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Simultaneous multi-electrode array recording and two-photon calcium imaging of neural activity

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Abstract

A complete understanding of how brain circuits function will require measurement techniques which monitor large scale network activity simultaneously with the activity of local neural populations at a small scale. Here we present a useful step towards achieving this aim: simultaneous two-photon calcium imaging and multi-electrode array (MEA) recordings. The primary challenge of this method is removing an electrical artifact from the MEA signals that is caused by the imaging laser. Here we show that artifact removal can be achieved with a simple filtering scheme. As a demonstration of this technique we compare large-scale local field potential signals to single-neuron activity in a small-scale group of cells recorded from rat acute slices under two conditions: suppressed vs. intact inhibitory interactions between neurons.

Keywords

electrophysiology; two-photon imaging; multi-electrode array; calcium imaging; spontaneous activity; local field potential; acute slice; cortex; rat

Introduction

A central concept in neuroscience is that coordinated interactions among groups of neurons encode and process information in the cortex. Experimental investigation of such network-level brain dynamics requires measurement tools capable of monitoring many neurons simultaneously. Over the past two decades, two-photon calcium imaging (2PCI) and multi-electrode array (MEA) recording have emerged as essential tools for this endeavor. Several recent reviews discuss more details of 2PCI (e.g. Kerr and Denk, 2008; Gobel and Helmchen, 2007; Svoboda and Yasuda, 2006; Yuste, 2005) and MEA measurements (e.g. Murthy and Fetz, 1996; Csicsvari et al., 2003; Nicolelis and Ribeiro, 2002). Considering the advantages and drawbacks of these two techniques, we propose here that combining them is a productive strategy for advancing studies of brain circuit function. More specifically, a major advantage of MEAs is that they often span millimeters of brain tissue providing a view of the large-scale activity of a neural population with sub-millisecond temporal resolution. However, MEAs cannot reveal which neurons are inactive, nor the cell types and exact locations of active neurons. In contrast, an inherent strength of 2PCI is that the relative locations and often the cell-type of the neurons that are loaded with calcium indicator dye are obtained, whether active or not. However, 2PCI is not capable of monitoring a neural

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population with as large a spatial extent as MEAs and has relatively poor temporal resolution (one action potential often causes a transient change in calcium signal lasting 100s of ms). Commercially available microscopes used for 2PCI are also limited in temporal resolution, typically attaining image frame acquisition rates from 1–25 Hz for a 512×512 pixel image. We note, however, that faster imaging technologies are rapidly developing (e.g. Reddy & Saggau, 2005). Moreover, other optical indicators of neural activity, such as voltage sensitive dyes offer higher temporal resolution, but at the cost of relatively low signal to noise ratio compared to calcium sensitive dyes. Here we demonstrate that a useful approach is to combine MEA and 2PCI measurements. In this way, we obtain simultaneous information on the dynamics of many single cells as well as the larger network in which they are embedded.

The primary challenge encountered when combining MEA and 2PCI is an electrical artifact in the MEA signals caused by the imaging laser. This artifact does not seem to arise for conventional fluorescence microscopy techniques (e.g. Syed et al., 2004). However, conventional imaging is often not feasible in the cortex where greater imaging depths require two-photon imaging. Riera et al. (2010) developed a technique to remove the laser artifact based on subtracting the frame-triggered average artifact waveform. However, their technique requires a microscope which outputs a trigger signal for each imaging frame. Here we present a new method to remove the laser-induced artifact from the MEA signals based on filtering in the frequency domain. Our method requires no trigger signals, and thus works independently of the type of microscope used.

We expect that this technique can be applied in diverse experimental studies. For a few examples, in vivo or in vitro studies comparing unit, multi-unit, or LFP signals to neural or glial calcium signals would all be feasible with this technique. Indeed, Riera et al. (2010) successfully used their technique to compare in vivo astrocytic calcium signals to neural signals recorded with a linear shank MEA. Importantly, they also used spike sorting to recover unit activity from the MEA signals in spite of the laser artifact. However, Riera et al. (2010) did not image neural calcium activity, nor did they show that the artifact could be removed for imaging frame rates above about 2 Hz. Higher frame rates are crucial for observing neural calcium signals, which occur on timescales as low as 100 ms. Here, we demonstrate our method in acute slices of rat cortex. We show that the artifact can be effectively removed from LFP as well as extracellular unit activity signals. We find that higher imaging frame rates (15–20 Hz) result in a less obtrusive artifact (smaller amplitude and more sparse frequency content), which facilitates its removal. We use our method to compare LFP signals recorded over a 1.6×1.6 mm² area to simultaneously recorded calcium signals of 7 single neurons within a small part of that area.

