Sex and Violence in Neuroscience

Attendance at the annual Society for Neuroscience meeting, held 13 to 18 November in Toronto, Ontario, set a new record and reached a total of more than 13,000. Research topics ranged from mechanisms that regulate ion channel activity, nervous system development, and the neural bases of behavior, to the mysteries of Alzheimer's disease, schizophrenia, Parkinson's disease, epilepsy, and AIDS dementia. The following briefings describe advances in understanding how sex hormones may modify certain cognitive skills, how normal brain signaling mechanisms may go awry and cause nerve cell death, and how many cells appear to contain the genetic agents of their own destruction.

Sex Hormones Linked to Task Performance

The controversial suggestion that sex hormones help to shape the cognitive abilities of women and men has been given another boost. New studies by Doreen Kimura and Elizabeth Sampson of the University of Western Ontario in London support the notion that increased levels of female sex hormones correlate with relatively higher performance of fine motor skills and relatively lower performance on spatial reasoning tasks. The researchers caution that individual women and men vary greatly in their ability to perform these skills, but that taken as a group, performance of certain skills appears to segregate on the basis of sex hormone levels.

"We conclude that changes in female sex hormone levels can be associated with changes in cognitive performance," said Kimura at the meeting. She and Hampson are currently evaluating whether daily fluctuations in testosterone affect cognitive function in men.

"While other studies have examined the effects of female sex hormones on movement skills and spatial tasks separately, Kimura's studies pull things together," says Bruce McEwen of Rockefeller University in New York. Although the new work does not explore novel questions, it does show that improvements in motor skills occur in combination with decreased spatial abilities.

The Ontario researchers studied a total of 200 women in two general age groups—some who had regular menstrual periods and others who were postmenopausal. The idea was to compare performance on a variety of tests within the same group of women when their sex hormone levels were highest and lowest. In both groups the results showed the same pattern. Women perform best in fine motor tasks when their sex hormone levels are high and best in spatial reasoning tasks when their hormone levels are low.

Blood levels of the female sex hormones, estrogen and progesterone, are lowest midway through the menstrual phase and are highest about 2 weeks later in the mid-luteal phase of the cycle. "Women in the menstrual phase perform significantly better in spatial tasks," said Hampson. In contrast, the same group of women, aged 25 to 39 years, tended to perform worse in the spatial tasks in the mid-luteal phase of their cycles. The researchers use a variety of spatial reasoning tests such as picking out which complex line figures contain a simpler line figure and identifying which line figures could be achieved by rotating a given line figure in space.

Postmenopausal women who receive estrogen therapy tend to perform fine motor tasks better on the days when they receive the hormone than on the days when they do not, Kimura reported. In one kind of motor skill test, for instance, the women repeated a series of fine movements as often as they could in a given amount of time. "The effect of the estrogen is selective," said Kimura, because only fine motor skills and not other kinds of skills showed significant improvement. Changes in mood did not appear to be associated with changes in task performance in either the menstruating or postmenopausal women, she said.

The sex hormones are made by the ovaries and testes. They enter the brain through the bloodstream readily because, as steroids, they are derivatives of cholesterol and pass through cell membranes easily. Estrogen and testosterone clearly affect brain development during a critical period of fetal life and apparently have a later effect during another critical period after birth, says McEwen. This much has been known for approximately 30 years. But sorting out how or whether sex hormones have more direct effects on cognitive function during adulthood has been more difficult, which is one reason why researchers view the new work as significant.

The ways in which steroid hormones affect brain function is determined by two major factors. One is the distribution of their receptors in the brain. Sex hormone receptors are located in the nuclei of cells and more recently researchers have also identified steroid hormone receptors in the plasma membranes of nerve cells. The second factor is the distribution enzymes that convert the hormones to their common active metabolite, β-estradiol. Which of these, or other, neurobiological effects of sex hormones accounts for the changes in cognitive function reported by Kimura and Hampson remains to be determined.

