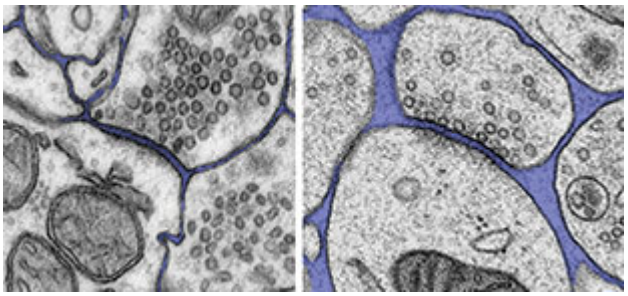
A common tissue fixation method distorts the true neuronal landscape.

By Kerry Grens | October 1, 2015



TIGHT SQUEEZE: Chemical fixation compacts synapses in a mouse brain (left), compared to freezing, which maintains the extracellular space (blue; right).
GRAHAM KNOTT

The paper
N. Korogod et al., "Ultrastructural analysis of adult mouse neocortex comparing aldehyde perfusion with cryo fixation," *eLife*, 4:e05793, 2015.

The fix
Soaking brain tissue with chemical fixatives has been the go-to method of preserving specimens for decades. Yet few neuroscientists take into account the physical distortion that these chemicals cause. And even among those who do pay attention, "we don't really know in quantitative terms how much really changes," says Graham Knott, a morphologist at the École Polytechnique Fédérale de Lausanne in Switzerland.

Shrinkage
Comparing fresh to fixed tissue, Knott and his colleagues found that chemical fixation shrank the tissue by 30 percent. "It raises the question of, 'What on earth is going on if it shrinks that much?'" says Knott. To find out, they turned to an alternative preservation approach, rapid freezing and low-temperature resin embedding, which was shown in the 1960s to better capture the natural state of the brain. Using a high-pressure version of this cryo-fixation technique, they observed neurons swimming in extracellular space and smaller astrocytes than are seen in chemically fixed samples.

Reality
NIH investigator Kevin Briggman says Knott's technique offers a much more accurate snapshot of the brain. An added bonus is that the elbow room around neurons afforded by cryo fixation makes it easier for automated methods to count cells or analyze structures. The only problem, he adds, is that, in contrast to chemical fixation, "you can't freeze a whole mouse brain."

The compromise

Briggman and Knott don't advocate doing away with fixatives. Rather, Knott says, scientists who use them should consider their effects when interpreting data. "We need to use models that pay very careful attention to how tissue has reacted to chemicals."