Materials and Methods

Acute slice preparation

Sprague-Dawley rats (Taconic Farms, Germantown, NY), 16–22 days old, were anesthetized with Pentobarbital Sodium Injection (Nembutal) and sacrificed according to standard NIH protocols. 400 µm thick coronal slices including primary motor or somatosensory cortical areas were made using a Leica FT1000S Vibrotome under ice cold slicing solution bubbled with carbogen (95% O₂, 5% CO₂). The slicing solution contained (in mM) 205 sucrose, 0.5 CaCl₂, 7 MgSO₄, 3.5 KCl, 0.3 NaH₂PO₄, 26.2 NaHCO₃, 10 D-glucose.

Suppressed Inhibition Protocol

Slices were stored for 1–3 hours in normal ACSF after slicing. The normal ACSF used here contained (in mM) 124 NaCl, 3.0 CaCl₂, 1 MgSO₄, 3.5 KCl, 0.3 NaH₂PO₄, 26.2 NaHCO₃, 10 D-glucose, and saturated with 95% O₂ and 5% CO₂ (310 ± 5 mOsm). During recordings the slice was perfused with a modified ACSF (hereafter called ACSF2) with KCl concentration raised to 10 μ M and 50 μ M picrotoxin added (Sigma P1675). Picrotoxin is a GABA_A receptor blocker. Under perfusion of ACSF2, the slice exhibited spontaneous bursts of activity every 2–30 seconds, which were always quite large in amplitude and spatial extent. Returning to normal ACSF perfusion restored the inactive state of the slice. This process was repeatable for as many times as we wished and was highly reliable from slice to slice and animal to animal, independent of age between 15 and 64 days old. This protocol was not intended to represent normal operating conditions of the cortex. Rather, it provides a convenient experimental control condition under which strongly correlated neural activity is ensured. The storage solution was room temperature and all recordings were done at 35 °C.

Intact Inhibition Protocol

The slices were stored for 1–3 hours in room temperature modified artificial cerebrospinal fluid (ACSF3) bubbled with carbogen. In the ACSF3, Na was replaced with choline to prevent excessive neural activity during storage. ACSF3 contained (in mM) 124 choline-Cl (Sigma), 3.0 CaCl₂, 1 MgSO₄, 3.5 KCl, 0.3 NaH₂PO₄, 26.2 NaHCO₃, 10 D-glucose, and saturated with 95% O₂ and 5% CO₂ (310 ± 5 mOsm). While in ACSF3, the slice was inactive when monitored with a MEA. About 5–10 minutes after beginning perfusion in normal ACSF, the slice resumed activity displaying spontaneous bursts of activity occurring sporadically with diverse spatial size and LFP amplitude. Compared to the disinhibited condition, the bursts were much smaller in amplitude and spatial extent, providing an experimental model that more closely resembles intact cortical activity. The storage solution was room temperature and all recordings were done at 35 °C.

Multi-cell bolus loading of calcium sensitive fluorescent dyes

Before eliciting spontaneous activity we loaded a local population of 10 – 100 cells with calcium sensitive fluorescent dye, as shown in Fig 1a. We found that both Oregon Green 488 BAPTA-1, AM (Invitrogen 06807) and fluo-4, AM (Invitrogen F14201) were similarly effective. Employing the technique developed by Stosiek et al (2003), called multi-cell bolus loading, we delivered the dye to the cells by pressure injection into extracellular space through a glass pipette. We found that multi-cell bolus loading was more effective than bath loading of dye as used in other in vitro calcium imaging studies (e.g. MacLean et al., 2005). First, a small amount (about 20 μ L) of dye solution was loaded into the pipette using a microfiller. Then, a tiny volume of the dye solution was injected into the tissue by manually applying pressure with a syringe for 1–3 minutes. To avoid pipette tip clogging and excessive injection flow rate which may damage the tissue, the injection progress was monitored periodically with epifluorescent or halogen lamp illumination and a digitally enhanced CCD camera.

