Mapping of excitatory and inhibitory postsynaptic potentials of neuronal populations in hippocampal slices using the GEVI, ArcLight

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Received 16 May 2018, revised 18 August 2018
Accepted for publication 20 September 2018
Published 16 October 2018

Abstract
To understand the circuitry of the brain, it is essential to clarify the functional connectivity among distinct neuronal populations. For this purpose, neuronal activity imaging using genetically-encoded calcium sensors such as GCaMP has been a powerful approach due to its cell-type specificity. However, calcium (Ca^{2+}) is an indirect measure of neuronal activity. A more direct approach would be to use genetically encoded voltage indicators (GEVIs) to observe subthreshold, synaptic activities. The GEVI, ArcLight, which exhibits large fluorescence transients in response to voltage, was expressed in excitatory neurons of the mouse CA1 hippocampus. Fluorescent signals in response to the electrical stimulation of the Schaffer collateral axons were observed in brain slice preparations. ArcLight was able to map both excitatory and inhibitory inputs projected to excitatory neurons. In contrast, the Ca^{2+} signal detected by GCaMP6f, was only associated with excitatory inputs. ArcLight and similar voltage sensing probes are also becoming powerful paradigms for functional connectivity mapping of brain circuitry.

Keywords: ArcLight, GCaMP, GEVI, GECI, hippocampus, synaptic excitation, synaptic inhibition

(Some figures may appear in colour only in the online journal)
Introduction

Neuronal circuit activity is a delicate balance between activation and inhibition. For example, Parkinson’s disease results from a shift towards inhibition in the output of the Basal Ganglia circuit, while a shift towards activation can result in Tourette syndrome [1, 2]. Improving the ability to monitor both types of activities in a population of cells will enable a better understanding of neuronal circuits including their computational abilities, plasticity, and related pathologies.

Neuronal activity is directly related to the membrane potential [3]. Activation is due to the depolarization of the plasma membrane reaching a threshold causing the neuron to fire an action potential. Hyperpolarization of the plasma membrane increases the barrier to reaching that threshold potential resulting in inhibition. Calcium imaging is an excellent method for monitoring neuronal activation since there is typically a large increase in the Ca$^{2+}$ concentration inside the cell once an action potential is fired. However, since subthreshold membrane potential changes do not normally affect internal Ca$^{2+}$ levels, synaptic activity would be better resolved by voltage imaging.

Neuronal circuits are composed of a great diversity of neurons which can be classified into distinct types based on anatomy, intrinsic properties, and the genes expressed [4–9]. To clarify the functional connectivity among this diversity of neurons, fluorescent imaging with genetically encoded voltage indicators (GEVIs) would be ideal since transcriptional promoters can be chosen to restrict fluorescence of the probe to specific cell types [10].

Genetically-encoded calcium indicators (GECIs) such as the GCaMP family of sensors have been utilized to visualize the neuronal activities from specific cell types [11–17]. However, there are potential drawbacks to calcium sensors for imaging neuronal activities. In some cell types, action potentials do not generate calcium influxes [18, 19] while in other cell types, ligand gated receptors can cause Ca$^{2+}$ influx [20]. Ca$^{2+}$ transients from internal sources are not necessarily related to plasma membrane potential changes [21–23]. These factors and the relatively slow Ca$^{2+}$ flux make Ca$^{2+}$ imaging a complement but not a substitute for voltage imaging.

GEVIs are becoming a powerful approach to study neuronal activities in the brain. GEVIs have lagged behind GECIs in part due to the multiple membrane potential changes during different neuronal activities. While an action potential is roughly a 100 mV depolarization of the plasma membrane, excitatory post synaptic potentials (EPSPs) depolarize the membrane potential by only 10 to 20 mV. Inhibitory post synaptic potentials (IPSPs) hyperpolarize the voltage across the plasma membrane by 5–10 mV. However, recent improvements in the signal size, speed, and the voltage sensitivities of GEVIs enable direct observation of the changes in neuronal membrane potential [24–33]. These properties directly affect the type of neuronal activities a GEVI can optimally resolve [34].

The goal of in vivo imaging in freely behaving mice using GEVIs is starting to come to fruition [35, 36]. However, the optical signal is still near shot-noise limited requiring sophisticated data processing to deconvolute the voltage-dependent optical signal from physiological noise sources such as blood flow and respiration. Robust population signals from head-fixed recordings of mouse olfactory bulb [37, 38] and the barrel cortex [39] using the GEVI, ArcLight [40], showed only depolarizing responses.

