Video Article

Two-photon Calcium Imaging in Neuronal Dendrites in Brain Slices

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Abstract

Calcium (Ca^{2+}) imaging is a powerful tool to investigate the spatiotemporal dynamics of intracellular Ca^{2+} signals in neuronal dendrites. Ca^{2+} fluctuations can occur through a variety of membrane and intracellular mechanisms and play a crucial role in the induction of synaptic plasticity and regulation of dendritic excitability. Hence, the ability to record different types of Ca^{2+} signals in dendritic branches is valuable for groups studying how dendrites integrate information. The advent of two-photon microscopy has made such studies significantly easier by solving the problems inherent to imaging in live tissue, such as light scattering and photodamage. Moreover, through combination of conventional electrophysiological techniques with two-photon Ca^{2+} imaging, it is possible to investigate local Ca^{2+} fluctuations in neuronal dendrites in parallel with recordings of synaptic activity in soma. Here, we describe how to use this method to study the dynamics of local Ca^{2+} transients (CaTs) in dendrites of GABAergic inhibitory interneurons. The method can be also applied to studying dendritic Ca^{2+} signaling in different neuronal types in acute brain slices.

Video Link

The video component of this article can be found at https://www.jove.com/video/56776/

Introduction

The contribution of a neuron to network activity is largely determined by the dynamic nature of the synaptic inputs it receives. Traditionally, the predominant method of characterizing synaptic activity in neurons relied on somatic whole-cell patch-clamp recordings of postsynaptic currents evoked by electrical stimulation of axons of passage. However, only activity of the proximally located synapses is truthfully reported in this case[1]. In addition, to assess the synapse-specific mechanisms, recordings from pairs of neurons and from dendrites at a specific location have been used to target the synapses of interest and the mechanisms of dendritic integration, respectively. A major breakthrough in the field of synaptic physiology was achieved through a marriage of optical and electrophysiological techniques. Two-photon excitation laser scanning microscopy (2PLSM) in combination with Ca^{2+} imaging and optogenetic tools can reveal tremendous details of the dynamic organization of synaptic activity at specific neuronal connections in brain slices *in vitro* and *in vivo*.

Several key advantages made 2PLSM stand out from the conventional, one-photon excitation microscopy[2]: (1) due to a nonlinear nature of two-photon excitation, the fluorescence is generated only in the focal volume, and all emitted photons represent useful signals (no need for pinhole); (2) longer wavelengths, used in 2PLSM, penetrate the scattering tissue more efficiently; in addition, scattered photons are too dilute to produce two-photon excitation and background fluorescence; (3) photodamage and phototoxicity are also limited to the focal plane. Therefore, despite the high cost of 2-photon systems in comparison with conventional confocal microscopes, the 2PLSM remains a method of choice for high-resolution investigation of neuronal structure and function in thick living tissue.

The first application of 2PLSM in scattering tissue was to image the structure and function of dendritic spines[3]. 2PLSM in combination with Ca^{2+} imaging has revealed that spines function as isolated biochemical compartments. Since in many neuronal types there is a one-to-one correspondence between spines and individual synapses[4], two-photon Ca^{2+} imaging soon became a useful tool reporting the activity of individual synapses in intact tissue[5,6,7,8]. Furthermore, 2PLSM-based Ca^{2+} imaging was successfully used to monitor the activity of single calcium channels and the nonlinear interactions between the intrinsic and synaptic conductances, as well as to assess the state- and activity-dependent regulation of Ca^{2+} signaling in neuronal dendrites[5,6,7,8,9,10,11].

