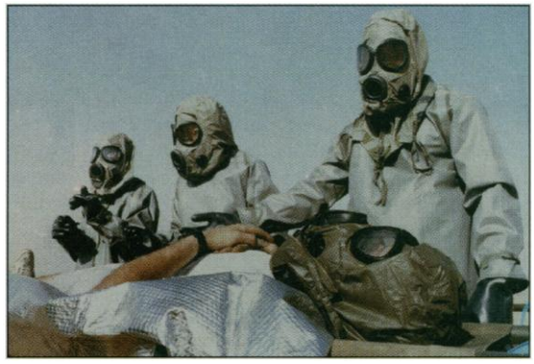
The Big Easy Serves Up a Feast To Visiting Neuroscientists

From 25 to 30 October, New Orleans was host to 24,000 neuroscientists at the 27th annual meeting of the Society for Neuroscience. With more than 14,000 presentations, the offerings were as rich as a New Orleans gumbo, spiced with new tidbits about topics ranging from prion proteins to Gulf War syndrome.

Rat Model for Gulf War Syndrome?

At the close of the 1991 Persian Gulf War, thousands of soldiers returned home apparently healthy, but reporting subtle problems ranging from muscle aches and fatigue to learning deficits or confusion. The U.S. government has officially attributed the collection of symptoms, known as Gulf War syndrome, to wartime stress, but that conclusion remains contentious. Many veterans, and a few researchers, point to another suspect: exposure to organophosphates (OPs), ingredients of chemical weapons and insecticides that were present in the Gulf.

But while OPs in high concentrations are extremely toxic, there has been little evidence that the low doses to which the Gulf War veterans were presumably exposed are harmful. Now, neuropharmacologists Jerry Buccafusco, Mark Prendergast, and Alvin Terry of the Medical College of Georgia and the Veterans Administration Medical Center in Augusta, and their colleagues, have found the first hints in an animal model that low-levels of OPs produce long-term problems in cognitive areas of the brain.



WIN MCNAMEE, POOL/AP PHOTO

At risk? The nerve gases these Gulf War soldiers prepared for may cause a learning deficit.

Buccafusco reported at the meeting that in a study of rats, OP treatment reduced the numbers of a receptor that enables nerve cells to respond to the neurotransmitter acetylcholine in an area of the brain—the hippocampus—known to be involved in learning and memory. The animals also developed learning deficits. And both the receptor loss and the learning problems lasted for weeks after the chemicals were gone.

While the result won't solve Gulf War syndrome, it is, says Philip Bushnell, a toxicologist at the Environmental Protection Agency in Research Triangle Park, North Carolina, "the first time anyone has seen cognitive deficits persisting this long after low-level exposure." The "interesting thing" about the work, he adds, is the correlation between the persistence of those deficits and the reduction in the number of the acetylcholine-binding "nicotinic" receptors, which a large body of evidence has shown to be important in cognitive function.

The idea that low OP doses might have subtle effects on the brain emerged in the 1960s when anecdotal reports suggested that agricultural and industrial workers experience memory and concentration problems after chronic, low-level exposure to organophosphates. Those reports attracted little attention, however, until similar symptoms cropped up among some Gulf War vets, and researchers began investigating the cognitive consequences of low-level OP exposures. But none of these efforts revealed deficits or brain changes that lasted beyond the period of exposure until this year, when Buccafusco's team did their study.

The researchers injected each of 70 rats with either a salt solution or a low dose—one that would produce no acute symptoms—of an organophosphate called diisopropylphosphorofluoridate (DFP) every day for 2 weeks. When they then evaluated the animals in the Morris Water Maze—a test of spatial memory that involves finding a hidden platform in a pool of water—they found that the DFP-treated rats took up to 30% longer than the saline-injected controls did to learn to navigate the maze. This learning deficit persisted for 3 weeks after the last DFP injection. It also appeared to spare long-term memories: Rats that had learned the maze before the DFP exposures performed as well as control rats.

In addition, the team has discovered a biochemical change in the brain that may explain the behavioral deficit. OPs block an enzyme that breaks down the neurotransmitter acetylcholine, causing the chemical to build up in the synapses between nerves and their targets. Because such increases may lead to compensatory decreases in the neurotransmitter receptors, the researchers looked at how DFP affects the brain concentrations of the two types of acetylcholine receptor: the muscarinic receptor, so called because it also binds the chemical muscarine, and the nicotine-binding nicotinic receptor.

