Innovation is a form of purposeful discovery behavior that exploits the unexpected, uses imagination, and provides 1 avenue of new solutions to complex human health needs. It is through this lens that 2 examples are described in which innovative approaches have been used to dissect the complexities of stroke pathophysiology. The first example focuses on one of the most fundamental genetic factors relevant to the brain and ischemic injury: biological sex. Much might be gained by understanding the details of sex-specific pathobiology, if the field is to develop therapies that work well in patients of both sexes. The second example surrounds brain–spleen cell cycling after stroke which is fundamental to our evolving understanding that stroke is a systemic disease, rather than solely a lesion of the brain. Although much work remains, it is now apparent that brain–spleen cell cycling is temporally specific, varies in intensity, and involves cell players that are of much wider lineages than originally thought. In the future, it is likely that innovation will need to turn to big data, particularly if our field is to tackle the daunting questions that most greatly matter to unraveling brain injury. The huge availability and growth rate of biomedical data, handled in a shared but coherent environment, offers an opportunity to further vitalize stroke research.

Looking at Stroke Research Through the Lens of Innovation

The Willis Lecture Award recognizes the contributions of Thomas Willis who is widely regarded as the father of modern neurology. This 17th century clinician scientist was arguably the first to correlate bedside observations of neurological disease with anatomic constructs of the brain and its circulation. What is most striking about his career is its natural seeking of innovative solutions and novel answers to poorly understood problems. It is Willis as an innovator that makes him so compelling a historical model for those who seek new discoveries about stroke today.

Innovation is typically defined as creativity with a purpose. The word, innovation, crops up daily in our conversations now, an activity that seems almost self-evident in its desirability. But innovation is not necessarily a matter of technical capability. It is a purposeful discovery behavior that exploits the unexpected, uses imagination, and provides 1 avenue of new solutions to complex human health needs. It is through this lens that the 2014 Willis Lecture re-examined the impact of 2 stories of stroke research that unfolded through the combined efforts of investigators from many disciplines, frequently executed through collaborative team science. The first story surrounds one of the most fundamental genetic factors relevant to the brain and ischemic injury: biological sex. Several recent reviews are available for deep dives into the data surrounding this topic. The second exemplar, of brain–spleen cell cycling, is fundamental to the concept that stroke is a systemic disease, rather than a lesion solely of the central nervous system.

Sex Matters in Cell Death

Literature spanning decades has cataloged sex differences in brain structure, neurochemistry, and cerebral vasculature in animals. More recently, postmortem studies and neuroimaging using single-photon emission computed tomography, positron emission tomography, and structural and functional MRI (MRI and functional MRI, respectively) have unlocked sex differences in humans. There are not only many similarities, but also important differences between the brains of men and women in health and disease. Simple examples include the larger overall size brain in men than in women and a higher percentage of gray matter in women and of white matter in men. Global cerebral blood flow is higher in women than in men, at rest and during cognition, and there are sex-specific regional flow differences. The brains of both sexes are neurochemically distinct in dopaminergic, serotonergic, and GABAergic neurotransmission, and recent work is uncovering significant sex differences in cerebral gene expression and epigenetic regulation. Most striking are the differences in the structural connectome of the brain between the sexes. For example, at the supratentorial level, men have greater within-hemispheric connectivity, as compared with greater between-hemispheric connectivity in women. In the cerebellum, the opposite is true. The suggestion is that male brains are structured for greater connectivity between areas controlling perception and motor function, whereas female brains are better connected for communication. These structural differences have clear implications for stroke survivors, both in terms of the functional deficits experienced and the potential for re-connecting damaged neural pathways.
It has been recognized for many years that stroke rates are higher in men versus women globally\(^\text{10}\) and that this sexually dimorphic epidemiology persists until ages well beyond the menopausal years.\(^\text{11}\) In children, sex differences in stroke risk and pathobiology are evident even before puberty.\(^\text{12}\) Although the importance and mechanisms of female gonadal steroids have been heavily studied in the injured brain, the aggregate data suggest that hormones do not fully account for male versus female cerebrovascular disease patterns. An alternative explanation is that chromosomal, genetic sex (XX versus XY based) acts on a molecular platform established early in development by sex steroids.

The jump from the known epidemiology to a new understanding began by simply asking if outcomes in experimental models of brain injury also reflected this sexual dimorphism, and if so, how deeply did sex differences penetrate into mechanisms of cell death. Early data from standard focal and global cerebral ischemia models in animals suggested that favorable outcomes occurred more frequently in women than in men, even in the presence of comorbidities, for example, diabetes mellitus and hypertension.\(^\text{2,13,14}\) These data pointed the way toward the need to solve technical demands that would allow the use of sex-stratified, in vitro cell systems in stroke research. The new approach allowed the challenge of the long-held assumption that the mechanisms and outcomes of injury in culture are independent of the sex of the cells.

