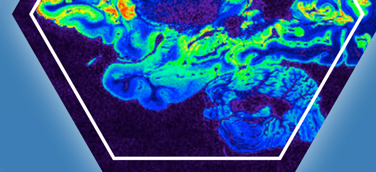
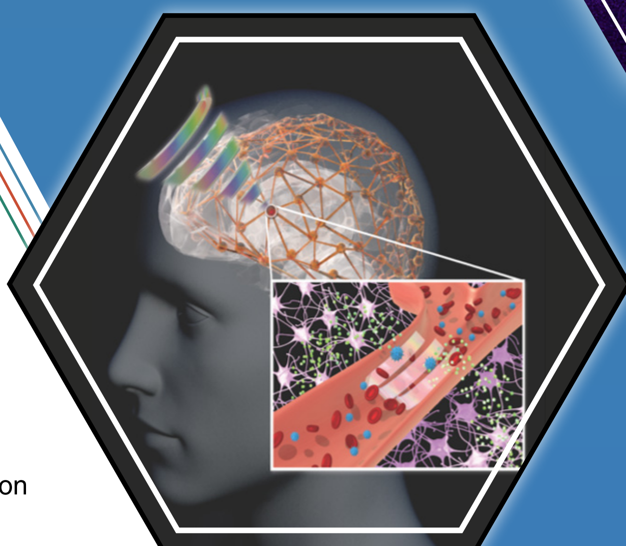
FEBRUARY 2022

Toolmakers Newsletter

ISSUE 05

In this issue

BRAIN INITIATIVE

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Welcome to the Toolmakers Newsletter!

Welcome to the first *Brain Research Through Advancing Innovative Neurotechnologies®* (BRAIN) Initiative Alliance Toolmakers Newsletter of 2022!

In this issue, we share with you four more exciting tool advancements in neuroscience: carbon fiber electrode arrays by Dr. Paras Patel and Dr. Cynthia Chestek; CaMPARI and Voltron by Dr. Eric Schreiter; a noninvasive ultrasonic drug uncaging technique by Dr. Raag Airan; and Gray Matter Research's microdrive recording systems for measuring neural activity by Dr. Baldwin Goodell. Let's explore the latest breakthroughs from these BRAIN investigators!

Images Below: Neurons expressing Voltron and labeled with different Janelia Fluor dyes (Left to right: JF_{503}, JF_{549}, JF_{585}, JF_{635}) Scale bar: 20 µm. Credit: Abdelfattah et al., 2019, Science.

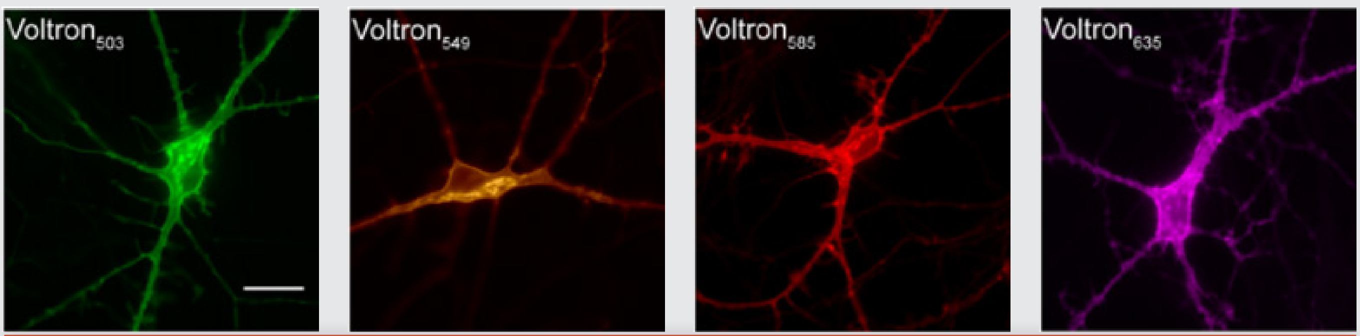
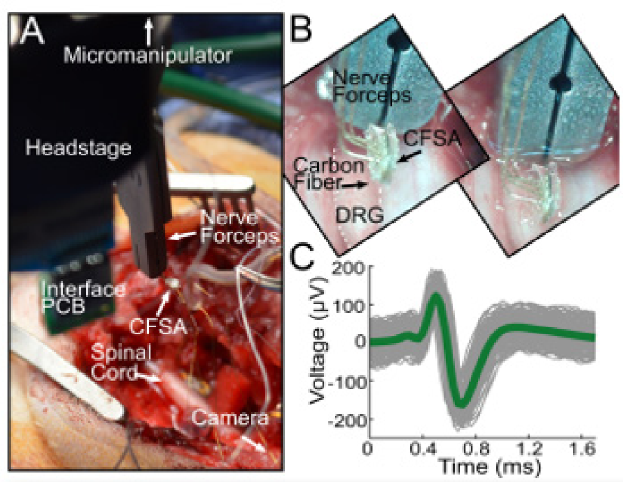


