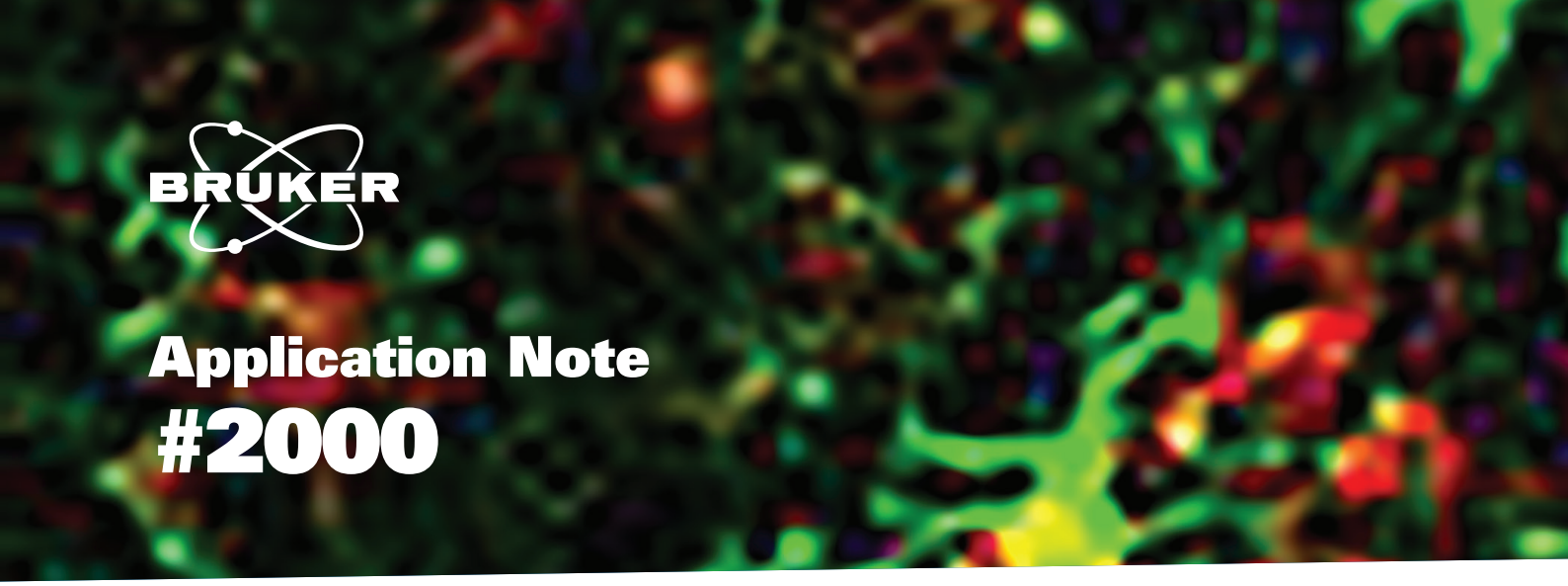

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Application Note
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Webinar Recap: In vivo Imaging of Microglia as the Brain's Thermostat for Tuning Neuronal Activity

Understanding the interaction between neurons and glia is critical to answering the most pertinent questions in neuroscience research. To understand brain cell dynamics, researchers must be able to image brain sections deeply and in vivo. Bruker's Ultima Investigator™ multiphoton microscope is well-suited for neuroscience applications such as this, featuring a base system specifically optimized for in vivo studies while supporting deep, fast, and sensitive imaging. This webinar recap summarizes the presentation given by Dr. Mario Merlini, assistant professor at the Institute of Blood and Brain at the University of Caen-Normandie, about his use of multiphoton microscopy and other techniques to address crucial research questions regarding the role of microglial cells in tuning neuronal activity in live mice.

