An optogenetic neural recording system and its application to study the mouse visual cortex

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***Abstract*— In this paper we present an optogenetic neural recording system and its application to the study of the mouse visual cortex. We used a 32 x 32 fast and custom-designed imaging chip and system specifically designed to operate with voltage sensitive dyes. The system can operate up to 1000 frames per second, delivering a peak SNR performance of 63 dB, weighs less than 10 grams and consumes 12 mW of power. We used this device to perform simultaneous electrical stimulation of area V1 and optical recording using a voltage sensitive dye in areas V1, LM and more in the mouse visual cortex. We also performed electrical stimulation in V4 and recorded optical signals using a calcium sensitive dye. We report experimental results on both calcium and voltage sensitive dye experiments that potentially help disambiguate the organization of the mouse visual cortex, revealing its complex structure.**

I. Introduction

Currently, mammalian neuronal processing of information is not well understood due to the inability to monitor widespread electrical activity across a large area of the cerebral cortex (10 mm or more) at a task relevant rate (1000 Hz or more). Functional magnetic resonance imaging and positron emission tomography allow three-dimensional recording of brain metabolism, but are indirect measures of neuronal activity, have low temporal/spatial resolution and require head fixation. Implantable microelectrode arrays allow high speed recording of neuronal activity in awake, unrestrained, and mobile animals but spatial resolution is limited and the electrodes damage the brain tissue [1]. Muti-photon [2] and confocal [3] fiber-optic-tethered laser-scanning have produced high resolution images of neurons in head fixed animals, but lack the sensitivity, speed and field of view to record the relatively small changes in fluorescence produced by optical probes across a particular functional domain of cortex.

Due to the inability for these methods to perform such experiments, little is known about the interaction between the V1 and higher areas in the visual cortex of rodents [4]. Imaging techniques of brain activity can help to understand the visual cortex of rodents, in particular given the recent rapid increase in the availability of knockout animals ready for visualization of cortical activity with fluorescent reporters and probes.

With genetically-encodable optical fluorescent probes, neuronal activity can be observed with high spatial and temporal resolution over a wide area of the cortex. While commercial-off-the-shelf wide field epi-fluorescence systems in use today can be used for in vivo animal experiments, such systems are too bulky and consume too much power to be used for awake and freely moving animals.

In this paper, we present experimental results of our custom miniature imaging system designed for freely behaving animals (Figure 1). We conducted experiments on the mouse visual cortex, using both voltage sensitive dyes (VSD) and calcium sensitive dyes (CSD). The contribution of this paper is to show how our system can aid in answering higher-level neuroscience questions such as the complex interactions between the different areas of the mouse visual cortex. These questions can only be explored with a high-speed, wide-field recording system that can capture neuronal activity in awake, freely-moving animals in their ”natural” state.

II. Imaging System

We use a miniature custom-designed high-speed and low-noise image sensor named *NeuroView* to image neural activity in rodents. The CMOS image sensor has a resolution of 32 x 32, compared to 80 x 80 or 128 x 128 of other scientific imaging systems. While the resolution is slightly lower than that of a scientific CMOS imaging system (100 x 100 to 256 x 256), it is a good balance for image quality and speed given the limitations of the particular CMOS process used. Each pixel is a modification of the 3- transistors (3T) active pixel sensor (APS) pixel structure, similar to the one presented in [5]. The sensitivity of each photodiode is 8.124 V/(lux·s) and the conversion gain is 0.141 μV/e$^-$.

The CMOS image sensor and control electronics fit in a head mountable package (Figure 1-B). Each photodiode in our system has a large well depth of 7.126 Me$^-$ to reduce the kT/C noise, which is a major component of temporal noise. The resulting peak SNR performance of 63 dB ($>$ 10 bits) is comparable to that of a much larger, research-grade image sensor made for fixed-animal biological experiments, which typically have data output of 12 bits. While scientific CMOS imaging systems weigh several pounds and consumes tens of