Calcium is a ubiquitous intracellular second messenger, and its subcellular spatiotemporal organization determines the direction of physiological reactions, from changes in synaptic strength to the regulation of ion channels, dendrite and spine growth, as well as cell death and survival. Dendritic Ca^{2+} elevations occur via activation of multiple pathways. Action potentials (APs), backpropagating to the dendrites, open voltage-gated calcium channels[12] and produce relatively global Ca^{2+} transients (CaTs) in dendrites and spines[13]. Synaptic transmission is associated with activation of postsynaptic Ca^{2+}-permeable receptors (NMDA, Ca^{2+}-permeable AMPA and kainate), triggering synaptic CaTs[6,14,15]. Finally, supralinear Ca^{2+} events can be generated in dendrites under certain conditions[11,12,13,16].

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Two-photon Ca^{2+} imaging in combination with patch-clamp electrophysiological recordings employs synthetic Ca^{2+}-sensitive fluorescent indicators, which are typically delivered through the patch electrode during whole-cell recordings. A standard method for quantification of Ca^{2+} dynamics is based on the dual indicator method[17,18]. It uses two fluorophores with well separated emission spectra (*e.g.*, a combination of a red Ca^{2+}-insensitive dye with green Ca^{2+} indicators, such as Oregon Green BAPTA-1 or Fluo-4) and has several advantages when compared to the single indicator method. First, a Ca^{2+}-insensitive dye is used to locate small structures of interest (dendritic branches and spines) where Ca^{2+} imaging will be performed. Second, the ratio between the change in green and red fluorescence ($\Delta G/R$) is calculated as a measure of $[Ca^{2+}]$, which is largely insensitive to changes in baseline fluorescence due to fluctuations in $[Ca^{2+}]_0$[17,18]. Furthermore, fluorescence changes can be calibrated in terms of absolute Ca^{2+} concentrations[19].

A general concern when running two-photon Ca^{2+} imaging experiments in acute slices is cell health and stability of the image acquisition due to the high laser power typically used. Additionally, in Ca^{2+} imaging experiments, there is concern about the perturbation and substantial overestimation of subcellular Ca^{2+} dynamics due to the fact that Ca^{2+} indicators act as highly mobile exogenous Ca^{2+} buffers. Thus, the choice of the Ca^{2+} indicator and its concentration depends on the neuronal type, the anticipated amplitude of CaTs, and on the experimental question.

We adapted the method of two-photon Ca^{2+} imaging for investigation of Ca^{2+} fluctuations in dendrites of GABAergic interneurons[9,10,11,20,21,22]. While the bulk of early Ca^{2+} imaging studies were done in principal neurons, inhibitory interneurons showcase a large variety of functional Ca^{2+} mechanisms that are distinct from those in pyramidal cells[20,23,24]. These interneuron-specific mechanisms (*e.g.*, Ca^{2+} permeable AMPA receptors) may play specific roles in regulating cell activity. While dendritic Ca^{2+} signaling in interneurons is a tempting target for further investigation, two-photon Ca^{2+} imaging in dendrites of these cells presents additional challenges, from a thinner diameter of dendrites and lack of spines to a particularly high endogenous Ca^{2+} binding capacity. As our research interests focus on the study of hippocampal interneurons, the following protocol, while applicable to different neuronal populations, was adapted to deal with those challenges.

This protocol was executed with a commercial confocal two-photon microscope, which was equipped with two external, non-descanned detectors (NDDs), electro-optical modulator (EOM), and a Dodt scanning gradient contrast (SGC), and installed on an optical table. The microscope was coupled with a Ti:Sapphire multiphoton laser mode-locked at 800 nm (> 3 W, 140 fs pulses, 80 Hz repetition rate). The imaging system was equipped with a standard electrophysiology rig, including a perfusion chamber with temperature control, a translating platform with two micromanipulators, a computer-controlled microelectrode amplifier, a digitizer, a stimulation unit, and data acquisition software.

Protocol

All experiments were performed in accordance with the animal welfare guidelines of the Animal Protection Committee of Université Laval and the Canadian Council on Animal Care.

