of molecular clock on ISR, XBP1-GFP signals will be verified in aggregate expressing flies without molecular clock. **2.** ***What candidate ISR related genes drives the selective vulnerability of clock neurons against protein aggregate?*** Using the publicly available RNASeq data and bioinformatics tools, the PhD student will look for candidate genes in the cellular stress related pathways. The identified genes and the key ISR genes will be knocked down in the fly clock neurons via somatic-CRISPR and RNAi technology and evaluate the effect of the knockdowns on ER stress level in the clock neurons and circadian behaviour. **3. *Does arrhythmic ISR gene expression exacerbate cellular stress in neurodegeneration?*** Using smFISH/RNAscope technology, we will verify daily expression rhythm of ISR genes in the fly brains between wildtype and clock-gene mutant flies. The student will test if loss of the ISR gene rhythm modifies ISR levels flies expressing protein aggregates (reported by ATF4-dsRed). Techniques that will be undertaken during the project:*Drosophila* Genetics, Video-based behaviour assay, *Ex vivo* brain imaging,  Bioinformatics on RNA-Seq, Immunohistochemistry BBSRC Strategic Research Priority: Understanding the Rules of Life - Neuroscience and behaviour |
| **References** |
| 1. Nature Reviews Neurology. 13, 477 (2017). 2. Disease models & mechanisms. 7, 445–458 (2014). 3. Cell Reports. 27, 59-70.e4 (2019). 4. PLOS ONE. 8, e75774 (2013 |