A witty yet professional title describing the tremendous expansion in our ability to record brain activity [large networks of neurons], made possible by combining electronics with improved sensitivity (sCMOS) and engineered proteins with enhanced signal (GCaMP6).

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This abstract briefly summarizes the work we have been doing and describes the main points we wish to emphasize in this paper. To summarize: we are introducing methods for recording neural activity [via calcium imaging] in awake behaving mice at a scale and [temporal] resolution substantially greater than anything reported previously. [This is a leap, puts us beyond a critical threshold as we might now observe all communication within entire brain regions and achievably process and use the information in real-time].

The achievement is made possible not by any radical new idea or invention, but by combining the latest incremental improvements in semiconductors [imaging/sCMOS] and synthetic biology [GCaMP6] with classic optics … image processing, parallel processing, cheap memory and digital storage…

Data are distilled to easily referenced signals representing individual cell activity over time [without losing access to more complex representations of measured calcium activity the thousands of cells measured at one time].

The video processing routine and cell identification routines are entirely automated.

The technique is widely applicable (despite the small size of the mouse's brain). That is to say it's not restricted to cell-type, promoter, brain-region, etc. Striatal cholinergic interneurons… stem cells… cortex…

Introduction

Let me introduce you to related techniques for recording neural activity once I've motivated the efforts of neuroscientists for doing this at all. In particular, let me highlight what an abysmal failure neuroscience has been in the past several decades; we know little more now about how the brain functions and dysfunctions than we did at the turn of the nineteenth century. Assuming our most basic model is correct, that neurons *communicate* with each other to process and store information, we still have yet to understand their language. Every effort to learn this language thus far could be compared to examining a Rosetta Stone finely ground to dust, ones to two grains at a time. Enough with the motivation.

Electrophysiology led to multielectrode. Optical imaging has its downsides. Intrinsic signal is indirect (like the BOLD signal in fMRI), flavoprotein autofluoresence is also indirect (representing changes in metabolism and signal SNR is miniscule. Calcium-sensitive and voltage-sensitive dyes require tremendous effort, patience, and skill, then they're toxic. Let's also compare to two-photon… thoroughly, and in the discussion…

Methods

Animal Preparation and Surgery:

During a craniotomy mice are transfected with GCaMP6f with variable [synapsin] promoter using adenovirus injected through a really large blunt-tip needle. A round glass window at the bottom of a stainless-steel cannula is cemented to the skull after sucking a core of cortex away and jamming the cannula down to the level of the hippocampus.

Microscope:

The camera is "Scientific" CMOS (sCMOS); the optics are inexpensive (and mostly stolen from other labs); the mechanics are also stolen. I'm sorry it's been so long, Jerome! Fluorescence excitation is done with a 5W LED (LedEngin, 470nm). The flatness of the illumination fields is non-critical, as these variations can be corrected for in software…

Acquisition:

VR? The computer is pretty decent, and is the same (or similar) as used for processing. We also spent a significant amount of time developing head-plates and a holder that provided a very rigid coupling to the base of the microscope, minimizing movement of the imaging plane (then didn't use them).

Video Processing:

Data formats

Homomorphic filtering

Motion correction

Normalization

Cell detection (automated ROI selection). Blobbing in single frames then combining.

Visualization (& analysis?):

The RegionOfInterest class.

Results

Optical characteristics of microscope:

Raw signals:

Motion

Physiological signals (heart-rate, respirations, flavoprotein autofluorescence.

Bleaching of fluorophores.

Effects of image processing operations:

Cell detection parameters and performance:

ROI average intensity traces:

Discussion