A deep look into the microglial-neuronal interactions in live mice with chronically impaired microglia motility

Through the use of two-photon (2P) microscopy in the early 2000s, it was found that a subtype of glia known as microglia has the unique ability to interact with neurons by altering their cytoskeletons. The motility of the microglial cytoskeleton is believed to be related to its immunological function. Microglia are known to affect neuronal function through mechanisms such as synaptic remodeling and altering activity,[1,2,3] noradrenergic signaling,[4,5] and ATP or adenosine mediated inhibitory feedback.[6] The recent publication by Merlini et al. [7] explores the basis of microglial-neuronal interaction by conducting experiments aimed to answer a range of research questions. These include: what happens to neuronal function when microglial motility is chronically blocked; is there a need for microglia to dynamically interact with neurons; why microglial dynamics respond and adapt to neuronal activity; is there a link between neuronal hyperexcitability and chronically impaired microglia dynamics; why chronic inhibition of microglial parenchymal surveillance and directed motility induces brain hyperexcitability; and why is hyperexcitability stimulus dependent?

Experimental techniques

Mouse lines with chronically impaired microglia motility (Mg^{PTX}) were developed using microglia-specific inhibition of Gi signaling via Pertussis toxin expression. A line of Mg^{PTX} mouse with constituent expression of GFP-positive microglia was also developed for fluorescent imaging. Mice were transfected or infected with the red calcium indicator jRCaMP1b. The following sections detail some of the techniques used to answer numerous research questions.

Visualizing parenchymal surveillance and directed motility in chronically impaired PTX mice without stimulation. 2P microscopy was used to visualize baseline differences between wildtype (WT) and Mg^{PTX} mice in the ability of their microglia to (1) perform parenchymal surveillance and (2) respond via directed motility toward tissue injury caused by laser ablation from the microscope.

Measurement of seizures and associated phenotypes of Mg^{PTX} mice. The rate of seizures in WT and Mg^{PTX} mice was measured when mice were placed in new environments. Seizures were also induced via the delivery of pilocarpine to the mice, and the cycling time of seizures was measured with EEG recording. Dendritic spine density and synapse density were measured with 2P and electron microscopes (EM) respectively.

Visualizing microglial dynamics and physiologically evoked neuronal excitation in awake mice.To capture real time microglial-neuronal interactions in an awake mouse, Bruker's Ultima Investigator multiphoton microscope was used to create a video in which microglial imaging is immediately followed by whisker stimulation in intervals repeating from zero to 35 minutes, totaling 40 minutes of collection time (see Figure 1). With the flexibility of the Ultima investigator base system, an awake WT or Mg^{PTX} mouse was head-fixed under the lens for in vivo imaging. First, Z-stacks of microglea and jRCaMP1b positive neurons were collected at 5 minute intervals in the absence of whisker stimulation. The dual wavelength capability of the Ultima Investigator was utilized to excite the GFP-expressing microglia at 940 nm and the jRCaMP1b neuronal calcium at 1100 nm. The neuronal images from this round of Z-stacks were used in the subsequent step for colocalization of the microglia activity with the neuronal activity captured under stimulation. In the next step, imaging in five-minute intervals was used to collect single-plane images of neuronal calcium/jRCaMP1b under automated whisker stimulation with an automated whisker stimulation system; for this, spiral scanning of the single plane was used at 12 Hz. For timelapse imaging and whisker stimulation, MATLAB was used to provide commands to PrarieLink™ software and an Arduino motor for stimulation respectively. Z-stacks from both timelapse collections were combined to show the microglial activity and the one-plane fast neuron activity images over time.

Photostimulation for glutamate uncaging. RuBi-Glutamate uncaging was performed to test how motility-impaired microglia respond to, or uptake, glutamate in comparison with WT microglia in mice. 2P microscopy was used to photostimulate the caged glutamate at 800 nm and to collect the image of the GFP-expressing microglia and neuronal Ca^{2+} signaling at 940 nm and 1100 nm.

Rescuing microglial motility phenotype. To determine if the impaired microglial dynamics phenotype in Mg^{PTX} mice could be rescued, the Mg^{PTX} mice were subject to cranial bathing with the activator of Rho/RAC/Cdc42, a downstream player in the Gi signaling pathway.

Major findings

Using these techniques, Dr. Merlini and colleagues acquired several compelling answers to their research questions:

- Microglia parenchymal surveillance (baseline motility) is stunted. Additionally, directed motility toward tissue injury via laser ablation is abolished in Mg^{PTX} mice (see Figure 2).
- Mg^{PTX} mice have a severe spontaneous seizure phenotype that increases mortality rate. More specifically, this occurs when mice are placed in new environments and neurons are hyper-excited without microglia interacting with them. Mg^{PTX} mice also have increased susceptibility to pilocarpine-induced seizures correlating with hypersynchronized EEG activity. While both Mg^{PTX} and WT mice exhibited similar EEG levels, Mg^{PTX} mice cycled through seizures stages much faster than WT.
- Chronic inhibition of microglial motilities does not affect dendritic spine density (measured by 2P), synaptic density (measured by EM), and non-evoked, baseline EEG activity.

