

Of Mice and Humans



What animal research means to neurology.

BY GINA SHAW

Back in the 1930s, patients receiving early rabies vaccines occasionally developed neurologic complications so severe that the situation was called a “neuromyolytic catastrophe.” About 1 percent of recipients of the vaccines—which had been grown in duck embryos—developed fevers, altered mental status, seizures, and limb weakness. Sometimes the reaction was fatal.

When the brains of people who died after these reactions were examined, the changes in the brain tissue didn’t resemble rabies, as doctors were expecting. Instead, they resembled a severe form of multiple sclerosis (MS).

Scientists wondered if it was possible that the patients had had an allergic reaction to the brain tissue from the duck embryos in the vaccine, and more tantalizingly, if a similar “allergic reaction” was going on in MS itself.

“The proof of the pudding was when they were able to take duck brain tissue without any rabies in it and recapitulate the disease, first in nonhuman primates, and then rodents,” says Richard Ransohoff, M.D., director of the Neuroinflammation Research Center at the Cleveland Clinic in Ohio. That disease model of MS became

known as experimental autoimmune encephalomyelitis (EAE), and it has provided some of the most important insights into the origins, pathology, and treatment of MS. In fact, EAE is one of the oldest animal models known to exist of any human disease.

“Our entire theory of MS has been in large part shaped by experience with EAE,” says Dr. Ransohoff.

Before virtually any drug manufactured today is tested in a human being, it is first studied in animals—usually mice or rats, and sometimes when necessary in other mammals like primates. Scientists can’t learn everything about how a drug will behave in human beings by studying it in animals, but they can glean many important insights about effectiveness, side effects, and dosage levels.

Even before new treatments are developed, research on animals has proven essential to understanding just how complex disease processes like Alzheimer’s, Parkinson’s, and MS are, what their underlying causes might be, and the best approaches for developing new treatments.

Animal research has been instrumental in developing treatments for multiple sclerosis, stroke, Parkinson’s, and other neurologic disorders.

“For almost every disease in neurology, I could identify animal studies going on now that are making a difference in how we diagnose, understand, and treat it,” says Jasper Daube, M.D., professor of neurology at the Mayo Clinic in Rochester, MN.

“Combine these studies with other research in animals—some 15, some five, some two years ago—they all add up to giving us the treatments we have, or at least identifying the underlying abnormality.”

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MULTIPLE SCLEROSIS AND ANIMAL RESEARCH

For neurologic diseases in particular, certain insights and advances would be virtually impossible without animal models like EAE.

"In most neurologic diseases, the peripheral tissues are normal, and studying them doesn't tell you very much," says Dr. Ransohoff. "You can get your hands on blood or spinal fluid, but you can't get brain tissue until people die, unless they have a biopsy, which is very invasive and not commonly done for most neurologic conditions. And people who die of a neurologic disease, like MS, do so after many years. That means that the brain tissue at that point is not representative of what's happening in the early to middle part of these diseases, when most of us think they are most treatable."

It's also almost impossible to study neurologic disease effectively by looking at cells in the laboratory. "The minute you take one of these networked brain cells, rip it out of the brain, and plate it on plastic or glass and feed it serum or growth factors, you've made it abnormal," Dr. Ransohoff says. "In many cases, you've destroyed everything that characterizes how the cell works in the intact brain. So we need living organisms to study these diseases."

Powerful regulations are in place that protect animals used in medical research. More than 50 federal laws exist to protect animals, but the Animal Welfare Act and the Health Research Extension Act of 1985 are the primary laws governing the use of animals in research. Many states also enact their own animal-research laws.

"The animals are treated well, housed well, and there are strict rules about the extent to which you can cause pain during any phase of research," says Dr. Ransohoff. "And whistle blowers who report violations of these regulations are also well protected by law."

But some animal rights organizations are now pushing to have animals given the same legal standing as humans, which would effectively ban all animal research since animals cannot give "informed consent."

Perhaps because of this pressure, many advocacy groups for people with neurologic conditions don't talk much about the advances made possible by research using animals. "If you go to the Web sites of many of these groups, they will rarely say what's being done in animal research for that condition," says Dr. Daube. "I've asked some of them if they could highlight

the importance of animal research, and their concern is the same—they might turn off some donors. But I think they're wrong. Some groups that are more public about animal research, like the Parkinson's Foundation, haven't been damaged by this openness, and it gives patients and family members a sense of how essential this research is."

In MS, for example, the vast majority of our current understanding of the disease has been worked out in the EAE animal model and then validated in human patients.

"Although not everything about the mouse immune system is the same as human, most things are," Dr. Ransohoff explains. "These models are now very refined, using inbred mice for which we know all their genetics. So we can select or modify individual genes and study exactly how that gene participates in the MS/EAE disease process."

The model isn't perfect. It's harder to study neurodegeneration—the long secondary progressive phase of MS—in the EAE model, perhaps because mouse and human brains differ more significantly than do mouse and human immune systems, and because of the short mouse lifespan.

But some of the most important treatments for MS available today have been worked out entirely using the EAE model. A recent example is natalizumab. In 1992, a paper in the medical journal *Nature* identified natalizumab's molecular target, along with the antibody that could be developed into a drug to suppress EAE. Almost exactly 10 years later, the pivotal clinical trials of natalizumab began.