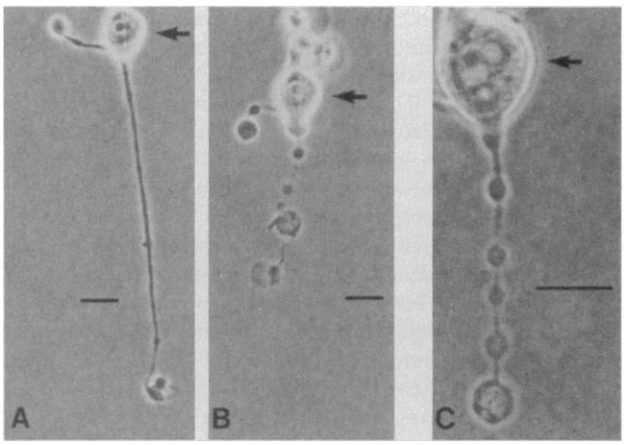
More Dimensions for Glutamate Toxicity

Certain populations of nerve cells in the brain appear to exist in a delicate balance between normal stimulation and a level of overstimulation that can kill them. A chief chemical culprit in this scenario is the amino acid glutamate, which acts as a transmitter that excites many kinds of brain neurons. New data presented at the recent meeting support the notion that nerve cell toxicity due to excess glutamate occurs by more than one mechanism and depends on more than one kind of receptor. Researchers are just beginning to sort out which of these toxic interactions may play a role in neurodegenerative brain disorders.

Glutamate is excitatory and potentially toxic because it binds to specific sites in nerve cell membranes and triggers the influx of sodium, potassium, and sometimes calcium ions, depending on the receptor site that is activated. In the past several years, researchers have emphasized that too much glutamate activation of one class of receptors—called NMDA receptors because they also bind *N*-methyl-D-aspartate—can kill nerve cells. Cell death is due, at least in part, to an uncontrolled rise in intracellular calcium ions.

Several groups of researchers have evidence that NMDA receptor overstimulation and nerve cell death occur in an ischemic attack, for example, when a stroke or heart attack diminishes the blood supply to the brain (see *Science* 15 January 1988, p. 254, and 6 February 1987, p. 632.). But new studies show that glutamate can kill cells by other mechanisms as well.

One alternative mechanism of glutamate toxicity results from "oxidative stress," says Joseph Coyle of The Johns Hopkins Uni-



Neuronal-glial hybrid cell *dying within minutes (A,B,C—enlarged) at the end of a 24-hour exposure to toxic levels of glutamate.* [*T. H. Murphy* et al., Brain Res., ***444***, *325 (1988)*]

versity School of Medicine in Baltimore, Maryland. He, Timothy Murphy, and Ronald Schnaar, also of Johns Hopkins, find that adding high concentrations of glutamate to cultures of neuronal-glial hybrid cells blocks a normal function of another amino acid, cystine.

"Glutamate inhibits the transport of cystine into these cells," says Coyle. "This causes a time-dependent reduction in the production of glutathione, a major reductant in cells." If cells can no longer convert enough cystine to glutathione they begin to die within 8 hours from damage by oxidizing free radicals such as superoxides ($O_2\cdot$), hydrogen peroxide (H_2O_2), and hydroxyls ($OH\cdot$). These oxidants are produced during normal metabolic reactions and they damage cell membranes, DNA, and proteins. Glutathione inactivation of such oxidants is one of several protective mechanisms that normally exist in cells.

Coyle and his co-workers find that this mechanism of glutamate toxicity is not dependent on NMDA receptors, because their line of hybrid cells does not express these receptors. Instead, glutamate competes with a membrane transport system that normally exchanges extracellular cystine for intracellular glutamate. This transporter selectively binds the glutamate analog quisqualate, an indication that this mechanism of toxicity depends on a separate class of membrane binding sites.

Another aspect of glutamate-related toxicity to brain neurons occurs because it activates a different kind of membrane receptor—the kainate binding site. "Some cells in the forebrain are hypersensitive to non-NMDA toxicity," says Dennis Choi of Stanford University. "These cells contain the neuropeptides somatostatin and neuropeptide Y and also NADPH-diaphorase, which makes a convenient marker for identifying the cells." While most cortical neurons are very sensitive to NMDA toxicity, these cells are relatively insensitive. Instead they are killed more easily by kainate, another chemical analog of glutamate.

