Neurofunctional Techniques

Lesson 5

23 October 2026

Introduction to scientific papers

Structure of the exam

1. **Paper presentation in small groups (5-6 students 2 hours)**
   Presentation + questioning: 20 points (+1 bonus)

   a) **Presentation (10 min per student, total 60 min)**
      - **What was known?**
      - **What is the gap?**
      - **What are the main findings?**
      - **Are the techniques appropriate?**
      - **What are the broader implications?**

   b) **Questioning on individual figures and techniques used (10 min per student, total 60 min)**

2. **Questionnaire on the Moodle platform.**
   10 multiple choice questions. 1 point each. Each question has only one correct answer
   Moodle questionnaire: 10 points.

One major objective of the course

Being able to understand a 2026 neuroscience paper

- **There are 3 papers, which I will now briefly introduce**

- **We will dedicate the next lesson on Friday 30 to discussing them**
  It is important that all of you read the papers before the lesson and come prepared with questions ask questions!

- **You will need to :**
  - **Choose 1 paper based on your personal interests**

  - **Form 6 groups** of 5-6 students each possibly **by Oct 30**
    (Marta and Tommaso: please help coordinate this)

  - **Each group will present 1 paper at the end of the course (Nov 27, Dec 4 or Dec 11)**

  (Clearly, more than one group may choose the same paper.)

Why did I choose these papers?

- **They address fundamental questions in contemporary neuroscience:**
  - Memory engram formation, allocation and retrieval
  - How neuronal circuits encode and store memories

- **They combine experimental techniques covered in this course:**
  - *In vivo* calcium imaging
  - Optogenetics and chemogenetics
  - Genetic targeting and neuronal activity tagging

- **They represent contemporary neuroscience research:**
  - Published in leading journals (*Cell, Neuron, Science*)
  - Relatively recent research (2017 - 2024)

Do we have the conceptual and methodological tools to understand, critically evaluate and appreciate these studies?

What is a memory engram?

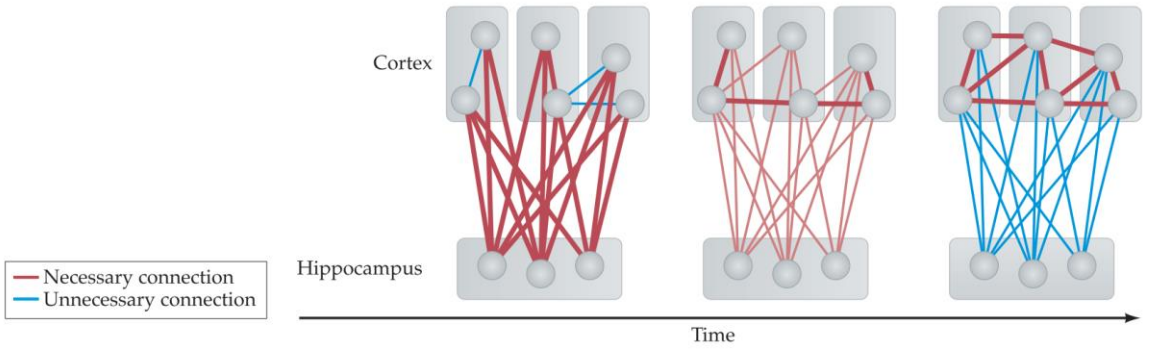
A memory engram is a population of neurons that undergoes lasting changes during learning and contributes to the storage, consolidation and retrieval of a specific memory.

Three fundamental questions:

- **Allocation:** Which neurons are recruited into a memory engram?
- **Development:** How do the mechanisms of engram formation change during brain maturation?
- **Retrieval:** How are engram neurons reactivated to recall a memory?

How can we identify and manipulate memory engrams?

We will explore this in greater detail after introducing optogenetics and chemogenetics.

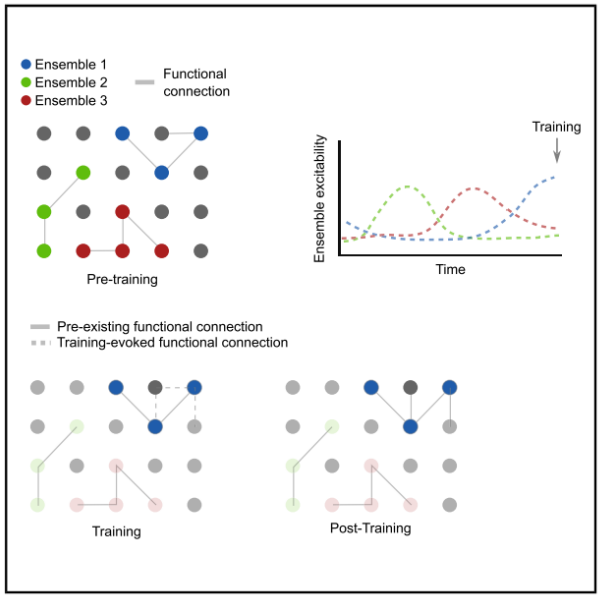


1

Neuron

Excitability mediates allocation of pre-configured ensembles to a hippocampal engram supporting contextual conditioned threat in mice

Graphical abstract



Highlights

- Active neurons in hours before experience are preferentially allocated to engram ensemble
- Active neurons in hours before experience are necessary for subsequent memory retrieval
- Functionally connected neuronal ensembles detected days before conditioning event
- Learning modifies functional connectivity between neurons in engram ensembles

Authors

Andrew J. Mocle, Adam I. Ramsaran, Alexander D. Jacob, ..., Blake A. Richards, Paul W. Frankland, Sheena A. Josselyn

Correspondence

sheena.josselyn@sickkids.ca

In brief

Neurons are allocated to an engram ensemble during an event. Mocle et al. found that engram neurons showed endogenously elevated activity hours before an event, but pre-existing functional connectivity days before an event, suggesting that small sub-ensembles of active neurons are allocated as groups and that their functional connectivity is modified by learning.