ANIMAL MODELS OF STROKE

When a person has a stroke, a blood vessel gets blocked off, because a clot either forms in the brain or travels there from another part of the body. When that vessel is blocked, it delivers little to no blood, oxygen, or glucose to the brain. That sets off something called the "ischemic cascade"—a series of biochemical reactions that damage and destroy brain tissue.

But how do these reactions happen? You can't study them directly in human patients, not least because you'd have to actually cause a person to have a stroke in order to watch them from the beginning.

"Animal modeling of stroke has been critical to our understanding of the ischemic cascade," explains Marc Fisher, M.D., professor of neurology at the University of Massachusetts Medical School and author of *Handbook of Experimental Neu-*



rology: *Methods and Techniques in Animal Research* (Cambridge University Press, 2006). “It’s given us a pretty reasonable understanding of how the tissue dies in ischemic stroke. If you understand that, then you can start to think about treatments that might interfere with the process.”

Tissue plasminogen activator (TPA), the “clot-busting” drug that can reverse the effects of a stroke if used appropriately within three to four and a half hours of the onset of symptoms, was initially studied in an animal model. “Our insights from the animal stroke model, showing that TPA has a beneficial effect, were very important in thinking about how the clinical model might take place,” says Dr. Fisher.

Animal research has also provided important insights about the other side of the coin—the potential drawbacks of TPA. “There are deleterious effects of TPA on tissue, which we’ve learned from studying animals,” Dr. Fisher says. “We’ve also learned that after you restore blood flow to the brain—either using TPA, or mechanically by taking the clot out of the vessel—the rush of blood to the injured tissue can actually cause further damage, called ‘reperfusion effects.’ This is something we need to understand better.”

More recently, improved ability to do small-animal imaging with MRI has allowed stroke researchers to look directly at the evolution of stroke damage in a living animal. “We can label our clots so that they are easy to see on imaging, and look at the temporal course of the damage and how quickly TPA works in dissolving the clot,” says Dr. Fisher. “We can then relate that to reperfusion effects in the tissue. It will also allow us to compare other thrombolytic [clot-dissolving] agents to TPA to see if we can dissolve clots faster and more extensively with fewer damaging effects, such as hemorrhage [bleeding].”

Dr. Fisher and his colleagues have recently published animal-model studies showing that giving high-flow oxygen might be able to extend the time window in which TPA can be safely and effectively given to stroke patients.

INSIGHT INTO PARKINSON’S

A number of different animal models have been used to study the origins and evolution of Parkinson’s disease. Perhaps most commonly used, and the one that has yielded the greatest insights, has been the “MPTP” model. MPTP is a chemical that causes permanent symptoms of Parkinson’s disease by killing certain neurons in the brain. Used in mice, rats, and sometimes primates, the MPTP model has allowed researchers to study the effects of a wide variety of genes that are associated with human Parkinson’s.

“Most of the current therapies for Parkinson’s symptoms

developed over the years were all first tested in the experimental mouse, mostly the MPTP model, before being given to people,” says Serge Przedborski, M.D., Ph.D., Ph.D. professor of neurology and pathology at Columbia University Medical Center in New York and a leading Parkinson’s researcher. “It gives us a ballpark idea, before clinical trials, if the treatment is going in the right direction or not.”

Perhaps an even more exciting direction made possible by MPTP animal research has been the development of deep brain stimulation (DBS) for Parkinson’s disease. DBS uses a surgically implanted, battery-operated device called a neurostimulator—like a pacemaker for the brain—to send an electrical signal to the areas of the brain that control movement, blocking the abnormal nerve impulses that lead to Parkinson’s symptoms. Although it’s not a cure, DBS has significantly reduced symptoms for many people with Parkinson’s.

“DBS had been used for years in people with epilepsy, but the idea to use it in Parkinson’s disease came from work that had been done on the chemical neuroanatomy of the brain in MPTP monkeys,” explains Dr. Przedborski. “Scientists observed that once the neurons in the brain that made dopamine were destroyed, these monkeys given MPTP had hyperactivity in a certain area of the brain called the subthalamic nucleus. They found that if you used a toxin to destroy this nucleus, the Parkinson’s symptoms in the monkey disappeared. From that, they theorized that if you implanted a needle to jam this hyperactivity with high-frequency electric stimulation, you’d get the same effect. And that’s exactly what happened—creating a treatment that has been the second most significant advance in the management of Parkinson’s since the discovery of levodopa. If you eliminate the MPTP monkey, this never would have happened.”

What’s more, the lessons that have been learned from animal models in one neurologic disease can often be “cross-fertilized” into other brain and central nervous system disorders. For example, Dr. Ransohoff points out, researchers are taking lessons learned from the EAE model and from basic neuroscience and performing cross-over experiments in an effort to understand how inflammation works in other chronic neurodegenerative diseases.

“If we could get a handle on which aspects of inflammation are helpful and which are harmful, we might have tools in our arsenal of treatments that could be applied to many forms of neurodegeneration,” he says. “This kind of research is something that only animal models can help us do.” NN



For more information on animal research, see [RESOURCE CENTRAL](#) on page 33.