

ON THE BRAIN

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ON THE BRAIN

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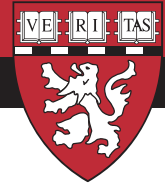
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Harvard Scientists Explore Brain Research in Interactive Videoconference

IN A UNIQUE attempt to share their expertise, three of Harvard's leading brain researchers participated in an interactive videoconference last fall, discussing how the brain and nervous system work and how the brain's trillions of connections allow us to see, think, feel and speak.

The videoconference, sponsored by the Harvard Alumni Association, was held Dec. 1 at The Harvard Club of New York City and broadcast via satellite to the Harvard campus in Cambridge and to a third location in Chicago.

In addition to Harvard president Lawrence H. Summers, the panelists included Carla Shatz, the Nathan Pusey Professor of Neurobiology at Harvard Medical School; Joshua Sanes, a professor of molecular and cellular biology and the Paul J. Finnegan Family Director of the Center for Brain Science; and Steven Pinker, the Johnstone Family Professor of Psychology. Harvard provost Steven E. Hyman moderated the proceedings.

A Leader in Brain Research

Neurobiology is a relatively young field. Harvard established the first neurobiology program in the country in 1966 and over the past 40 years has contributed greatly to our knowledge of the nervous system and how it works. Harvard's long and distinguished history of neuroscience research and education, both at the basic science and clinical levels, combined with its strengths in physics, chemistry, bioengineering, psychology, evolutionary biology and economics, will uniquely catalyze the new field of systems neuroscience. Last year, Harvard established the Center for Brain Science, which will employ these and other disciplines to focus on the

components of the nervous system to bridge the gap between molecular and behavioral science in an effort to better understand how neuronal activity affects behavior.

In introducing the brain researchers at the "Building Connections: Exploring the Mind and Brain" conference, Summers spoke about the rapid advances made in neuroscience over the past few decades, including how living nerve cells generate, conduct and process electrical signals; the mechanisms that transform a cluster of near-identical cells into a complicated neural network; and the way sensory information is processed and coded as it moves from the eye to the brain.

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(L-R) Dr. Joshua Sanes, Dr. Steven Pinker, Dr. Carla Shatz, and Harvard provost Steven E. Hyman. (Photo by Elena Olivo)

Blocking Protein in Inner Ear May Be Key to Restoring Hearing

MORE THAN 25 million Americans suffer from some form of hearing loss. The most common causes – age, exposure to loud noises, viruses and bacteria, tumors and certain medications – all share one thing in common: the death of tiny hair cells in the inner ear that convert vibrations into electrical signals that the brain decodes as sound. Once these hair cells die, they cannot regenerate.

Now, researchers at Harvard Medical School and Massachusetts General Hospital have isolated a cell-cycle regulator that prevents these hair cells from regenerating, a finding that could some day lead to new treatments for deafness and hearing loss. This regulator, the retinoblastoma gene (Rb1), has a number of functions depending on its location. For example, a mutation of Rb1 causes tumors in the retina. In other parts of the body, it helps cells to differentiate. In the inner ear, the protein produced by the gene – retinoblastoma protein or pRb – is responsible for halting the regeneration of hair cells.

“We are all born with thousands of these hair cells, which are sensitive to insult – noise, infections, certain antibiotics,” says Zheng-Yi Chen, an assistant professor at HMS and MGH. “These hair cells die easily. The retinoblastoma protein prevents other

hair cells from entering the cell cycle to proliferate.”

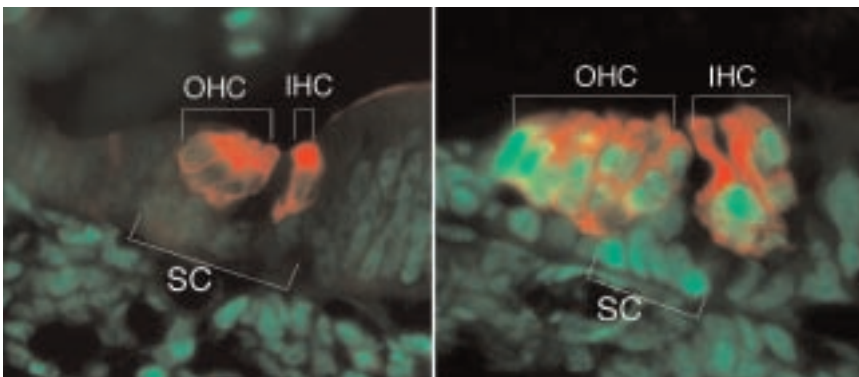
Dr. Chen and his colleagues made this discovery by studying the embryonic development of the inner ears of mice using oligonucleotide microarrays to determine when genes are switched on and off. They found that Rb1 was poorly expressed in the first 12 days of embryo development, but was up-regulated from day 14 on, when the full complement of inner hair cells had been established. This suggests that pRb, which has been shown to block progression of the cell cycle, may prevent the growth and regeneration of inner hair cells. To test the theory, Chen teamed up with Philip Hinds at Tufts–New England Medical Center, who had developed knockout mice in which the Rb1 gene is removed in the inner ear. In these mice, the researchers found greater numbers of hair cells in both the utricle, the ear’s balance structure, and the cochlea, the part of the inner ear that senses sound.

