Simultaneous 2-photon calcium imaging in dendritic tufts receiving feedback inputs, and cell bodies of the same neurons receiving feedforward inputs (D3.1.1 – SGA2)

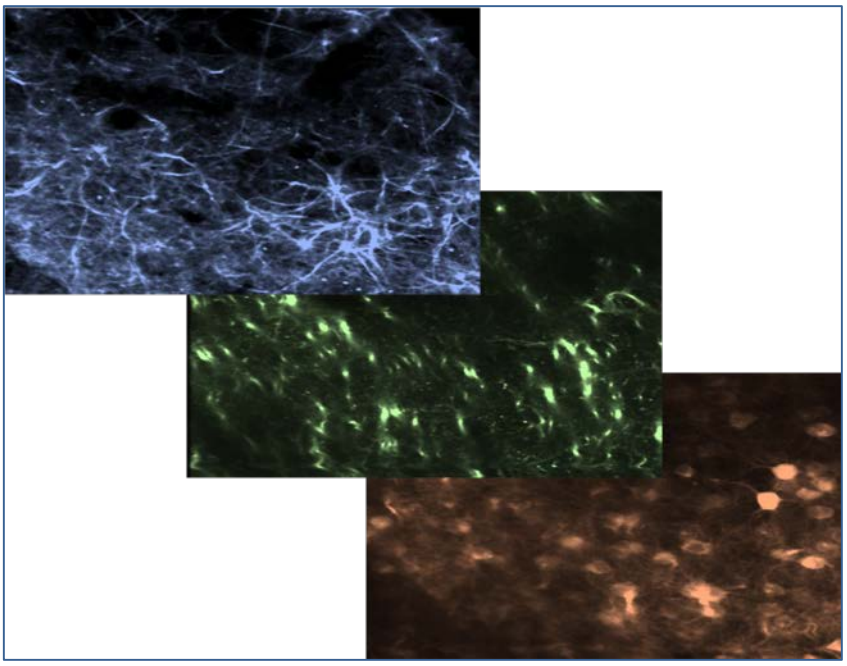

Figure 1: Calcium signals imaged in dendritic tufts, apical dendrites and somata of Layer 5 neurons in mouse visual cortex

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Description in GA:	Data collected and cross-species comparisons in visual cortex incorporated (T3.1.2)
Abstract:	We established an approach to simultaneously record calcium signals in dendritic tufts and apical dendrites, or apical dendrites and somata of Layer 5 pyramidal neurons in awake, behaving mice.
Keywords:	Dendritic integration, visual responses, primary visual cortex.
Target Users/Readers:	Neuroscience community, HBP partners.

Table of Contents

Table of Figures

1. Imaging Neuronal Activity in Dendritic Tufts, Dendrites and Cell bodies using Two-Photon Calcium Microscopy in Awake, Behaving Mice

1.1 Introduction

The ability of the brain to produce complex behaviour requires accurate sensory processing. Sensory networks such as the visual system are strongly driven by bottom-up or feedforward information. Importantly, however, top-down or feedback projections from thalamic nuclei and cortical areas can strongly modulate feedforward signals, a process that allows placing visual inputs in context and comparing them to predictions the brain makes about the environment (Larkum, 2013). Although it is thought that cortical feedback is crucial for accurate sensory perception and awareness, it remains unclear how feedforward and feedback information is integrated at the level of individual neurons. It is believed that feedforward sensory information inputs mostly enter dendrites near the cell body, while feedback information about what is to be expected would enter dendrites in further away, near the surface of the cortex (the dendritic tuft). This wiring would allow the integration of feedforward and feedback information at the single cell level. In order to test this hypothesis directly, we established a two-photon imaging approach allowing the simultaneous measurement of calcium responses in the dendrites near the cortical surface and in the cell body of the same neurons. This approach can be used in mice that are awake and perform a visual task.