1. Preliminary Preparation (Optional: Prepare 1 Day in Advance)

1. Prepare three types of artificial cerebrospinal fluid (ACSF) solution (Normal, Sucrose and Recovery Solutions, 1 L each; see **Table 1**). Adjust the osmolality of the solutions to 300 ± 10 mOsm[25] and cool the ACSF-Sucrose down to a near-freezing point (0-4 °C).
   NOTE: The use of a low-sodium cutting solution (ACSF-Sucrose) helps preserve the viability of neurons in the superficial layers of acute slices[26].
2. Prepare 0.75 mL of patch-solution containing a red fluorophore (*e.g.*, Alexa-594) and a green synthetic calcium indicator (*e.g.*, Oregon Green BAPTA-1; see **Table 1**). Include biocytin (see **Table 1**) in the patch-solution if *post hoc* morphological identification of recorded cells is required.
3. Adjust the osmolality of the solution to 280 ± 5 mOsm and pH to 7.35 ± 0.5. Keep the solution in the refrigerator or on ice at all times.
   NOTE: Choice of the calcium indicator should be predicated on its dynamic range and on the nature of the investigated Ca^{2+} events.
4. Using a micropipette puller, prepare patch pipettes from borosilicate glass capillaries (see **Table of Materials**) with a ≈ 2 µm tip (pipette resistance of 3-5 MΩ) and stimulation pipettes from borosilicate theta-glass capillaries (see **Table of Materials**) with a 2-5 µm tip.
   NOTE: The puller settings to make pipettes of a given diameter and shape will be determined by the puller filament type and the ramp test results for a given type of glass.

2. Hippocampal Slice Preparation

1. Anesthetize the mouse (P13-20; the strain and sex of the mouse is determined by the experimental question being addressed) with 1 mL of isoflurane placed onto a paper towel within an inhalation narcosis chamber. When the animal is deeply anesthetized, as evidenced by a lack of movement and slow respiration, remove it from the narcosis chamber and decapitate using a guillotine.
2. Expose the skull by cutting the skin with a small pair of scissors from the back of the skull to the nose. Use a small pair of mini bone rongeurs to make a hole at the bregma level. Then use the scissors to make a caudal-to-rostral cut along the sagittal suture. With the rongeurs grasp the back of each skull flap and lift upward and outward to remove the skull covering each hemisphere.
3. After the brain is fully exposed, undercut the optic nerves with a small spatula. Remove the brain from the skull and put it into a Petri dish containing continuously oxygenated, cold ACSF-Sucrose (see **Table 1** for sucrose concentration).
   1. If tissue from older mice is required, perform an intracardiac perfusion using a syringe (25G) filled with 20 mL of ice-cold ACSF-Sucrose prior to the brain extraction to obtain good quality slices.
4. Prepare hippocampal slices from the extracted animal brain.
   1. Let the brain chill for around 2 min. Place a piece of filter paper on an ice-packed Petri dish, transfer the brain onto the filter paper. Dissect the two hemispheres.
   2. Glue the dissected hemispheres (the orientation is determined by the experimental goal to obtain coronal, transversal, or horizontal slices) onto a vibratome table and immerse it into the vibratome tray filled with ice-cold oxygenated ACSF-Sucrose. Make 300-µm thick slices at a temperature of about 1 °C by using a vibratome cooler or placing the ice around the vibratome tray.

3. Using a flat paint brush, accumulate the slices containing the hippocampus (up to 6 slices per hemisphere) in a bath containing oxygenated ACSF-Recovery heated to 37 °C.
4. Leave the slices in the ACSF-Recovery bath for at least 45 min.