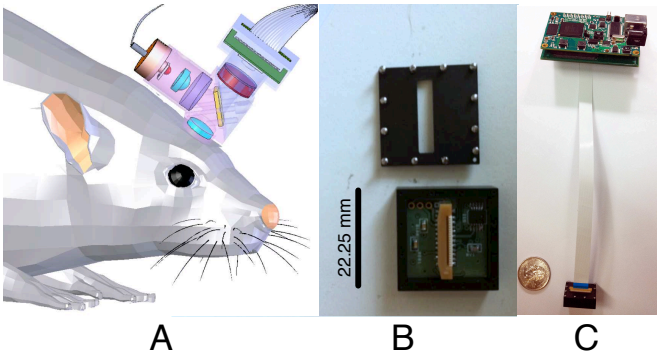

Fig. 1. (A) Head-mountable microscope (B) Our imaging system, placed in a housing to connect to the microscope, measures 22.25 mm x 22.25 mm. (C) Complete imaging system, with a quarter for size comparison. The head-mountable imaging system is connected to the data capture board (about the size of a credit card) via a 20-wire flex cable.

watts of power, our imaging system weighs a few grams and consumes only 12 mW of power.

The photodiode saturates at 120 lux at 500 frames per second (fps) with a SNR of 63 dB. For reference, off-the-shelf scientific CMOS image sensors had a SNR of 55 to 72 dB during in vivo experiments. The peak quantum efficiency (QE) was only about 25%. This is much less than scientific CMOS imagers which typically have up to 80% QE. This discrepancy is due to the fact that the CMOS process used is not optimized for optical imaging applications. Figure 1-A shows our current prototype of miniature microscope for the fluorescent imaging system. Also Figure 1-C shows the entire data-collection system built around the custom camera NeuroView.

III. Voltage Sensitive Dye Imaging

Our experiments involve the stimulation of one area of the cortex, while observing the resulting activity over a wide area of the cortex. This kind of experiments can clearly highlights the advantages of wide-field epi-fluorescence imaging: with high spatial and temporal resolution, the propagation of action potentials can be clearly observed on large, multiple cortical areas.

One example is a voltage sensitive dye (VSD) experiment on mouse visual cortex to investigate the effect of intracortical electrical stimulation in V1 and surrounding visual areas. The visual cortex of a mouse was transdurally stained with RH 1691. An electrode was inserted into V1 (250 um) and a train of 10 cathodic-first bi-phasic rectangular pulses of 200 μs phase duration and amplitude of 75 μA were applied at 200 Hz to the electrode. Further details of the experiment protocol is described in [6].

The change in fluorescence in the visual cortex was recorded with MiCAM Ultima at 500 fps to confirm the biological validity of the output. Then, we replaced MiCAM Ultima with our imaging system and ran the same experiment at 250 fps. Both imaging systems gave comparable results, as shown in Figure 2. The observations of V1 response to train stimuli are similar to those obtained in [7], [8] - it is clear that the cortical

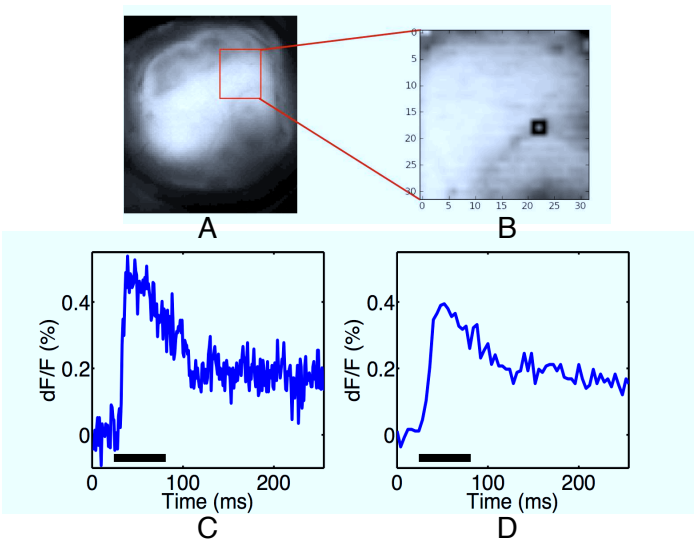

Fig. 2. (A) V1 and its surrounding areas as seen with MiCAM Ultima. The red box in A is the area imaged by our imaging system (B). The black-bordered box in B is the site where the changes in fluorescence is recorded by MiCAM Ultima (C) and our imaging system (D) as an average of three runs. The black bar in the graphs represents stimulation train length.

activity can remain hundreds of milliseconds even after the simulation has ended (Figure 2).

