

# An in vivo electrophysiological approach to the classification of interneurons in the behaving animal.

## Background

My doctoral work explored the complex relationship between anatomy, behaviourally relevant brain-network states and the activity of specific neural cell classes (pyramidal cells and inhibitory interneurons). To understand how the cooperative activity of different neuronal types contributes to the encoding of behaviourally relevant information in the brain is one of the greatest challenges of Systems Neuroscience. It necessitates an understanding of the “chronocircuitry” of the brain (coined by Professor Peter Somogyi). This term describes brain function at two interrelated levels. The first is the structural context, the connectivity of cell classes within an anatomical framework complete with an understanding of information-input and information-output structures. The second is a description of cell firing patterns in time, the unique profile of each cell’s input and output and its relation to the behaviour of the animal and ongoing brain-network activity.

Towards this understanding, recent work under anaesthesia (using the juxtacellular labelling technique) has identified cell type-specific firing patterns to a number of oscillatory brain network states in the rat hippocampus, a structure that is thought to be crucial to the encoding of episodic and spatial experience. Network states that have been explored include the theta (5-12 Hz), gamma (30-70 Hz) and the ripple (180-250 Hz) oscillations.

In contrast to anaesthesia, however, there is currently no reliable means by which single cells can be recorded and labelled (for identification) in waking/behaving or sleeping conditions. Although similar network patterns to those observed under anaesthesia can be identified during waking and sleep, it is unknown whether specific cells types maintain their theta and ripple modulated firing properties across all states. The key issue in this regard is that the anaesthetic protocol used to describe the anaesthetised firing properties of cell types (urethane + Ketamine/xylazine, or UKX anaesthesia) is known to alter important oscillatory network characteristics such as rhythm frequency and the phase locking of cell activity to these oscillations. For example, under UKX anaesthesia the theta oscillation slows to between 4 and 6 Hz.

## **Approach**

The basis of my doctoral work was to simultaneously record the firing properties of large populations of cells (using the tetrode recording technique) in waking, sleep and anaesthetised conditions. Cells were then putatively assigned to classes according to their firing properties under UKX anaesthesia and then changes in these firing properties assessed across conditions.

## **General Findings**

Through these studies I have shown that the population theta phase locking of both interneurons and pyramidal cells remained similar in behaving and anaesthetised conditions, even across changes in theta frequency. The relationship between cell excitation and theta phase, however, was found to alter under anaesthesia. In contrast to theta, interneurons exhibited considerable variability in the preservation of their gamma phase preferences from the waking to the anaesthetised state, some cells firing almost on opposite phases to those observed in waking conditions. This rearrangement in population discharge, however, was found to vary as a function of interneuron type; putative PV+ basket cells, for example, retaining the strongest theta and gamma phase locking of all classes, consistent with their hypothesised role as a rigid, “clock-work” mechanism by which theta and gamma oscillations are expressed in area CA1 of the hippocampus. Finally with regards network oscillatory states, interneuron firing patterns to ripple oscillations were broadly preserved across states, although some exceptions were observed in relation to interneuron class.

In the final part of my thesis, I showed that the identification of interneuron-specific behavioural correlates in the waking animal, specifically in relation to direction-sensitive and novelty-induced firing rate changes, may aid the development of protocols for classifying interneurons recorded in un-anaesthetised, behaving conditions.

## **Summary**

In brief, my work has shown that UKX anaesthesia exerts differing effects according to interneuron type and that these effects change as a function of oscillatory network state. Overall, this suggests that caution should be taken when viewing anaesthetised brain states as analogous in some form to either waking or sleep conditions. The identification of novel, cell type-specific, behavioural correlates also suggests new strategies for classifying interneurons through using already widely available technological approaches.