3. Whole-cell Patch-clamp Recordings

1. Set a hippocampal slice in place in a bath positioned under the objective (magnification: 40x, numerical aperture: 0.8) of a laser-scanning two-photon microscope. Continually perfuse the bath with oxygenated ACSF-Normal heated at 30-32 °C at a rate of 2.5-3 mL/min.
2. Use infrared differential interference contrast (IR-DIC) or Dodt-SGC microscopy to locate a cell of interest using its size, shape, and position.
   NOTE: Depending on the targeted neuron population, a transgenic mouse model can be used to easily locate cells of interest. In this case, this step can be substituted with use of a fluorescent light source.
3. Fill a stimulation pipette with an ACSF-Normal solution containing the red fluorophore (*e.g.*, Alexa-594).
4. Set the stimulation pipette (connected to an electrical stimulation unit) on top of the slice so that the tip is in the same region as the cell of interest.
5. After filling the patch pipette with patch solution and attaching it to a head stage, set it over the slice so that its tip is directly above the cell of interest.
6. Fit a syringe into a three-way stopcock and connect it to the patch-pipette through the plastic tube. Inject constant positive pressure into the patch pipette (≈ 0.1 mL) using the syringe. Keep the stopcock in "close" position.
7. Activate the amplifier control software module and switch to voltage-clamp mode by clicking on the "VC" button. Open the electrophysiology data acquisition software (*e.g.*, clampex) for acquisition of electrophysiological signals and click on the "Membrane Test" icon to have it continually send out a square voltage pulse (5 mV, 10 ms), which is necessary to monitor changes in the pipette resistance.
8. Lower the patch pipette until it is right on top of the targeted cell. Upon making contact with the cell membrane, remove the pressure by turning the stopcock into the "open" position.
   NOTE: Contact with the cell membrane is detected when a small increase in the pipette resistance (≈ 0.2 MΩ) is seen and a small indentation on the cell is caused by positive pressure.
9. Apply a slight negative pressure to the patch pipette using an empty syringe fitted into the stopcock until the pipette resistance provided by the software reaches 1 GΩ. In the 'Membrane Test' window of the data acquisition software, clamp the cell at -60 mV.
10. Continue applying negative pressure until the cell is opened and the whole-cell configuration is achieved. Detection of this cell state is based upon the sudden change in the pipette resistance (from 1 GΩ to 70-500 MΩ depending on the interneuron type) and the appearance of large capacitive transients in the 'Membrane Test' window.

4. Two-photon Ca^{2+} Imaging

1. If interested in the excitatory postsynaptic responses, add the $GABA_A$ receptor blocker gabazine (10 µM) and the $GABA_B$ receptor blocker CGP55845 (2 µM) in the ACSF bath.
2. In amplifier control software module, set the patch configuration to current-clamp by clicking on the "CC" button and record the cell's firing pattern in response to somatic injections of depolarizing current (0.8-1.0 nA, 1 s). Wait for at least 30 min for the cell to be filled with the indicators present in the patch solution.
   NOTE: The cell's firing pattern and active properties can be used to identify the cell's subtype; *e.g.*, fast-spiking cells. It is important for the indicator concentration to stabilize through diffusion before starting to record CaTs (see **Table 2** for troubleshooting).
3. AP-evoked CaTs
   1. Using the image acquisition software, start acquiring images. Locate a dendrite of interest using the red fluorescence signal. To ensure a visible response, first, choose a proximal dendrite (≤ 50 µm) as the efficiency of AP backpropagation may significantly decline with distance in GABAergic interneurons[22].
   2. Using the 'rectangular tool' in the image acquisition software, zoom in on the targeted region and switch to the "*xt"* (linescan) mode. Position the scan across the dendritic branch of interest. With the laser intensity controllers in the acquisition software, set the two-photon laser at a minimal power level where baseline green fluorescence is just slightly visible, to avoid phototoxicity.
   3. In the electrophysiology data acquisition software 'Protocol' window and image acquisition software, create a recording trial of the desired duration containing a somatic current injection of the desired amplitude.
      1. Using the image acquisition software, click the "Start record" button and acquire the fluorescence continuously for 1-2 s. Repeat the image acquisition 3-10 times. Wait at least 30 s between single scans to avoid photodamage. If acquiring linescans at multiple points along a dendrite, take them in random order to avoid order effects.