1

Neuron

Article

Excitability mediates allocation of pre-configured ensembles to a hippocampal engram supporting contextual conditioned threat in mice

Question:
How neuronal excitability and pre-existing network organization influence engram allocation

They address this with:

- ***In vivo*** **calcium imaging**
- **Activity-dependent genetic tagging**
- **Optogenetic coupled to behavioral analyses**

2

NEUROSCIENCE

A shift in the mechanisms controlling hippocampal engram formation during brain maturation

Adam I. Ramsaran[1,2], Ying Wang[1,3], Ali Golbabaei[1,4], Stepan Aleshin[5], Mitchell L. de Snoo[1,4], Bi-ru Amy Yeung[1,3], Asim J. Rashid[1], Ankit Awasthi[1], Jocelyn Lau[1,3], Lina M. Tran[1,3,6], Sangyoon Y. Ko[1,3], Andrin Abegg[1,7]†, Lana Chunan Duan[1,4], Cory McKenzie[1,2], Julia Gallucci[1]‡, Moriam Ahmed[1], Rahul Kaushik[5,8], Alexander Dityatev[5,8,9], Sheena A. Josselyn[1,2,3,4,10], Paul W. Frankland[1,2,3,4,11]*

The ability to form precise, episodic memories develops with age, with young children only able to form gist-like memories that lack precision. The cellular and molecular events in the developing hippocampus that underlie the emergence of precise, episodic-like memory are unclear. In mice, the absence of a competitive neuronal engram allocation process in the immature hippocampus precluded the formation of sparse engrams and precise memories until the fourth postnatal week, when inhibitory circuits in the hippocampus mature. This age-dependent shift in precision of episodic-like memories involved the functional maturation of parvalbumin-expressing interneurons in subfield CA1 through assembly of extracellular perineuronal nets, which is necessary and sufficient for the onset of competitive neuronal allocation, sparse engram formation, and memory precision.

2

NEUROSCIENCE

A shift in the mechanisms controlling hippocampal engram formation during brain maturation

Question:

How the mechanisms governing hippocampal engram formation change during brain development

They address this with:

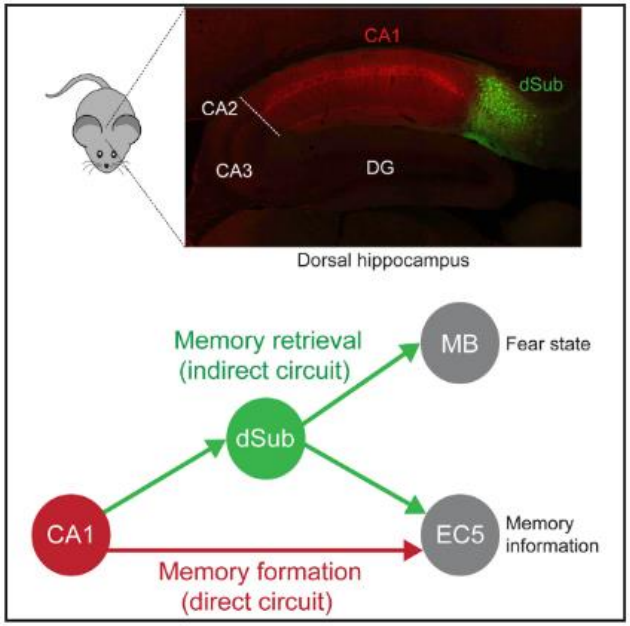
- ***In vivo* chemogenetics coupled to behavioral analyses**
- **Molecular manipulations**

3

Cell

Distinct Neural Circuits for the Formation and Retrieval of Episodic Memories

Graphical Abstract



Highlights

- dSub and the circuit, CA1 → dSub → EC5, are required for hippocampal memory retrieval
- The direct CA1 → EC5 circuit is essential for hippocampal memory formation
- The dSub → MB circuit regulates memory-retrieval-induced stress hormone responses
- The dSub → EC5 circuit contributes to context-dependent memory updating

Authors

Dheeraj S. Roy, Takashi Kitamura, Teruhiro Okuyama, ..., Yuichi Obata, Atsushi Yoshiki, Susumu Tonegawa

Correspondence

tonegawa@mit.edu

In Brief

Episodic memories are formed and retrieved through distinct hippocampal pathways.