Brain slice is a valuable way to investigate neuronal network through GEVs because of the lack of physiological motion artifacts as well as the accessibility to the deeper regions in the brain. In spite of the advantages of brain slices, examples of voltage imaging in brain slice using GEVIs have been primarily proof of principle recordings in the original report of a newly developed probe. hVOS [41–43] and VSFP-Butterfly [25] were able to show fluorescent signals in response to field stimulation. hVOS exhibits a linear fluorescence sensitivity to physiological voltage range but requires the application of an exogenous quenching compound, dipicrylamide. VSFP-Butterfly is a FRET probe, which offers the advantage of a ratio-metric probe enabling the removal of motion induced signals due to respiratory and cardiovascular artifacts in vivo. In slice the advantage of a ratiometric recording is of lesser value since movement artifacts due to respiration and blood flow do not exist. Even though VSFP-Butterfly’s voltage-dependent change in fluorescence was small (0.5% ΔR/R), clear optical signals could be seen demonstrating the usefulness of GEVIs in cortical brain slices [44].

For these reasons, we chose brain slice to assess the ability of the GEVI, ArcLight [40], to report different types of neuronal activities from a population of cells in the CA1 region of the mouse hippocampus. This region of the hippocampus is an excellent testing ground for reporting both depolarizations and hyperpolarizations of neuronal plasma membranes since the CA1 circuitry exhibits feed-forward inhibition as a consequence of excitatory synaptic inputs rapidly followed by inhibitory inputs [45] while ArcLight has a broad voltage range that can respond to both hyperpolarization and depolarization of the plasma membrane.

Materials and methods

Animals

Experiments were conducted in compliance with the rules and regulations established by the Animal Care and Use Committee of Korea Institute of Science and Technology under the ethical permission (approval No. 2017-031). Ca$^{2+}$/calmodulin-dependent protein kinase II (CaMKII) α-Cre mice (#005359; Jackson Laboratory, Bar Harbor, ME) are bred in specific pathogen free animal facility. Both male and female mice were utilized for the experiments.

AAV delivery

For the viral delivery of ArcLight or GCaMP6f into mice CA1 hippocampus, associated adeno virus (AAV) was injected into
adult mice by conventional method [46]. In brief, Adult mice over 4 weeks old were first anesthetized by the intraperitoneal injection of 250 mg kg\(^{-1}\) 2,2,2-Tribromomethanol (Sigma, St. Louis, MO) or by 1.5% to 3% isoflurane. The mouse was then mounted on a stereotaxic device (51730D, Stoelting, Wood Dale, IL) and ~2 mm diameter craniotomies were created by a surgical drill (K1070; Foredom, Bethel, CT) at 1.7 mm posterior to bregma, 1.2 mm lateral to midline bilaterally. Glass-capillary injection pipette (25 µm tip inner diameter) filled with 25 NaHCO\(_3\), 3 Na-pyruvate, 1 ascorbic acid, 1 MgCl\(_2\), 25 NaHCO\(_3\), 75 sucrose, 1 ascorbic acid, 7 MgCl\(_2\), 0.5 CaCl\(_2\), 2.5 KCl, 0.3 NaH\(_2\)PO\(_4\), 1.25 NaH\(_2\)PO\(_4\), 25 NaCl was used to inject the ACSF. The mice were then incubated at 36 ± 1 °C by a temperature controller (TC-344B, Warner Instruments, CT). After injection, the scalp was sutured and treated with povidone-iodine. Mice were transferred back to the home cage warmed on an electric blanket with free access to the water containing 1.6 mg/ml aceatinophen (Children’s Tylenol, Johnson & Johnson, NJ) until fully recovered. The mice were subjected to the experiments at least 2 weeks after the injection.

