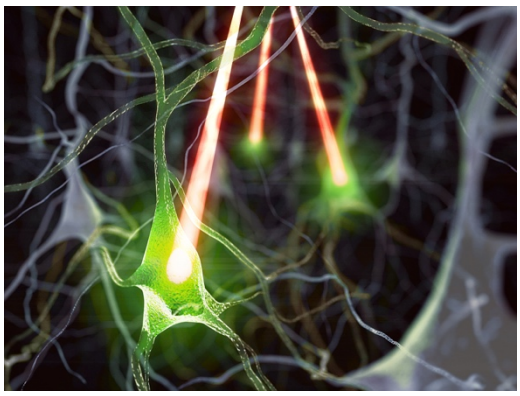
FINAL REPORT: Marie Curie IIF 328048 - ManipulateNeuralCode



Neurons in the brain store, process, and transmit information via electrical impulses. How does the spatiotemporal pattern of activity in the brain drive perception, or enable performance of an action? Such fundamental questions have yet to be answered despite substantial recent progress in neuroscience. We have developed novel approaches to answer these questions, in order to reveal how our brains work to perform complex computational feats surpassing what human engineers can create. Such understanding may give us deep insights into the workings of the cerebral cortex, and will aid the fight against debilitating disorders of the brain.

Previous research on how electrical activity drives behaviour has taken two complementary approaches: correlating neuronal activity with what is happening in the environment, and stimulating neuronal activity while recording behavioural responses. In the first approach, many of the basic calculations performed by an animal's sensory system can be characterized by altering the environment and recording the response of neurons in the brain. Recent technical advances now enable recording of movies showing the activity of large groups of neurons at once. Such data are often highly informative, but nonetheless cannot reveal whether the recorded activity is responsible for one's perception, or may have just coincidentally occurred at the time of the recording.

The second approach is to stimulate neuronal activity and record responses from an animal. While these experiments complement the first approach by providing more direct evidence, they show only that activating certain neurons is sufficient. For example, a neuron that is sufficient to drive an action in the lab may not necessarily be the neuron that is normally active when the animal behaves naturally. This subtle difference is crucial in understanding the neural code because the goal is to understand how the brain works under normal conditions, not how it is able to work under artificial conditions in the laboratory.

The goal of this project was to bring these two approaches together by closing the loop between recording and manipulating neural activity to understand how it underlies behaviour. We have developed an optical approach – published recently in the high-profile journal Nature Methods (Packer et al, 2015) which allows us to use light to both record and manipulate the activity of many neurons simultaneously on the level of individual electrical impulses. We developed a novel all-optical method for reading and writing activity in neural circuits with single-cell resolution and single action potential precision *in vivo*. The strategy relies on coexpression of an optogenetic probe (C1V1) and a genetically encoded calcium sensor (GCaMP6s), independently targeted by dual two-photon laser beams for readout and manipulation of activity. We have demonstrated fast readout of large fields of view up to 0.5 mm square during simultaneous multi-neuronal photostimulation with minimal crosstalk between stimulation and recording channels. A spatial light modulator allows tens

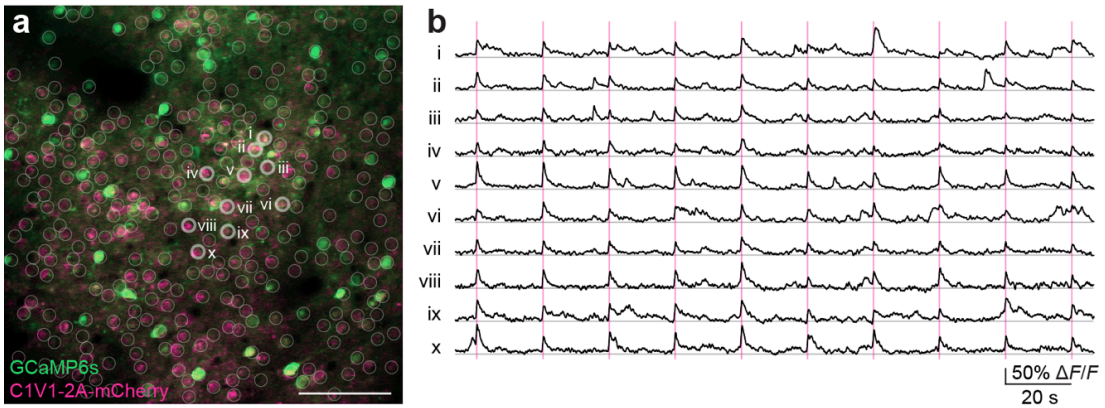


Fig. 1. All-optical interrogation in cortical layer 2/3 of awake mice. Left: 10 out of ~200 neurons expressing GCaMP6s and C1V1 are targeted for simultaneous two-photon photostimulation (scale bar, 100 µm.) Right: Each neuron responds robustly on each photostimulation (purple vertical bars).

of user-selected neurons to be targeted for spatiotemporally precise optogenetic activation. Targeting of one to 20 neurons in a chronic, non-invasive fashion works robustly and reliably in awake and behaving animals (**Figure 1**).

