New Clues to Autism

Speakers: Daniel H. Geschwind, MD, PhD, University of California, Los Angeles; Eric Peterson, PhD, University of Colorado; Brendon Nacewicz, University of Wisconsin, Madison; Aysenil Belger, PhD, University of North Carolina—Chapel Hill, and James S. Sutcliffe, PhD, Vanderbilt University, Nashville, Tennessee

New research has found that some of the brain abnormalities and behaviors associated with autism also are present in the parents and siblings of individuals with the disorder. Rare mutations on a specific gene appear to be risk factors for autism. Scientists have also discovered that avoidance of eye contact with other people is not, as previously believed, a primary cause of the social impairments observed in autism.

All of these studies promise to shed light on more effective methods of diagnosis and treatment. Autism affects as many as 24,000 children born in the U.S. each year. It is a brain disorder that impairs a person’s ability to think, feel, communicate, and relate appropriately to the outside world. These behaviors can range from mild to severely disabling, but their impact on the lives of individuals with the disorder and on their families is almost always devastating. People with autism often have other debilitating brain disorders, including attention-deficit/hyperactivity disorder, obsessive-compulsive disorder (OCD), epilepsy, and depression.

Although the exact cause of autism is unknown, experts believe that it is associated with abnormal brain developments that emerge in part as a result of genetic factors. A better understanding of what exactly goes wrong in the developing brains of autistic children might be able to help scientists identify the genes involved with autism and thus to generate better methods of diagnosing and treating the disorder.

Many areas of the brain have been implicated as an etiologic factor, but pinpointing specific brain structures has proved challenging. Brain development and cognitive maturation are difficult to control in clinical studies of autistic children.

Researchers at the University of Colorado, Colorado State University, and the University of Denver decided to study the brains of parents of autistic children to see whether they also had abnormalities associated with the disorder. If they did, those abnormalities might be the heritable ones involved in the emergence of autism.

For the study, 40 parents of autistic children and 40 age-matched and sex-matched controls received magnetic resonance imaging (MRI) brain scans. Scientists identified many brain regions in which “the autism parent group was either smaller or larger than in the brains of the adults with a negative family history,” reports Eric Peterson, PhD. These areas include the cerebellum, which plays a role in cognitive thinking (speech, learning, emotions, and attention) and in motor function, and the basal ganglia, a brain region associated with compulsive and ritualistic behavior. People with autism often have difficulty with changes in their routines and can develop patterns of repetitive behaviors.

These findings offer hints as to which brain abnormalities might be heritable with autism. One of the next steps, says Dr. Peterson, is to confirm the findings in studies on pairs of twins when only one twin has autism and to use functional brain imaging for family members to see whether the malfunctioning of those same brain regions is also heritable.

At the University of Wisconsin-Madison (UW), researchers found that the non-autistic brothers of people with autism show the same avoidance of eye contact as their autistic siblings when they viewed images of strangers and family members. Abnormal eye contact is common in people with autism, and it is often one of the behaviors first noticed in children with the disorder. The non-autistic brothers, like their autistic siblings, had smaller-than-normal amygdalas, an area of the brain involved in understanding emotional facial expressions and in feeling fear in social situations.

Using computerized technology, the UW researchers measured the amount of time that three groups of participants, 6 to 18 years of age, spent looking at the eyes of people in pictures. The groups included nine boys with autism, nine non-autistic brothers of individuals with autism, and nine boys with typical development. Intelligence quotient (IQ) scores were similar for the non-autistic brothers (average, 120) and the control group (average, 119) but were lower for the autistic group (95). Nevertheless, the autistic subjects and the non-autistic brothers were most similar when it came to eye contact: both groups showed decreased eye contact in relation to the control group. The low incidence of eye contact occurred even when the
boys were shown pictures of family and friends. Thus, the behavior did not reflect inherited shyness.

The researchers also examined brain structures in all three groups. The brothers of individuals with autism had smaller amygdalas than the control group. In fact, amygdala volumes were similar to those of their autistic siblings.

“This suggests that other brain systems must be able to compensate for this abnormality in the non-autistic brothers,” says Brendon Nacewicz, one of the study’s authors. “Multiple brain systems must therefore be affected to develop the full syndrome of autism.”