Slice preparation and drug application

300 µm thick acute coronal brain slices (≈1.7 mm posterior to bregma) were made after the deep anesthesia of the mice with halothane (Sigma). Isolated brain slice was sliced by Vibratome (VT-1200, Leica, Nussloch, Germany) in a cold, high-sucrose, artificial cerebrospinal fluid (ACSF) solution containing (in mM): 87 NaCl, 2.5 KCl, 25 D(+)-glucose, 1.25 NaH\(_2\)PO\(_4\), 25 NaHCO\(_3\), 75 sucrose, 1 ascorbic acid, 7 MgCl\(_2\), 0.5 CaCl\(_2\), pH 7.4, by gassing with 95% O\(_2\)/5% CO\(_2\). Slices were then placed in an interface chamber filled with ACSF containing (in mM): 125 NaCl, 2.5 KCl, 25 D(+)-glucose, 1.25 NaH\(_2\)PO\(_4\), 25 NaHCO\(_3\), 3 Na-pyruvate, 1 ascorbic acid, 1 MgCl\(_2\), 2.5 CaCl\(_2\), pH 7.4, oxygenated with 95% O\(_2\)/5% CO\(_2\). Slices were incubated at 36 °C for at least 1 h. During the experiment, a slice was placed on the microscope stage chamber with continuous perfusion of ACSF at 1–2 ml min\(^{-1}\). The temperature of ACSF on the recording chamber was adjusted to 33 °C ± 0.5 °C by a temperature controller (TC-344B, Warner Instruments, CT) throughout the experiment. When required, the following drugs were dissolved in ACSF for perfusion: 50 µM D-AP5, 40 µM CNQX, 40 µM bicuculline and 1 µM tetrodotoxin (TTX) (all from Sigma). A minimum of 10 min of drug perfusion was done to ensure proper delivery.

Neuronal activity imaging

A high-speed CCD camera (Neuro CCD, RedShirtImaging, Decatur, GA) was connected to the c-mount port of an upright epi-fluorescence microscope (Slicescope, Scientifica, East Sussex, UK) in combination with a GFP filter set (GFP-3035D-OMF, Semrock, Rochester, NY) and a 10 × water immersion objective lens (UMPlanFL N; NA = 0.3, Olympus, Tokyo, Japan). Through the conventional EPI light source port, 8.8 mW of 460 nm LED light (UHP-Mic-LED-460, Prizmatix, Givat-Shmuel, Israel) was applied to illuminate the whole field of view (1.7 mm diameter, 3.7 mW mm\(^{-2}\)).

The fluorescent signal of ArcLight was acquired with an 80 × 80 pixel CCD camera sampling at 1 kHz from the dorsal hippocampal CA1 region (500 µm × 500 µm). The spatial resolution before spatial filtering was 6.25 µm/pixel. All the acquired fluorescent signals were processed as fractional fluorescence change values [%ΔF/F = ((Fx − F0)/F0) × 100] with NeuroPlex (RedShirtImaging, Decatur, GA) using an offline Butterworth low-pass filter (value 50). All the frame-subtracted ArcLight/GCaMP6f mapping images shown in this report are processed with 3 × 3 mean iteration 1 long-pass filter provided by Neuroplex.

Region of interest for spatial averaging of fluorescent signals

To obtain the fluorescence signal traces from each stratum in CA1 region, each stratum in the entire field of view was selected as a region of interest and spatially averaged. To eliminate the non-synaptic stimulation artifact, an area with a radius of 100 µm from the stimulating electrode tip was excluded from spatial averaging (figure 2(A)). All the fluorescent signal traces in this paper are generated as described above.

Field potential recording

During the imaging, field potential was simultaneously monitored by an ACSF-filled glass capillary pipette (=1 µm tip diameter, 5 MΩ) connected to a patch-clamp amplifier Multiclamp 700B (Molecular devices, San Jose, CA). To prevent the baseline drift of the voltage, 0.1 Hz high-pass filter was applied during the recording. Obtained field potential was analyzed by Clampfit (Molecular Devices, San Jose, CA). To measure the fEPSP peak, Clampfit’s waveform statistics function was used.

Electric stimulation

Test pulses ranging from 20–300 µA, (50 µs single square pulses) were applied every 20 s to the Schaffer collateral through a bipolar tungsten electrode (30201, FHC, Bowdoin, ME) that was connected to an isolator (model DS3, Digimeter Ltd, Hertfordshire, UK). The stimulus timing was triggered by the patchclamp amplifier.

Confocal imaging of fixed slice preparation

After finishing the imaging experiments, brain slices were fixed in 2% paraformaldehyde (PFA; Electron Microscopy Sciences, Hartfield, PA) in phosphate-buffered saline (PBS, pH7.4; Life technologies, Waltham, MA) overnight at 4 °C. The brain slices were then washed three times over 1 h in PBS. The brains were immersed in EverBrite Mounting Medium with DAPI and then sealed with a coverslip using CoverGrip Sealant (both from Biotium, Fremont, CA). We acquired single optical sections of confocal images by a laser scanning confocal microscope (A1R; Nikon, Tokyo, Japan) using 488 nm laser and the green fluorescent protein filter set.
Statistics
All the error bars represent standard error of the mean (SEM). Means are presented as mean ± SEM. For the comparison of input–output (I–O) curves in figure 2, we used repeated-measures two way ANOVA and Turkey post-hoc test. For comparison of means from multiple groups in figure 3, one-way ANOVA followed by the Fisher post-hoc test was performed. To test the significance of Pearson’s correlation coefficient in figure 2, T-test was used. In this report, the significance level of 0.05 was applied to all comparisons. All the ANOVA analyses were performed using OriginPro (OriginLab, Northampton, MA).

