MICROSCOPY

Windows on the brain

A comparison of two surgical preparations for two-photon *in vivo* microscopy of the mouse brain highlights the necessity for careful experimental design and proper controls.

The availability of transgenic mouse lines with fluorescently labeled neurons has made two-photon fluorescence microscopy a powerful tool for studying the morphological and functional dynamics of the microstructure of the mammalian brain. But unfortunately, even the two-photon microscope cannot penetrate the skull.

For short-term studies, it is possible to cut a hole in the skull of an anesthetized mouse and image for several hours. Many processes, however, require much longer experiments. Covering the hole with a glass window allows repeated imaging through this open-skull window over several months. Alternatively the intact skull can be mechanically thinned in a small region. "This methodology is now becoming very widespread, and it has become an essential technique for analysis of plasticity and disease processes *in vivo*," says Ole Ottersen, an expert in transcranial imaging at the University of Oslo.

One of the processes being examined using these techniques is the appearance and disappearance of tiny spines on neuronal dendrites. These dendritic spines form one half of a synapse between the dendrite and another neuron, and are thought to mediate neuronal connectivity.

Unfortunately, as sometimes happens, results obtained by different labs are quite dissimilar. Wen-Biao Gan from New York University, who uses the thinned-skull method, says, "I was very bothered by the discrepancy between my results showing low levels of spine turnover and those of Karel Svoboda, who uses the open-skull window technique." Gan thus performed a side-by-side comparison of the two techniques to try and explain the difference.

Gan found that when he used the open-skull window method he obtained results similar to Svoboda's. When he examined the brains at the end of the experiments however, he saw that the open-skull mice displayed activated astrocytes and microglia indicative of trauma to the brain, whereas the thinned-skull mice showed no such inflammation. The spine turnover results correlated with the evidence of trauma indicating that the inflammation may be responsible for the increased spine turnover. This simple explanation may not be sufficient though.

Joshua Trachtenberg, who has worked with Svoboda and is a longtime friend of Gan, says, "I'm glad Wen-Biao did this. I think it is important that people push and push in our field, otherwise people get lazy." Although inflammation may explain the results of Gan's comparison, he does not believe that inflammation resulting from the open-skull technique is responsible for the different results from the two labs. Instead, the different transgenic mouse lines used may be responsible for the discrepancy. Gan only used his own line in the comparison.

"Everybody wants to write this off as being unimportant, but I think it is fundamentally important," says Trachtenberg. "When I do the thinned-skull method on the GFP mice [that Svoboda uses] I get the same result that has been published with the open-skull GFP mice. I think there may be some evidence that the YFP [in Gan's mouse line] is labeling a different set of neurons."

David Linden from Johns Hopkins University believes the technique and the resulting trauma may be responsible for some of the disparate results but cautions that it depends on how it is performed. "[Gan's] goal was to explicitly compare his conditions to Svoboda's, … so Gan wasn't trying to optimize the open-skull method," says Linden. Although the open-skull window has distinct advantages such as better optical quality and a much larger number of imaging sessions, according to Linden it is sensitive to pressure on the brain surface and infection.

"What it probably means is that if you use an open-skull prep you need to make a small hole, be very concerned about putting minimum pressure on the brain, wait for at least several weeks before taking your images and perhaps even give chronic antibiotics" says Linden. He emphasizes that in the future, "everybody is going to have to calibrate their prep themselves to be confident that the inflammatory cascade is over in their own hands, with their own surgery, experimental conditions and region of the brain."

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RESEARCH PAPERS

Xu, T.-H. *et al.* Choice of cranial window type for *in vivo* imaging affects dendritic spine turnover in the cortex. *Nat. Neurosci.* **10**, 549–551 (2007).