Image Right: Carbon fiber silicone array (CFSA) experimental setup in the dorsal root ganglia (DRG). (A) Surgical setup showing the CFSA, held by nerve forceps, and interface polyimide circuit board (PCB) connected to a headstage lowered to the DRG with micromanipulator. (B) CFSA, held by nerve forceps, as seen by the camera before (top) and after (bottom) insertion into the DRG. (C) Spontaneous neural cluster and mean waveform shape (green line) recorded in the DRG with the CFSA (mean Vpp = 298.8 ± 15.5 µV, signal to noise ratio (SNR) = 6.5). Credit: Welle et al., 2021, *IEEE Transactions on Neural Systems and Rehabilitation Engineering*.



On the front cover: **Top Right Hexagon:** An image still from a video of the C. elegans brain, including every nerve and muscle fiber, being reconstructed by serial-section electron microscopy. Credit: Daniel Witvliet, University of Toronto and Harvard University, 2020. **Top Central Hexagon:** Four-week-old rat cortical neurons labeled for dendrites (red), axons (green), and nuclei (blue). Credit: Karthik Krishnamurthy, Davide Trotti, Piera Pasinelli, Thomas Jefferson University, 2020. **Bottom Right Hexagon:** A pseudo-colored image of high-resolution gradient-echo MRI scan of a fixed cerebral hemisphere from a person with multiple sclerosis. Credit: Govind Bhagavatheeshwaran, Daniel Reich, National Institute of Neurological Disorders and Stroke, National Institutes of Health, 2016. Bottom Central Hexagon: Nanoparticles for ultrasonic uncaging of neuromodulatory drugs in the nervous system. Credit: Airan Lab, 2021.

Carbon fiber electrodes for electrophysiological or dopamingeric recordings – Dr. Paras Patel and Dr. Cynthia Chestek

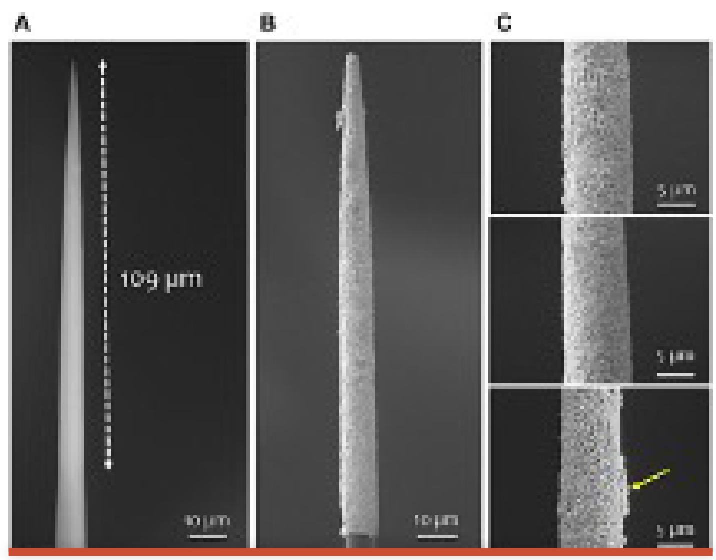
At the University of Michigan, Drs. Paras Patel and Cynthia Chestek and their team have developed carbon fiber electrode arrays that can be optimized for either electrophysiology or the detection of dopamine in rodents and aplysia. High density carbon fibers arrays are used to record electrophysiological activity and detect dopamine in deep brain structures and are localized within the tissue during histological analysis.