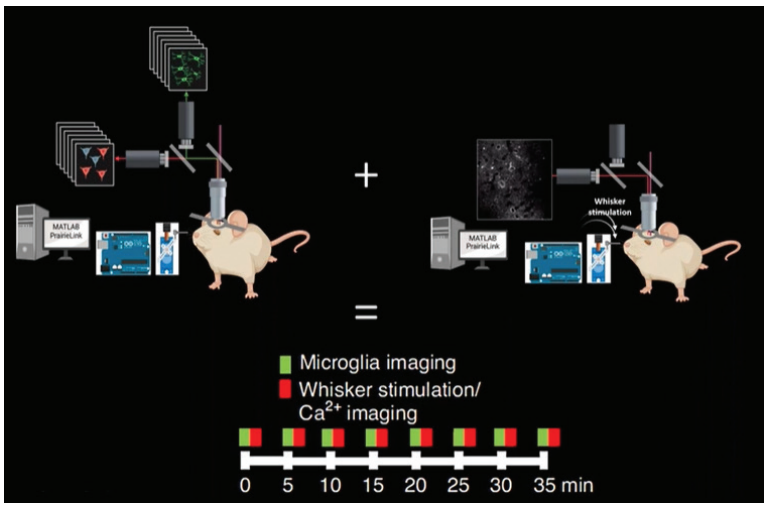

FIGURE 1.

Schematic of in vivo 2P imaging of microglia and neuronal Ca^{2+} transients before and during whisker stimulation in awake GFP-expressing mice.

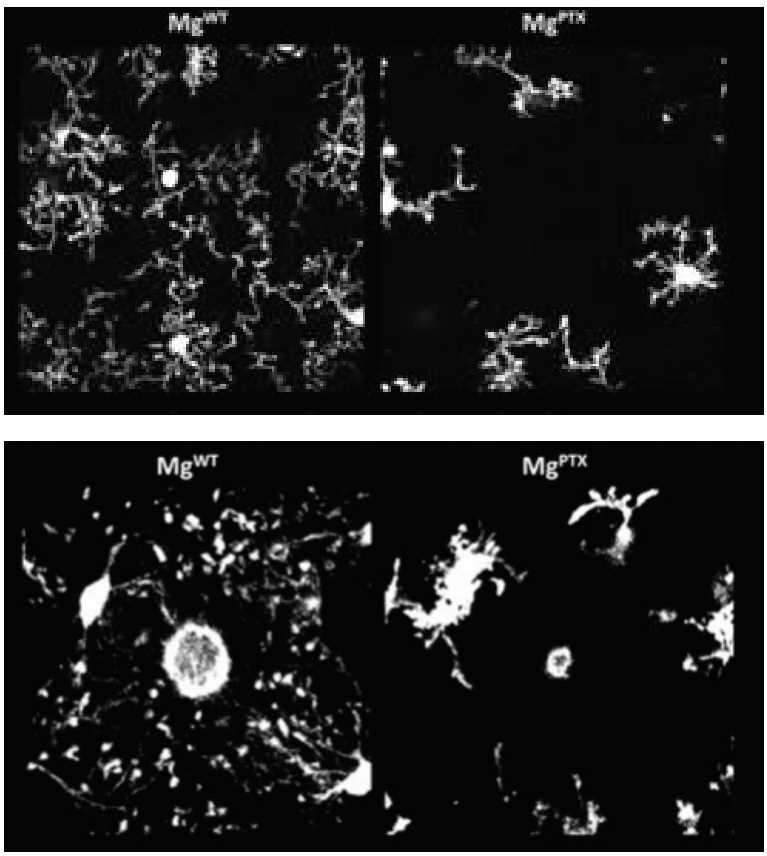

FIGURE 2.

In vivo 2P time-lapse imaging of (top) cumulative microglial surveillance and (bottom) microglial-directed process motility toward laser ablation.