Why the brain should have so many built-in ways to destroy itself because of glutamate toxicity is somewhat of a puzzle, but more and more receptor types for glutamate are being identified. Carl Cotman of the University of California at Irvine joins other researchers in proposing an updated list. Neurons in the mammalian brain have at least five different kinds of glutamate receptors, he says—NMDA, quisqualate, kainate, a population that binds L-2-amino-4-phosphonobutyrate or AP4, and a different quisqualate receptor that is linked to the breakdown of phosphatidyl inositol, a membrane lipid. This fifth receptor type may also require a G protein that binds guanine nucleotides when it is activated. The transport molecule described by Coyle and his collaborators belongs to a separate class of membrane sites.

Both Cotman and Coyle believe that several common mechanisms of toxicity may emerge out of this confusing array of binding sites for glutamate. "One idea is that calcium is a common final end point for glutamate toxicity," says Cotman. "NMDA receptors can induce toxic levels of intracellular calcium by themselves." Other, non-NMDA glutamate receptor types may further enhance calcium toxicity when they are stimulated at the same time as NMDA receptors, he says. Cotman and his colleagues and other investigators, are finding that abnormalities in NMDA receptor sites may be associated with neurological disorders such as epilepsy and Huntington's disease.

Another general mechanism for toxicity may be due to an unrestrained rise in oxidizing agents within a cell. "Oxidative stress may be part of a final common element of quisqualate and kainate toxicity," says Coyle. "There may be many different processes going on that lead to oxidative stress and cell death."

How these membrane sites function during normal neuron-to-neuron signalling or brain development, and how they contribute to nerve cell damage during an ischemic attack is an area of intense investigation.

Cells Without Growth Factors Commit Suicide

The idea that nerve cells, and probably most other cell types, require growth factors for normal development and differentiation is not new. But within the past 2 or 3 years, several groups of researchers have approached the issue from a very different perspective. Instead of asking why cells need these factors, they are asking why cells die without them. The surprising answer seems to be that without growth factors, cells produce "killer proteins" that cause their death.

"Our hypothesis is that neurons don't just peter out and die when they are deprived of nerve growth factor, they commit suicide," says Eugene Johnson of Washington University Medical School in St. Louis, Missouri. This is "physiologically sound," he says, because nerve cells proliferate too much during the development of the nervous system and their number needs to be cut back. Later in adulthood similar mechanisms may account for increased cell death in neurodegenerative diseases such as Parkinson's, Alzheimer's, and amyotrophic lateral sclerosis, a motorneuron disorder.

In their new work Johnson, David Martin, and Tat Suro Koike, also of Washington University, evaluate the response of cultured rat sympathetic neurons to the withdrawal of nerve growth factor (NGF). The cells die within 48 to 72 hours after taking NGF out of the culture medium. But death can be inhibited if messenger RNA or protein synthesis is blocked, which indicates that the cells must be producing proteins that ultimately kill them. Johnson and his co-workers have not yet identified these proteins but they agree with other researchers that cells are actually programmed to die and that growth factors somehow prevent the genetic program from being read.

Similarly, Ronald Oppenheim of Bowman Gray School of Medicine at Wake Forest University in Winston-Salem, North Carolina, has new evidence that nerve cells produce killer proteins during normal cell death in vivo. In the developing chick embryo, about 50% of both spinal cord motorneurons and dorsal root ganglion neurons, which lie in groups outside the spinal cord, normally die when the embryo is 5 to 12 days old. But if Oppenheim, David Prevette, and Michael Tytell, also of Bowman Gray, inject the embryos with cycloheximide to prevent protein synthesis, they can prevent most of these nerve cells from dying, at least for a while. "It is a block of naturally occurring nerve cell death," says Oppen-

heim. Additionally, injecting cycloheximide blocks motorneuron death due to the removal of a developing limb bud that produces a muscle-derived growth factor necessary for motorneuron survival (*Science*, 13 May, p. 919).

The notion that growth factors prevent the production of killer proteins is not restricted to the nervous system, however. Essentially the same phenomenon appears to occur in insect muscle cells and in rat prostate epithelial cells.