Article

Cell

Distinct Neural Circuits for the Formation and Retrieval of Episodic Memories

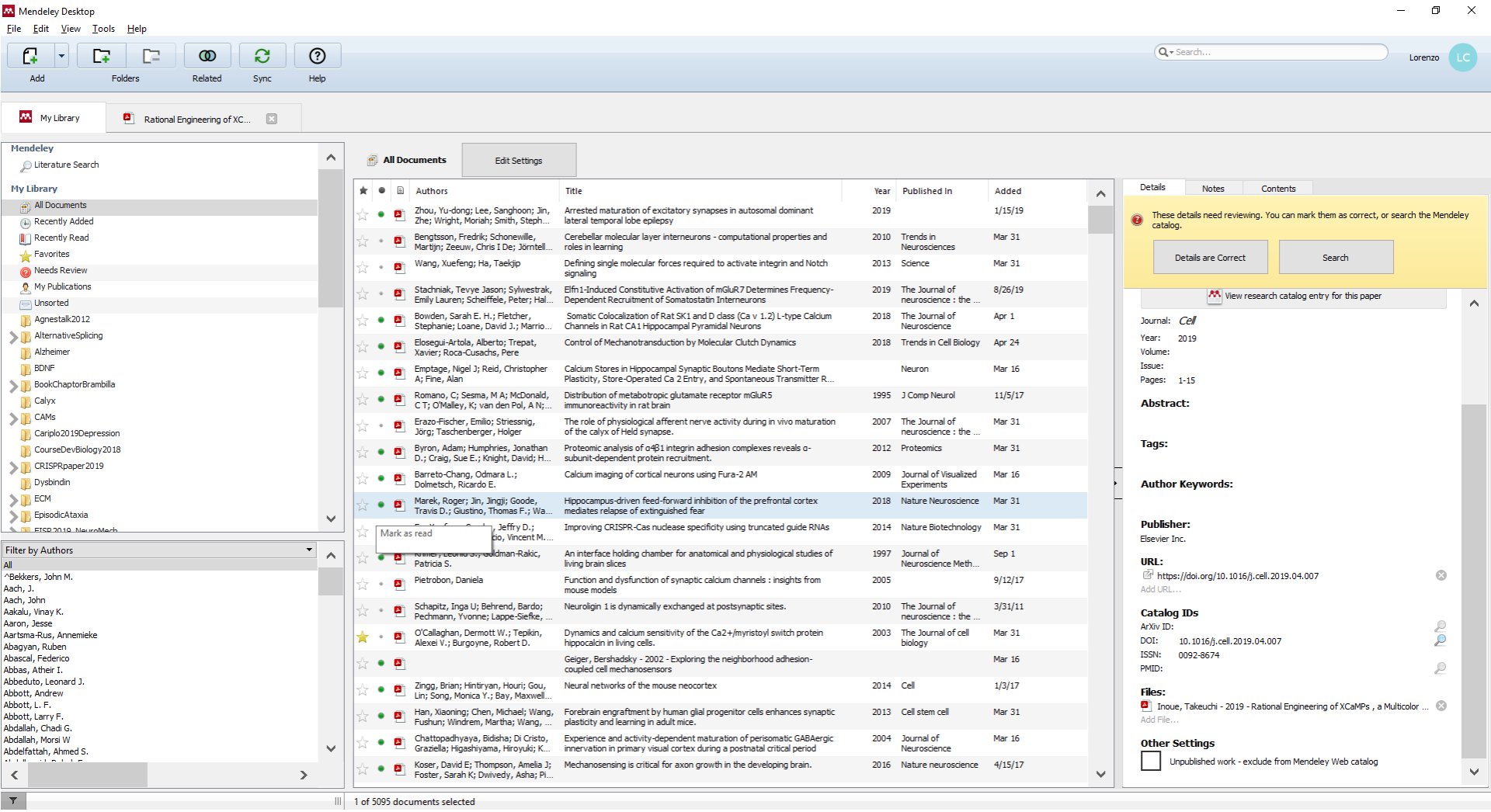
Question:

Engram retrieval: How are stored memories recalled?

They address this with:

- ***In vivo* optogenetics coupled to behavioral analyses**
- ***In vivo* calcium imaging**

Use a software to organize your library



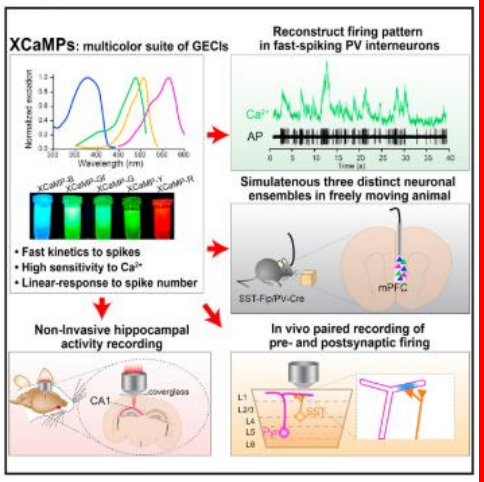
Mendeley Desktop
File Edit View Go Tools Help
Select Pan Note Highlight Color Zoom Zoom To Fit Fullscreen Sync Help
My Library
Rational Engineering of XC...