- While WT mice exhibit a massive increase in parenchymal surveillance by microglia in response to whisker stimulation versus no whisker stimulation, this phenomenon is completely abolished in Mg^{PTX} mice. The parenchymal surveillance looks identical under 2P imaging whether whiskers are stimulated or not in Mg^{PTX} mice (see Figure 3).
- When WT mice were stimulated, there were significantly more microglial process extensions and retractions compared to the unstimulated WT mice. However, in Mg^{PTX} mice there was no difference between stimulated or unstimulated mice in their number of process extensions and retractions. Interestingly in analysis of processing velocity of the microglia, there is no difference between WT and Mg^{PTX} mice in whether they are stimulated or not stimulated. This shows that these cells are not dead because even though the motility is impaired, the velocity is unchanged. Furthermore, the cells still have the proper machinery, but they are not able to modify the cytoskeleton to extend processes and react to stimuli.
- While WT microglia have a preference for contacting evoked (versus non-evoked) neurons from whisker stimulation, this preference is lost in Mg^{PTX} mice (see Figure 4).
- Neuronal excitation induces intraneuronal Ca^{2+} accumulation and increases decay time of intraneuronal Ca^{2+} transients in Mg^{PTX} mice. Therefore, there is a continuous Ca^{2+} increase with an impairment in neuronal ability to return to baseline levels of Ca^{2+} in Mg^{PTX} mice, resulting in increased Ca^{2+} decay time.
- Compared to WT mice, evoked neuronal activity is hypersynchronized, or essentially simultaneous, in Mg^{PTX} mice. Meaning, more neurons are hyperexcited, or firing, at the same time.

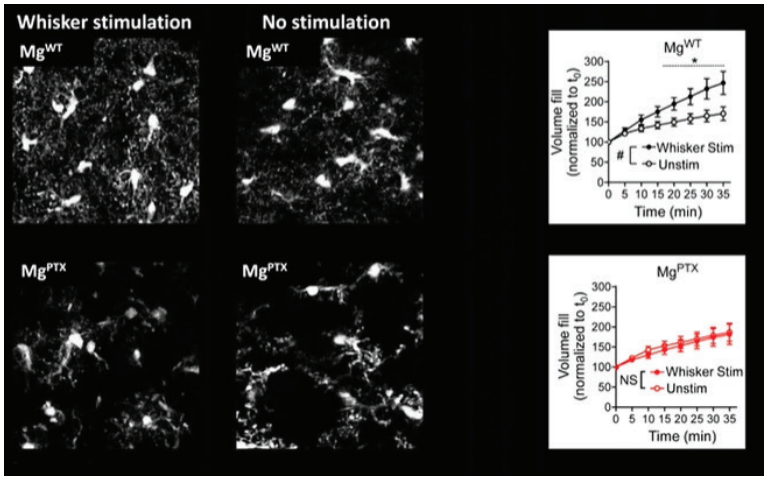

FIGURE 3.

In vivo 2P imaging (left) and quantification (right) of cumulative microglia surveillance after whisker stimulation in awake WT and Mg^{PTX} mice.

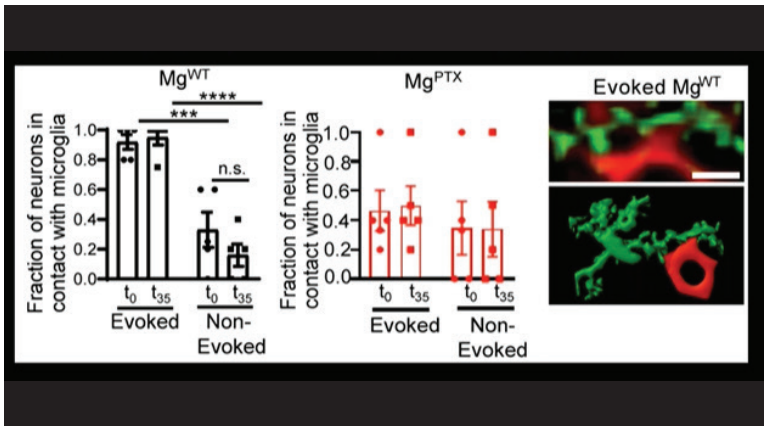

FIGURE 4.