We have used this method to stimulate the same neuronal population in different behavioral states. Crucially, we have been able to activate functional ensembles of neurons by targeting photostimulation to only those neurons that exhibit a particular sensory-evoked response (**Figure 2).** This approach extends the optogenetic toolkit beyond the specificity obtained with genetic or viral approaches, enabling high-throughput, flexible and long-term optical interrogation of functionally defined neural circuits with high spatiotemporal resolution and precision in the mammalian brain *in vivo.*

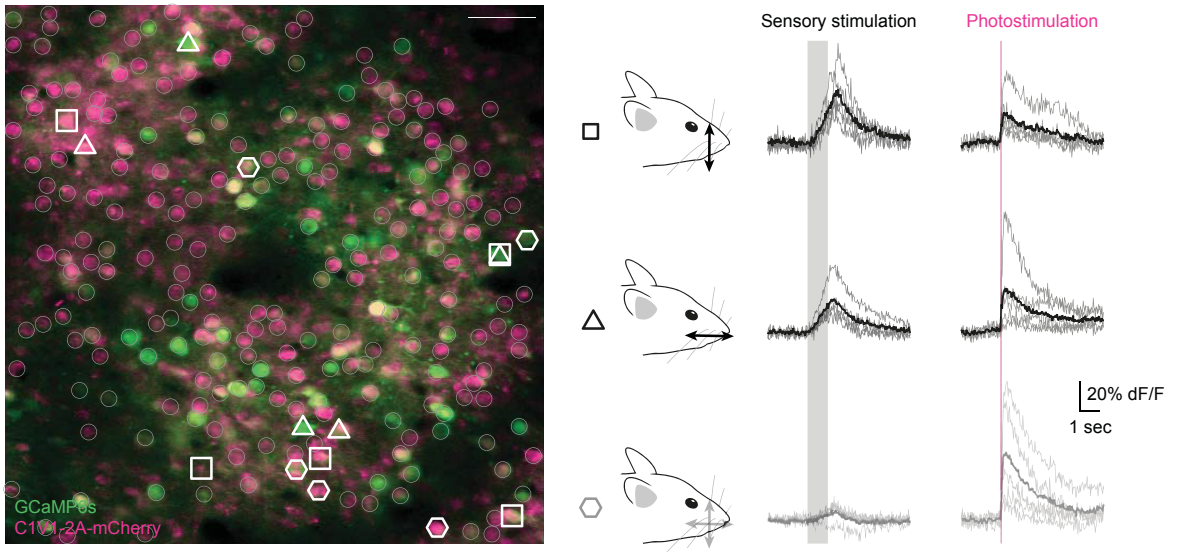


Fig. 2. Targeting neurons for optogenetic manipulation based on their individual functional signatures *in vivo*. Left: Neurons coexpressing GCaMP6s (green) and C1V1- 2A-mCherry (pink) in the C2 barrel of mouse somatosensory cortex (scale bar, 50 µm). Groups of individually identified neurons were selected for photostimulation based on their response to dorsal-ventral and rostrocaudal whisker stimulation (right). Five neurons that responded differently or not at all to sensory stimulation (gray shading) were simultaneously photostimulated (pink line).

Combining optogenetics and calcium imaging, two powerful optical technologies for reading and writing activity in large populations of neurons, has been a longstanding goal of the neuroscience community since optogenetics burst onto the scene a decade ago. Such a combination, applied *in vivo* with sufficient spatiotemporal selectivity to record from and activate single spikes in single neurons, would open the door to a range of experiments to probe the nature of the neural code, or how neurons process and store information, during behavior. In particular, being able to target neurons based on their functional signature, rather than simply their genetic identity, would enable one to replay and manipulate natural patterns of activity in neural circuits. We are confident that our approach will enable many new and groundbreaking neuroscience experiments, and is thus likely to appeal to a wide audience. In particular, we believe that this approach will enable many of the "dream experiments" outlined in our recent review of optogenetic targeting (Packer et al., *Nature Neuroscience*, 2013), which seek to deepen our understanding of normal brain function by exploring the cellular basis of the neural code. In addition, the unprecedented resolution and scale of this approach may have an impact beyond basic neuroscience in the clinical realms of neurology and psychiatry. For example, these techniques could be applied to understand dysfunction in disease models of many debilitating afflictions, such as anxiety, depression, addiction, autism, and movement disorders that affect millions of people (Tye and Deisseroth, *Nature Reviews Neuroscience*, 2012).

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