IV. Calcium Sensitive Dye Imaging

In the experiments in the previous section, VSDs were used, due to their response times (in the order of milliseconds or less). However, it is thought that the major signal source for VSDs is postsynaptic potentials rather than action potentials [9]. Calcium sensitive dyes (CSD) can be used to study direct effects of action potentials in neurons, due to the fact that an action potential opens voltage-dependent Ca^{2+} channels and causes $[Ca^{2+}]i$ to increase in the cell somata [10]. Excitability, neurotransmitter release, associativity, plasticity and gene transcription are all controlled by Ca^{2+}[11]. With CSDs, we can discriminate the propagation of excitatory signals that induce super-threshold activation, which helps to reveal the plastic changes of the synaptic transmission.

We used our imaging system to demonstrate the spatiotemporal properties of the $[Ca^{2+}]i$ changes evoked by layer 4 stimulation in primary visual cortical slice preparations by means of Ca^{2+} imaging to study the propagation of action potentials in the primary visual cortical neuronal networks. Detailed preparation protocols can be found in [13].

An electrode inserted into Layer 4 and a signal with 100 μs phase duration and amplitude of -40 μA/+100 μA were applied. $[Ca^{2+}]i$ transients were induced by the electrical stimulation of layer 4 of the visual cortex. Redshirt Imaging's NeuroCCD-SM256 was used to initially verify the validity of the experimental output. NeuroCCD was configured to run at 360 fps at a resolution of 64 x 64. Then, data was collected using our imaging system at 25 fps during the same experiment.

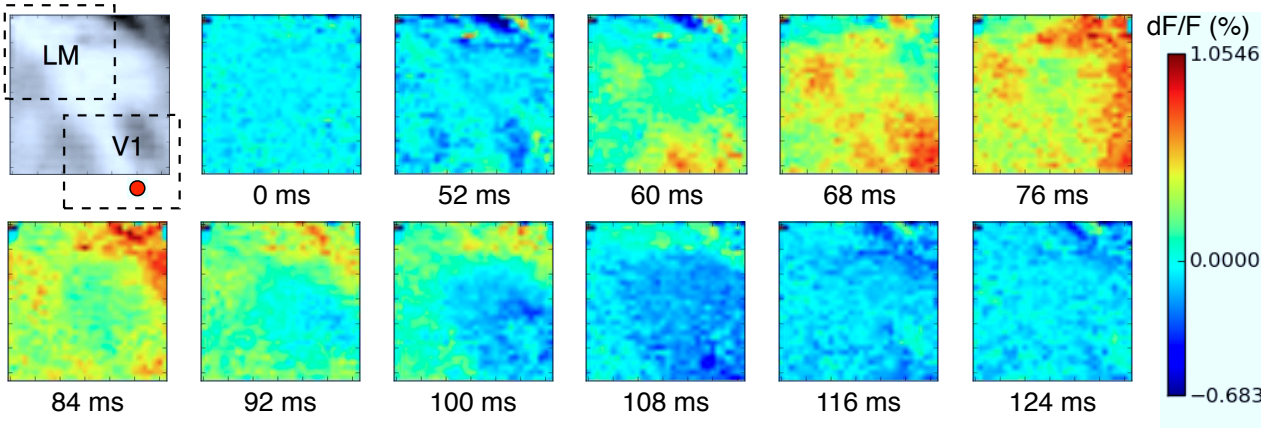

Fig. 3. Data from a VSD experiment showing the effect of simulating V1, collected with our imaging system. When V1 is stimulated at the location of the red dot, neuronal activity spreads from V1 to LM regions of the visual cortex. The change in fluorescence detected is as high as 1.05%.