Results
Expression of ArcLight in comparison with GCaMP6f in CA1 hippocampus
For the imaging of neuronal population activities, excitatory neurons in CA1 hippocampus which are known to be CaMKII positive [47–49] were targeted for expression. To observe their activities, ArcLight or the GECI, GCaMP6f, were expressed in a cre-dependent manner in the hippocampal CA1 region of CaMKII α-cre mouse via AAV injection (figure 1(A)). Two weeks after the AAV injection, ArcLight and GCaMP6f showed broad expression in the entire CA1 region. The expression pattern of the membrane bound GEVI versus the cytosolic GECI resulted in near negative images of one another (figure 1(B)). Because ArcLight is a membrane-targeted sensor, stratum oriens (str. ori.) and stratum radiatum (str. rad.) which have denser membrane concentrations due to branching dendrites exhibited higher fluorescence than the stratum pyramidale (str.pyr.; figure 1(B) left). Conversely, GCAMP6f, a cytosolic protein, primarily exhibited soma-filling expression in str. pyr., with lesser fluorescence in the dendritic branches from individual neurons in the str. rad. (figure 1(B) right).

Association between fEPSP and Population ArcLight signal
We monitored the fluorescence changes of CA1 neurons in response to Schaffer collateral electrical stimulation with a high-speed CCD camera sampling at 1kHz (figure 1(A), right). Upon Schaffer collateral stimulation, a depolarizing reduction of the ArcLight fluorescence (figure 2(A3)) was observed followed by an increase in fluorescence that is associated with the hyperpolarization of the plasma membrane (figure 2(A4)). We measured the changes in the ArcLight signal size induced by electric stimuli in parallel with a field potential recorded from the pyramidal cell layer (figures 2(B) and (C)). When the stimulus intensity was increased, the peak amplitude of the field excitatory postsynaptic potential (fEPSP) increased as did the depolarizing ArcLight signal and a subsequent hyperpolarizing ArcLight signal (figures 2(D)–(F)). The depolarizing ArcLight signal in the str. rad. and the fEPSP amplitude were significantly correlated with each other (figure 2(F); $r = 0.679$, $p = 0.003$). The spatially-averaged, depolarizing ArcLight signal in the str. rad. was significantly larger than in the str. pyr. or the str. ori. layers (figure 2(D); $n = 6$, $p < 0.0001$). The hyperpolarizing ArcLight signal was also seen in all the CA1 strata, and the spatially-averaged hyperpolarizing ArcLight signal in the str. pyr. was larger compared to the str. rad. (figure 2(E); $n = 6$, $p = 0.015$).

Comparison between ArcLight and GCaMP for synaptic input mapping
Figure 3(A) shows the ArcLight signal of the CA1 excitatory hippocampal neurons along with the application of neurotransmission blockers. During control conditions (figure 3(A)), ArcLight showed a biphasic fluorescent signal. In the str. rad., ArcLight showed the largest depolarizing signal among the strata. The depolarizing signal peaked at 20ms post stimulation with less than 1ms deviation ($n = 6$, stimulus = 250 µA). A subsequent hyperpolarizing ArcLight signal was observed in the entire CA1 sub-region. After the application of glutamate receptor blockers, CNQX and D-AP5, the depolarizing ArcLight signal was significantly blocked (figures 3(A) and (C) left panel; $n = 6$, str. ori., $p = 0.00002$; str. pyr., $p = 0.000008$; str. rad., $p = 0.00002$), but the hyperpolarizing ArcLight signal was still present. This hyperpolarizing ArcLight signal was significantly reduced by GABA A receptor blocker bicuculline in all layers (figures 3(A) and (C) right panel; $n = 6$, str. ori., $p = 0.0007$; str. pyr., $p = 0.00004$; str. rad., $p = 0.00001$). The ArcLight signal in all three layers was completely blocked by the application of a voltage-gated sodium channel blocker, TTX which indicates that all the ArcLight signal detected here are from synaptic events and not the result of direct stimulation. The signal partially recovered after 2hr of washout. From these results, we conclude that ArcLight was able to detect both excitatory and inhibitory postsynaptic potentials in hippocampal CA1 circuit.