This work has many uses—the team implemented tip sharpening techniques for better penetration into neural structures. These techniques can be used in animal behavioral studies that require acute or chronic electrophysiological recordings, dopamine detection in the brain, or acute electrophysiology in the peripheral nervous system. Scientists typically chronically implant the array into a region of the brain associated with reward-related behavior to perform behavioral tasks (e.g., nose poke for rewards, narcotic self-administration) and receive high density recordings of dopamine signals to better understand dopamine kinetics.

This work was first published in the *Journal of Neural Engineering* in 2020. Since then, Drs. Patel and Chestek and team have published additional papers outlining the expanded capabilities of their carbon fiber electrode arrays. They introduced high density compensation fiber (HDCF) arrays, which are more amenable to different brain regions and animal models. They have also experimented with adding platinum iridium as a coating material for the carbon fiber arrays, enabling additional microstimulation capabilities (see image).

The team hopes to continue distributing these electrodes to existing collaborators and expand to new labs, with an overall emphasis on electrode customization per user needs. They are also developing even smaller devices using soft substrates that will increase the longevity of implants in soft tissue.

Image: Scanning electron microscopy (SEM) of platinum-iridium carbon fibers (PtIr-CF) with an exposed surface of 109 µm (A) before and (B) after the PtIr coating; (C) SEM images of three different PtIr-CF. Credit: della Valle et al., 2021, *Frontiers in Nanotechnology.*



> One potential target area is microstimulation of the retina to restore vision. Another is the peripheral nervous system to enable sensory information input, also through stimulation, for those with prosthetic limbs.
>
> *– Dr. Paras Patel*

Fluorescent indicators for neuronal activity: CaMPARI and Voltron – Dr. Eric Schreiter

Dr. Eric Schreiter and his team at Janelia Research Campus recently developed Calcium Modulated Photoactivatable Ratiometric Integrator (CaMPARI) and the chemigenetic voltage indicator Voltron, two fluorescent reporters of neuronal activity.

CaMPARI is a fluorescent protein-based calcium integrator for permanently marking neuronal activity. CaMPARI irreversibly changes a neuron's color from green to red when calcium is elevated during a light pulse. The more calcium is present in the neuron, the more the neuron will change colors. The team has experimented with CaMPARI in fruit flies to identify neurons that were activated in response to specific odors. Dr. Schreiter and his team have also been working to further develop CaMPARI. They developed CaMPARI2—the second generation of CaMPARI molecules. CaMPARI2 has higher contrast, dramatically improves color brightness, and allows the use of immunocytochemistry of fixed tissue to visualize neurons that were marked *in vivo*. Additionally, the team made an erasable version of CaMPARI called rsCaMPARI, which can mark multiple populations of active neurons.

Voltron is a chemigenetic fluorescent voltage indicator that records *in vivo* electrical activity over long periods. It combines fluorescent dyes with an engineered multipart protein that alters the dye's intensity when specific neurons are switched on. Voltron is used with light-sheet microscopy and other light microscopes to help detect neural signals throughout the brain. So far, Voltron has been used for *in vivo* voltage imaging in mice, zebrafish, and fruit flies. In lab testing, Voltron made it possible to watch neurons light up in the spinal cord of developing zebrafish. Dr. Schreiter and his team are now developing Voltron2, a new version of Voltron that improves the sensitivity of voltage imaging.

Both CaMPARI and Voltron record neuronal activity *in vivo* in model organisms and trace neurons, but they operate at opposite ends of the temporal regime. While CaMPARI traces neurons based on calcium activity level and captures static snapshots of calcium that scientists can read out later, Voltron measures voltage and allows scientists to follow action potentials, some of the fastest signals in the brain.

Dr. Schreiter and his team are distributing CaMPARI and Voltron as broadly as possible through open repositories to enable new biology. "We engineered these tools to be useful for monitoring the activity of many neurons in the brains of animals to connect brain activity with behavior," says Dr. Schreiter, "but we honestly never know what a new tool will enable down the road."

Video: Dr. Eric Schreiter describes the CaMPARI and Voltron tools he has worked on at Janelia Research Campus.