A stock solution of 90% DMSO and 10% Pluronic F-127 was prepared and used for multiple day's experiments. It was sterile-filtered with a 0.2 μ m pore size filter and stored at room temperature for up to a week. For each experiment, 50 μ g of dye was dissolved in 10 μ L of DMSO/Pluronic stock. Then, 90 μ L of ACSF was added for the following final injected dye solution: 90% normal ACSF, 9% Dimethyl Sulfoxide (DMSO, Sigma D2650), 1% Pluronic F-127, dye to a concentration of 400 μ M.

During and for 15–20 minutes following dye injection, the slice was perfused in the same fluid as used for slice storage at 35 °C. The waiting period after injection was chosen to allow excess dye solution to wash out of the extracellular tissue. The wash-out was monitored every few minutes with epifluorescence or two-photon imaging.

As other authors have noted, we found that the younger the animal, the higher the efficacy of dye loading. The age of the rats in these experiments was chosen to take advantage of this fact, but avoid developmental issues in young animals.

Two-photon imaging

Our two-photon microscope is a modified Zeiss 310 confocal upright microscope, retrofitted by LSM Technologies (Etters, PA, U.S.A.). Two-photon excitation is provided by a femtosecond pulsed Ti-sapphire laser (Coherent Chameleon Ultra). We adjusted the laser to output 850 nm light at 2.3 W. The laser power was attenuated with an acousto-optical modulator (MT110-A1,5-IR, Quanta Tech Inc), so that the power preceding the microscope optics was 200–500 mW. We use a Zeiss 40×, water-immersion objective suitable for infrared light (IR ACROPLAN 40×/0.8 W). The laser power that the tissue experienced was 10–40 mW.

The laser beam was scanned with the original galvonometers of the Zeiss 310. The temporal resolution of imaging was dependent on the spatial extent of the imaged region and the spatial resolution. For low spatial resolution imaging (about 1 pixel/ μm^2) and small imaging regions (e.g. 20×128 pixels including 5–10 somas) we achieve temporal resolutions around 30 frames/s. For higher spatial resolution (about 4 pixels/ μm^2) imaging of the same region we achieve about 15 frames/s. Since we are interested in using calcium signals as an indicator of neuronal spiking, we average over all the pixels covering a given soma and therefore increasing the number of pixels/soma raises the signal to noise ratio. Considering that typical somatic calcium transients associated with spikes last 0.5 to 2 seconds, we typically chose to image at a rate of 15 frames/s.

The Zeiss 310 was retrofitted with an additional photomultiplier tube (PMT) for two-photon imaging (a nondescanned detector). A dichroic mirror (675DCXR) was used to direct the fluorescent signal to the PMT. Two optical filters were placed between the objective and the PMT to reduce contamination of the fluorescence signal by laser and other light sources: 1) a bandpass optical filter with center wavelength 510 nm, half band width of 40 nm, and 2) a low-pass filter with cutoff at 680 nm. The optical path length between the back of the objective and the PMT was 15 cm.

The calcium signals were processed as follows. A Matlab-based semi-automatic soma detection algorithm was used to determine which pixels cover each cell. Briefly, this entailed, subtracting a spatially-smoothed version of each image from itself and then selecting pixels with intensities that were above a threshold. Next, any group of “connected” above-threshold pixels was used to define a region of interest (ROI). Manual inspection and intervention was used to separate merged ROIs of closely spaced cells as necessary. To calculate the fluorescence time trace of a cell, the intensities of all pixels inside of the ROI were averaged at each time point and then smoothed over a 3 point sliding time window (1 point backward, 1 point forward in time). The trace was then normalized by a baseline fluorescence trace defined by an interpolated, low-pass filtered fit to local minima within consecutive 50 point subsections of the trace.