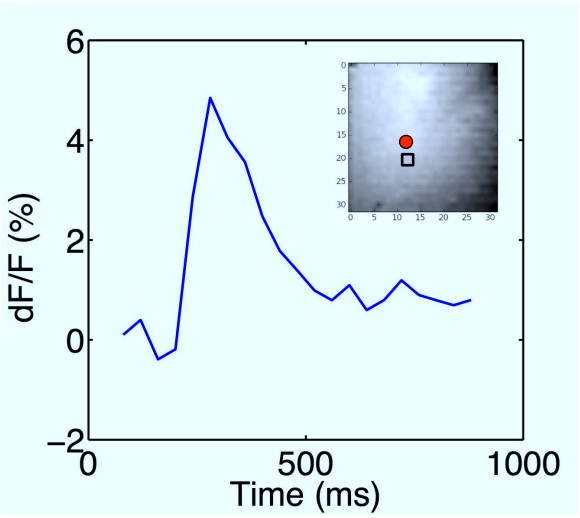

Fig. 4. A CSD experiment in the V4 region of a slice of the visual cortex. The image at the top right corner of the graph is from our imaging system. The electrode and the area of stimulation (red dot) can be clearly seen. The graph shows dF/F in the area marked by the black square for a single run.

The two systems performed similarly, other than the slower frame rate of NV2. The slower frame rate is not too detrimental, as calcium dye transients are in the order of tens of milliseconds. The neuronal activity observed by our imaging system in Figure 4 and Figure 5 were comparable of that in [14], and can be used to determine the spatiotemporal properties of the $[Ca^{2+}]i$ changes evoked by layer 4 stimulation in primary visual cortical slice preparations by means of Ca^{2+} imaging to study the propagation of action potentials in the primary visual cortical neuronal networks.

V. Conclusion

We have demonstrated a miniature imaging system that can be used in head-mounted microscopes to measure neuronal activity in the mouse visual cortex. The imaging system measures 22.25 mm x 22.25 mm and weighs less than 10 g, which is less than any other scientific imaging system available today. The imaging system is capable of recording as low as a 0.1% change in fluorescence at hundreds of fps using VSD and CSD. Data obtained with our miniature system shows that it is comparable to that of the non head-mountable scientific imaging systems.

TABLE I
Comparison of Imaging Systems

	MiCAM Ultima	NeuroCCD	This work
Weight (g)	250	$> 2{,}000$	< 10
Power (mW)	> 100	51,000	12
Resolution	100 x 100	256 x 256	32 x 32
Peak SNR (dB)	> 70	80	63
Well Size (Me^{-})	10	0.7	7.126
Shutter	Global	Global	Global
Max FPS	10,000	100	1000

VI. Acknowledgments

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References

[1] R. Biran, D. Martin, and P. Tresco, "Neuronal cell loss accompanies the brain tissue response to chronically implanted silicon microelectrode arrays," *Experimental Neurology*, vol. 195, pp. 115–126, 2005.

[2] F. Helmchen, M. Fee, D. Tank, and W. Denk, "A miniature head-mounted two-photon microscope: high resolution brain imaging in freely moving animals," *Neuron*, vol. 31, no. 1, pp. 903–912, 2001.

[3] B. Flusberg, A. Nimmerjahn, E. Cocker, E. Mukamel, R. Barretto, T. Ko, L. Burns, J. Jung, and M. Schnitzer, "High-speed, miniaturized fluorescence microscopy in freely moving mice," *Nature Methods*, vol. 5, no. 1, pp. 935–938, 2008.

[4] Q. Wang and A. Burkhalter, "Area map of mouse visual cortex," *The Journal of Comparative Neurology*, vol. 357, no. 3, pp. 339 –357, 2007.

[5] J. Park, V. Pieribone, J. Verhagen, C. vonHehn, and E. Culurciello, "High-speed fluorescence imaging system for freely moving animals," in *IEEE International Symposium on Circuits and Systems, ISCAS '09*. Taipei, Taiwan: IEEE, May 2009, pp. 2429 – 2432.

[6] Y. Okazaki, T. Fehervari, H. Sawai, and T. Yagi, "In vivo optical measurements of the activities of mouse striate and extrastriate cortices evoked by intracortical electrical stimulation," in *25th Symposium on Biological and Physiological Engineering BPES2010*, 2010.