Cell

Resource

Rational Engineering of XCaMPs, a Multicolor GECI Suite for *In Vivo* Imaging of Complex Brain Circuit Dynamics

Graphical Abstract



Authors

Masatoshi Inoue, Atsuya Takeuchi, Satoshi Manita, ..., Karl Deisseroth, Kazuo Kitamura, Haruhiko Bito

Correspondence

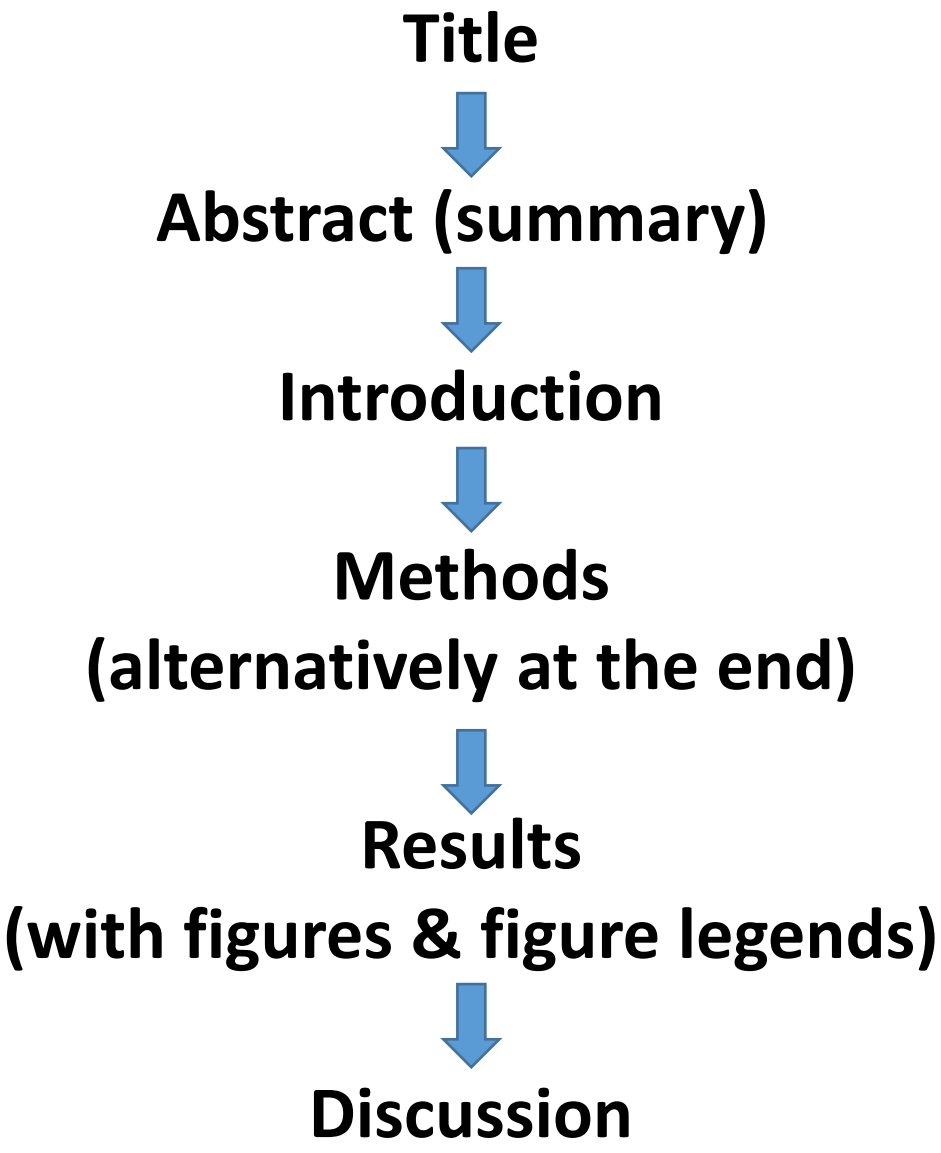
hbito@m.u-tokyo.ac.jp

In Brief

Quadricolor suite of genetically encoded calcium indicators for multiplex recording in the brain.

Highlights

- Developing XCaMPs, a spectrally resolved, quadricolor (BGYR) suite of linear GECIs
- Resolving PV interneuron spike trains and non-invasive imaging of hippocampal CA1
- Cell-type-specific excitation-inhibition dynamics revealed by 3-color fiber photometry
- Local dendritic inhibition identified *in vivo* via paired axon and dendrite recording


Title
Abstract (summary)
Introduction
Methods
(alternatively at the end)
Results
(with figures & figure legends)
Discussion

Rational Engineering of XCaMPs, a Multicolor GECI Suite for *In Vivo* Imaging of Complex Brain Circuit Dynamics

A central goal of neuroscience is to elucidate how information is encoded and processed at the circuit, neuronal ensemble, single neuron, or subcellular structure resolution *in vivo*. Calcium (Ca^{2+}) imaging allows the tracking of population dynamics of neuronal activity at the cell soma levels as well as within neuronal compartments. Genetically encoded calcium indicators (GECIs) enable long-term, repetitive, and unbiased functional imaging (Gunaydin et al., 2014; Ziv et al., 2013). Recently, an array of GECIs were developed to expand the utility of this approach (Chen et al., 2013; Fujii et al., 2013; Inoue et al., 2015; Miyawaki et al., 1997; Nakai et al., 2001; Zhao et al., 2011). Rapid advances in sensor dynamic range and brightness were especially focused on single-wavelength GECIs, such as GCaMP. However, several design challenges still remain, especially in terms of response kinetics, signal linearity, sensitivity, and multicolor availability, which together currently present a substantial bottleneck to quantitatively understand complex activity dynamics of the brain circuits at the cellular and synaptic levels.