Figure 3(B) shows the GCaMP6f signal of CA1 excitatory hippocampal neurons treated with neurotransmission blockers. Compared to the ArcLight signal shown in figure 3(A), GCaMP6f’s Ca$^{2+}$ signal was larger, and monophasic in all the strata. In the str. rad., the Ca$^{2+}$ signal peaked 40ms post stimulation with less than 2ms deviation ($n = 5$, stimulus = 250 µA). The peak time for the GECI, GCaMP6f, fluorescent signal was significantly slower than the depolarizing signal from ArcLight ($p = 0.00009$; ArcLight, $n = 6$; GCaMP6f, $n = 5$; student’s t-test). Application of NMDA receptor blocker D-AP5 significantly reduced the Ca$^{2+}$ signal size in all the strata (figure 3(D); $n = 5$, str. ori., $p = 0.01$; str. pyr., $p = 0.003$; str. rad., $p = 0.008$). Additional CNQX and bicuculline application did not significantly affect the Ca$^{2+}$ signal size compared with AP5 treated signal. The rest of the Ca$^{2+}$ signal was completely blocked by TTX (figures 3(B) and (D)). 1hr after the washout, the Ca$^{2+}$ signal was partially recovered. These data suggest that, GCaMP’s Ca$^{2+}$ signal was mainly mediated by NMDA receptors. GCaMP6f failed to register the hyperpolarization event observed with ArcLight.
Discussion

This study has demonstrated the ability of the GEVI, ArcLight, to map multiple activities of the neuronal circuits in the CA1 region of mouse hippocampus. ArcLight was able to report both excitatory and inhibitory inputs from a population of neurons. In contrast, the Ca²⁺ signal detected by GCaMP6f was not able to indicate any GABAergic inputs.

It is known that approximately 11% of the neuronal population in the hippocampal CA1 region are interneurons [50, 51] with each of them locally projecting GABAergic inputs to several hundreds of excitatory neurons [4, 52]. ArcLight was able to spatially discriminate the network of both depolarization and hyperpolarization in the hippocampal CA1 circuitry in a manner consistent with known circuitry [52, 53].

In excitatory neurons, the depolarizing signal of ArcLight was confirmed to be mediated by glutamate receptors since the NMDA receptor blocker, D-AP5, and the AMPA receptor blocker, CNQX, both reduced the depolarizing ArcLight signal (figure 3(C), left panel). The hyperpolarizing ArcLight signal was confirmed to be induced by GABAergic inhibitory inputs, because it was reduced by the GABA_A receptor blocker, bicuculline (figure 3(C), right panel). GCaMP6f detected excitatory inputs and did not show any signals associated with inhibitory inputs. Since the choice of the method that can detect inhibitory signals from defined neuronal populations is very limited, GEVIs will be valuable tools to further investigate the circuitry of such regions that inhibition is playing key roles [53–56].

Most of the current connectome approaches have one common, but critical limitation: they rely solely on structural mapping and therefore cannot inform us about the functionality of identified synaptic connections [57, 58]. It is well documented that the presence of structural synaptic elements (i.e. presynaptic bouton adjacent to postsynaptic process) alone does not confirm functional connections, and there are structured synaptic connectivities that are not consistent with Peters’ rule. Synaptic connections are not necessarily proportional to the degree of cellular overlap [59–61]. It is therefore crucial to extend current connectome mapping approaches to incorporate functional probing of synaptic connections among defined neuronal populations. For this purpose, the combination of optogenetic photostimulation and activity imaging with GEVIs will facilitate the resolution of neuronal circuit activities.