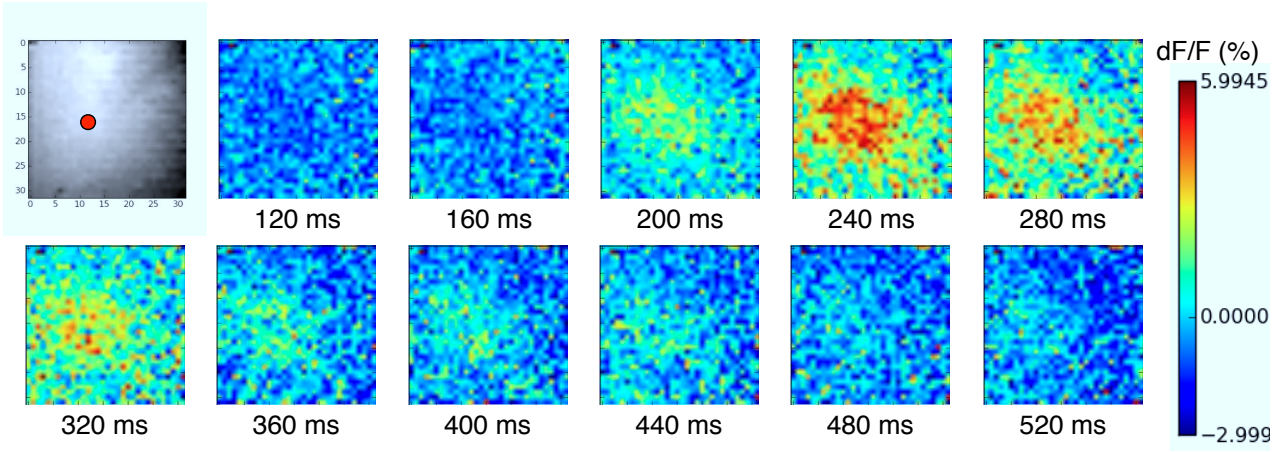

Fig. 5. Data from a CSD experiment showing the effect of simulating layer 4 of the visual cortex (at the red dot). The change in fluorescence detected by our imaging system is as high as 5.99%.

[7] E. Schmidt, M. Bak, F. Hambrecht, C. Kufta, and D. O. P. Vallabhanath, "Feasibility of a visual prosthesis for the blind based on intracortical micro stimulation of the visual cortex," *Brain*, vol. 119, pp. 507–522, 1996.

[8] T. Fehervari, M. Matsuoka, H. Okuno, and T. Yagi, "Real-time simulation of phosphene images evoked by electrical stimulation of the visual cortex," in *Neural Information Processing. Theory and Algorithms*. Springer Berlin / Heidelberg, 2010, vol. 6443, pp. 171–178.

[9] A. Grinvald, E. Lieke, R. Frostig, and R. Hildesheim, "Cortical point-spread function and long-range lateral interactions revealed by real-time optical imaging of macaque monkey primary visual cortex," *Journal of Neuroscience Methods*, vol. 14, pp. 2545–2568, 1994.

[10] D. Smetters, A. Majewska, and R. Yuste, "Detecting action potentials in neuronal populations with calcium imaging," *Methods*, vol. 18, no. 2, pp. 215 – 221, 1999.

[11] M. Berridge, "Neuronal calcium signaling," *Neuron*, no. 21, pp. 13–26, 1998.

[12] C. Hansel, A. Artola, and W. Singer, "Relation between dendritic ca2+ levels and the polarity of synaptic long-term modifications in rat visual cortex neurons," *European Journal of Neuroscience*, vol. 9, no. 11, pp. 2309–2322, 1997.

[13] M. Osanai, N. Yamada, and T. Yagi, "Long-lasting spontaneous calcium transients in the striatal cells," *Neuroscience Letters*, vol. 402, no. 1-2, pp. 81 – 85, 2006.

[14] M. Osanai, S. Tanaka, Y. Takeno, S. Takimoto, and T. Yagi, "Spatiotemporal properties of the action potential propagation in the mouse visual cortical slice analyzed by calcium imaging," *PLoS ONE*, vol. 5, no. 10, p. e13738, 10 2010.