C O L U M B I A U N I V E R S I T Y
IN THE CITY OF NEW YORK

DEPARTMENT OF BIOLOGICAL SCIENCES

Dr. Peter Stern

Senior Editor

Science

Thursday, March 24, 2016

Dear Peter,

We submit our manuscript "**Imprinting and recalling cortical ensembles *in vivo***" for consideration as a *Science* report.

In this paper we used two-photon optogenetic stimulation with single cell resolution to "play the cortical piano" with the cortex without opening the skull of an awake behaving mouse. When we were characterizing the reliability of the population responses to repetitive photostimulation, we discovered that recurrent activation of a given group of neurons creates a neuronal ensemble that is imprinted in the cortical circuitry. These ensembles can be recalled from single neuron stimulation, demonstrating pattern completion and pointing out that the cortex is an attractor neural network. Moreover, changes in the functional connectivity between these ensembles persist for at least a day. So we can "write" activity into the cortex *ad libitum* and our imprinted "melodies" persists!

In addition, our work also beautifully demonstrates Hebbian plasticity in the cortex of an awake animal. Although the Hebbian synaptic plasticity hypothesis has inspired neuroscientists for decades, the creation of artificial Hebbian ensembles has been technically challenging, until now where we can directly build and see these Hebbian ensembles.

We think that our paper is novel and significant for a broad scientific community because it is a technical tour de force that demonstrates the possibility to reprogram neuronal microcircuits with single cell resolution. As you know, direct electrical stimulation of specific brain areas has been used as an alternative to treat movement disorders (Brice and McLellan, 1980), neuropsychiatric disorders (Mayberg et al., 2005; Williams and Okun, 2013) and epilepsy (Bergey, 2013). However, deep brain stimulation requires invasive surgery that can lead to chronic inflammation, neurodenegeration or adverse effects. The development of optogenetic tools has shown the potential to rescue Parkinsonian symptoms stimulating specific areas in a less invasive manner (Gradinaru et al., 2009). However, current optogenetic experiments involve the stimulation of large volumes of neurons whose precise spatial location is unknown. Two recent publications have demonstrated the potential of two-photon optogenetics for simultaneous manipulation and recording of cortical neurons with single cell resolution (Rickgauer et al, 2014; Packer et al. 2015). However, manipulation of neuronal ensemble dynamics with single cell

901 NWC Building, MC4822 550 W 120th Street New York, NY 10027
212.854.2354 • Fax 212.854.4619 • rmy5@columbia.edu

COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK

DEPARTMENT OF BIOLOGICAL SCIENCES

resolution has never been reported. Here we go beyond and have advanced the optical techniques and population analysis to modify and target specific subpopulations of neurons with single cell resolution. The identification of physiologically relevant neuronal ensembles combined with two-photon optogenetics with single cell resolution could be used systematically to reorganize the association between targeted groups of neurons. The reprogramming of neuronal ensembles offers the possibility to alter behavior or treat pathological disorders at the microcircuit level with single cell resolution.

None of the material in our manuscript has been published or is under consideration elsewhere.

All experimental procedures were carried out in accordance with the US National Institutes of Health and Columbia University Institutional Animal Care and Use Committee.

If the manuscript is accepted all the data will be available upon request.

As reviewers we recommend Ed Fetz, Tobias Bonhoeffer, Moshe Abeles, Karl Deisseroth, Rui Costa, Gyorgy Buszaki, Viviana Gradinaru, Kay Tye, Jose Carmena, David Tank, Christopher Harvey, Takao Hensch, John Donoghue, Fritjof Helmchen, Mark Schnitzer, Michael Long, WenBiao Gan, Alison Barth, Ed Callaway, Michael Stryker, Sonja Hofer or Susumu Tonegawa.

Unfortunately Karel Svoboda or Michael Hausser would be in conflict of interest.

Sincerely,

Rafael Yuste, M.D. Ph.D.
Professor of Biological Sciences and Neuroscience
Director, NeuroTechnology Center
Co-Director, Kavli Institute for Neural Circuitry
Columbia University
New York, NY 10027

Luis Carrillo-Reid, Ph.D.
Department of Biological Sciences
Columbia University
New York, NY 10027