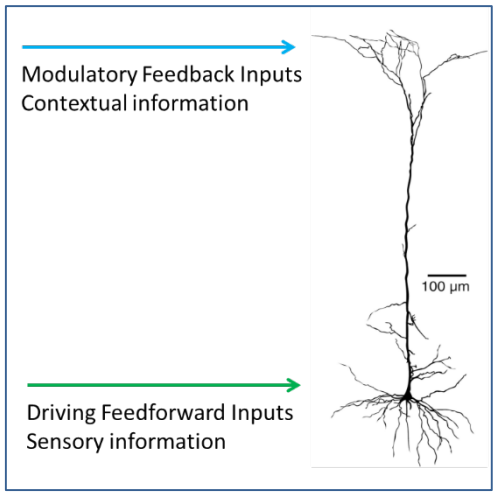

Figure 2 Sources of input to a layer 5 pyramidal neuron

1.2 Approach and Results

To enable us to visualise responses of dendrites and cell bodies of neurons, we needed to label only a fraction of the neurons with a fluorescent protein that changes its fluorescence intensity during activity. If all neurons are labelled, it is impossible to identify individual dendrites or to know to which cell body they belong. The activity-dependent fluorescent protein we used is GCaMP6F, a green fluorescent protein that increases its fluorescence when it binds to calcium, which enters the neuron when it responds to synaptic inputs. The neurons we labelled where those situated deep in the visual cortex, so called layer 5 pyramidal neurons. These neurons have the shape shown in Figure 2, with their dendritic tuft at the cortical surface, and the cell body half a millimetre deeper. To achieve this, we made use of a viral vector that drives expression of GCaMP6F, which we injected in a transgenic mouse line that allows the selective labelling of layer 5 pyramidal neurons (RBP4-cre) (Figure 3). By injecting a low concentration of the virus, sparse labelling can be achieved allowing the imaging dendritic tufts, the cell bodies, and the dendrite that connects these: the "apical dendrite".

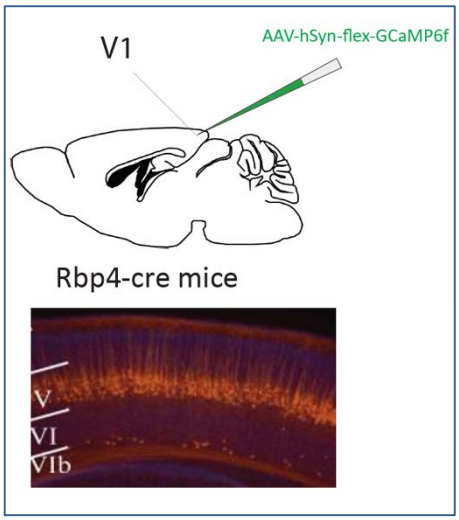

Figure 3 Labelling layer 5 neurons

Next, a window was placed in the skull of these mice, above

V1 making it possible to perform two-photon microscopy in this brain region while the mice were watching a screen on which visual stimuli were shown. Two-photon microscopy is a technique that makes it possible to perform fluorescence microscopy deep in the cortex, without causing damage to the tissue.

First, visual stimuli were presented in order to map to which part of the screen the imaged cells were responsive (receptive fields, "RF mapping") (Figure 4). Next, we showed optimal visual stimuli (black and white bars in different orientation ("orientation tuning") and natural images. While these images were presented, calcium imaging was performed using a lens that could be rapidly focused ("Optotune lens"), allowing us to simultaneously image at 2 depths, at a frequency of 15 Hz. We then compared calcium responses in the dendritic tuft with those in the apical dendrite, and the responses in the apical dendrite and the cell body.