At the University of North Carolina–Chapel Hill (UNC), researchers made the striking discovery that although people with autism tend to avoid looking directly at faces, when they do focus on faces, their brains respond in ways similar to those of people who do not have autism. This finding suggests that behavioral interventions may help these people improve their ability to interact socially.

Using functional magnetic resonance imaging (fMRI), a noninvasive technique, Gabriel Dichter, PhD, and Aysenil Belger, PhD, asked subjects to perform a task that required them to attend to certain items in the environment while disregarding other items. The participants were shown pictures of arrows (non-social items) and faces (social items). They were then asked to report the direction (left or right) in which the arrows pointed.

With both social and non-social items, the autistic participants showed markedly less activity than the controls in the “executive” regions of the brain (including portions of the frontal lobes), where specialized tasks (e.g., sifting through complex information, selecting task-appropriate responses or inhibiting task-inappropriate ones) take place. With the social items, however, the results were surprising. Responses in the executive areas differed in the two groups, but responses in the areas of the brain that process faces—including the fusiform gyrus—were remarkably similar.

This new research, conducted with support from the National Institute of Mental Health (NIMH) Studies to Advance Autism Research and Treatment (STAART) Network and the UNC Neurodevelopmental Disorders Research Center, has exciting implications.

“Since the brain seems capable of responding to faces when attention is directed toward faces, purely behavioral interventions that instruct individuals with autism to look at faces may help both to increase the brain’s responsiveness to social cues and to improve the quality of social interactions,” Dr. Dichter says.

Researchers at Vanderbilt University report that several rare mutations within a specific gene (the serotonin transporter, or SERT) are risk factors for autism. These findings may eventually make it possible to test autistic children for these gene variants, just as children can be tested for cystic fibrosis, a disease that is linked to a single gene but is triggered by many different mutations. Evidence suggests that early diagnosis and intervention (before age 3) results in better outcomes for autistic children.

The SERT gene regulates serotonin levels in the brain. About 25% of people with autism have elevated levels of serotonin, and selective serotonin reuptake inhibitors (SSRIs) have helped to ease the symptoms of autism in some patients.

Previous research attempting to link the SERT gene to autism had been promising but inconclusive.

“Either this was not the gene, or there had to be different genetic variants that were acting differently in different people,” says James Sutcliffe, PhD.

Hoping to find rare mutations involved in a person’s risk of developing autism, Dr. Sutcliffe and his colleagues took DNA samples from 120 families likely to possess a genetic risk factor on chromosome 17 (where the SERT gene resides). They found 19 different SERT mutations (or variants) in families with more than one male with autism. (The finding that families with multiple males with autism had these variants is consistent with the higher prevalence of autism among males; four times as many males as females are affected by the disorder.)

Four of the 19 SERT mutations were in “coding” regions, or areas of the gene that are translated into protein. “These variants were significantly associated with increased rigid-compulsive behaviors,” notes Dr. Sutcliffe.

Such behaviors are common in autism and of related disorders, such as OCD.

These investigators also discovered that two intracellular signaling pathways in the brain—p38MAPK and PKG—“go haywire” in the presence of mutated SERT genes. This might explain how SERT mutations disrupt serotonin signaling in autism. Drugs that target these pathways are being investigated for the treatment of inflammation, cancer, and other disorders unrelated to autism.

However, it is still unclear which aspects of the autism syndrome or phenotype are related to a particular genetic risk factor and whether these risk factors are specific for autism or cause more general brain dysfunction.

**Tau as a Target for Alzheimer’s Disease**

**Speakers:** Gail V. W. Johnson, PhD, University of Alabama; Eva-Maria Mandelkow, MD, PhD, Max Planck, Germany; Frank M. LaFerra, PhD, Department of Neurobiology and Behavior; University of California, Irvine; and Virginia M.-Y. Lee, PhD, Center for Neurodegenerative Disease Research, University of Pennsylvania, Philadelphia, Pennsylvania

New studies are elucidating the role of the protein tau in Alzheimer’s disease (AD) and other neurodegenerative diseases and its possible use as a target for therapy.

Scientists are determining how tau misfolds and clumps to form neurofibrillary tangles, which are a characteristic feature found in the brains of patients with AD. Other work shows that early changes in tau that precede the formation of the tangle are tied to early AD symptoms and that a vaccine may be effective against tau’s ill effects in patients with early AD.

