

VIEWPOINT

Analyzing postmortem brains for autism? Proceed with caution

BY MANUEL CASANOVA

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Some years ago, I received an email from Jane Pickett, director of brain resources and data for what was then the Autism Tissue Program (now **Autism BrainNet**). Jane was excited: She had collected a brain weighing well over 1,700 grams — notably larger than that of the average adult.

“It could shed light on the nature of macrocephaly in autism,” she said. **Macrocephaly**, or a large head, sometimes accompanies autism. Although I hated to disappoint her, I explained that the increased size was likely due to a postmortem artifact: After death, the molecular pump that maintains the cell’s balance of electrolytes shuts down. In this state, inappropriate handling of the brain allows water to diffuse freely into cells, causing the brain to swell. The observed macrocephaly, which was not apparent during this person’s life, was therefore not a likely consequence of autism, but a change that affected the tissue after death.

These sorts of artifacts are pervasive in postmortem brain samples. Brain weight, for example, can be affected by many variables, including the individual’s cause of death, the duration of a terminal illness and the method of tissue fixation¹.

For nearly 20 years I was a medical examiner in Washington, D.C., and director of brain banks at both Johns Hopkins University in Baltimore and the National Institute of Mental Health. During that time, I examined every piece of donated tissue and found that only 1 in 10 brains was of sufficient quality to be added to the collection.

Researchers who study postmortem brains must identify and control for artifacts. And any scientist who relies on the results of postmortem studies must learn to recognize when the data may be tainted by death itself.

Identifying artifacts:

Scientists are aware that brain tissue can change after death, but these changes are largely unacknowledged in the literature².

In a 1998 study of the neuropathological abnormalities associated with autism, researchers reported that four of the six brains they examined were larger than normal³. However, three of these brains also showed evidence of postmortem swelling. When swelling occurs during life, the brain pushes through openings in the skull. But these brains showed no herniation, suggesting that the swelling took place after death.

The researchers also noted that one of the brains was soft to the touch and contained numerous bacteria. These conditions are sure signs of putrefaction, and the samples should never have been included in any study.

Improper preservation can present another opportunity to introduce artifacts. Because the brain contains a large amount of water, freezing a harvested sample too slowly allows ice crystals to form. These jagged shards can cut through delicate cell membranes, destroying cells' structure and dispersing their contents. Such changes can confound studies that require quantifying the molecular contents of particular cell types, such as comparisons of gene expression.

In addition to postmortem changes, the process of dying itself can alter the brain. In 2007, I conducted a survey of the material collected by the Autism Tissue Program⁴.

Of the 35 brains from people with autism, 11 belonged to individuals who had drowned and 23 were from people who had died from a variety of causes, many of which involved a loss of circulation followed by restoration of blood flow to the brain. Episodes of 'ischemia and reperfusion' generate reactive chemicals that damage neurons, particularly in the membranes that make up the brain's white matter (nerve fibers and their insulating sheaths).

So, the appearance of inflammatory cells in this white matter is more likely to reflect how the individuals died than anything to do with autism. Seizures — which are common among autistic people — can trigger the same type of inflammatory reaction. Autism researchers should therefore be skeptical that these kinds of changes are scientifically meaningful.

Quality assurance:

Recognizing and controlling for potentially misleading data requires a concerted effort from those who work with or rely on results generated using postmortem tissue. The process of quality assurance begins with the tissue banks.

During my years as a medical examiner, we subjected samples to gross and microscopic examination, and we made careful records regarding conditions that might have compromised the tissue's condition. We also obtained detailed histories of the donors, including blood samples and

information on diagnosis, medications and cause of death. This record-keeping may seem excessive, but a donor brain with no history is essentially useless.

Thorough assessment of brain morphology requires expertise in neuropathology. Even those in closely related fields, such as neuroanatomy, do not necessarily have the training to recognize pathological changes, much less distinguish them from postmortem artifacts.

However, even the brain bank's quality assurance can sometimes fall short. In a 2010 note in the Autism Tissue Program archives, investigators remarked that, on examination, the tissue they had received "... showed extensive degradation." Even more alarming, the samples went on to be used in additional studies even after this cautionary note became part of the bank's published records.

Ultimately, it falls to those conducting the studies — and those reading them — to exercise vigilance when it comes to interpreting results derived from postmortem tissues⁵. As with any study, controls should match samples in terms of age and sex, history of seizures, medication use and condition prior to death. In postmortem studies, the tissues must also have been handled the same way following autopsy.

Using various methods, researchers should confirm sample quality and determine whether the tissue is suitable for the experimental design. Consulting a neuropathologist could help with these assessments.

Despite the challenges of working with postmortem tissues, postmortem brain studies still offer the surest path to quantifying the changes that take place in the brains of people with autism⁶. Brain imaging techniques do not come close to matching the resolution of postmortem tissue examination. Ultimately, the key to treating autism rests in our ability to accurately read the neuropathological clues etched in the brain after death.

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