They found that DFP immediately depressed levels of both receptor types in brain areas involved in cognition, including the hippocampus, striatum, and parts of the cortex. The muscarinic receptor quickly rebounded in all areas after the DFP treatment was stopped. But the numbers of nicotinic receptors in the hippocampus stayed low, bottoming out at 30% of baseline levels about a week after the treatment and remaining significantly depressed until the experiment was ended another 2 weeks later.

Those results hint that DFP might be causing learning problems by somehow depressing the numbers of nicotinic receptors. To test the idea, the Georgia researchers gave nicotine, which activates the receptor, to another 12 of 24 DFP-exposed rats just before they were plopped in the water maze. The results: The nicotine-treated rats performed just as well as rats that had received only saline injections.

Buccafusco is the first to concede that it's too soon to draw any firm connection to Gulf War syndrome. He points out, for example, that neither his experiments nor anyone else's can duplicate the chemical exposures of Gulf War veterans because it's been impossible to determine exactly what they were. "We don't know whether the troops were exposed to the same levels of organophosphates in the same time course as our rats. Nor do we know what other chemicals they were exposed to," Buccafusco says. But even if the findings are totally irrelevant to the Gulf War, he adds, with the widespread use of pesticides and the worldwide stockpiling of nerve agents, studying the effects of OPs will be highly relevant in the future.

–Ingrid Wickelgren

Protective Role for Prion Protein?

With the award of the Nobel Prize last month to University of California, San Francisco, neuroscientist Stanley Prusiner, prions have been in the news a lot lately. But in spite of the work by Prusiner and others implicating these rogue proteins in a variety of fatal brain diseases, including the United Kingdom's bovine spongiform encephalopathy (BSE or "mad cow disease"), the story has some gaping holes. One

of the largest: No one knows the function of the normal prion protein, known as PrP^c. Data presented at last month's neuroscience meeting by neurobiologist David Brown of the University of Cambridge in the United Kingdom may help remedy that.

His new findings suggest, Brown says, that PrP^c protects neurons by binding copper ions. When they run free, these reactive ions are highly toxic to cells. If the normal prion protein does help sequester the ions, the abnormal prion folding and clumping thought to cause BSE and other prion diseases could cause nerve cell death by interfering with this protective function.

Researchers who study copper binding are all abuzz about Brown's results. "All my friends in neuroscience e-mailed me [after Brown's talk] to ask what is going on with this," says Jonathan Gitlin, an expert on copper-binding proteins at Washington University in St. Louis. Gitlin himself says that PrP^c could well play a role in protecting nerve cells from copper ions.

But Brown's theory that a loss of the copper binding contributes to prion disease is more controversial. Many prion researchers believe that the destructive effects of the malformed prion protein, rather than loss of normal PrP^c function, cause the diseases. As prion researcher Adriano Aguzzi of the University of Zurich in Switzerland points out, mice that make no PrP^c because the gene has been knocked out do not get them.

Because those mice don't show obvious ill effects from the loss of PrP^c, Brown took a different route to identifying the role of the normal protein. Previous studies by other researchers had shown that a fragment of PrP^c binds copper in the test tube. Working in collaboration with Hans Kretzschmar of the University of Göttingen in Germany, Brown went on to see what effect this copper binding might have on brain cells.

In his first experiments, he cultured brain neurons from normal mice and from mice lacking a functional PrP^c gene. He found that cells without PrP^c are much more susceptible than the cells from the normal mice to poisoning by copper sulfate. Brown then went on to show that a peptide containing the copper-binding site of PrP^c could protect the mutant cells from copper's toxicity.

Further evidence that PrP^c may protect cells from copper came in experiments indicating that the protein binds the metal in living brains. PrP^c is normally found in the membranes of brain cells, and Brown reported that membranes prepared from the brains of normal mice "have a lot of copper"—almost 20 times more than those from the knockout mice. Because PrP^c is concentrated in the membranes of synapses, the specialized gaps where neurons are chemically coupled to one another, Brown postulated that it may serve to sop up copper ions released into synapses when neurons fire. In support of that idea, he showed that copper diminishes electrical activity in cerebellar neurons from the knockout mice that lack PrP^c, but not in cerebellar neurons from normal mice.