Scientists also report that findings from other neurodegenerative diseases such as Parkinson’s disease (PD) can help advance research on AD. They hope that these studies will lead to the development of therapies that target the pathological processing of tau as a treatment for AD. They anticipate that if they can better understand the early stages of tau malfunction, then perhaps they will be able to delay AD progression.

One of the hallmark neuropathological characteristics of...
AD is neurofibrillary tangles. These tangles are composed primarily of tau, a natural component of nerve cells that helps to regulate the movement of nutrients along the microtubule tracks. In patients with AD, tau becomes abnormally modified; this impairs the protein’s ability to bind to microtubules and to move essential nutrients through nerve cells. Instead, tau binds to itself, resulting in the classic tangles.

It has now been observed that tau can exert its toxic effects even before tangles form. AD is marked not only by these tangles, which contain misformed tau, but also by lesions called amyloid plaques. These plaques develop outside the nerve cells and contain amyloid beta peptide. These two types of lesions interact to produce the symptoms of AD.

Recognizing these events, Frank M. LaFerla, PhD, developed mice with mutations in genes that caused the formation of both amyloid plaques and neurofibrillary tangles. His mouse model of AD contained three mutant human genes: (1) amyloid precursor protein (APP), (2) presenilin-1 (PS1), and (3) tau.

Mutations in APP and PS1 can increase the production of amyloid beta and harm the body’s ability to clear it, allowing it to accumulate inside and outside nerve cells and to interfere with nerve impulses. Tau mutations cause a rare form of dementia characterized by the loss of nerve cells and the presence of neurofibrillary tangles similar to those in AD.

The mice with the mutant human genes developed malfunctions in amyloid beta and tau in a way that mimics what happens in the brains of human beings with AD. The researchers were then able to test the ability of anti-AD therapies to reduce the effects of amyloid beta-containing plaques and tau-containing neurofibrillary tangles.

The LaFerla team found that vaccinating the mice with amyloid beta in early-stage disease reduced not only the amount of amyloid beta in their brains but also the quantity of early tau formations. However, the amyloid beta vaccine did not reduce the more mature neurofibrillary tangles that develop later in the disease.

“The findings in these mice appear to agree with data from Alzheimer’s disease patients who received amyloid beta immunotherapy,” says Dr. LaFerla. “These findings suggest that concomitantly treating both plaques and tangles may provide the most effective means for treating Alzheimer’s disease patients.”

Scientists are also exploring similarities between AD and other neurodegenerative diseases and are looking for clues as to how AD might develop. With AD, PD, and other age-related neurodegenerative disorders, proteins misfold and accumulate as “trash” in the brain. While tau-containing neurofibrillary tangles develop in AD, alpha-synuclein accumulates as structures (called Lewy bodies) in the brains of patients with PD.

“Because Alzheimer’s disease patients often develop Parkinson’s disease and vice versa and because similar misfolded proteins accumulate in both disorders, the diseases may be linked by a common mechanism,” says Virginia M.-Y. Lee, PhD.

Studies from Dr. Lee’s laboratory show that alpha-synuclein and tau can synergize each other’s clumping: that is, alpha-synuclein may initiate or facilitate tau tangle formation in AD. Indeed, clumps containing alpha-synuclein are often present in the brains of both AD and PD patients.

**Advances in Treating Drug Abuse**

**Speakers:** Eric J. Nestler, MD, PhD, University of Texas Southwestern Medical Center; Zheng-Xiong Xi, MD, PhD, National Institute on Drug Abuse, Baltimore, Maryland; Abraham Zangen, PhD, Weizmann Institute for Science, Rehovot, Israel; Matt W. Feltenstein, PhD, Department of Physiology & Neurosciences, Medical University of South Carolina, Charleston; and Fanny F. Botreau, PhD, Concordia University, Montreal, Canada.

Novel approaches to blocking the activity of certain neurotransmitters might be able to help users of illegal drugs quit the habit and prevent relapses after they have stopped using drugs, scientists report. Researchers have discovered that applying electrical stimulation to the brain has helped to prevent drug addiction and relapse in animal studies.