While the fluorescent signals shown in this report indicate that ArcLight can report both excitatory and inhibitory neuronal activities in a population of cells, further validation of these population signals is required. Of interest is the ability to resolve subthreshold activity from action potentials. At single cell resolution, this is a trivial matter. The speed of the fluorescent response as well as the size of the optical signal can easily differentiate an action potential from synaptic activity. Even ArcLight which has a relatively slow response time (10 ms....
Figure 2. ArcLight signals from excitatory neurons in CA1 hippocampus. (A) Excitatory neurons’ activity map in CA1 hippocampus evoked by 250 µA electric stimulation at Schaffer collateral. (A1) Bright field image of a recorded hippocampal slice (grey) overlaid with colored region of interest for spatial averaging (str. ori., blue; str. pyr., red; str. rad., green). Tip location of the stimulating electrode is indicated as S. Field potential recording electrode’s tip is indicated as R. (A2) Heat map of ArcLight’s fluorescence before the stimulus. (A3) Heat map of the depolarizing ArcLight signal. (A4) Heat map of the hyperpolarizing ArcLight signal. (B) fEPSP traces evoked by different stimulus amplitudes, three trials average. (C) Traces of evoked ArcLight signal induced by different stimulus intensities, 4 trials average. Traces were spatially averaged in each stratum indicated by the left panel in (A). (D) The stimulus-response correlation of depolarizing ArcLight signal. (E) The stimulus-response correlation of hyperpolarizing ArcLight signal. (F) Correlation between the fEPSP slope and the depolarizing ArcLight signal peak of the str. rad. (n = 6). All the error bars in the figures are SEM. Values in (D) and (E) are statistically compared with repeated-measures two way ANOVA and Turkey post-hoc test (significance indication: *p < 0.05, ***p < 0.0001.) Vertical dotted line over the traces in (B) and (C) indicate the timing of stimulus.
Figure 3. Comparison of the evoked field fluorescent signals between ArcLight and GCaMP in hippocampal slices. (A) Top row: ArcLight signal evoked by 250 µA electric stimulation, spatially averaged in entire region of each stratum. Middle row: map of depolarizing ArcLight peak signal. Bottom row: map of hyperpolarizing ArcLight peak signal. Each column represents the results of drug application shown on top. Asterisks indicate the position of stimulating electrode tip. Each trace and map are the averages from 10 trials. (B) Top row: GCaMP6f signal evoked by 250 µA electric stimulation, spatially averaged in entire region of each stratum. Bottom row: map of peak Ca2+ signal. Each column indicates the effect of drugs shown on top. Asterisks again indicate the position of stimulating electrode tip. Each trace and map are the average from 10 trials. (C) Left: average of depolarizing ArcLight signal peak and the effects of drugs (control, APV, CNQX and Bic: n = 6, washout: n = 5). Right: average of hyperpolarizing ArcLight signal peak and the effects of drugs (control, APV, CNQX and Bic: n = 6, washout: n = 5). All the bars represent means ± SEM. Asterisks in figures (C) and (D) indicate a significant difference compared with control with critical values less than p < 0.05, tested by one-way ANOVA followed by Fisher’s post-hoc test. # indicates the significant difference at p < 0.05 between the specific pairs. Non-significant differences are indicated with ns. Vertical bars under the traces in (A) and (B) indicate the timing of stimulus.
compared to other GEVIs can resolve action potentials in neurons firing at over 30 Hz [27]. However, when imaging neuronal circuit activity from a population of cells, the optics may no longer resolve single cell fluorescence resulting in the summation of the output from multiple neurons. The lag of the fluorescent signal from ArcLight when compared to the field potential recording may be due the slower kinetics of ArcLight. However, the larger volume of cells being reported by ArcLight may also contribute to this lag. The temporal variance of the fluorescent signal would be higher for a larger volume of cells, but it is not clear to what degree that variance affects the signal. Future comparisons to other GEVIs especially those with faster kinetics should clarify this issue. By imaging brain slice of the CA1 region of the mouse hippocampus, the consequences of the different physical characteristics of a GEVI (speed, signal size, voltage sensitivity) can be tested at the circuit level which will facilitate the interrogation of neuronal circuits and the interpretation of the optical signals seen in behaving animals. Taken together, we conclude that ArcLight and similar probes are becoming a powerful paradigm for functional circuitry mapping of the brain.

Disclosures

All authors declare no conflicts of interest.

Acknowledgements/Funding sources

We thank Hong Hua Piao, Arong Jung, Sung Moo Lee, Tristan Geiller, and Bok Eum Kang for technical assistance related to this work. We thank Vivek Jayaraman, Douglas S Kim, Loren L Looger, and Karel Svoboda from the GENIE Project, Janelia Research Campus, Howard Hughes Medical Institute for allowing us to use GCaMP6f through UPenn Vector core. Research reported in this publication was supported by: Korea Institute of Science and Technology (KIST) grants 2E26190, 2E26170, and the National Institute of Neurological Disorders And Stroke of the National Institutes of Health under Award Number U01NS099691. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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