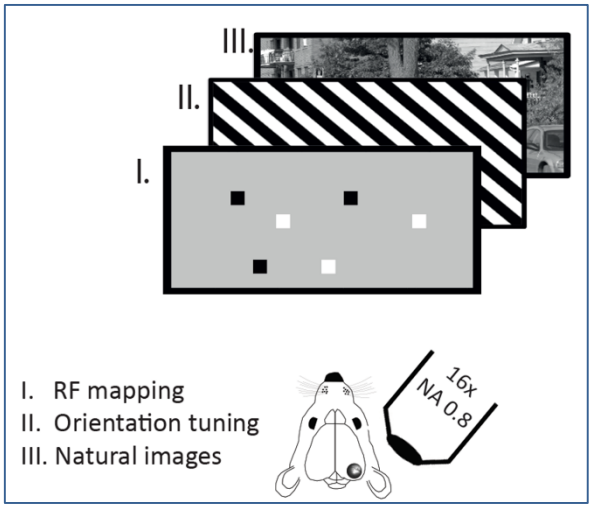

Figure 4 Visual Stimulation

This revealed that calcium responses in the apical dendrite and the soma were similar (Figure 5: green and red trace mostly overlap), meaning that the apical dendrite signal could be used as a proxy for the signal in the cell body. In contrast, the calcium responses in the tuft and apical dendrite differed significantly, with many tuft responses (blue trace) that were not observed in in the apical dendrite (red trace).

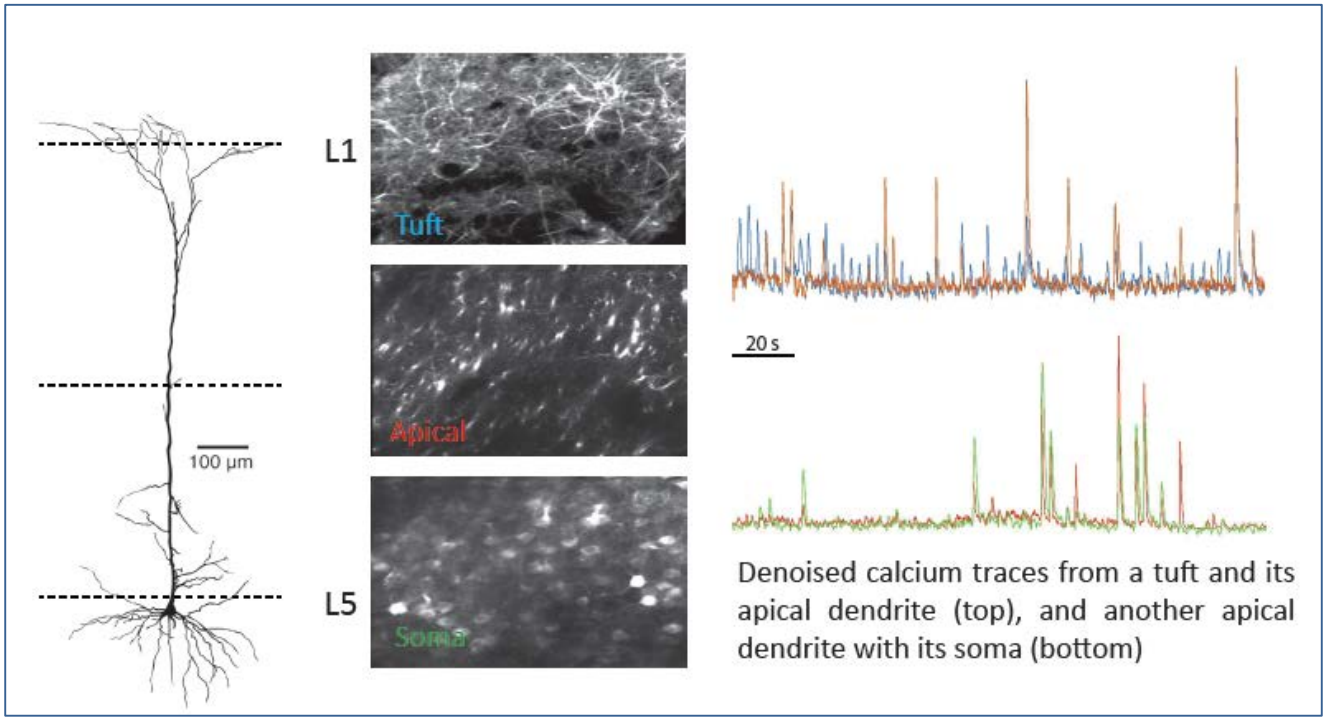

Figure 5 Calcium imaging in tufts, apical dendrites and cell bodies during visual stimulation

