Further Examples of Microscopy-Based Projects in Animal BioSciences

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At FoM 2013, we presented examples of microscopy-based projects in Animal Biosciences. We present here some further examples in that field:

**Understanding early mechanisms of Protein Misfolding Diseases (PMDs)**

Alzheimer's, Parkinson's, Huntington's and Prion diseases are all examples of PMDs associated with progressive aggregate accumulation of abnormal proteins. To further understand PMDs, primary hippocampal neuronal cells were isolated from two mouse lines which support or curtail protein aggregate formation/misfolding mechanisms. Characterisation of this system involved: time-lapse phase-contrast to follow neuronal growth and confocal microscopy using neuronal/glial markers (MAP2 and GFAP), allowing for detailed visualisation of cultured neurons. Confocal images were analysed with IMARIS software. Development and detailed characterisation of this system is essential to provide a reproducible in-vitro model to investigate protein misfolding interactions within the brain.

**Dynamic cellular photoperiodic changes in pars tuberalis and hypothalamus in sheep**

We examined changes in pars tuberalis (PT) and median eminence (ME) cells in sheep under short (SP) and long photoperiods (LP). ChromograninA (CHGA), a packaging protein, was expressed in PT thyrotroph cells. Confocal microscopy revealed CHGA localised to granules and increased in SP compared to LP. Western blot, EM and RNA-seq confirmed this, suggesting SP is defined by a storage state switching to a secretory state in LP. Synaptic changes at the ME interface of the hypothalamus/pituitary with photoperiod, changes in Gonadotropin-releasing hormone (GnRH) neuronal distribution and tanycytes and their relationships were observed between photoperiods. In LP the end-feet of the ME tanycytes overwhelm nerve terminals, potentially prohibiting GnRH release and reproductive changes. In SP, tanycytes are less packed and GnRH nerve contact reduced, potentially allowing GnRH release, driving reproductive circuits from the pituitary (LH, FSH) to activate the gonads. Results indicate that PT and hypothalamus are highly responsive to photoperiodic changes.

**Development and differentiation of tissue macrophages**

Macrophages perform multiple roles in embryonic/postnatal development, requiring constant turnover. However, their replenishment source is controversial. We aim to study macrophage origins by imaging transgenic mouse embryos that have eCFP+ (yolk sac-derived) or eGFP+ (haemopoietic) macrophages. A variety of imaging techniques are being used to detect macrophage migration patterns from onset to tissue entry. Fluorescence stereomicroscopy allows us to identify the time point corresponding to the onset of macrophages; and confocal / 2-photon in vitro and in vivo imaging strategies can detect macrophage migration.

**High Resolution Microendoscopy (HRME) for point of care pathology (POP)**

Potential clinical and veterinary clinical applications for fibre-based micro-endoscopy systems are numerous, including tumour detection, monitoring response to topical therapies (e.g. siRNA delivery), lesion detection, guided biopsy and/or resection in various accessible organs (skin, oropharynx, ear, etc.) We built a fibre-optic fluorescence microscope to specifications from a group at Rice University, Houston [1]. We intend to examine the potential for this system to benefit clinical veterinary medicine and surgery. We present here results from the in vitro validation of this instrument, by adaptation of the condenser of an inverted fluorescent microscope to include the fibre and comparison/correlation of mirror images from the microscope and micro-endoscope. [2]