“These hair cells,” says Chen, “are produced early in embryonic development. The number stays the same throughout life. In the knockout mice, the hair cells continue to divide, producing more and more hair cells. More importantly, these regenerated hair cells are functional.”

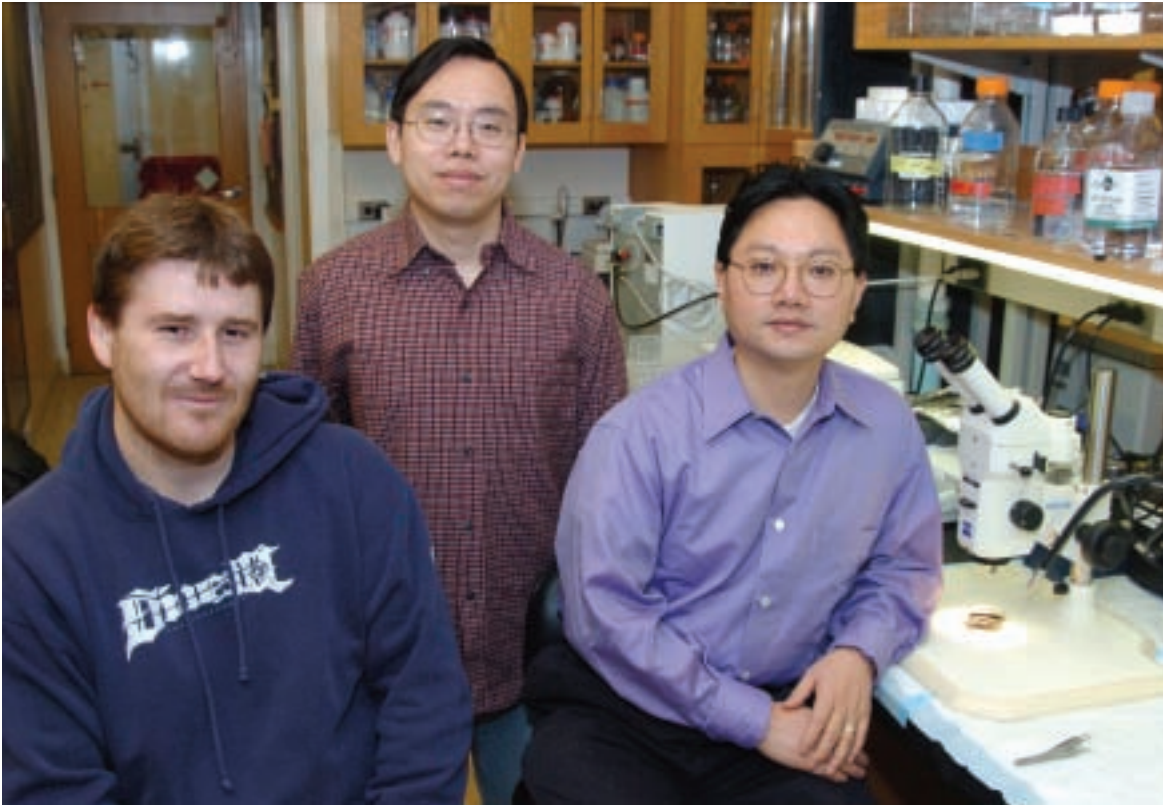
Chen’s research is one of the major findings made by HMS researchers, including David Corey, who identified a key protein in the inner ear that transforms sound waves into electrical impulses, and Stefan Heller, who discovered that the inner ear is a source of progenitor cells.

Chen and his colleagues hope to use their findings to develop strategies to block the function of Rb1 in order to get hair cells back into the cell cycle in adult animals and ultimately in humans. Because the long-term consequences of permanently turning off the Rb1 gene are unknown, scientists are examining two approaches to temporarily turn off the gene, allowing hair cells to re-enter the cell cycle, and then turn the gene back on so it can continue its other functions, especially tumor suppression.

The first approach involves using a chemical compound in which a given type of cell with Rb gene expression can be grown. The compound, which inhibits the growth of pRb, degrades over time so the gene can be turned back on. While such



Retinoblastoma prevents hair cell division. In the mouse embryo at left, the cell division marker PCNA (light green) is absent in the three outer hair cells (OHC), one inner hair cell (IHC), and support cells (SC) of the inner ear, indicating that these cells are not dividing. Ablating the retinoblastoma gene, however, causes rampant cell cycling and a surge in PCNA, shown at right. (Image courtesy of Zheng-Yi Chen)



(L-R) Dr. Cyrille Sage, Dr. Mingqian Huan and Dr. Zheng-Yi Chen. (Photo by Steve Gilbert)

a compound has yet to be identified, it is increasingly popular among scientists to screen for such compounds to block the function of certain proteins because the compounds could potentially be used as drugs.