After the *Manduca* moth emerges from its pupal case at the end of metamorphosis, the muscle cells needed for that process die within 36 hours. "These are giant muscle fibers," says Lawrence Schwartz of the University of Massachusetts in Amherst. "The triggers for muscle cell death are hormonal and new genes for killer proteins are expressed when the hormones are withdrawn." James Truman of the University of Washington in Seattle, and Brian Kay of the University of North Carolina in Chapel Hill collaborated in earlier phases of the research and Schwartz is now trying to clone the genes that code for what he describes as "endogenous cell death products."

In their studies of epithelial cell death in the rat prostate gland, Debra Wolgemuth, Zahra Zakeri, and Ralph Buttyan of Columbia University in New York and Richard Lockshin of St. John's University in Jamaica, New York, reported earlier this year that a "reactive cascade" of gene activity is associated with cell death (*Molecular Endocrinology*, volume 2, page 650). The epithelial cells require testosterone, the male sex hormone produced in the testes, as a growth factor and will die within 5 days after a rat is castrated.

The Columbia researchers analyzed specific RNAs at 1-day intervals after castration and noted a significant increase in a particular temporal sequence of RNAs for the cellular oncogenes c-*fos* and c-*myc* and a heat shock protein. Interestingly, the same genes are active in many cultured cell types during proliferation and differentiation. The researchers do not yet know if the increased mRNAs that occur as prostate cells are dying represents increased gene expression or the stabilization of existing mRNAs. But they note that c-*fos* gene expression is linked to the degradation of phosphatidyl inositol, a membrane lipid, and increases in intracellular calcium ions.

Whether similar genetic mechanisms account for nerve cell death following the withdrawal of nerve growth factor is still unknown. Johnson, Oppenheim, and Schwartz, however, are beginning to explore that question.

■ **DEBORAH M. BARNES**

New Active Faults in L.A.

A University of Southern California seismologist has confirmed that two potentially destructive but deeply buried faults run beneath downtown Los Angeles, Beverly Hills, and Dodger Stadium. Egill Hauksson had been working on tracing buried active faults in the bedrock beneath the sediments of the Los Angeles basin when the magnitude 5.9 Whittier Narrows shock struck 20 kilometers east of downtown Los Angeles (*Science*, 8 January, 1988). No fault rupture broke the surface. Analyses of the rupture's seismic waves showed that the fault responsible is 11 to 16 kilometers below the surface. That was all the proof anyone needed to confirm suspicions of there being more to the earthquake hazard than meets the eye.

Hauksson recently completed his analysis of 200 far smaller earthquakes in the Los Angeles basin. About half of those were more or less vertical ruptures clustered about the three major strike-slip faults of the basin. These are vertical faults whose opposing faces slip by each other in a northwest-southeast direction, just as the San Andreas does. But about one-third of the small quakes were of the thrust type, in which a crustal block is shoved over a second block along a steeply inclined fault. These events clustered along two trends, including the Elysian Park fault, a buried thrust fault whose existence had been inferred from geological studies (see map).

The buried thrust faults seem to be capable of generating earthquakes, but to estimate the hazard, seismologists must know how much seismic energy is being stored on the faults and how it will be released. For limits on the energy being stored, Hauksson takes two estimates of the rate at which the motions of the Pacific and North American plates are compressing the basin in the direction of the thrusting. Thom Davis, a Los Angeles consultant, has estimated from studies of deformed sediments filling the basin that the rate of compression is 10 millimeters per year.

On the other hand, geodesists using very long baseline interferometry can only estimate that compression is progressing at 3 ± 6 millimeters per year. Thus, the strain requiring release could be zero, which is unlikely given the seismic evidence and the mountains shoved up around the basin, or it could be in a league with that building on the nearby San Andreas. Hauksson assumes enough energy has been stored to produce a magnitude 7.5 earthquake, whose energy release is equivalent to that of 30 events of magnitude 6.5. To this uncertainty must be added the imprecision inherent in identifying the size of the fault segments that will fail in individual earthquakes, whose magnitude will depend on segment length.

These faults have produced only one damaging earthquake during the 200 years of the historical record, which may be the quiet before the storm. Although emergency response planning has included the possibility of buried faults, the adequacy of current building codes must be reconsidered. ■ **RICHARD A. KERR**