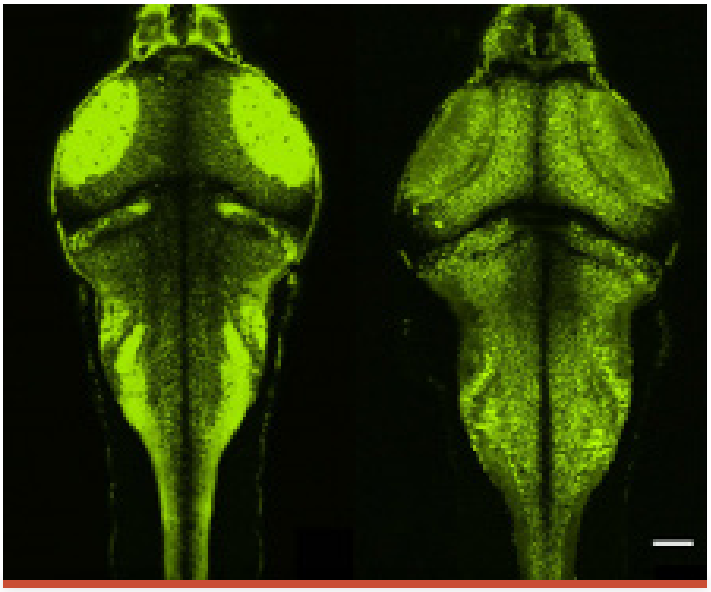


Image: Confocal image of transgenic (left) Voltron and (right) Voltron-ST zebrafish (4 dpf) labeled with JF525 dye. Scale bar: 50 µm. Credit: Abdelfattah et al., 2019, *Science.*

Nanoparticles for ultrasonic uncaging of neuromodulatory drugs in the nervous system – Dr. Raag Airan

Dr. Raag Airan's research at Stanford University centers around developing novel, noninvasive drug delivery systems to facilitate more precise control of neural activity, which may eventually lead to new, more precise treatments for brain diseases in humans. Dr. Airan's team primarily uses focused ultrasound to precisely deliver drugs to the brain with high spatial and temporal resolution. A few years ago, they developed a noninvasive ultrasonic drug uncaging technique that uses nanoparticles to effectively deliver a wide variety of drugs. The technique involves infusing nanoparticles intravenously, and then applying targeted ultrasound to activate drugs in specific brain areas (see image). The particles may be loaded with various neuromodulatory agents.

In medical practice, this drug uncaging technique could one day be used to help validate critical mediators of epilepsy for patients undergoing evaluation for epilepsy treatment. It also has the potential to help psychiatrists better understand what brain regions contribute to a patient's condition. In a therapeutic sense, it may be able to knock down pathologically overactive brain regions while the patient undergoes standard of care, such as cognitive behavioral therapy.

Dr. Airan describes these future implications as "adding a tool to the neurologist or psychiatrist's armament, allowing them to maximize the therapeutic actions over the side effects of a wide variety of neuropsychiatrically active drug compounds."

Dr. Airan's lab has also validated a production and storage scheme that will set the stage for clinical translation from animal models to humans by creating a fool-proof protocol that is scalable, stable, and optimized so that any lab can easily make their own nanoparticles. While the pandemic made it challenging to continue, Dr. Airan and his team have been using the time to educate themselves on the process of clinical translation.

"There are a lot of i's to dot and t's to cross in taking a formulation that has been validated in rats and preparing it to be produced at scales and to the standards necessary for human administration," says Dr. Airan. The extra research time paid off—the team is now on track to move toward regulatory approval with their system in the next one to two years!

> " With this effort, we aim to be able to target delivery of the anesthetic propofol to brain regions of interest with millimeters of spatial precision and seconds of temporal precision.
>
> *– Dr. Raag Airan*

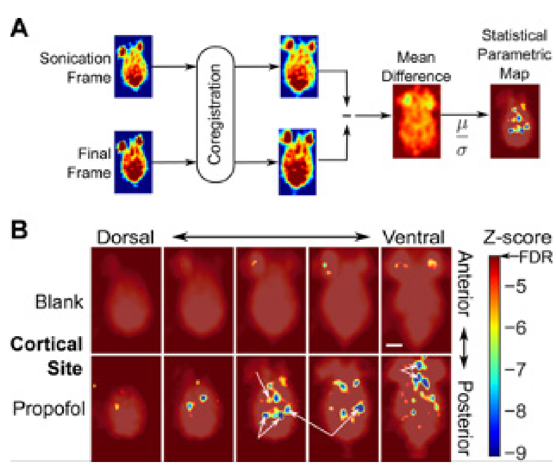


Image: Ultrasonic propofol uncaging maps functional connectivity of the cortex. (A) Analysis scheme for statistical parametric mapping of the whole-brain effects of targeted ultrasonic propofol uncaging. (B) Functional connectivity maps for cortical target sonication of animals receiving (top) blank or (bottom) propofol-loaded nanoparticles. Credit: Wang et al., 2018, *Neuron*.