Multi-electrode array recording

Simultaneous with two-photon imaging we used a multi-electrode array to measure local field potential in the slice. The MEA system was purchased from Multi Channel Systems

and included a 60-electrode array (200/30-Ti), headstage (MEA1060-2-BC), filter amplifier (FA60SBC), and PCI-bus data acquisition card (MCCard 64). The electrodes were arranged in an 8×8 grid with no corner electrodes, 200 μm inter-electrode spacing, and 30 μm electrode diameter. After amplification, voltages down to about 5 μV were distinguishable from noise. Measurements were recorded at 1 kHz sample rate. As shown in Fig 1c, the slice was placed on the MEA so that we recorded activity from cortical layers II–V. The slice was held stationary on the MEA by the weight of a small platinum wire bent into a U-shape with thin ($<1\mu\text{m}$ diameter) nylon fibers spanning the U like a harp.

Spike sorting

Unit activity was recorded with the MEA with 25 kHz sample rate. Putative extracellular spike waveforms were extracted by the following three steps using Matlab: 1) band-pass filtering the raw data between 100 and 3000 Hz, 2) identifying the times of negative voltage deflections which drop below -4 standard deviations (SD), 3) saving the -1 to $+3$ ms around the threshold crossing time. For the data we analyzed, the -4SD threshold was chosen to obtain all potential spikes and resulted in a significant number of ‘noise’ waveforms as well. This allowed us to be certain that the unit was well isolated from the noise. The extracted waveforms were sorted using the Matlab toolbox MClust (v3.3, developed by A.D. Redish et al.).

Results

Two-photon calcium imaging reveals single-spikes and spike bursts

In Fig 1 we show a group of spontaneously active neurons labeled with calcium sensitive dye (Oregon green). The calcium dynamics of the cells marked in Fig 1a were diverse, including brief transients lasting several hundred milliseconds and fluctuating bursts lasting for many seconds (Fig 1b). This activity was recorded under standard ACSF perfusion (the ‘intact inhibition’ protocol in Methods). To get a better understanding of what type of neural activity the calcium signals reveal, we performed whole cell patch clamp recordings of single cells (Fig 1c). With step current injections, we induced 1, 2, or 4 action potentials in the cell and recorded the resulting calcium signals (Fig 1d). Thus, for a given neuron, the amplitude of the calcium signal reflects the spike rate. However, considering the variability of dye concentration from neuron to neuron (an unavoidable result of extracellular loading of dye), we cannot quantitatively assess the relative spike rates from different cells.

Laser artifact

The biggest challenge encountered when combining MEA and 2PCI measurements was that the laser used for two-photon imaging caused a large amplitude voltage artifact in the MEA signal. For example, Fig 2 shows MEA recordings from an acute coronal slice (Fig 2a) of rat cortex with the imaging laser off (Fig 2b) and with it on (Fig 2c). We note that the artifact disappears when the laser beam is physically blocked while keeping all other aspects of the imaging and MEA apparatus unchanged. This indicates that the artifact is truly light-induced, rather than some electronic cross-talk between the microscope and the MEA system. In Fig 2c, the imaging frame rate was 27 Hz and the imaged region spans the 4 electrodes with the largest amplitude artifact. The electrodes neighboring the imaging region also exhibit an artifact, but with smaller amplitude. The laser artifact amplitude was dependent on the imaging frame rate and the laser power. To gain a quantitative understanding of these effects we performed control experiments with only ACSF and no brain tissue present (Figs 2d–g). Here we imaged a region spanning only one electrode (Fig 2d). First we measured the artifact for varying frame rate with the imaging focal plane parallel to, but 200 μm from the MEA surface (as typical in our experiments). Here, the laser wavelength was fixed at 800 nm, and laser power was fixed at 64 mW. All laser power

measurements were taken preceding the microscope optics, following the AOM. We varied the frame rate by changing the aspect ratio of the imaging region as illustrated in Fig 2d; the large and small regions corresponded to 4 and 20 Hz respectively. The artifact voltage time series for these two frame rates are shown in Fig 2e (red, blue respectively). We found that the standard deviation of the artifact amplitude decreased as the imaging frame rate increased for rates <12 Hz (Fig 2f). Above 12 Hz, the artifact amplitude did not change significantly with imaging rate. Next we recorded the standard deviation of the artifact voltage for varying laser power (fixed: frame rate 10 Hz, wavelength 800 nm). Using an acousto-optical modulator to vary the power from 0 to 160 mW, we found that the artifact grew approximately linearly with the laser power (Fig 2g). However, other than the amplitude, the waveform of the artifact was similar for different laser power (e.g. green trace in Fig 2e). The artifact was present for all laser wavelengths between 700 and 1050 nm.