The direct reason for setting up this imaging approach was to allow us to be compare visual responses driven by feedforward inputs or feedback inputs in different species. In humans, such responses have been recorded using functional MRI, which provides layer-specific information but no information about the responses of individual neurons (Smith and Muckli, 2010). In macaques, electrophysiological recordings are typically used. This provides information about individual cell responses, but not about the responses in the tufts or dendrites. Thus, if we perform experiments in mice that were previously performed in humans and macaques, we can see how responses in the tufts or cell bodies in the mouse visual system relate to electrophysiological or fMRI responses in

macaques and humans. This provides cross-species, multi-level information about how the visual system integrates feedforward and feedback information.

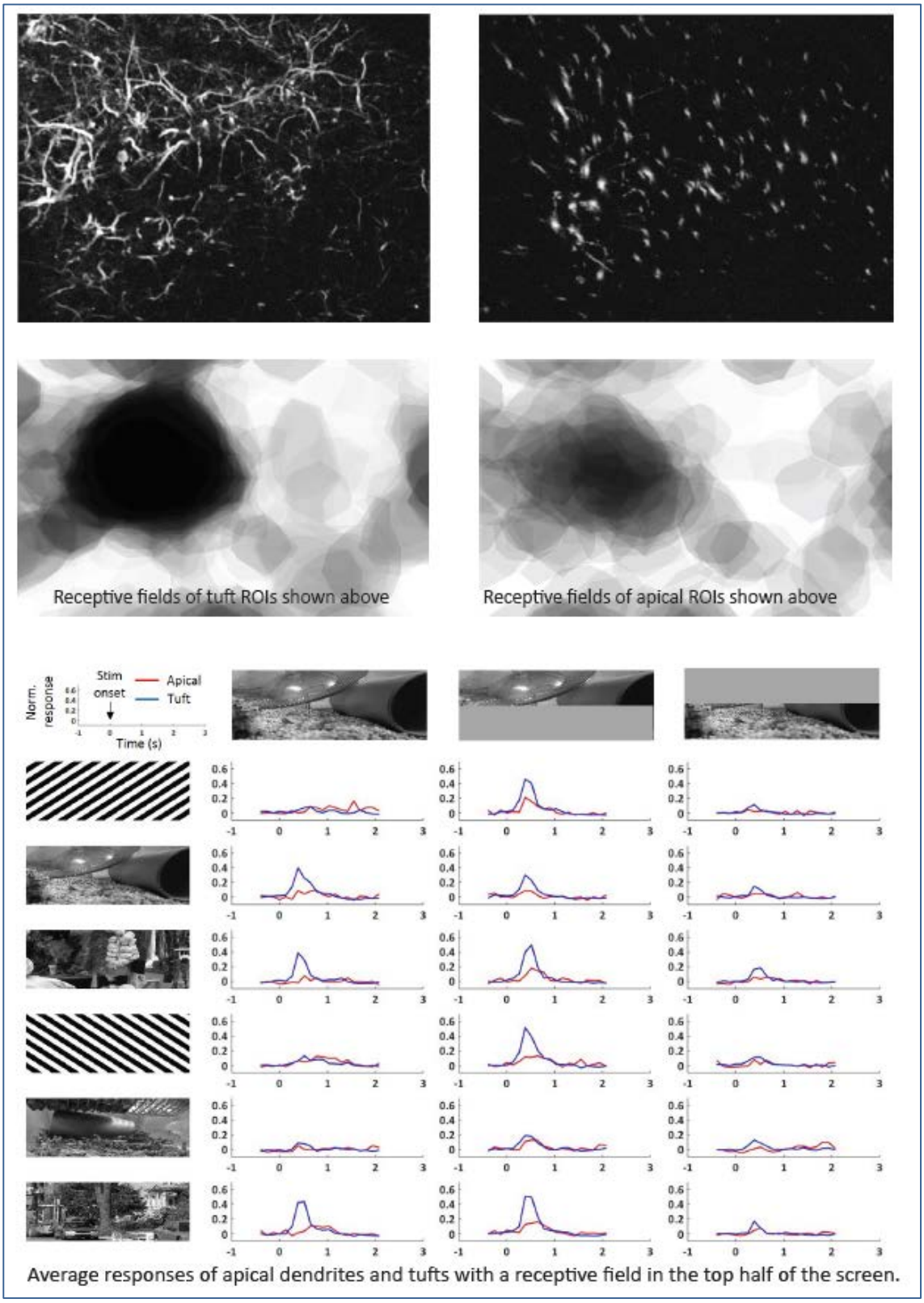

Figure 6 Responses of tufts and apical dendrites to full and partially occluded natural images and gratings

Top panels: two-photon calcium imaging in dendritic tufts (left) and apical dendrites (right) of layer 5 pyramidal neurons. Middle panels: receptive field mapping of the dendritic tufts (left) and apical dendrites (right). Lower panel: Averaged response traces of dendritic tufts (blue traces) and apical dendrites (red traces) to various visual stimuli, as indicated in the left row next to the traces. Images are shown in full, or with the lower or upper part occluded (as indicated in the panels above the traces).

We therefore initiated experiments in which we adapted an experimental paradigm, previously used in human subjects and macaques, for mice. This paradigm is aimed at separately inducing sensory and contextual responses in the visual cortex. Images are shown to the subjects, but sometimes, a part of the image is occluded. When the whole image is shown, the visual cortex receives sensory inputs. However, when the partially occluded image is shown, the part of the visual cortex that responds to this part of the image does not receive a sensory input. However, fMRI experiments revealed that this part of the visual cortex still responds, and that it is possible to determine which image was shown based on this response. This suggests that the non-occluded part of the image causes contextual information about this image to be transferred to the part of the visual cortex that is not directly stimulated. This is probably feedback information. In mice, we repeated this experiment and full images and partially occluded images were shown while we recorded calcium responses in the dendritic tufts (possibly receiving contextual feedback inputs) and the apical dendrite (mimicking the response of the cell body, that reacts to the sensory input).

