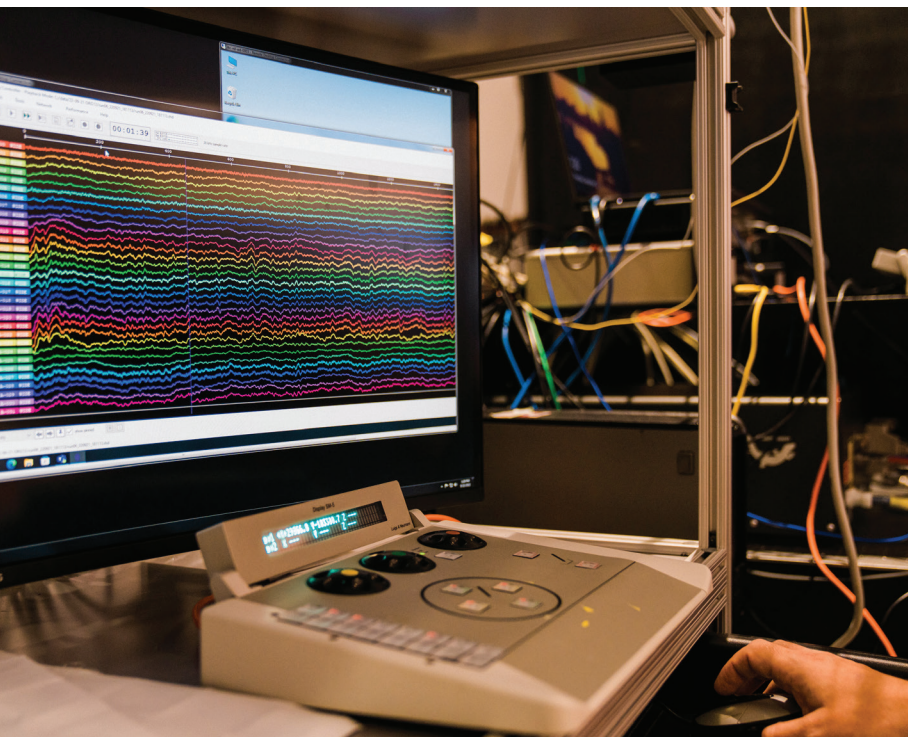
Research Highlight #2009

Madison Wilson and Martin Thunemann, Ph.D.
University of California, San Diego, and Boston University

Studying human cortical organoids in the mouse retrosplenial cortex with two-photon imaging and electrophysiology

Organoids are becoming an increasingly useful tool to study various neurological phenomena. Recently, a collaboration between neuroscientists and engineers at Boston University, University of California San Diego, and Salk Institute successfully integrated human cortical organoids in the adult mouse retrosplenial cortex. Two-photon (2P) imaging showed vascularization of the transplanted organoids and electrophysiology recordings showed a response to visual stimuli. For this study, Madison Wilson and Martin Thunemann share first authorship and both are excited by the implications of their collaborative study on evaluating the development, maturation, and functional integration of human neuronal networks within the mouse brain.

> "Our lab is curious about the functional aspect of implanting organoids in the mouse brain. After an external stimulus, does the organoid not only physically connect but also start to form functional responses as well? That was the baseline motivation for our work, and we also wanted to use both imaging and electrical recordings because you get different trade-offs. With electrical measurements, you get those very fast millisecond timescale recordings, but with imaging, you're able to see a wider field-of-view and a good overall picture of what's happening to the organoid."
> – Madison Wilson



Electrophysiology recordings.
Image courtesy of Christopher McIntosh.

ABOUT THE RESEARCHERS

Madison Wilson is a 4th year Ph.D. student in Electrical and Computer Engineering at the University of California, San Diego. She previously obtained a B.S. from the University of Illinois at Urbana Champaign in electrical and computer engineering and psychology, with a concentration in behavioral neuroscience. She is currently a member of Duygu Kuzum's Neuroelectronics Group, which is interested in researching the interface of nanoelectronics and the brain.

Martin Thunemann is a research assistant professor (BME) at Boston University College of Engineering. He studied biochemistry at Eberhard Karls University of Tübingen, Germany, and joined Anna Devor's lab as a post-doctorate at the University of California, San Diego in 2015. The Neurovascular Imaging Laboratory moved to its current location at Boston University in 2020 and specializes in imaging neuronal, glial, vascular, and metabolic activity in the brains of living and behaving experimental animals.