Most importantly, the kinetics of current GECIs remain too slow to resolve high-frequency spike rates of individual neurons. For example, fast-spiking (10–100 Hz), parvalbumin (PV)-positive interneurons, whose firing frequency and spike sequence are implicated in the regulation of gamma oscillations and place field shape (Hu et al., 2014), cannot be accurately estimated *in vivo*. Genetically encoded voltage indicators (GEVIs) show potentials

Abstract (summary)

1) It needs to be succinct (always with word limit)

Introduction

1) It must be focused (i.e. structured like a funnel)
2) It must identify the scientific gap/question
   b) Convince the reader the gap/question is important
   c) Convince the reader you know how to address the question / fill the gap

Rational Engineering of XCaMPs, a Multicolor GECI Suite for *In Vivo* Imaging of Complex Brain Circuit Dynamics

Masatoshi Inoue,[1,2,14] Atsuya Takeuchi,[3,14] Satoshi Manita,[4,14] Shin-ichiro Horigane,[1,5,6] Masayuki Sakamoto,[1] Ryosuke Kawakami,[7,11] Kazushi Yamaguchi,[7] Kouhei Otomo,[7] Hiroyuki Yokoyama,[8] Ryang Kim,[1] Tatsushi Yokoyama,[1] Sayaka Takemoto-Kimura,[1,5,6,10] Manabu Abe,[9] Michiko Okamura,[1] Yayoi Kondo,[1] Sean Quirin,[2] Charu Ramakrishnan,[2] Takeshi Imamura,[11] Kenji Sakimura,[9] Tomomi Nemoto,[7,12] Masanobu Kano,[3,13] Hajime Fujii,[1] Karl Deisseroth,[2] Kazuo Kitamura,[3,4] and Haruhiko Bito[1,13,15,*]

[1]Department of Neurochemistry, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan
[2]Department of Bioengineering, Stanford University School of Medicine, Stanford, CA 94305, USA
[3]Department of Neurophysiology, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan
[4]Department of Neurophysiology, Faculty of Medicine, University of Yamanashi, Chuo, Yamanashi 409-3898, Japan
[5]Department of Neuroscience I, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Aichi 464-8601, Japan
[6]Department of Molecular/Cellular Neuroscience, Nagoya University Graduate School of Medicine, Nagoya, Aichi 466-8550, Japan
[7]Laboratory of Molecular and Cellular Biophysics, Research Institute for Electronic Science, Hokkaido University, Sapporo, Hokkaido

Extending the color palette of GECIs that are capable of *in vivo* AP recording would create flexibility in the optophysiology and optogenetics toolbox and facilitate quantitative paired recordings of distinct neuronal ensembles that together compose complex brain circuit dynamics (Livet et al., 2007; Tsien, 2005). To achieve this goal, here, we introduce a suite of multicolor (blue, green, yellow, and red), fast, and AP-resolvable GECIs, which enable the interrogation of brain information processing and dynamics *in vivo* with unprecedented spatial and temporal resolution. Biochemical comparisons of previously published GCaMPs revealed that mutations introduced during the transition from G-CaMP2 (Hill coefficient ~3.8; Tallini et al., 2006) to G-CaMP4.1 (Shindo et al., 2010; Hill coefficient ~2.1) created a unique circumstance whereby enhancement of signal amplitude was accompanied with improved linearity. Because of this, we reasoned that a G-CaMP4.1 backbone might provide a much more favorable template space for further re-designing of sensor linearity than that of any other green GECIs, such as GCaMP6f (Hill coefficient ~3.1). Consistently, replacement of

Abstract (summary)

1) It needs to be succinct

   (always with word limit)

Introduction

1) It must be focused

   (i.e. structured like a funnel)

2) It must identify the scientific gap/question

   b) Convince the reader the gap/question is important

   c) Convince the reader you know how to address the question / fill the gap

to tracking action potentials (APs) in single neurons *in vivo*; however, large-scale population voltage imaging with single-cell resolution still remains a challenge in the mammalian brain due to insufficient kinetics and dynamic range (Gong et al., 2015; Hochbaum et al., 2014; Yang et al., 2016). Therefore, improving GECI kinetics and sensitivity still provides the best chance to quantitatively record from mixed neuronal ensembles with fast-spiking activity.

Additionally, currently available colors of GECIs for *in vivo* imaging are limited to green and red for AP detection (Akerboom et al., 2013; Chen et al., 2013; Dana et al., 2016; Inoue et al., 2015; Zhao et al., 2011). A major technical difficulty for expanding the GECI color palette lies in the fundamental difference in the chemical flexibility of the green and red fluorophores. All GCaMPs variants consist of a circularly permuted fluorescent protein (cpFP), the Ca^{2+}-binding protein calmodulin (CaM), and a Ca^{2+}/CaM-sensing peptide, such as the commonly used M13 fragment from smooth muscle myosin light chain kinase. Despite the similarity in these domain structures, successful mutagenesis screens in green GECIs cannot be easily translated to red fluorescent protein (RFP)-based variants and vice versa.