At the electrodes adjacent to, but outside the imaged region, the artifact also grew with laser power, but was smaller in amplitude by about 2 orders of magnitude (inset in Fig 2g). This artifact on the nearby electrodes was due to the spread of unfocused light after it passes the focal plane. Considering that we used an objective with numerical aperture NA=0.8, the laser beam was expanded to fill the back aperture of the objective, and ACSF for the working fluid, the light cone of the imaging laser subtends a half-angle of approximately 38°. Thus, with a $200 \times 200 \mu\text{m}^2$ imaged area at a focal plane 200 microns above the MEA surface, the unfocused light at the MEA surface is expected to illuminate a region that is about $500 \times 500 \mu\text{m}^2$ with lower intensity light at the boundaries. This is consistent with our observations of the spatial spread of the artifact. This is further supported by the fact that when the distance between the focal plane and the MEA surface is increased, the spread of the artifact-affected region also increases.

Although our aim was not to determine the physical origins of the artifact, others have suggested a photoelectric effect might be the cause (Riera et al., 2010). Another possibility is a photovoltaic effect, similar to solar cell technology. For example, Xing et al. (2008) recently showed that 632 nm, 7 mW continuous laser light induces a voltage at the junction between silicon and titanium-nitride. This effect may be related to the artifact we observe because our recording electrodes are made of titanium-nitride and insulated with silicon-nitride.

Recovering local field potential signal from the artifact

Our first aim was to recover the local field potential signal from the laser artifact. We achieved this using a carefully designed band-stop filter to remove the artifact. Our method for recovering the MEA signal was possible because the frequency content of the laser artifact was isolated in discrete narrow bands, which may be removed while maintaining much of the original signal. As demonstrated in Fig 3, the artifact was comprised of frequencies at the imaging frame rate F and integer multiples of F . Therefore, we removed the artifact with multiple band-stop filters with center frequencies of F , $2F$, $3F$, and $4F$ and 4 Hz width for each band. We used a phase-neutral, second order Butterworth band-stop filter (*butter* and *filtfilt* functions in Matlab). Second, we low-pass filtered the data with a cut-off frequency of $4F$ (phase-neutral, 4th order Butterworth). In Fig 3a we show an LFP recording from one electrode obtained during imaging, before (blue) and after (red) removing the artifact. The corresponding power spectra are shown in Fig 3b.

To quantitatively assess the effectiveness of our filtering method we used a surrogate signal (Fig 3c, blue). First, a clean LFP signal was recorded from a brain slice with the laser turned off. Next, the laser artifact was recorded with the MEA, but with no brain tissue present. The surrogate signal was generated by adding together the clean LFP signal and a pure laser artifact signal. By filtering the surrogate signal, we recovered the signal shown in Fig 3c

(red). The corresponding power spectra are shown in Fig 3d compared with the power spectrum of the original, clean LFP data (green).

The surrogate data allowed us to quantitatively compare our filtering scheme to three alternative filtering schemes: 1) a single low-pass filter with a cutoff at F , 2) a pair of band-stop filters at F and $2F$ plus a low-pass at $2F$, and 3) three band-stop filters at F , $2F$, and $3F$ plus a low-pass at $3F$. The error (mean squared difference) between the recovered and the original data were 35.5 , 26.7 , $23.4 \mu V^2$, respectively. Our method (4 band-stop filters) results in $23.1 \mu V^2$, which approaches the ‘best possible’ error of $21.2 \mu V^2$. Note that the best possible error is not zero because of the noise level that is present in the original data even without a laser artifact. The best possible error was computed by comparing the original data to a low-pass filtered version of itself.