4. Synaptically-evoked CaTs
   1. Locate a dendrite of interest using the red fluorescence signal.
   2. Set the stimulation pipette on the surface of the slice above the dendrite of interest. Slowly lower the stimulation pipette into place, minimizing movement to avoid disturbing the whole-cell configuration. Position the pipette at 10-15 µm from the dendrite.
   3. To visualize the location of synaptic microdomains in aspiny dendrites, in the image acquisition software switch to the '*xt'* (linescan) mode and position the line along the dendritic branch of interest.
   4. In the 'Protocol' Window (of electrophysiology data acquisition software) and image acquisition software, create a recording trial that triggers the stimulation unit after the trial start.
   5. Using the acquisition software, click the "Start record" button to scan along the dendrite continuously for 1-2 s. Repeat acquisition 3-5 times, waiting 30 s between scans to prevent photodamage.
      NOTE: The length of scan can be adjusted depending on the evoked event's kinetics, but should be minimized to prevent photodamage (see **Table 2** for troubleshooting).
   6. Repeat step 4.5.5 in different conditions (*e.g.*, different intensity/length of stimulation, introduction of a pharmacological blocker, *etc.*) depending on the specific question being addressed.

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7. Stop the acquisition when the cell shows signs of deteriorating health: depolarization below -45 mV, increase in the baseline Ca^{2+} level, morphological deterioration of dendrites (*e.g.*, blebbing, fragmentation; see **Table 2** for troubleshooting).
8. To obtain preliminary information on the cell's morphology and keep a record of the location of the stimulation pipette, acquire a *Z*-stack of the cell in the red channel. Using the acquisition software in '*xyz*' mode, set the upper and lower stack limits to image the entire cell with all processes included. Set the 'step size' at 1 µm and initiate the stack acquisition using the "Start record" button.
9. When the *Z*-stack acquisition is complete, use the "Maximum projection" option in the software to superimpose all focal plans of the stack and verify the quality of acquisition. Then, slowly retract the patch pipette out of the slice.
10. To fix the slice for *post hoc* morphological identification, quickly remove the slice from the bath using a flat paint brush, and place it between two filter papers, in an ACSF-filled Petri dish. Replace the ACSF with a 4% paraformaldehyde (PFA) solution and leave the dish in a 4 °C room or refrigerator overnight.

5. Immunohistochemistry for *Post Hoc* Morphological Identification of Recorded Cells

NOTE: After 1 night of fixation in PFA, the slice can be stored in phosphate buffer (PB) with sodium azide (0.5%) for up to 1 month.

1. Put the slice in Tris-buffered saline solution (TBS) with Triton X-100 (0.3%) and perform 4 washes (5-10 min each).
2. Put the slice in TBS-Triton 0.3% with 10% normal goat serum (NGS) for 1 h.
3. Put the slice in TBS-Triton 0.3% with 1% NGS and a Streptavidin-conjugated fluorophore (*e.g.*, Alexa Fluor 546-conjugated Streptavidin, 1:200) for overnight incubation.
4. Wash 4 times (5-10 min) in TBS.
5. Mount slices on microscope slides and image using a confocal microscope. To have a complete cell reconstruction, acquire a *Z*-stack (step size: 1 µm) using a 20x objective. For imaging an Alexa-546-conjugated label, use a 543 nm laser and a 515-560 nm detection filter set.

6. Analysis of CaTs

1. Extract the fluorescence values from the regions of interest (ROIs) in the linescan images from the red and green channels and average the values of the multiple repeats for each condition to reduce noise.
   NOTE: When linescan acquisition is performed along the dendrite, it is possible to extract data from multiple ROIs, which may correspond to dendritic microdomains (2-5 µm), and thus allowing the study of the Ca^{2+} signal compartmentalization.
2. For each ROI, express the changes in Ca^{2+} amplitude as:
   $$\frac{\Delta G}{R} = \frac{G - G_0}{R}$$
   NOTE: Here G is the fluorescence value from the green channel; G_0 is the baseline fluorescence value from the green channel during the pre-stimulation period; R is the fluorescence value from the red channel[18]. As two-photon excitation is highly localized and does not generate a significant background, subtraction of background fluorescence is not required.