Consistently, although signal dynamic range and brightness of green GECIs, such as GCaMP6, were massively improved (Chen et al., 2013), their highly cooperative Ca^{2+} binding, with Hill coefficients ranging from 2 to 4, turned out to be difficult to correct. This implies that fluorescence signals drastically change over a narrow range of Ca^{2+} concentration, with non-linear stimulus response properties relative to spike number (Rose et al., 2014). Conversely, we previously developed a sensitive and fast red color GECI, R-CaMP2 (Inoue et al., 2015), which could image synaptic and somatic population activities *in vivo* with a linear stimulus response based on its low (near 1) Hill coefficient (Inoue et al., 2015; Gaffield et al., 2016; Kim et al., 2016). In spite of our success to create a powerful red GECI through the use of a mutated Ca^{2+}/CaM-sensing domain (mutated ckkap sequence) of a neuronal protein CaMKK-α/β, which we have extensively studied in the past (Bito et al., 1996), we were unable to transplant this domain into either a GCaMP6f or a GCaMP6s backbone of green GECIs (Inoue et al., 2015).

Extending the color palette of GECIs that are capable of *in vivo* AP recording would create flexibility in the optophysiology and optogenetics toolbox and facilitate quantitative paired recordings of distinct neuronal ensembles that together compose complex brain circuit dynamics (Livet et al., 2007; Tsien, 2005). To achieve this goal, here, we introduce a suite of multicolor (blue, green, yellow, and red), fast, and AP-resolvable GECIs, which enable the interrogation of brain information processing and dynamics *in vivo* with unprecedented spatial and temporal resolution. Biochemical comparisons of previously published GCaMPs revealed that mutations introduced during the transition from G-CaMP2 (Hill coefficient ~3.8; Tallini et al., 2006) to G-CaMP4.1 (Shindo et al., 2010; Hill coefficient ~2.1) created a unique circumstance whereby enhancement of signal amplitude was accompanied with improved linearity. Because of this, we reasoned that a G-CaMP4.1 backbone might provide a much more favorable template space for further re-designing of sensor linearity than that of any other green GECIs, such as GCaMP6f (Hill coefficient ~3.1). Consistently, replacement of G-CaMP4.1's M13 calmodulin-binding sequence with new mutants of the ckkap sequence derived from CaMKK-α/β successfully generated a series of linearly responding green GECI with Hill coefficient near 1. The signal-to-noise ratio (SNR) and spectral properties were further optimized through linker diversification. We then further expanded the color palette into yellow and blue. Mutagenesis in the ckkap sequence in R-CaMP2 further boosted the performance of red GECI to an unprecedented level. Thus, we created next-generation "XCaMPs" (with hyphened suffix for color: XCaMP-B for blue, XCaMP-G for green, XCaMP-Y for yellow, and XCaMP-R for red). *In vivo* recording using combinations of XCaMPs demonstrated that XCaMPs represent new multiplexable GECIs, with previously unattained high SNR, linear property, and high-frequency spike resolution suitable for *in vivo* interrogation of complex neural dynamics.

RESULTS

Rational Design of Multicolor Calcium Indicators XCaMPs

To advance our hypothesis that a G-CaMP4.1 backbone would serve as an excellent template space for further advancing sensor linearity, we mutated GCaMP3 (Tian et al., 2009), GCaMP5G (Akerboom et al., 2012), GCaMP6f, GCaMP6s (Chen et al., 2013), and G-CaMP4.1 (Shindo et al., 2010) as starting GECI backbones and replaced the M13 fragment with ckkap. Consistent with our original hypothesis, ckkap-modified G-CaMP4.1 showed the highest $\Delta F/F$ and brightness under saturating Ca^{2+} concentrations, so we selected it as a template for further optimization. We introduced mutations into both the linker sequences to promote brightness and dynamic range ($\Delta F/F$) and into the ckkap sequence at five residues that are divergent between CaMKK-α and CaMKK-β (Figures 1A and 1B). To augment sensitivity to cytoplasmic Ca^{2+} rises, we added a nuclear export sequence (NES) to the N terminus of candidate sensor proteins. After screening, we chose three prominent indicators, hereafter termed XCaMP-G, XCaMP-Gf, and XCaMP-Gf_o ("f" for fast; "f_o" for fast-original), which showed promising characteristics for further characterization. These sensors had absorption and emission spectra nearly identical to GCaMP6f. Yet all showed a slightly but significantly blue-shifted wavelength of the excitation maximum (Figures 1C and 1D; Table S1). Furthermore, all XCaMP-G class indicators showed superior biophysical properties (Figures 1 and S1; Table S1): all had high Ca^{2+} binding affinities (K_d = 100–200 nM) and low Hill coefficients in the range of 1 or 2 (Figures 1E–1G; Table S1), although GCaMP6f and GCaMP6s showed much higher binding cooperativity (higher Hill coefficient; approximately 3). XCaMP-G also showed >2-fold increase in Ca^{2+}-free fluorescence as compared with GCaMP6f and GCaMP6s, and XCaMP-Gf and XCaMP-Gf_o showed much stronger improvement in Hill coefficient with minor distinction in *in vitro* properties (Table S1). These suggest that XCaMP-G might be beneficial for identifying subcellular neuronal structures, such as dendritic spines and axonal arbors, at resting basal brightness in the absence of spikes, whereas XCaMP-Gf and -Gf_o may be better suited for measurements of fast spikes. XCaMP-G (487 nm) and -Gf_o (488 nm) had noticeably