Recovering unit activity signal from the artifact

Our next aim was to apply our method to recover extracellular unit activity during imaging. For such signals, we must remove the artifact from the 100 to 3000 Hz frequency band. As shown in Fig 3, the artifact is predominately composed of discrete frequency bands at F , $2F$, $3F$, etc, where F is the imaging frame rate. However, in the 100–3000 Hz band we must remove not only these peaks, but also peaks at the line scan frequency of the microscope, f_{line} and its integer multiples $2f_{line}$, $3f_{line}$, etc. Each imaged frame is composed of multiple line scans, typically acquired at a rate $f_{line} = 730$ Hz. The blue power spectra in Fig 4a (and inset) reveal the peaks associated with F and f_{line} . Since spike waveforms are fast sharp deflections, their frequency content is broadband. As demonstrated in the inset of Fig 4a, the majority of the spectrum, i.e. the range of frequencies between the artifact peaks, was uncorrupted by the artifact. Thus, removing the artifact should leave the spike waveforms largely intact.

In Fig 4b–e, we demonstrate that we are able to recover unit activity signals from the laser artifact. Following the same strategy that we implemented in Fig 3, first we constructed a surrogate signal by adding together a clean unit activity signal with a pure laser artifact signal. Both signals were recorded with the same MEA system as discussed above at 25 kHz sample rate. The imaging frame rate $F=16$ Hz. The first step in recovering the signal was a multi-band-stop filter which removed all peaks between 60 Hz and 1600. Each band-stop width was 1 Hz. Next, a high-pass filter with 100 Hz cutoff was applied. Finally, a low-pass filter with 1400 Hz cutoff was applied twice. All filters were implemented using Matlab (*filtfilt* and *butter* functions) as described above. Although, an upper cutoff of 3 kHz is commonly used for study of unit activity, we found that the band from 100–1400 was sufficient for recovering most of the original signal. We confirmed that it is possible to remove all artifact peaks in the 100 to 3000 Hz band, but the filtering became computationally intensive (e.g. requiring 41 seconds to filter 10 seconds of data running Matlab on a PC with a 3.2 GHz CPU).

Fig 4b shows the original unit activity (green) compared with the recovered signal (red). The corresponding power spectra are shown in Fig 4a with the same colors. Fig 4c demonstrates that the artifact-corrupted signal, before recovery (blue), is much larger in amplitude than the original and recovered signals, emphasizing the necessity for a good strategy to remove it. Finally, we demonstrated that the recovered signal is amenable to spike sorting with quality comparable to that of the original signal. Figs 4d and e show original and recovered sorted clusters respectively. These data were obtained from a 300 second duration recording, including the occurrence of about 200 spikes. Figs 4b and c show short sections from this long recording.

Demonstration of simultaneous 2PCI and MEA recording

In Fig 5, we demonstrate simultaneous two-photon calcium imaging and micro-electrode array measurements of spontaneous neural activity in acute cortex slices from 16–22 day old rats. More detail is provided in the Methods. We compared activity under conditions of suppressed versus intact inhibitory signaling. In the case of suppressed inhibition, GABA_A signaling was blocked with Picrotoxin (50 μ M) bath-applied to the tissue and KCl concentrations were elevated (10 mM). In the case of intact inhibition, activity was recorded under perfusion of standard artificial cerebral spinal fluid. In both cases, the slice exhibits spontaneous activity apparent both at the millimeter-scale network level with the MEA and in micrometer-scale cortical circuits using 2PCI, though the character of the network dynamics is dramatically different. We imaged the activity of 10–100 single neurons which were located in an approximately $100 \times 200 \mu\text{m}^2$ region, while the MEA spanned a much larger region of $1.6 \times 1.6 \text{ mm}^2$. Thus, our measurements relate the activity of a local population of cells to the collective activity of the larger network, in which they are embedded.

Figs 5b–d present an example recording during activity with inhibition suppressed. The MEA recording (Figs 5c,d) reveals large amplitude bursts in the local field potential that most often span the entire region of the measurement. A similar character of widely synchronized activity is observed at the single cell level as revealed by 2PCI (Fig 5b). For every LFP burst, there is a corresponding calcium signal from nearly every cell in the imaging region. In times between LFP bursts, the cells are inactive.