Brown proposed that PrP^c could have a more indirect role as well: It might pass the copper to other proteins, which could ferry it to enzymes inside the cell that need it for their activity. These include superoxide dismutase, which protects cells from damage by oxidizing chemical species that can form in cells from normal activities, such as energy metabolism. Indeed, he and Kretzschmar found less resistance to oxidative damage in neurons from PrP^c knockout mice compared to those of normal mice.

Even though prion researchers generally blame the abnormal protein for the cell death seen in prion diseases, Aguzzi says Brown's findings raise the possibility that the loss of a normal function could also contribute in ways that have been missed or somehow compensated for in the knockout mice. If so, the normal prion protein may end up stealing some of the attention its malignant counterpart is now getting.

–Marcia Barinaga

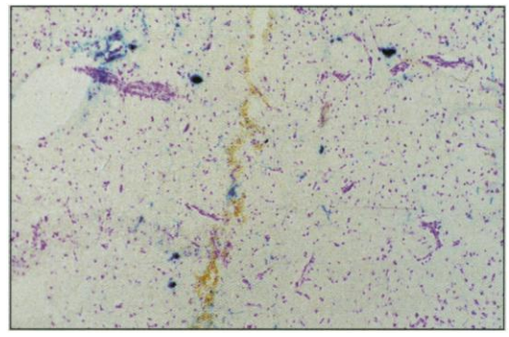
Heat Shock Protein Linked to Stroke Protection

Just a few years ago, neurologists believed they could do little to treat strokes. But recent work has shown that if they act quickly to restore blood flow with a clot-busting drug, they can limit the brain damage from strokes—and the accompanying deficits in memory, speech, or mobility (*Science*, 3 May 1996, p. 664). Now, neuroscientists Midori Yenari, Sheri Fink, Robert Sapolsky, Gary Steinberg, and their colleagues at Stanford University have identified a molecule that could lead to a new kind of stroke therapy.

As Yenari reported at the meeting, the researchers found that introducing the gene for a heat shock protein (HSP)—so-called because it is produced in response to increased temperatures or other stresses—into the brains of rats reduced the number of neurons that died after their blood supply was cut off. "It's a very exciting result," says Dennis Choi, a neurologist at Washington University in St. Louis. "It provides good evidence that heat shock protein has its arms around neuroprotective levers in the cell." While the technical obstacles of converting the finding into a therapy for stroke are formidable, adds Choi, "it's not beyond the pale."

The Stanford researchers undertook the study because earlier work had shown that HSP concentrations increase in the brain after experimentally induced strokes. Although the proteins are known to protect cells against damage caused by various stresses, "people didn't know whether they were doing anything useful" in stroke, Sapolsky says.

To find out, the Stanford team inserted the gene for HSP 72, which is particularly abundant in times of stress, into a modified version of the genome of herpes simplex virus (HSV), which can infect nerve cells. The researchers then injected this vector into the brains of 11 rats in a region called the striatum. They also injected another eight rats with a control HSV vector that did not contain the HSP gene.



M. YENARI, R. SAPOLSKY, *ET AL.*

Rescued. Rat brain neurons given a gene for heat shock protein 72 (blue stain) survive an experimental stroke.

After 12 hours, the team induced strokes in all the rats by temporarily choking off the artery that feeds the striatum on one side of the brain. Two days later, when they assessed the damage in the affected area, they found that only 60% of the striatal neurons carrying the control vector had survived, while 95% of those carrying the HSP-expressing vector were still alive. The researchers hypothesize that the HSP might be protecting neurons by binding to key cellular proteins and thus helping them keep their shape and function after a stroke.

Experts caution that a comparable gene therapy for human stroke patients is a long way off, at best. Experimental neurologist Howard Federoff of the University of Rochester Medical Center in New York points out, for example, that no known viral vector can spread widely enough in the much bigger human brain to cover the region typically damaged during a stroke. In addition, Sapolsky's team has yet to show that they can protect neurons by injecting the HSP gene after a stroke occurs. Other experiments by the Stanford group have shown that genes inserted into the HSV vector become active about 2 hours after the injections, with peak activity some 10 hours later. It's not yet clear whether that is soon enough for the additional HSP to ward off cell death.

Even if it isn't, it might be possible to use the gene therapy preventatively when the risk of stroke is high, as in certain surgical procedures. Alternatively, investigators could design small-molecule drugs that stimulate extra production of the HSP or mimic its cell-sparing effects.

–Ingrid Wickelgren