Representative Results

Using the protocol presented here, we obtained CaTs evoked by somatic current injection and by electrical stimulation in dendrites of oriens/alveus interneurons in the CA1 area of the hippocampus. After patching a neuron, identified based on its shape and position, we acquired linescans across a proximal dendrite at multiple points at given distances from the soma (**Figure 1A**). We observed a decrease in the amplitude of CaTs induced by backpropagating APs as the distance from the soma increased (indicative of reduced AP amplitude or a distance-dependent decline in calcium channel distribution, **Figure 1B**). After immunohistochemistry (using Streptavidin-conjugated Alexa Fluor 546), we were able to retrieve the biocytin labeling of the cell and identify the recorded neuron as a bistratified cell with an axon occupying stratum oriens and stratum radiatum (**Figure 1C**). In the second experiment, a stimulation pipette was brought to a distal dendritic site. Scanning along the dendrite, we were then able to assess the amplitude of the postsynaptic Ca^{2+} signals in a given dendritic branch (**Figure 2A-C**) in response to electrical stimulation of axons of passage. The postsynaptic CaTs differed between neighboring dendritic microdomains (segment 1 to 6; **Figure 2D**), which could be associated with a spread of Ca^{2+} signal from the zone of initiation and/or different postsynaptic mechanisms involved.

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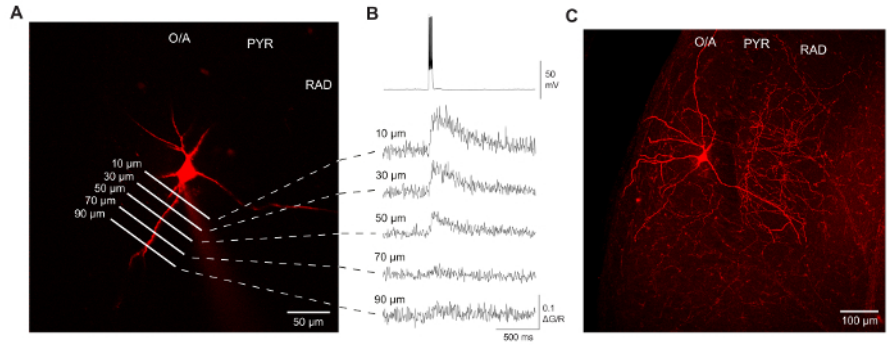

Figure 1: Representative example of AP-evoked Ca^{2+} transients. (**A**) Maximum projection of a two-photon *Z*-stack (13 µm) of a patched interneuron filled with Alexa-594 (20 µM). White lines denote the location and length of linescans. (**B**) Traces showing the CaTs elicited in the locations shown in A. Top trace shows the short AP train generated through somatic current injection during the recording trial. Time scale is the same on all traces shown. (**C**) Maximum projection of a confocal *Z*-stack showing the biocytin labeling of the cell. O/A: Oriens/Alveus; PYR: Stratum Pyramidale; RAD: Stratum Radiatum. Please click here to view a larger version of this figure.