Figure 6 illustrates this experiment. We recorded responses in tufts (left) and apical dendrites (right). We identified the part of the screen to which the imaged cells responded. Then natural images and oriented bars were presented. Mostly, the entire images were shown, but also images in which the lower or upper part of the image was occluded. As the receptive fields of the imaged neurons were in the upper part of the screen, occluding this area would cause an absence of feedforward sensory input, while contextual feedback information could still be provided.

The figure shows responses of the tufts (blue traces) and apical dendrite (red traces) of an example neuron, to the different (occluded) images. Responses to the image of which the upper part is occluded (right column) are clearly weaker. However, tuft responses (blue traces) can still observed. So far, these results match observations in human subjects in which responses in the top layer of the visual cortex can be still be observed with fMRI when only the part of the image is shown that does not directly stimulate this part of the visual cortex.

2. Impact

We have demonstrated that we can image neuronal responses in dendritic tufts, apical dendrites and cell bodies using two-photon calcium imaging in awake, behaving mice. This approach is an important tool for testing the hypothesis that feedforward inputs are modulated by feedback inputs providing contextual information, a theory known as dendritic amplification and an important cornerstone of research in SP3 (Larkum, 2013). Currently, this approach is being applied in a paradigm that has also used in humans and macaques, involving the presentation of visual stimuli that are partially occluded (Component C2021). Results in human subjects suggest that when the partially occluded images are shown, our visual system fills in the missing part. Our newly developed technique now allows us to study how such filling in works within neurons in the visual system, and whether this indeed involves dendritic amplification.

By April 1st, 2020 the data will be available here:

https://kg.ebrains.eu/search/instances/Dataset/684eff17-358f-431d-849a-8b81332a1f19

3. References

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