Complementary Publications

Wilson, M.N., Thunemann, M., Liu, X. et al. Multimodal monitoring of human cortical organoids implanted in mice reveal functional connection with visual cortex. *Nat Commun* 13, 7945 (2022). https://doi.org/10.1038/s41467-022-35536-3.

Thunemann et al. Deep 2-photon imaging and artifact-free optogenetics through transparent graphene microelectrode arrays. *Nat Commun* 9, 2035 (2018). https://doi.org/10.1038/s41467-018-04457-5.

Microelectrode Arrays for Imaging and Recording

To be able to image and record simultaneously, Madison spoke of how their lab used graphene microelectrode arrays implanted into the adult mouse head. Graphene is a beneficial material as it is conductive and transparent so you do not get the light-induced artifacts that you would with conventional metals like gold. What they hoped to accomplish by surgically implanting the microelectrode arrays and creating cranial windows, a process that makes each mouse in the study extremely valuable, was to see if these electrodes enable the integration of electrical recording with multiphoton imaging and optogenetic stimulation. Experiments were also run to measure local field potentials (LFP) or spiking activity after delivering light pulse stimuli to the mouse's eye.

> "We looked at the spiking activity in response to LED stimuli to the eye to see if there was a difference. These kinds of analyses supported our conclusion that there was some functional electrical activity in the organoid that was mature enough and connected enough such that it was able to respond as the native brain was responding."
> – Madison Wilson

Earlier research using this multimodal imaging approach showed that electrical recordings, multiphoton imaging, and optogenetic stimulation were successfully integrated by these graphene microelectrodes [Thunemann et al., 2018]. Additionally, the organoids generated a functional response to sensory stimuli.

Integration of Organoids into the Mouse Cortex

Another consideration while implanting these organoids was their ability to be perfused and receive oxygen and nutrients. This led to the implantation of their organoids into the restrospinal cortex for a higher likelihood of successful integration into the big vascular bed found there. Martin further spoke of how studies done with organoids in vivo, versus in culture, are unique for the ability of the host to provide oxygen and how this impacted the structure of their experiments.

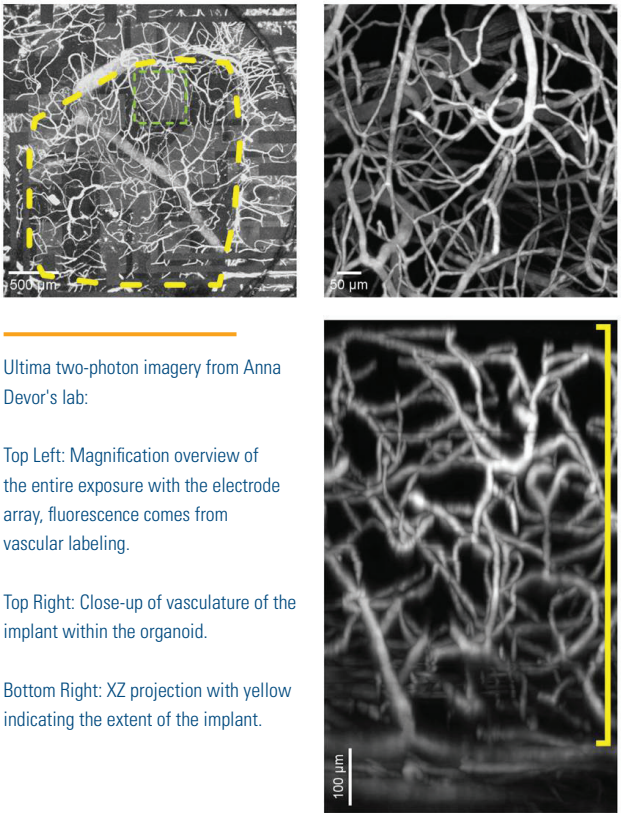
> "Our idea was if we implant these organoids into a mouse brain which is immunodeficient, meaning it doesn't reject the human cells, the mouse will start to grow vessels into this human cell mass, and they will start to interact. We hope, and I think it's clear from a couple of other papers, that the mouse brain environment is very nurturing for these organoids to develop further than they would do in culture. What we did after the implantation was measure the activity of the mouse brain and the implanted organoids with electrophysiology, which was what we used the graphene electrodes for. At the same time, because the electrodes are chronically implanted but transparent, we use two-photon microscopy to look at the vasculature."
> – Martin Thunemann

This experimental setup enabled the visualization of the mouse vessels growing into the human cell mass and perfusing with blood, meaning that their organoids were successfully vascularized by the host blood vessels. They further supported these findings by staining human cells and saw that the mouse vasculature was starting to perfuse them.

