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**Animal Welfare** **Anaesthetic**

# Determination of Loss of Consciousness in Rodents

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**Key words**
Animal welfare, 3Rs, Anaesthesia, Analgesia, Behaviour, Neuroscience, Electrophysiology

**Aim of the study**
The original aim of this project was to investigate the use of evoked neurophysiological potentials to investigate the point of loss of consciousness in rodents undergoing general anaesthesia and euthanasia. Due to the COVID-19 pandemic approximately 6 months of time available for experimentation were lost and further restrictions upon resumption of work prevented the goals being reached. The aims and scope of the project were altered upon discussion with and mutual agreement of the FSVO. Material and methods

**Results and significance**
Characterising somatosensory evoked potentials:
This work was performed during the early stages of the project. Mice were implanted with a peripheral nerve stimulating electrode around the median nerve and high-density recording electrodes in the primary somatosensory cortex. Intrinsic optical imaging (BOLD signal) was optimised to increase the accuracy of brain implantation, therefore adding a refinement to reduce the number of animals required. After recovery from surgery, animals were allowed to move freely in the homecage and somatosensory potentials recorded across and between vigilance states (wakefulness, sleep, general anaesthesia). No changes in evoked potential activity were found during changes in vigilance, however viral tracing revealed important anatomical links between primary cortex and higher order cortical areas. Evoked activity in these higher order areas did change with changes in vigilance state, hence the need to record from highly precise brain areas was established. Interestingly, EEG activity, including measurements of theta activity, did not correlate with changes in vigilance. Insufficient data from higher order cortical areas were collected prior to restrictions to complete this part of the project.

Neurophysiology of escape behaviours:
Brain activity recording of local field potentials (LFP) were performed in mice during aversion avoidance testing using the light-dark box paradigm. Carbon dioxide and isoflurane were used as the aversive agents. The methodology was refined to allow accurate testing of aversion by training the animals to rest in the dark compartment. Predictably, mice left the dark chamber upon exposure to both agents. Further, second exposure to isoflurane resulted in enhanced aversion as previously reported in the literature. Full analysis of the data has not yet been completed; however, the aim is to correlate the neurophysiological fingerprint of escape behaviour, marked as the transition of the animal from the dark to light chamber. This can then be used to determine the motivation of other behaviours such as rearing/jumping, which may be interpreted as either aversion or exploration. One peer-review publication is expected from this work[1].

Anxiety behaviours during CAS in mice:
Part of the restricted working time was used to add additional experiments to a previous project. Performance on the elevated plus maze was investigated in mice exposed to carbon dioxide, mice or low atmospheric pressure. Additional experiments were required to answer reviewer comments and these have now been completed. On peer-review publication is expected from this work and the manuscript is in preparation[2].

Optimisation of general anaesthesia for craniotomy:
Mice underwent craniotomy using a variety of anaesthesia and analgesia regimens. Four treatment groups, meloxicam only, carprofen only, meloxicam and dexmedetomidine, carprofen and dexmedetomidine were sued. Mice were monitored intraoperatively with a customised to pulse oximeter and capnograph to investigate intraoperative stability and also by 24-hour, seven-day video recording to investigate recovery. The aim is to refine methods for reduction and refinement of mice used in neuroscience. One peer-review publication is expected investigating intraoperative stability and this manuscript is in preparation[3]. Video recordings are being used to formulate a machine learning based algorithm to detect behavioural end-points relevant to successful recovery from craniotomy. Importantly, this will also extend to prediction of animals requiring rescue analgesia or other interventions thus providing a further refinement to postoperative care. One further peer-review publication is expected from this work[4]. Additional data collected during the craniotomy includes intrinsic optical imaging (BOLD signal). Comparison of anaesthesia regimes will allow refinement of signal detection thus providing a refinement to mice undergoing experimental imaging. A graduate student is being recruited to analyse the data and a one further peer-review publication is expected from this work[5].

Depth of anaesthesia monitoring:
Additional work was performed during restricted periods on a collaborative basis developing a tool to measure depth of anaesthesia in mice, using machine learning algorithms. The algorithm reads EEG in realtime and determines the depth of anaesthesia based on a theoretical concentration of inhaled isoflurane, as determined by experimentation. One peer-review publication is expected from this work and the manuscript is in submission to Scientific Reports[6]. The FSVO is acknowledged for partial funding of this work.

Summary
Although the original aims of the project could not be realised due to unforeseen restrictions, the project was reorientated to answer questions aligned with the FSVOs primary research interest. Despite a significant foreshortening of the time available, six peer-review publications are expected from this work.

**Publications, posters and presentations**

[1] Gent, T.; Neurophysiological signatures of escape behaviour. Scientific Reports, (*target journal*).
[2] Steiner, Ar.; Renner, A.; Bettschart-Wolfensberger, R.; Gent, T. Atmospheric effects on anxiety testing in mice. Animals (Basel), (*target journal*).
[3] Schiele, A.; Bettschart-Wolfensberger, R.; Gent, T.; Refining anaesthesia and analgesia for craniotomy in mice; a multimodal approach. Animals (Basel), (*manuscript in preparation*).
[4] Publication on machine learning-based assessment of postoperative recovery. (*work is at too early a stage to predict, title, authors and target journal*)
[5] Publication on optimisation of anaesthesia for intrinsic optical imaging. (*work is at too early a stage to predict, title, authors and target journal*).
[6] Schmidt, D.; English. G.; Gent T.; Yanik, MF; von der Behrens, W.; Electrocorticography based monitoring of anaesthetic depth in mice. Scientific Reports (*in submission*). *Manuscript available online in BioRxiv: https://www.biorxiv.org/content/10.1101/2021.07.12.452032v1.full*

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