Neurotransmitters such as dopamine, and the receptors where they create their actions, help to regulate our sensation of reward—the “high” that keeps the cycle of drug use going. Studies over the past few years suggest that such neurotransmitters may play a role in addiction to marijuana, opiates, nicotine, and alcohol.

Blocking the dopamine D₃ receptor in animals diminishes the rewarding effects of marijuana and might be a potential therapy for marijuana abuse, report Zheng-Xiong Xi, MD, PhD, and his colleagues.

The investigators used the compound SB-277011A to block dopamine D₃ receptors in rats and examined how this affected the brain areas that register reward. They then measured dopamine levels in the brains of rats that were given marijuana alone and in rats that were first given the dopamine blocker and, next, marijuana.

In the first experiment, the investigators surgically implanted rats with an electrode in the medial forebrain bundle, a brain circuit tied to reward. The rats were allowed to administer electrical stimulation through the electrode, which delivered a strong rewarding effect.

Rats receiving marijuana needed less electrical stimulation to sustain the feeling of reward than rats not given marijuana, because the marijuana reward and the electrical stimulation reward combined to produce the overall reward effect. When rats received the dopamine blocker, the reward effect of marijuana was significantly lowered or eliminated, so that these animals needed more electrical stimulation to create the same effect.

In the second experiment, rats were injected with marijuana. Samples of neurotransmitters were collected from the nucleus accumbens, one of the brain’s major reward centers. Dopamine levels were 50% above normal in the rats given marijuana alone, but rats that were pretreated with SB-277011A and then given marijuana had no increase in dopamine levels.

“These findings strongly suggest the dopamine D₃ receptor is a potential target for therapies to fight drugs of abuse,” Dr. Xi says.

Dr. Xi and his co-investigators took a similar approach to show that blocking certain cannabinoid-like receptors in the brain inhibits cocaine’s rewarding effects. Like the dopamine D₃ receptors, these receptors for cannabinoid-like neurotransmitters (called CB₁ receptors) are involved in addiction to marijuana, opiates, nicotine, and alcohol; however, their

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function in cocaine addiction remains unclear. They appear to be involved in relapse to cocaine use, but their role in producing the direct rewarding effects (the high) of cocaine, sought by drug addicts, has been controversial.

The first experiment was similar to the work with the dopamine D3 receptor. Rats were implanted with an electrode in the medial forebrain bundle and were allowed to self-administer rewarding electrical stimulation. Animals that received cocaine needed less electrical stimulation to sustain the brain’s reward system, because the overall reward effect was the sum of the cocaine and the electrical stimulation effects. The rewarding effect of cocaine was diminished or abolished when the rats were given AM 251, a compound that blocks the CB1 receptor.

The investigators then studied the effects of AM 251 on the self-administration of intravenous (IV) cocaine. Each rat was surgically implanted with an IV catheter and was allowed to press a wall-mounted lever in its cage to receive cocaine. The experiment was set up so that the rats had to work harder for each subsequent cocaine infusion.

The rats eventually reached a point at which they stopped their drug-taking behavior because the amount of work needed was not worth the cocaine, called the “breakpoint.” Rats pretreated with AM 251 had a lower breakpoint, showing that the rewarding effect of cocaine was decreased in these animals.

“These findings suggest that the brain’s cannabinoid-like neurotransmitters are involved in cocaine’s rewarding effects,” Dr. Xi explains. “Future studies will look at whether AM 251 inhibits the rewarding effects of other addictive drugs and could lead to effective anti-addiction treatments for use in humans.”

In other work, scientists have shown that applying electrical stimulation to rats’ brains alone—without blocking receptors in the brain’s reward system—was enough to reduce the animals’ drug-seeking behavior.

Dino Levy, Abraham Zangen, PhD, and their associates trained rats to press a lever to self-administer cocaine via a catheter in their jugular vein. After 10 days of training, the rats self-administered a large amount of cocaine.

For the next 10 days, some of the rats received electrical stimulation for 30 minutes through miniature metal electrodes implanted in the prefrontal cortex or lateral hypothalamus; each of these brain areas is involved in reward and addiction. The rats were then exposed to the cocaine lever again. This time cocaine was not available, but cocaine-seeking behavior could be measured by the rate at which the rats pressed the cocaine-associated lever.