Results

Tell a (good) story and avoid:

a) **Describing your experiments in a boring way (we did this, then with this…). Remember that you do not need to follow the chronological order of your experiments.**

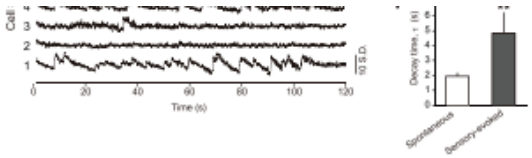
b) **Interpreting your results**

For each experiment try instead to give:

a) **The rational (Why?, always start with why)**

b) **A clear description (How?)**

c) **The conclusion the reader need to draw (but no interpretation!) → Next question**



after the cessation of the whisker stimuli, as shown by a significant slowdown of decay as compared with spontaneous activity (Figure 7F). These observations are in general agreement with neuronal properties of hippocampal CA1 neurons that responded to textures (Itskov et al., 2011) and sounds (Vinnik et al., 2012). Our findings underscore XCaMP-R's potential to enable a non-invasive deep hippocampal imaging using conventional two-photon microscopy and reveal the rapid emergence of persistent activity in cortical sensation-triggered active neuronal ensembles in hippocampus CA1.

DISCUSSION

In this study, we have rationally engineered XCaMPs, a next-generation GECI suite composed of sensitive and fast GECIs suitable for multispectral imaging. These features of XCaMPs are compatible, and likely will synergize, with recently developed optical engineering approaches for the fast readout of neuronal network activity. XCaMPs may also be compatible with rhodopsin-based optogenetic tools with large spectral separation. Of particular note is the massively improved time constants of XCaMP-Gf, such as $t_{over\ 3D}$ (3.5 ms *in vivo*; 1.9 ms in acute slices), which is particularly suitable for closed-loop optogenetic activation or inhibition control (Grosenick et al., 2015).

XCaMP suites allow us high-quality multiplexed recording of multiple cell types in awake animals. Previous simultaneous imaging of distinct inhibitory ensembles lacked any temporal resolution to disambiguate the timing of event-related activities in comparison to excitatory neurons. Our finding of temporal shift in mPFC PYR excitatory and both PV- and SST-inhibitory neurons is likely to be relevant in understanding how an initial relief from inhibition near the soma (preferentially innervated by PV-positive interneurons) is then followed by dendritic relief of inhibition (mainly through SST-interneurons), prior to triggering a cognition-related excitatory activity. Additionally, although the functional anatomical basis for cortical GABAergic inhibition is currently under active investigation in many laboratories, our *in vivo* paired recording results shed light on the spatiotemporal scale of dendritic computation underlying GABAergic inhibition

Discussion

Your results ←→ literature

1) **First paragraph: summary of your findings with methods used to achieve them (to show the solidity of your findings) and broader implications**
2) **The rest of the discussion can vary considerably from paper to paper**
   a) **How your findings align (or do not align) with the current body of knowledge**
   b) **Interpretation (draw conclusions from your and previous findings combined)**

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-Parvalbumin	Abcam	Cat# ab11427; RRID: AB_298032
Goat polyclonal anti-rabbit Alexa Fluor 647	Life Technology	Cat# A-21245 RRID: AB_2536101
Bacterial and Virus Strains		
AAV2/1-CaMKIIα-XCaMP-G	This study	N/A
AAV2/1-CaMKIIα-XCaMP-Gf_o	This study	N/A
AAV2/1-CaMKIIα-XCaMP-Y	This study	N/A
AAV2/1-CAG-XCaMP-R	This study	N/A
AAV2/1-CAG-DIO-XCaMP-Y	This study	N/A
AAV2/1-CAG-DIO-XCaMP-Gf_o	This study	N/A
AAV2/1-CAG-DIO-XCaMP-Gf	This study	N/A
AAV2/1-CAG-DIO-GCaMP6f	This study	N/A
AAV2/1-CAG-DIO-TagRFP	This study	N/A
AAV8-CaMKIIα-XCaMP-B	Stanford Gene Vector and Virus Core	N/A
AAVDJ-EF1α-fDIO XCaMP-Gf_o	Stanford Gene Vector and Virus Core	N/A
AAVDJ-EF1α-fDIO XCaMP-Gf	Stanford Gene Vector and Virus Core	N/A
AAV9-EF1α-DIO-XCaMP-R	Stanford Gene Vector and Virus Core	N/A
Chemicals, Peptides, and Recombinant Proteins		
Hoechst 33342	Thermo Fisher Scientific	Cat# H3570
Doxycycline	Sigma-Aldrich	D9891
Tamoxifen	Sigma-Aldrich	T5648
XCaMP-B	This paper	GenBank: MK770164
XCaMP-G	This paper	GenBank: MK770159
XCaMP-Gf	This paper	GenBank: MK770160
XCaMP-Gf_o	This paper	GenBank: MK770161
XCaMP-Y	This paper	GenBank: MK770163
XCaMP-R	This paper	GenBank: MK770162
GCaMP6f	This paper	N/A
GCaMP6s	This paper	N/A
Critical Commercial Assays		
Calcium Calibration Buffer Kit#1	Life Technologies	Cat# C3008MP
SYBR qPCR: SYBR *Premix Ex Taq* (Tli RNase H Plus)	Clonetech	Cat# RR420
NucleoBond® Xtra Midi EF	MACHEREY-NAGEL	740420
Experimental Models: Cell Lines		
Primary rat hippocampal neuron	This paper	N/A
HEK293T	ATCC	CRL-3216
DH5	Thermo Fisher	18258-012
KRX	Promega	L3002
Sf9	Thermo Fisher	12552014
Experimental Models: Organisms/Strains		
Mouse: PV-iCre-IRES-FLAG-EGFP	This paper	N/A
Mouse: SST-IRES-Cre (Ssttm2.1(cre)Zjh/J)	Josh Huang, Jackson Laboratory	Cat#013044