Consequently, studies performed in primary cell cultures (ie, cells obtained from sexed rodent embryos and grown in the absence of sex steroids in the culture media) were able to demonstrate and track the relative sensitivities of XX and XY cells to injury. Similarly, sex differences could be elucidated in experimental paradigms that use pharmacological probes to evaluate molecular signaling cell death pathways. The earliest example is from the study by Du et al\(^\text{15}\) with systematic observations that male and female neurons proceed to cell death via differing molecular pathways. XY neurons are more susceptible to glutamate- and peroxynitrite-(ONOO\(^-\)) induced injury, whereas XX neurons are more sensitive to staurosporine, triggering apoptosis. To simulate ischemic injury, we and others have exposed sex-stratified primary astrocytic or cerebral microvascular endothelial cell cultures to oxygen-glucose deprivation. In each case, XX cells tolerated oxygen-glucose deprivation to a greater extent than do XY cells (Figure 1).\(^\text{16,17}\) In part, XX astrocytes enjoy relative protection because of their capacity to express high levels of P450 aromatase, allowing the local synthesis of antioxidant estradiol. Studies of various neuronal populations suggest that sex differences are more varied than in astrocytes, with reports of high XY sensitivity in hippocampal neurons\(^\text{13}\) but high XX sensitivity when neurons are selected from cerebellar granular layers.\(^\text{19}\) More structured tissue cultures, such as those arising from hippocampal slices, demonstrate high XY sensitivity to oxygen-glucose deprivation relative to matched female slices.\(^\text{20}\)

Using sex-stratified animal and cell models, it became possible for the first time to understand how XX versus XY cells might rely on differing ischemic cell death molecular signaling or engage differing protective molecules in the face of injury. Such molecules include apoptosis-inducing factor, caspase 3, glutathione, both the neuronal and inducible isoforms of nitric oxide synthase, poly-ADP ribose polymerase, P450 aromatase, superoxide dismutase, and others.\(^\text{2}\) Many sexually dimorphic molecules have been identified through the use of knockout mice because the practice of using all male animals has lessened, but much work remains. For example, only a few studies have used tissue- and cell-specific knockouts to more carefully parse out sex differences. And few sex-specific mechanisms have evaluated for their hormone-dependent or -independent properties. Given the high failure rate of experimental neuroprotective therapies to advance care for patients of either sex, capitalizing on the sex specificity of death mechanisms could be a valuable first step toward personalized therapeutic interventions.

Finally, although the epidemiology of stroke incidence may be favorable to women versus men until advanced age, outcomes from stroke in women are not favorable. Once stroke occurs, women can sustain severe damage with high short-term mortality relative to men and experience considerable loss of quality of life.\(^\text{21–23}\) So much might be gained by understanding the details of sex-specific pathobiology.

**Brain–Spleen Cross-Talk After Stroke**

A second story of research innovation centers on the underestimated effect of dual supersystem activation (the central nervous system and the immune system) and how this interaction complicates cerebral ischemia. During the course of this work, we learned that stroke in humans and animals precipitates damage to immune organs and that spleen cells engage in cross-talk with the brain lesion. By way of context, it has been long recognized that patients with acute stroke may survive initial brain events, only to experience delayed recovery, or even death, because of infectious complications. The potential for aberrant systemic immune function in such complications has been described clinically and experimentally (for reviews, see Chamorro et al,\(^\text{24}\) Vogelgesang and Dressel,\(^\text{25}\) Offner et al,\(^\text{26}\) Emsley and Hopkins,\(^\text{27}\) and Chamorro et al\(^\text{28}\)). But the underlying immunology has been understudied, in part because the stroke field lacked neuroimmunologists at the experimental table. In this story, the jump from the known complications of stroke to a new understanding of process began through the questions of an immunology colleague about the state and function of the poststroke spleen, thymus, and lymph nodes in murine focal stroke models.\(^\text{29–40}\)