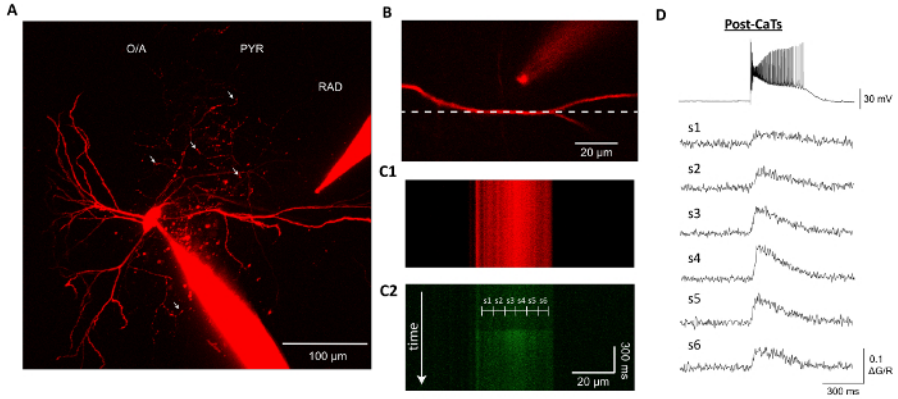

Figure 2: Postsynaptic Ca^{2+} transients evoked by a burst of electrical stimulation (3 stimuli at 100 Hz). (**A**) Maximum projection of a two-photon *Z*-stack (148 µm) showing a patched interneuron filled with Alexa-594. White arrowheads point to the location of axonal terminals within the pyramidal layer, suggesting the interneuron is a basket cell. (**B**) Single focal plane illustrating the dendritic branch where the stimulation electrode was positioned. Dashed line shows the location of the linescan shown in (C1 and C2). (**C**) Images illustrating the result of a linescan in the red (1; Alexa-594; 20 µM) and green (2; Oregon Green Bapta-5N; 300 µM) channels. The images were binned into smaller segments to analyze the spread of the response over 30 µm. (**D**) Traces showing the Ca^{2+} transients (CaTs) elicited in the segments displayed in C2. Top trace shows the voltage response to electrical stimulation recorded at the somatic level during the imaging trial. Please click here to view a larger version of this figure.

Solution	Component	Concentration (mM)
ACSF-Normal	NaCl	124
	KCl	2.5
	NaH_2PO_4	1.25
	$MgSO_4$	2
	$NaHCO_3$	26
	Glucose	10
	$CaCl_2$	2
ACSF-Recovery	NaCl	124
	KCl	2.5
	NaH_2PO_4	1.25
	$MgSO_4$	3
	$NaHCO_3$	26
	Glucose	10
	$CaCl_2$	1
ACSF-Sucrose	KCl	2
	NaH_2PO_4	1.25
	$MgSO_4$	7
	$NaHCO_3$	26
	Glucose	10
	Sucrose	219
	$CaCl_2$	1
K^+-based Patch solution	K^+-gluconate	130
	HEPES	10
	MgCl2	2
	Phosphocreatin di(tris)salt	10
	ATP-Tris	2
	GTP-Tris	0.2
	Biocytin	72
	Alexa-594	0.02
	Oregon Green-BAPTA-1	0.2

Table 1: Solution recipes. Compounds and concentrations for solutions used during the protocol.

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Problem	Solution
Unable to patch healthy neuron	Check for signs that the slices are unhealthy: shrunken or swollen cells, visible nuclei, etc. If so, discard the slices. Also verify the patch-pipette resistance and the patch-solution's osmolality; replace them if the values are not in the appropriate range.
Fluorescence signal from dendrites is low	Wait longer for the cell to fill. If an obstruction is keeping the patch-solution from diffusing into the cell, try to apply a small amount of negative pressure in the patch-pipette.
Electrical stimulation is not evoking a Ca2+ response	Check for the presence of an artefact in the electrophysiological recording. If absent, check for a short-circuit/stimulating unit malfunction. If present, raise the stimulation intensity or move the stimulation pipette closer to the dendrite. It is to be noted that the distance between the stimulation electrode and the dendrite should not exceed 8 um to prevent direct depolarization of dendrites when studying synaptic responses.
Evoked Ca2+ signal is too high/saturates the Ca2+ indicator	Reduce laser power. If the problem persists in multiple cells, use a different Ca2+ indicator, with a lower Ca2+ affinity.
Baseline Ca2+ signal is gradually increasing during the experiment	Wait for a longer period between individual scans. If the baseline is still increasing, stop acquisition; it indicates that the cell's health is likely declining.
Amplitude of Ca2+ signal decreases during scans	Reduce laser power or zoom out (if applicable).
Dendrite is fragmenting ("blebbing") after scanning	Reduce the laser power or zoom out. If blebbing is limited to the targeted dendrite, choose another dendrite and reduce laser power/ zoom out. If multiple dendrites are blebbing, stop acquisition.
Fluorescence signals are "drifting" out of the scan line after sweeps	Reduce movement in the slice by reducing the speed of ACSF perfusion. Before patching, make sure that the slice is strongly fixed in place by a net.