Figs 5e–g shows an example recording of spontaneous activity with inhibition intact. In contrast with disinhibited activity, the MEA reveals diverse spatiotemporal patterns of LFP activity (Figs 5f,g). The LFP fluctuations vary in location, amplitude, and spatial area. 2PCI (Fig 5e) also reveals a broad variety of activity patterns at the single-cell level both during and between periods of elevated LFP activity in the larger network. Surprisingly, there was little apparent correlation between the activity of single neurons and the LFP even when the LFP activity overlapped spatially with the imaged region. These measurements highlight the complex, multi-scale relationship between LFP signals and spiking neurons – a problem whose solution we hope will be facilitated with our method in future studies.

Limitations of the method

Although the MEA signals shown in Figs 3, 4, and 5 were successfully recovered, our method has several limitations. For example, non-removable artifacts can occur. To avoid, this problem we offer the following strategies. First, the artifact must not saturate the MEA recording amplifiers. This results in unrecoverable loss of information and also spreads the frequency content of the artifact into many harmonic side-band frequencies that are not possible to filter out. Choosing a sample rate for the MEA recording that is higher than the line scan frequency f_{line} also facilitates artifact removal. This is because undersampling the high frequency components of the artifact can result in extra frequency content that is difficult to filter out (e.g. see the small peaks between the large peaks in the power spectra shown in Fig 3 where the MEA sample rate was 1 kHz). Imaging frame rate above about 12 Hz is generally advantageous because of lower artifact amplitude (Fig 2f) and also because it provides a greater bandwidth of uncorrupted frequency spectrum. We note that this technique is particularly well suited to studies of specific frequency bands if the imaging frame rate is chosen to avoid artifacts in that band. For example, for comparing beta frequency (12–20 Hz) neural signals with gamma frequency (30–80 Hz) signals, $F=30$ Hz would be a good choice of imaging frame rate; there would be no artifact in the beta range and only one peak at 60 Hz in the gamma range. We also note that there is a brief, but unavoidable, broad-band artifact at the beginning and end of each 2PCI recording session

(visible in Fig 1c). This is likely due to transient acceleration of the scan mirror speed from rest to full speed, effectively ramping the frame rate up from zero to F. This is easy to avoid by excluding that period in the data processing.

We also emphasize that the spatial extent of the MEA recordings we employed was much larger than that of the imaged region. Indeed, in our demonstrations in Figs 3 and 4, the imaged regions were about the size of the recorded region from one MEA electrode. Nonetheless, since we were able to remove the laser artifact from the nearest electrode to the imaged region, we anticipate that future studies with larger imaged regions (or more closely spaced electrodes) will also benefit from our technique.

Conclusions

In summary, we present simultaneous recordings of LFP and somatic calcium transients using combined microelectrode arrays and two-photon imaging. This was achieved by carefully filtering the MEA data to remove a laser-induced artifact from the signal. The technique is effective for LFP signals as well as extracellular unit activity signals. Applying this technique to record spontaneous activity in acute slices of rat cortex, our initial findings support the idea that intact inhibitory signaling is crucial for maintaining diverse spatiotemporal network-level dynamics. With the complementary strengths of the MEA and 2PCI, we obtain a view of large-scale network dynamics with millisecond temporal resolution (LFP) as well as the detailed relative spatial locations and activity patterns in a local group of neurons (calcium imaging).

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Figure 1.

Calcium imaging of local populations of cortical neurons. (a) A snapshot of a population of neurons loaded with calcium sensitive fluorescent dye (Oregon Green). The calcium signals of the cells marked in yellow are shown in (b), revealing diverse activity patterns. (c) Whole cell patch recording of a single neuron intracellularly loaded with 100 μ M dye concentration. (d) Using step current injections, the patched cell shown in c was induced to fire 1, 2, and 4 action potentials while imaging the calcium signal. The amplitude of the calcium signal increases with the spike rate of the neuron.

Figure 2.