Evoked and non-evoked neuronal somata following whisker stimulation in contact with microglia in MgWT and Mg^{PTX} mice (left). In vivo 2P image (top right) and 3D reconstruction (bottom right) of microglia (green) contacting a jRCaMP1b-expressing neuronal soma (red) in the barrel cortex of a MgWT mouse during whisker stimulation.

- The microglial uptake of parenchymally released glutamate was impaired in Mg^{PTX} mice. Microglia did not respond with directed motility when RuBi glutamate was uncaged using photostimulation by the 2P microscope (see Figure 5).
- The stunted microglial behavior phenotype can be rescued by activating the Rho/Rac/Cdc42 complex downstream of the impaired Gi signal present in Mg^{PTX} mice. When microglia impairment was rescued with activation of Rho/Rac/Cdc42, there was a similar pattern of Ca^{2+} uptake to WT mice, as well as a Ca^{2+} decay time more like WT mice.

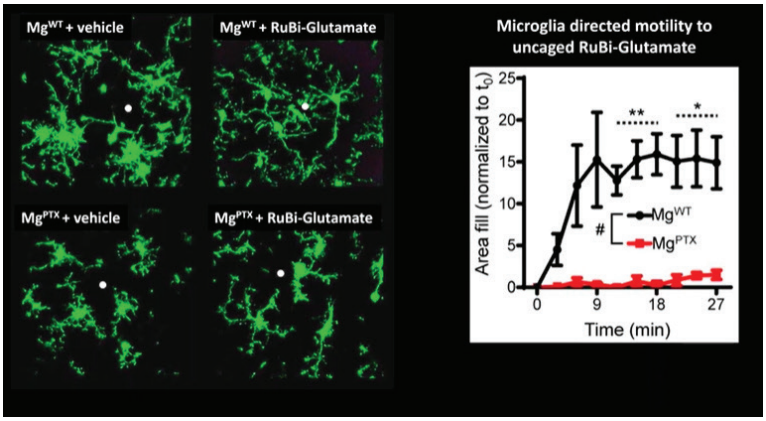

FIGURE 5.

In vivo 2P time-lapse images (left) and quantification (right) of microglial-directed motility in response to glutamate uncaging in the somatosensory cortex. Microglia areas filling around the uncaging site is shown as white dots.

Implications

This research addresses the complex question of why microglia dynamics respond and adapt to neuronal activity. One major takeaway is that microglia do this to maintain evoked neuronal activity within physiological limits, preventing hyperexcitability of neurons. Rather than acting as binary ON/OFF switches of neuronal activity, microglia act as modulatory, thermostat-like cells. This is a novel contribution to the previous understanding of microglial-neuronal interactions. Furthermore, microglial dynamics are highly different between spontaneous, suppressed, and evoked neuronal activity, shown in examples with anesthetized animals, evoked animals, and in vivo non-evoked animals;[1-7] as such, it is important to take into consideration the experimental set up when investigating microglial dynamics.

The Ultima Investigator 2P platform uniquely supported in vivo brain imaging in mice under stimulated and unstimulated conditions, which was important to investigate microglial dynamics in a realistic setting. Seemingly, microglia can sense the type of neuronal activity that occurs, which has major implications for neurodegenerative and neuropsychiatric disorders in which microglia lose their motility due to overactivation and these impaired microglia are not able to modulate neural activity. Regardless of whether the neurons that which the microglia fail to regulate are inhibitory or excitatory, the impairment will have profound effects in the overall neuronal circuitry.

Ultimately, prevention of neuronal hyperexcitability is required for healthy brain function and this research reveals the essential role of microglia in this process. The Mg^{PTX} mice developed by Merlini et al. can be used for future studies in microglia dynamics in neurological conditions caused by neuronal hyperexcitability including Alzheimer's disease, traumatic brain injury, epilepsy, autism, neuroinflammatory diseases and psychiatric disorders.

Acknowledgements

This app note is sourced from Dr. Mario Merlini's webinar titled "*In vivo* imaging of microglia as the brain's thermostat for tuning neuronal activity," which can be accessed by visiting: https://www.bruker.com/en/news-and-events/webinars/2021/In-vivo-imaging-of-microglia-as-the-brains-thermostat-for-tuning-neuronal-activity/watch-9qo8t.html

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