Rats that received the electrical stimulation did not press the lever to receive cocaine as much as the untreated rats. In another test, these rats were willing to work less to receive a subsequent dose of cocaine.

To understand how the electrical stimulation treatment affects addictive behavior, the researchers measured receptors to glutamate in the brain areas related to drug addiction. (Glutamate is a neurotransmitter thought to enhance the environmental cues associated with cocaine.) Electrical stimulation seems to normalize the levels of some glutamate receptors in some reward-related brain regions, whereas cocaine alters their levels.

“We show that by applying electrical stimulation to specific brain regions, it is possible to break, rearrange, or even reverse alterations caused by repeated drug use,” Dr. Zangen says. “These findings pave a way for a novel treatment strategy for reducing craving for drugs of abuse, and could help drug addicts quit the habit or prevent relapse.”

He suggests that this animal model might be applied to humans through deep transcranial magnetic stimulation, which uses alternating magnetic fields to stimulate areas of the brain noninvasively. Another option would be to surgically implant a stimulating device inside the brain, similar to deep-brain stimulation, which is used to treat Parkinson’s disease, he says.

One of the most difficult problems for the long-term treatment of cocaine addiction is the likelihood of relapse during abstinence. Triggers for relapse might be environmental cues or small doses of the drug that bring up memories of prior drug use. In animal models of relapse, these events can also re-instate cocaine-seeking behavior. Several studies suggest that these triggers may induce relapse by increasing levels of the neurotransmitter dopamine in the brain’s reward centers.

The antipsychotic agent aripiprazole (Abilify, Bristol-Myers Squibb/Otsuka) helped to lessen the drug-seeking behavior of abstinence laboratory rats when they were exposed to drug-associated cues or were given small doses of cocaine, compared with controls, in studies by Matt W. Feltenstein, PhD, and his colleagues. Aripiprazole stabilizes dopamine activity. It has a relatively low side-effect profile compared with some other drugs.

Another agent that might help prevent relapse in cocaine users is d-cycloserine, says Fanny F. Botreau, PhD. In experiments by Dr. Botreau and her team, rats were tested for the probability of returning to an environment where they had previously received cocaine. Some rats were given d-cycloserine after each of several so-called “extinction trials,” which were designed to extinguish the association between cocaine and the environment; other rats were not given the compound. Rats receiving d-cycloserine immediately after each extinction trial took significantly fewer days to stop preferring the cocaine-associated environment than did control rats.

“Our findings suggest that d-cycloserine or similar agents could be used to help human drug addicts extinguish the emotional responses and thoughts induced by environments and cues previously associated with drug use,” says Dr. Botreau. “These types of drugs could be used to avoid or at least reduce craving during the periods of detoxification and abstinence.”

Viral Vectors for Huntington’s and Parkinson’s Disease

Speakers: Anders Bjorklund, MD, University of Lund, Sweden; Beverly Davidson, PhD, University of Iowa; Nicole Déglon, PhD, Atomic Energy Commission, Orsay, France; Patrick Aeberscher, MD, École Polytechnique Fédérale de Lausanne, Switzerland; and Deniz Kirik, MD, PhD, University of Lund, Sweden

Techniques using stripped-down altered viruses (viral vectors) create improved animal models of disease and hold
promise for patients with serious neurological ailments.

The viral vector technique relies on the ability of viruses to invade cells and transfer genetic material. Whereas a cold virus would transfer cold-producing genetic material, the stripped-down viruses are altered to transfer genetic material that can reproduce the effects of a certain neurological disease in an animal model. Alternatively, in the case of drug therapy, they can transfer genetic material that can stop the biological mechanisms that underlie a neurological disease.

Our genes produce proteins that control brain development and function. In Huntington’s disease (HD), however, a faulty version of a gene, termed “huntingtin,” produces a flawed protein that causes the system to go awry. As a result, people with the faulty gene experience cell damage and destruction in the brain’s basal ganglia and cortex. This can affect coordination, thought, perception, and memory. Many patients experience involuntary movements of the arms, legs, body, and face. These symptoms are often accompanied by mood swings, depression, irritability, slurred speech, and clumsiness.