Distributed recording systems for measuring neural circuit activity – Dr. Baldwin Goodell

Dr. Baldwin Goodell is the owner of Gray Matter Research, a company established in 2007 to distribute equipment designed in the Gray Lab at Montana State University to other neuroscience researchers. For years, Gray Matter Research has been designing and manufacturing large-scale microdrive systems to record neural circuit activity in non-human primates that span the depth and breadth of the brain. These systems can significantly expand the scope, duration, and reliability of measuring neural circuit activity by enabling semi-chronic recording from large numbers of independently moveable microelectrodes.

These systems have many advantages—they are fully assembled and sterilized before shipping and ready for implantation upon delivery—perhaps why they are now being used in over 50 labs around the world. They can also collect large amounts of data while improving laboratory animal care by reducing the number of animals and implants used in an experiment. The microdrive systems were equipped with a few new improvements in 2021, including new shapes that allow more choices for users to try and reach the brain targets they're interested in.

> These systems are optimized to offer a larger number of electrodes. They also allow electrodes to travel longer travel distances and allow more flexible curve trajectories so they can enter specific brain areas. The systems register the electrodes to post-op scans which improves reliability.

"Another thing we're working on—our microdrives move single electrodes into the brain—they can only record one signal at a time right at the tip of the electrode," says Dr. Goodell. "We'd really like to start implementing these with polyelectrodes or laminar probes so that each actuator moves a probe that can record from several channels along the shank of the probe." Dr. Goodell and his team have been working to make that happen in hopes of using space more efficiently via channels to get more data out of the same area.

Gray Matter Research is also working on projects that complement their microdrive systems, like orthopedic implants for the skull made from titanium or biocompatible plastic. These implants feature ports designed so that microdrives can attach to them, with the goal of making surgery faster, easier, and more efficient.

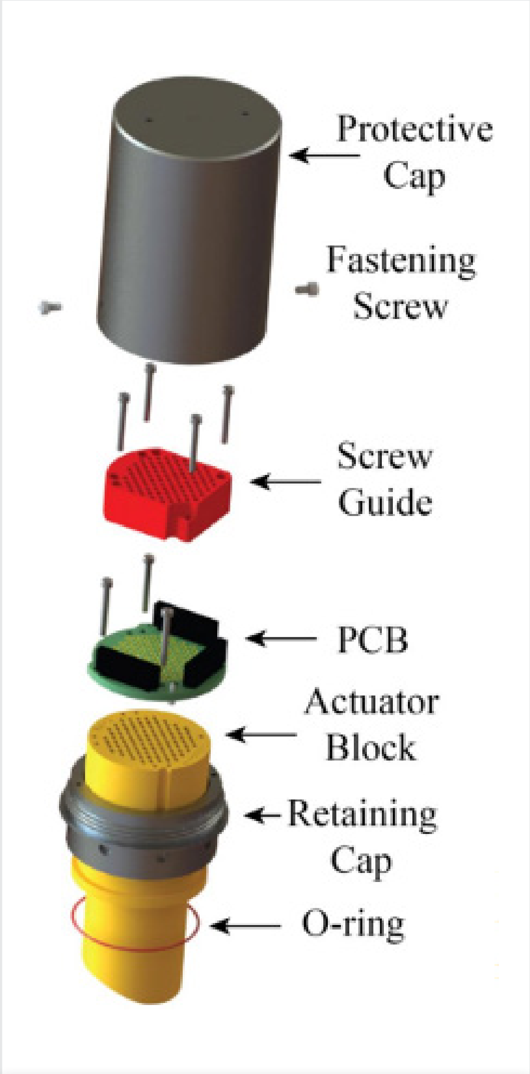


Image: Exploded view of Gray Matter Research's SC96 microdrive system. Actuators, including the lead screw, compression spring, shuttle and electrode, are not shown. PCB = printed circuit board. Credit: Gray Matter Research, LLC, 2020.

Excited by the potential of the tools in this issue?! Stay tuned for our next issue and explore more products of BRAIN Initiative discoveries in our Toolmakers' Resources page!