Biological Psychiatry: Celebrating 50 Years

Nanovesicles: A Novel Window Into Neuronal Functioning

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The human body may be thought of as composed, in the first instance, of the humors blood, phlegm, yellow bile, and black bile. Health is primarily the state in which these constituent substances are in the correct proportion to each other, both in strength and quantity, and are well mixed. Pain occurs when one of the substances presents either a deficiency or an excess, or is separated in the body and not mixed with the others.

—Hippocrates, *On the Nature of Man*

For thousands of years, physicians and scientists have strived to understand what is perhaps the most central question in all of medicine: how does the body coordinate its innumerable regulatory processes—from breathing and circulation, to temperature, sleep/wakefulness, appetite, and acid/base balance—and maintain homeostasis? While the Greeks (and ancient Egyptians before them) had localized human intelligence to the brain, it was more than 2000 years before humorism fell by the wayside and scientists began to appreciate the ways in which the central nervous system governs these processes.

The brain is well poised—and well protected—to serve in this role. The human body has evolved multiple levels of defense to keep it safe. The brain is encased within the skull and, further, within the thick dura. It is protected not only from external threats but also from internal threats. As early as 1885, scientists began to appreciate the concept of the blood-brain barrier, when Paul Ehrlich found that when he injected dye into the blood circulation of mice it did not stain the brain or spinal cord. While this was initially thought to be an impenetrable boundary, after the introduction of the scanning electron microscope in the 1960s scientists were able to visualize the unique cell–cell junctions that create the permeable—though highly selective—barrier.

While this boundary is crucial to protect this vital organ, it also poses a unique challenge: how can the brain coordinate the body's complex functions from within a quarantined space? Obviously, some communication may occur via direct electrical signaling—but this is only helpful for direct point-to-point communication between cells (e.g., to stimulate movement of a muscle). Most ways in which cells would ordinarily communicate (including through the release of neurotransmitters) are blocked: only a select group of cells are able to release a limited set of neuroendocrine signals that are able to cross the blood-brain barrier.

For researchers in psychiatry and neurology, this boundary is especially problematic: the same mechanisms that protect the brain from external and internal threats limit the tools we can use to study it. Whereas gastroenterologists or pulmonologists or oncologists may be able to directly obtain a biopsy specimen from diseased tissue, psychiatrists are generally forced to make do with indirect measures of brain function. For example, while it was clear by the 1980s that a primary aspect of posttraumatic stress disorder was dysregulation of the body's stress response system (including via the hypothalamic-pituitary-adrenal axis), there was no direct way to evaluate it; the best approach at the time was to collect 24-hour urine samples and measure cortisol excretion (1).

While our technology has improved dramatically over the past 20 to 30 years, we still struggle to directly assess cellular functioning. Our most cutting-edge research tools continue to rely on indirect signals that are generated by large populations of neurons (e.g., electromagnetic signals, as measured by electroencephalography or magnetoencephalography, and blood flow/metabolic signals, as measured via functional magnetic resonance imaging, positron emission tomography, or functional near-infrared spectroscopy). Under conventional circumstances, we still lack the ability to directly access cellular function in the brain. Only recently have scientists uncovered a different process—one that has been hidden in plain view for quite some time.

The first insight came from seemingly unrelated research conducted in the early 1980s. Researchers were studying the enigmatic process of red blood cell development, a process during which cells extrude their nucleus and lose most internal organelles and surface receptors. The hypothesis at the time was that surface receptors were likely being degraded by lysosomes inside the cell—the same as any other internal waste (2). Surprisingly, researchers discovered a different process that was taking place: the cell membrane would invaginate and form an internal, membrane-bound compartment (an endosome); the compartment was then filled with multiple intraluminal vesicles; these, in turn, were filled with a variety of endoplasmic materials; and, finally, this compartment would merge with the cell membrane and release the vesicles outside the cell. At the time, these vesicles—now termed exosomes—were assumed to primarily act as waste disposal. For more than 20 years, this is where things stood. Over the past decade, research into exosomes has exploded.

One of the first new findings was that exosomes are far more prevalent than initially thought. They may be produced in a wide range of cell types, including epithelial cells, adipocytes, and tumor cells. They can then be found in many accessible body fluids, including blood, cerebrospinal fluid, urine, saliva, and breast milk (3). Of particular interest to neuroscientists is that exosomes both are produced by neurons and can also cross the blood-brain barrier—meaning that they represent an additional means of traversing the brain's natural quarantine.

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The next major finding was the discovery that the material inside of exosomes is not merely waste: to the contrary, a seminal paper in 2007 showed not only that exosomes may contain nucleic acids (as well as proteins and lipids) but that they may also play an active role in cellular signaling (4,5). For example, exosomes have been shown to modulate immune functioning, with both immunosuppressive and immune-activating effects (6).

While researchers are still working to understand the scope and significance of exosome signaling, particularly with respect to the brain, this work has already opened new dimensions of research. One crucial finding has been that each exosome contains surface membrane proteins that represent a distinct cellular "fingerprint"—thus, from peripheral blood, it is possible to extract and isolate those exosomes that come specifically from neurons, astrocytes, microglia, and oligodendrocytes (4). The content of these vesicles then offers a unique window into the parent cell's functioning. For example, the protein synaptopodin is essential for normal synaptic structure and is known to be taken up by damaged neurons after neurologic injury. Accordingly, measuring synaptopodin levels from peripherally drawn neural exosomes may offer both a real-time means of assessing the extent of brain damage (akin to measuring troponin levels to assess damage to the heart after myocardial ischemia) and also a way of tracking improvement with levels of treatment (7). Another active area of investigation relates to microRNAs (miRNAs)—a class of small, noncoding RNAs that regulate gene expression at the post-transcriptional level (8) and that are thought to be involved in a range of medical (e.g., cancer and cardiovascular disease) and psychiatric illnesses (e.g., depression, bipolar disorder, and schizophrenia). As with so many other things, research into the role of miRNAs in psychiatric illness has historically been limited by the impermeability of the blood-brain barrier—on their own, miRNAs cannot cross. Looking inside of exosomes, though, may offer a crucial glimpse at this aspect of neural functioning, with significant implications for both health and disease.

There are other areas of medicine that have similarly struggled due to the inaccessibility of target tissue—most notably, fetal development. We know that in utero exposure to substances or stress can impair neurodevelopment, but historically there has not been any way to assess this in real time—until now. A recent observational study with pregnant women, some of whom had heavy alcohol consumption, showed that fetal neural exosomes can cross both the fetus's blood-brain barrier and the placental barrier and then be isolated from the mother's blood; moreover, they could also be used as a marker of neuronal damage, thereby offering a real-time metric of fetal brain health (9).

If exosomes are involved in normal physiological signaling, it may also be that they play a role in disease states. For example, it is well known that the pathogenesis of Alzheimer's disease includes the overproduction of amyloid beta and hyperphosphorylated tau proteins. Yet how this process begins—and how it continues to develop over time—remains a mystery. Recent research has shown that despite the beneficial effect of facilitating intercellular communication, exosomes may also play a role in propagating the disease process—an idea with major implications for both future research and treatment (10).

Since the origin of our field, the greatest historical barrier to psychiatric research has been the inaccessibility of brain tissue. While we are only beginning to understand the scope and function of exosomes, emerging data suggest that they might offer a sort of noninvasive "liquid biopsy" of the brain—a direct measurement of neuronal cellular functioning. Such data have the potential to transform the way we diagnose, monitor, and, one hopes, ultimately treat neuropsychiatric disorders.

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