Two-photon imaging laser causes a voltage artifact in the MEA signals. (a) Photomicrograph of an acute coronal slice of rat cortex (one hemisphere) placed on a planar 59-electrode MEA. (WM - white matter). Black dots are the recording electrodes, black lines are insulated leads. The red square shows the imaged region during the recording shown in c. (b) Local field potential (LFP) signals recorded from the slice with the imaging laser turned off. Each box displays 2 seconds of LFP recorded from a single electrode; the spatial arrangement of boxes matches that of the MEA electrodes shown in a. (c) LFP recordings from the same slice shown in b, during imaging (27 Hz frame rate). The laser power was 70 mW. The red box indicates the imaged region. The artifact is present on nearby electrodes outside the imaged region as well, due to spread of unfocused light. (d) We quantified the artifact amplitude with no slice present (ACSF only) and an imaging region that included only one electrode. Imaging frame rate was varied by changing the aspect ratio of the imaging region from 512×512 (4 Hz) to 512×102 (20 Hz). (e) Artifact voltage time series for 4, 10 and 20 Hz frame rates (red, green, blue, respectively). The laser power was 60, 120, 60 mW respectively. (f) The artifact amplitude (standard deviation) decreased with increasing frame rate. (g) The artifact amplitude increased linearly with laser power (tested at 10 Hz frame rate). The power was measured after the AOM and before the microscope optics. All tests at 800 nm laser wavelength.

Figure 3.

Removing artifact from local field potential signals. (a) MEA signal recorded during imaging (blue). The artifact was removed revealing the underlying LFP signal (red). (b) Power spectra of the MEA signal before (blue) and after (red) removing the artifact. The frequency content of the artifact is primarily discrete bands corresponding to the imaging frame rate F and integer multiples $2F$, $3F$, etc. Artifact removal entails band-stop filtering to remove the corrupted frequency bands. (c) Artifact removal method was tested using a surrogate signal (blue) generated by adding together a clean LFP signal (laser off) with a pure laser artifact (no neural activity). Our filtering scheme was used to recover the original LFP signal (red). (d) Power spectra of original (green), surrogate (blue), and recovered (red) signals.

Figure 4.

Removing the artifact from unit activity. (a) Power spectra of the laser artifact (blue), the original signal (green), and the recovered signal (red). The spectra shown in the orange frame (right) is a expanded view of the orange box. The artifact consists of frequencies which peak at multiplies of the imaging frame rate F and the line scan frequency f_{line} . To recover the signal, all artifact frequency peaks between 60 and 1600 Hz were removed with band-stop filters. (b) Time series of the original (green) and recovered (red) unit activity signal. The asterisk indicates the spike that is shown in c. (c) Time series of original signal (green), original signal plus artifact (blue), and recovered signal (red). Note that without removing the artifact, the spike signal (asterisk) is not apparent. (d) Spike-sorted original data. (e) Spike sorted recovered data. The sorting is still possible, because recovery filtering cause only minor changes to the extracellular spike waveforms.

Figure 5. Simultaneous two-photon imaging and multi-electrode array recording reveals activations of small groups of neurons as well as the larger network in which they are embedded. (a) Two-photon image of cells loaded with calcium sensitive fluorescent dye overlaid with a diagram of some of the MEA electrodes. The red box indicates the imaged region for panel e. The letter labels A–G indicate the recording sites of the LFP signals shown in panels f and g. (b) 2PCI recordings from 7 neurons under disinhibited conditions. (c) LFP recorded from 7 electrodes simultaneously with the calcium signals shown in a before removing the laser artifact. The red dashed line marks the start of imaging. (d) LFP signal recovered after removing the artifact from the MEA recordings. Disinhibition results in network wide synchrony. The MEA recordings show that the synchrony spans millimeters; the 2PCI shows that nearly every neuron participates in each burst. (e) With inhibition intact, 2PCI reveals that strong synchrony among a local population of 7 neurons is rare. (f) LFP recorded from 7 electrodes simultaneously with the calcium signals shown in c before removing the artifact. The letter labels indicate which electrode recorded the signal in panel a. (g) The recovered LFP signal. In spite of the apparent lack of synchrony in the 2PCI signals in c, the LFP still exhibits bursts of network activity spanning 100s of microns, although with much smaller amplitude than observed for disinhibited conditions.