Table 2: Troubleshooting table. Solutions to common problems that may arise during a Ca^{2+} imaging experiment.

Discussion

The method shown here demonstrates how the combination of two-photon Ca^{2+} imaging and patch-clamp electrophysiology can be used for studying dendritic Ca^{2+} signaling in neuronal dendrites in acute brain slices. This method allows for monitoring of both the local Ca^{2+} elevations evoked by electrical stimulation or backpropagating AP in dendritic segments, and the cell's somatic response. This makes it an excellent tool to study how various parts of the dendritic tree integrate inputs and communicate with the soma. But given the length of the experiment, particular attention needs to be paid to the cell health by limiting the use of laser power for imaging so as to avoid the photodamage of dendrites. This can be also achieved by decreasing the zoom level, decreasing the duration of scan, and increasing the scan speed during image acquisition. Imaging interneuron dendrites introduces additional challenges. As dendrites of these cells have no spines, scanning along the dendrite is recommended to facilitate the localization of postsynaptic Ca^{2+} microdomains. In addition, given a high endogenous Ca^{2+} binding capacity, Ca^{2+} elevations in interneuron dendrites are smaller and slower than those generated in the dendrites of pyramidal cells. This requires acquiring several linescan images for averaging as well as a sufficiently long duration of individual linescans in order to describe the Ca^{2+} signal kinetics appropriately.

Local electrical stimulation using bipolar theta-glass electrode represents a useful tool for characterization of dendritic postsynaptic Ca^{2+} microdomains. However, it may still activate different "en passant" axons located in a given region. The number of inputs activated in this case is unknown and can be only estimated using additional computational tools. Two-photon glutamate uncaging[27], which allows for simultaneous stimulation of multiple individual synapses, represents a good alternative for eliciting local dendritic Ca^{2+} responses. This method is widely used for study of principal cells, which have clearly defined excitatory postsynaptic structures (spines), but is not well-suited to the study of interneurons, which overall have a low spine density. Uncaging glutamate along the interneuron dendrite as it is applied to principal cells would unavoidably activate multiple extrasynaptic mechanisms of Ca^{2+} signaling[9,10], making it difficult to interpret the observations.

In the last decade, advances in optical technologies offered improved temporal resolution for two-photon Ca^{2+} imaging. One of the most promising is random-access microscopy (RAMP) through acousto-optic deflectors (AODs)[28,29]. Unlike conventional raster scanning, RAMP scans selected points of interest at high frequency, which is enabled by the fast speed of inertia-free AODs. It is ideal for the simultaneous study of multiple dendritic branches, as it is not limited to points laid out in a linear pattern. However, the higher temporal resolution offered by the AODs may be unnecessary when imaging Ca^{2+} events lasting hundreds of milliseconds within the same focal plan. In addition, the location of scanning points within the same structure of interest may be difficult to control due to micro-drifts in slice preparations associated with a high perfusion rate, which is necessary for good slice quality. Hence, the high temporal resolution (500-700 Hz) linescan-based acquisition method presented in this article is still preferable when imaging Ca^{2+} signals in individual dendritic branches.

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Disclosures

The authors have no competing financial interests or other conflicts of interest.

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