To answer these novel questions, we and others began to evaluate how focal cerebral ischemia altered the structure
and function of lymphoid tissue outside of the brain. For the first time, spleens, thymi, and lymph nodes were examined alongside the brain in our standard mouse stroke models. We found that ischemic brain injury leads to biphasic consequences. First, there is a rapid splenic activation within 6 hours of the brain insult in which splenocytes produce massive internal levels of inflammatory chemokines and cytokines (eg, tumor necrosis factor-α, interleukin-6, interleukin-2, monocyte chemoattractant protein-1, and macrophage inflammatory protein-2), with similar changes occurring in lymph nodes and blood.29,30 Perhaps in response to sympathetic nervous system hyperstimulation, the activated spleen releases subsets of immune cells into the blood, for example, T and B lymphocytes, macrophages, and dendritic cells. Second, there is a delayed, but massive and progressive, splenic apoptosis in which the organ visibly shrivels in size, and its cells are selectively destroyed in their inflammatory milieu. By 96 hours after stroke, spleen cell numbers are drastically reduced relative to sham-treated mice or mice naive to injury. These splenic changes do not seem to directly influence early infarct size in mice, but the animal’s ability to respond to antigenic challenges is exhausted (a significant measure of immunosuppression). For example, T-lymphocyte response to antigens concanavalin A and anti-CD3 monoclonal antibody are all strongly decreased, likely attributable to reduced T-cell numbers and the increased suppressive activity of T regulatory cells. Some part of these processes may also occur in patients. Pilot studies suggest that daily spleen size changes after acute ischemic stroke, first a contraction then re-expansion, as measured by abdominal ultrasound. When individual patients were further analyzed, daily spleen volume changes were positively associated with clinical course.41

Subsequent studies deepened our understanding that cells of splenic origin enter the brain and amplify known local brain inflammatory processes arising from polymorphonuclear neutrophils and resident microglia. We coined this process as brain–spleen cell cycling after stroke (Figure 2). The evolving brain injury signals for the biphasic activation and atrophy of the spleen, followed by apoptosis and compromised immune function. Remaining intrasplenic cell subsets are released into the blood, followed by trafficking across a cerebral microvasculature replete with inflammatory display of adhesion molecules and chemokines. Interestingly, early studies now show that some of these processes are influenced by sex and sex steroids.33,35,39

A key assumption in the cycling hypothesis is that adaptive immune cells must be triggered through an encounter with brain-derived antigens, either in soluble form or as presented by macrophages or dendritic cells. Under ordinary circumstances, the central nervous system is generally immune privileged in that brain is isolated from the immune system by a functional blood–brain barrier. After injury, antigen-presenting cells may leave the brain and be transported by the blood or cerebrospinal fluid to lymphoid tissue, including the cervical lymph nodes. A unique observation is that brain-derived material, for example, myelin or subunits of...
the N-methyl-D-aspartic acid receptor, can be identified by immunoreactivity in the palatine tonsils and cervical lymph nodes of patients with acute stroke. Leakage of brain autoantigens such as myelin basic protein or myelin oligodendrocyte glycoprotein might produce important autoimmune responses. For example, using an adoptive transfer technique and myelin oligodendrocyte glycoprotein-reactive spleen cells, myelin oligodendrocyte glycoprotein-reactive splenocytes which secrete toxic Th1 cytokines (eg, interferon-γ and tumor necrosis factor-α) were transferred into severe combined immunodeficient mice. This maneuver resulted in increased infarct size, increased neurological deficits, and a higher accumulation of immune cells in ischemic brain in the treated mice relative to control animals.

Although much work remains, brain–spleen cell cycling may be temporally specific and varies in intensity, depending on the size of initial brain infarction. The cell players involved are of much wider lineages than originally thought, involving both innate and adaptive immune cell types, and may be controlled by different mechanisms. For example, a previously unknown role for B regulatory lymphocytes in experimental stroke is now widely recognized. B Regulatory cells, acting through interleukin-10–dependent protective mechanisms, diminish stroke volume, improve neurological damage, and reduce infiltration of inflammatory leukocyte subsets such as Gr1+ neutrophils, CD3+ T lymphocytes, and CD11b+CD45high macrophages after focal cerebral ischemia.

Embracing the Next Innovation

Watchers of innovation, and those of us who hold large ambitions for the future of stroke research, will readily seek bold new approaches across basic, translational, clinical, and population sciences. As shown by the 2 exemplatives offered here, innovation springs quickly when collaborators of different disciplines learn to speak each other’s language. Or when quixotic, out-of-the-box questions can rise to the forefront of one’s research. But in the future, innovation in stroke research will need to turn to big data, particularly if we are to tackle daunting questions that taunt us with difficult breakthroughs. The huge availability and growth rate of biomedical data, handled in a shared but coherent environment, offers an opportunity to further vitalize stroke research. Unlike the advances in cancer care and discovery, big data approaches are still rare in stroke studies at the bench or in the clinic. Envision the potential for innovation as we accumulate the stroke genome on a thumb drive, linked to clinical and epidemiological data, and coupled with poststroke brain connectome imaging. Perhaps even Thomas Willis would be intrigued!

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References


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Patricia D. Hurn

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