(Continued on next page)

Materials & methods

Ask yourself a question:

Can a knowledgeable person reproduce my data by reading them?

One possible way

how to proceed

when writing a

thesis/paper

Figures (do they make an interesting story?)

(can I watch the figures without reading the legend?)

↓

Figure legends

(can I read the legends without reading the main text?)

↓

Methods

↓

Results

↓

Introduction

↓

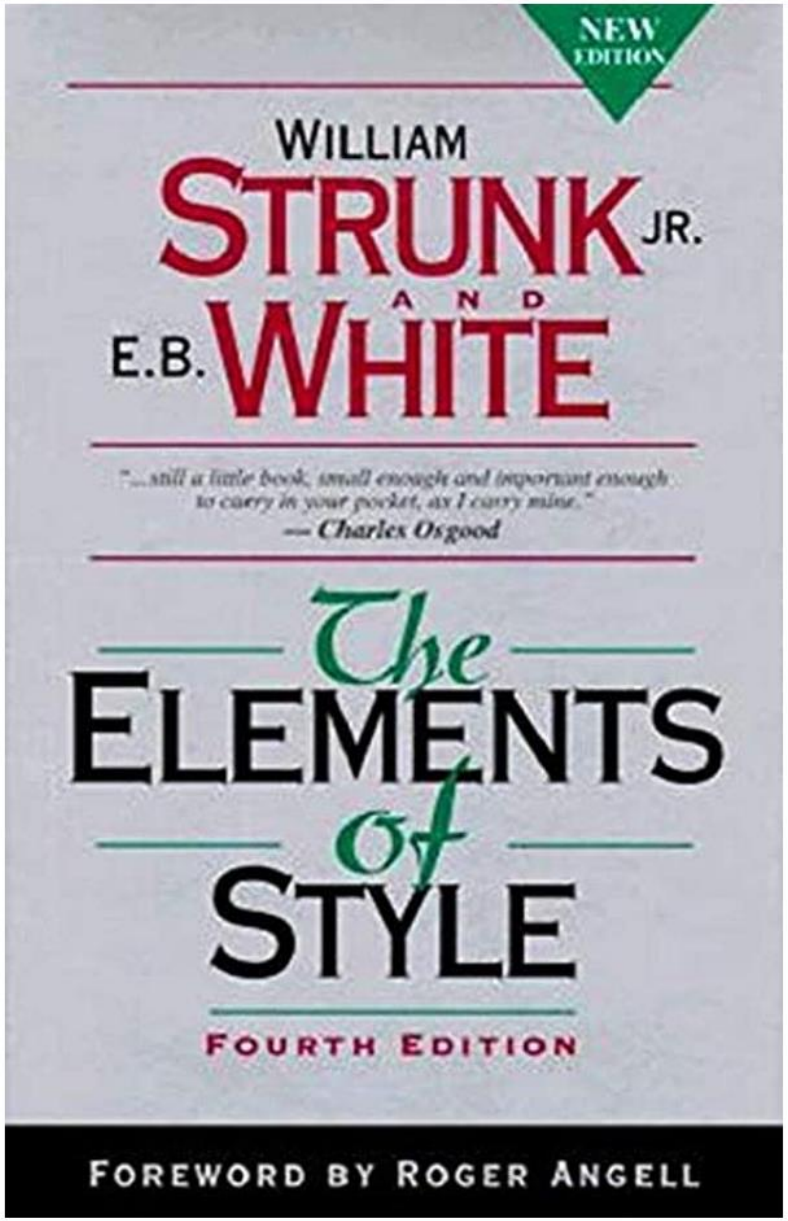
Discussion

↓

Abstract

Read papers

Write papers


NEW EDITION
WILLIAM STRUNK JR.
AND
E.B. WHITE
"...still a little book, small enough and important enough to carry in your pocket, as I carry mine."
— Charles Osgood
The ELEMENTS of STYLE
FOURTH EDITION
FOREWORD BY ROGER ANGELL


Essentials of Writing Biomedical Research Papers
Second Edition
An engaging and effective "nuts and bolts" approach to scientific writing
Handy chapter checklists summarize key points
Examples culled from actual research papers illustrate each discussed guideline
Review exercises enable the reader to apply guidelines firsthand
Mimi Zeiger