

Size Matters: Brain's Inner Matrix Is Twice as Large as Once Estimated

A CITY COULD NOT function without its streets. The brain, too, needs its conduits for communication, nutrient supply, and waste disposal—functions that are believed to be served by the so-called extracellular space (ECS). Scientists know that this space is filled with fluid containing proteins and salt, akin to spinal fluid and the matrix around most of the body's cells, but until now they've been uncertain about its exact size.

Since the 1970s a number of research groups have focused on the ECS, according to Charles Nicholson, Ph.D., Professor in the Department of Physiology and Neuroscience. He and his colleagues, however, are the first to measure it precisely. As it turns out, the ECS is twice as wide as previously estimated: between 38 and 64 nanometers wide, which is about one seventeen hundredth the diameter of the average human hair. The scientists presented their results in a recent issue of *The Proceedings of the National Academy of Sciences*.

Researching the brain's unexplored extracellular space, says Dr. Nicholson, helps to advance our knowledge of the brain's anatomy. One practical application

of this knowledge is drug development. For example, a promising drug must not only pass the blood-brain barrier—the membrane that keeps many blood-borne infections and toxins from crossing into the brain. It must also travel through the ECS. If a brain tumor drug is too bulky, it will not reach the tumor.

Once a drug is administered, many factors affect how it might reach its target. Yet the last leg of a drug's journey always involves diffusion, says Dr. Nicholson. Diffusion refers to the spread of molecules through a medium in which they randomly bump into other molecules or boundaries. "By studying how certain molecules diffuse, you can say something about the structure through which they are traveling," he says.

In research that draws on such diverse disciplines as neuroscience, physics, mathematics, pharmaceuticals, nanotechnology, and engineering, the team used special fluorescent probes to explore the ECS's unmapped terrain.

Despite its difference in size, the rat brain is actually an excellent model for studying the human brain, explains Dr. Nicholson. The scientists chose to work with live, anesthetized animals

because the ECS shrinks and changes after death.

The experiments were set up to compare diffusion of different-size molecules, such as dextrans, which are water-soluble polymers used in ophthalmic solutions.

Robert G. Thorne, Ph.D., a postdoctoral fellow and co-author of the study, also chose quantum dots, which are tiny semiconductor crystals. They have been engineered to emit light in a range of colors over an extended time period.

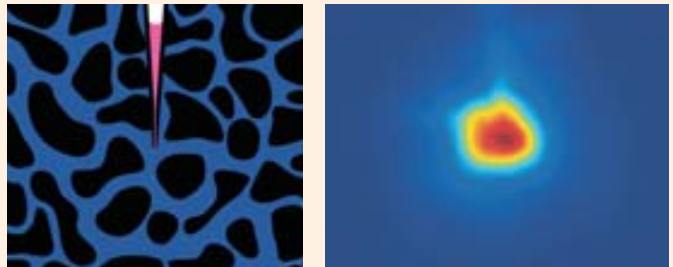
Quantum dots have been used since the late 1990s in many ways: in biology to tag molecules and in electronics to create light devices.

Using a thin pipette, the investigators delivered a few drops containing the probes to the ECS of rats. For the sake of comparison, probes were also added to an environment with known diffusion properties: a lab dish of

through the rat's cortex.

The experimental data were then analyzed mathematically with the help of software written by Dr. Nicholson. To explain how this analysis of diffusion data led to an estimate of ECS width, he uses an urban analogy. At rush hour in Times Square, people are not exactly making a beeline for the subway. "They have to follow the streets, and this delays them a certain amount," says Dr. Nicholson. That factor is called tortuosity. There is also much more crowding than if the commuters poured from their buildings into an open field. The crowding provides information about the volume of a given space. So the tortuosity and volume data help researchers calculate the dimensions of the ECS.

The experiments had their challenges. The probes



THE BRAIN'S ECS IS BEGINNING TO BE MAPPED: ITS DIMENSIONS ARE LARGER THAN PREVIOUSLY ESTIMATED. LEFT, A GRAPHIC RENDERING OF THE INTERCELLULAR MATRIX OF THE RAT'S NEOCORTEX. AN EXPERIMENTAL PIPETTE CAN DELIVER A FLUORESCENT PROBE TO THE TISSUE. RIGHT, A PSEUDOCOLOR IMAGE RENDERING OF THE FLUORESCENT PROBE'S DIFFUSION CLOUD.

agarose, a gel-like material consisting of seaweed extract. Much like the way a drop of food coloring would disperse in a glass of water, each probe created a diffusion cloud, which scientists imaged and analyzed. The quantum dots took 39 seconds to diffuse in agarose and 30 minutes to diffuse to the same extent

had to be kept from clumping and the quantum dots required a special coating to prevent them from disintegrating in the brain's salty, watery environment. The team is now testing various types of molecules to better understand the ECS, the brain's vital matrix. ●

—Vivien Marx