As the disease progresses, patients can have difficulty swallowing and can experience loss of balance, impaired reasoning, and memory problems. Death is commonly caused by a complication of the disease such as choking, or an injury related to a fall.

“Therapies for HD are often targeted at reducing the toxic effects or other properties of the flawed huntingtin protein,” says Beverly Davidson, PhD.

Dr. Davidson and her colleagues used a viral vector to deliver small fragments of genetic material to suppress the production of the flawed huntingtin protein.

“Our use of this technique, also known as RNA interference, is the first example of targeting the fundamental underlying problem in HD,” she says. “If we can get rid of the flawed protein, we should have a great impact on the disease.”

In the study, the technique reduced flawed huntingtin levels to 40% of pretreatment levels. In addition, the technique reduced clumps in cells. These clumps are normally formed by the flawed huntingtin protein. The reduction in protein levels correlated with improvements in movement problems seen in mice with HD, such as wavering when they walked or an inability to keep their balance on a rotating rod.

“The data suggest that even slowing down, rather than completely stopping, the production of the flawed protein can give the cells a chance to catch up and clear up the problems caused by mutant huntingtin,” Dr. Davidson explains.

The mouse model used by the researchers demonstrated disease symptoms very early on.

The researchers plan to test the therapy in a model that more closely resembles human HD in which the symptoms are more subtle and take many more months to appear. Other researchers have developed improved animal models with the aid of viral vector techniques.

Most recently, other researchers used a viral vector technique to create a non-human primate model of HD. First, they found that a viral technique, used in adult rats, boosted production of the flawed huntingtin protein in the brain area known to be affected by HD resulted in a selective and severe neuropathology characterized by huntingtin clumps in cells, brain cell dysfunction, and the cell death typical of HD. Non-human primates injected with the viral vector also showed behavioral deficits associated with HD.

In parallel with these studies, the researchers are also testing the use of viral vectors to treat pathological states. For example, they are developing a viral vector-based RNA interference technique, similar to Dr. Davidson’s, that may block or delay the appearance of HD symptoms. Other researchers have used viral vectors to create animal models of Parkinson’s disease (PD), which helped them identify ways to treat the movement disorder.

PD is characterized by slow movements, tremors, muscle rigidity, and gait and postural deficits. These symptoms are the consequence of the specific degeneration of brain cells that secrete the chemical dopamine in the substantia nigra brain area, which is involved in the control of voluntary and involuntary movements.

The pathological hallmark of PD is the presence of clumps in degenerating brain cells, called Lewy bodies. Several mutated genes have been linked to forms of PD, including two that affect a protein called alpha-synuclein.

“Although these mutations account for only rare cases of Parkinson’s disease, alpha-synuclein is also one of the primary components of the Lewy bodies, the pathological hallmark of PD, supporting a central role for alpha-synuclein in all forms of PD,” says Patrick Aebischer.

In a new study, Dr. Aebischer and his colleagues developed an animal model of PD that targets alpha-synuclein with viral vector approaches. Researchers injected mutated forms of the alpha-synuclein gene into rats in the brain region that is affected in the human disorder.

“By doing this, we developed a genetic model of PD that recreated the major pathological features of the disease such as the specific death of the dopamine-secreting brain cells in the substantia nigra brain area and the presence of intracellular clumps resembling Lewy bodies in the human disease,” says Dr. Aebischer. “These results were confirmed by the successful development of a similar PD model using another virus as gene carrier in both rats and non-human primates.”

In a second part of the work, researchers determined through studies of these animal models that increasing the expression of a gene termed Parkin, also linked to PD, significantly decreased the death of substantia nigra brain cells.

“This indicates that techniques that alter the activity of the Parkin gene may represent a promising approach for the treatment of PD,” Dr. Aebischer suggests.

As a next step, the researchers are testing a large number of small molecules in the animal models to identify any that could prevent the development of PD.

Another research group also used viral vectors to create improved animal models of PD. Deniz Kirik, MD, PhD, and his associates created a rat model and a monkey model of PD by using the viral vector technique to deliver the human alpha-synuclein gene to the dopamine-secreting brain cells located in the substantia nigra. They found that the affected cells were first functionally impaired and then slowly degenerated over several weeks. When the degenerative changes were most severe, behavioral impairments developed in the animals.