Research Protocol

Name of student: #### #########
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Title of Project: Analysis of the mouse somatosensory code *in vivo* during a whisker stimulation task

Background and Rationale for the Project: Understanding the code that neurons use to store and process information is one of the most highly sought after goals in neuroscience today, yet the answer has thus far remained elusive. This is largely due to the fact that behavioural processes are thought to result from the concerted activity of populations of cells too large to easily monitor and analyse simultaneously. However, modern experimental techniques allowing scientists to visualise and manipulate an ever-increasing number of neurons are yielding important clues as to the code behind their combined activity.

The mouse somatosensory system is an ideal model for understanding population activity. The neural substrate encoding somatosensory information gleaned from whisker stimulation is topographically organised into discrete columns of cells, known as barrels, which receive sensory input from individual whiskers[1]. These are reliably targeted by intrinsic imaging and accessible to large-scale recording methods such as calcium imaging[2]. Further to this, activity in somatosensory cortex is relatively sparse, making recording and analysing it a feasible goal[3]. Previous studies have therefore been successful in demonstrating that the mouse's somatosensory system is highly discriminatory in behavioural tasks[4]. However, as yet no systematic description of the distribution of neurons carrying information relevant to these behavioural tasks has been carried out, nor has the permanence of this distribution been assessed over long periods of time.

Therefore, this project aims to use 2-photon calcium fluorescence imaging *in vivo* to analyse the population activity in mouse somatosensory cortex in response to a simple whisker stimulation/behavioural response task. As a result, we hope to be able to fine-tune the topographical description of a somatosensory percept and ascertain the permanence of its neuronal representation over time.

Aims:
1. Visualise the calcium fluorescence resulting from action potential dynamics within barrel cortex in response to a whisker stimulation behavioural task.
2. Determine the percentage of cells that discriminate the features of the task and assess how this percentage varies laterally across the cortical surface and with cortical depth.
3. Monitor any variation in the distribution of discriminatory neurons over long periods of time.

Experimental Design: 5 male P35 mice will undergo headplate installation and intrinsic imaging-targetted injection of GCAMP5 calcium indicator into the superficial layers of the C-row barrels of somatosensory cortex.

Animals will be water-restriced to encourage adaptation to the training rig and learning of the behavioural task. Adaptation will consist of acclimatisation to the training rig, temporary clamping in the headplate holder and introduction to the water-reward apparatus. The behavioural task will consist of learning to lick a spout only in response to whisker stimulation. Correct licking during whisker stimulation will result in a water-reward and incorrect licking in the absence of whisker stimulation will result in an air-puff.

Once successfully trained, a craniotomy will be performed and a chronic cranial window will be inserted to give visual access to the C-row barrels. The calcium fluorescence resulting from neuronal action potential dynamics will then be recorded through a high-speed 2-photon microscope as the awake mouse is performing the behavioural task. Custom-built MATLAB software will be used to identify regions of interest (ROIs) in videos of calcium fluorescence that correspond to neuronal somata and extract the raw fluorescence data corresponding to each cell.

Within the GCAMP-expressing portion of each mouse's somatosensory cortex 5 $100um^2$ fields of view (FOVs) will be selected and population calcium fluorescence activity will be recorded from 100 – 300um below the cortical surface during the whisker stimulation task. We will then analyse the dynamics of each neuron by calculating the discriminability, reliability and strength of its activity. Discriminability will rank a neuron on a scale of -1 to +1 on the basis of its average response during stimulation periods relative to non-stimulation periods. Reliability will rank a neuron on the basis of the standard deviation of the amplitude of its responses during each stimulation (normalised to the standard deviation of its overall activity during the recording period). Strength will simply be a measure of the average amplitude of a neuron's response during stimulation.

With these measures we will determine the percentage of neurons that contain significant information to perform the task, analysing how this percentage varies with cortical depth. We will then also rank cells in each FOV as strong, weak and non-encoders and monitor any variation in their ranking over a period of 2 weeks to assess the permanence of the cellular representation of the task's features.

Three controls will be implemented to confirm that we image in the correct cortical location. Firstly, we will repeat intrinsic imaging after cortical injection and chronic window installation to ensure that GCAMP expression is present in the correct location. Secondly, we will compare the average population response of all cells in the putative barrel location with a neighbouring non-barrel location upon whisker stimulation. Thirdly, after the experimental period each mouse's whiskers will be trimmed to so as to leave only the C-row. We will then stimulate only these whiskers and verify the population activity in the putative C-row barrel. In order to verify that behavioural response is a result of somatosensory stimulation (as opposed to any other environmental cue) we will inject the GABA agonist muscimol into somatosensory cortex and assess the mouse's behavioural performance during the experimental task.

Justification: In order to have a chance of understanding the complexity of the neural code this project incorporates several crucial features. The model system is well characterised, easily-targettable and easily accessible to wide-field calcium fluorescence imaging. The behavioural task, and thus the percept, under scrutiny is simple ensuring the best possible chance of analysing the distribution of cells encoding its various components. Finally, GCAMP5 has a relatively long window of viability making it ideal for imaging population activity over longer periods of time as is proposed in this study.

Home Office/Ethical Approval: My project requires a Personal Individual License for Modules 1-3 and Module 4. This is already in place.

References:

[1]Woolsey TA & Van der Loos H 1970, The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. Brain Res. 17, 205–242

[2]Grienberger C & Konnerth A 2012, Imaging Calcium in Neurons, Neuron 73, 862–885

[3]O'Conner AH et al. 2010, Neural Activity in Barrel Cortex Underlying Vibrissa-Based Object Localization in Mice, Neuron 67, 1048-1061

[4]O'Connor DH *et al*. 2010, Vibrissa-based object localization in head-fixed mice, J. Neurosci. 30, 1947–67