Two-Photon Imaging of Vascularization

Martin Thunemann described the ability of this group to image mouse vasculature as a standard technique that he had experience in after using 2P imaging during his post-doctoral studies. For their study, dextrans were fluorescently labeled and injected into the bloodstream of the mice with a retro-orbital intravenous injection before being imaged by Bruker's Ultima two-photon laser scanning multiphoton microscope. Optical imaging of vascularization, along with post-mortem immunostaining, further supports the fact that the organoid grafts were vascularized by the host and integrated with the surrounding cortex.

> "We have very clear stainings of human cells that the mouse vessels are starting to perfuse. It's a bit like an injury that needs to be healed because when we put the human cells in, the mouse responds by starting to supply oxygen and nutrients to the organoids, which enables them to grow. We also see that the human cells survive for four months, six months, and eight months, so they are stably implanting, which is really good for us."
> – Martin Thunemann



Ultima two-photon imagery from Anna Devor's lab:

Top Left: Magnification overview of the entire exposure with the electrode array, fluorescence comes from vascular labeling.

Top Right: Close-up of vasculature of the implant within the organoid.

Bottom Right: XZ projection with yellow indicating the extent of the implant.

Future Studies in the Lab

As is the nature of many scientific studies, there are future directions that can be taken in the lab that add invaluable data to previous findings. Madison and Martin both mention how an additional study they are actively pursuing is labeling human cells with calcium indicators to expand the structures they can look at past blood cells during the mouse lifespan. The ability to express GCaMP in human organoids is a new development that has a lot of promising implications. Additionally, now that the lab is established and able to function without the unique challenges the global pandemic caused in 2020, they can study the mice that have already undergone surgery (headpost implantation, craniotomy, organoid implantation, and graphene microelectrode array implantation) for a longer time frame. Additionally, in their most recent paper, they looked at how anesthesia affects spontaneous neural activity and found that organoid activity was generally lower than the surrounding cortex, which is another area for further investigation.

> "We touched on the anesthesia response and mentioned that there's short-range connections over the cortex, but maybe not long-range projections. So, I think the thalamus would be an interesting area to study. If we implanted more than three months in vivo, could those long-range connections be established?"
> – Madison Wilson

Understanding Neurological Disorders

Organoids have many applications and, with help from collaborators in laboratories and universities, are becoming an increasingly rich resource. Therefore, ongoing developments include using a patient's stem cells to generate organoids that are specific to them. Afterward, they could screen drugs and measure their responses in the lab before being tested in humans. Another long-term goal of this research team is to establish a model system that can be used to study various neurological disorders, one of which is epilepsy.

> "One thing we are heavily working on is making sure the implantation of the organoid doesn't create too much variability between individual animals. We then want to look into organoids from people that have neurological diseases, and for us, one of the easiest ones would be epilepsy or anything showing epilepsy phenotypes. It's very easy for us to detect, either with imaging or with electrophysiology, because a seizure is a very clear event when it's happening. Going from there, we can think about more complicated disorders like schizophrenia."
> – Martin Thunemann

Furthermore, demonstrating visual responses in organoids creates a platform for studying various neurological injuries and potential restorative actions, such as transplantable neural prosthetics.

> "I think the demonstration of visual responses in the organoid is very promising. This is just the first step toward cell replacement therapy or neural prosthetics. Of course, the organoids are not at that level yet but the fact that it was able to form initial connections is very promising for the future of healing damaged brain regions."
> – Madison Wilson

Summary

This study done by Madison Wilson, Martin Thunemann, and their collaborators is an important investigation of the implantation of human-derived cortical organoids in mice using electrophysiology and 2P imaging. Their development and use of graphene microelectrode arrays enabled the integration of electrical recording with multiphoton imaging and optogenetic stimulation. This allowed them to measure spiking activity in response to visual stimuli and to observe functional connections with the surrounding cortex. This platform can be used for future studies aimed at better understanding and remediating neurological dysfunctions.

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