Another approach is called RNA interference, or RNAi. With this approach, scientists make a small RNA nucleotide that is introduced to and taken up by hair cells, combining RNA to the retinoblastoma gene product. This results in down-regulation of the gene. Like the chemical compound, RNAi degrades rapidly, Chen says.

These approaches, Chen says, will be targeted to certain hair cells in the inner ear that are more sensitive to damage than others. Hair cells at the base and apex of the cochlea, which control hearing at high and low frequencies, are extremely sensitive to insult, while those responsible for the middle ranges are more robust.

Assuming that regenerating hair cells using one or both of these methods can restore hearing, the

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Hair bundles function without retinoblastoma. Hair bundles in the inner ear, imaged by differential interference contrast microscopy (left), take up the dye FM1-43 (right) through the mechanotransduction channel. (Image courtesy of Zheng-Yi Chen)

Optic Nerve Regeneration May Lead to Restored Eyesight

WHILE MANY CELLS in the body can renew themselves after an injury, nerve cells in the central nervous system, which includes the brain, retina, optic nerve and spinal cord, cannot. In mammals, including humans, these nerve cells lose their ability to regenerate after neurons have formed their connections during development.

Because damage to the central nervous system is irreversible, researchers at Harvard Medical School are examining the molecular mechanisms that prevent CNS axon regeneration. Specifically, they are looking at whether successful regeneration of the severed optic nerve can restore visual function in animals. Damage to or diseases of the optic nerve and retina are among the leading causes of blindness.

During the early stages of mammalian embryo development, says Dong Feng Chen, HMS assistant professor of ophthalmology and a researcher at

Schepens Eye Research Institute, nerve fibers that form the central nervous system continue to grow and are capable of regenerating if injured. Then, at a certain point in development, these neurons lose their ability to grow new fibers.

Chen says that while scientists are uncertain why neurons in the CNS stop regenerating it is widely held that mammalian brains require more stability because they have higher cognitive function than lower vertebrates, whose neurons continue to regenerate. The lack of neuronal regeneration in the CNS creates this stability in our brains – but it also leaves us susceptible to injuries that cannot be repaired.

Keys to Regeneration

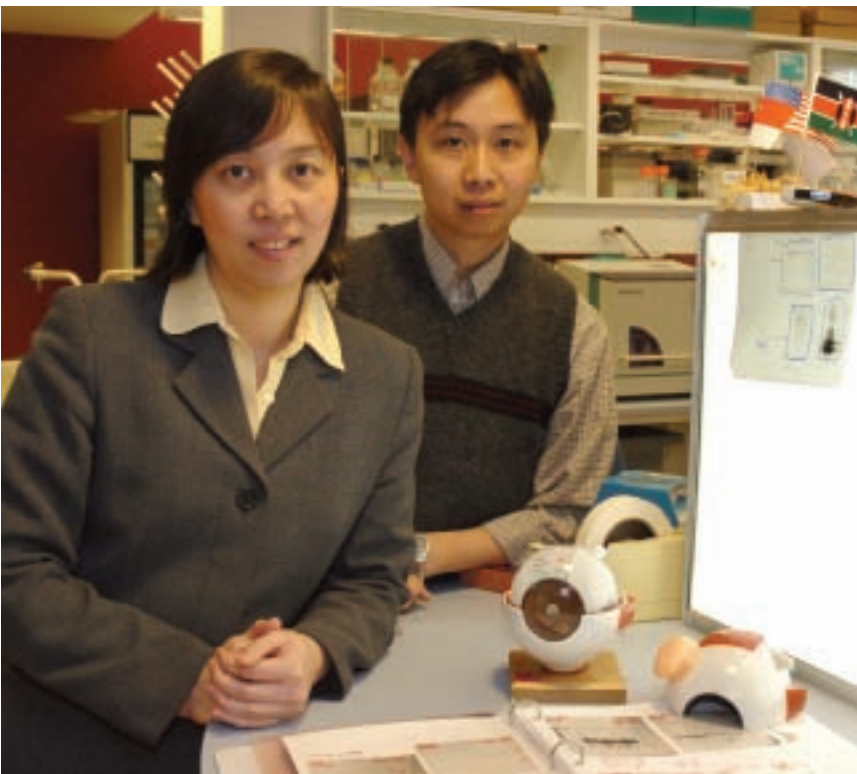
“Failure of nerve regeneration usually reflects both the loss of the intrinsic growth capacity of axons and the development of an inhibitory environment in the mature brain for such growth,” Chen says.

In her laboratory, Chen and research fellow Kin-Sang Cho have identified the Bcl-2 gene as the key regulator that supports the intrinsic regenerative capacity of optic nerve fibers. Bcl-2 is a well-known anti-apoptotic gene whose primary function is to support cell survival and block cell death. For some reason, this gene turns off when neurons form connections in the central nervous system.

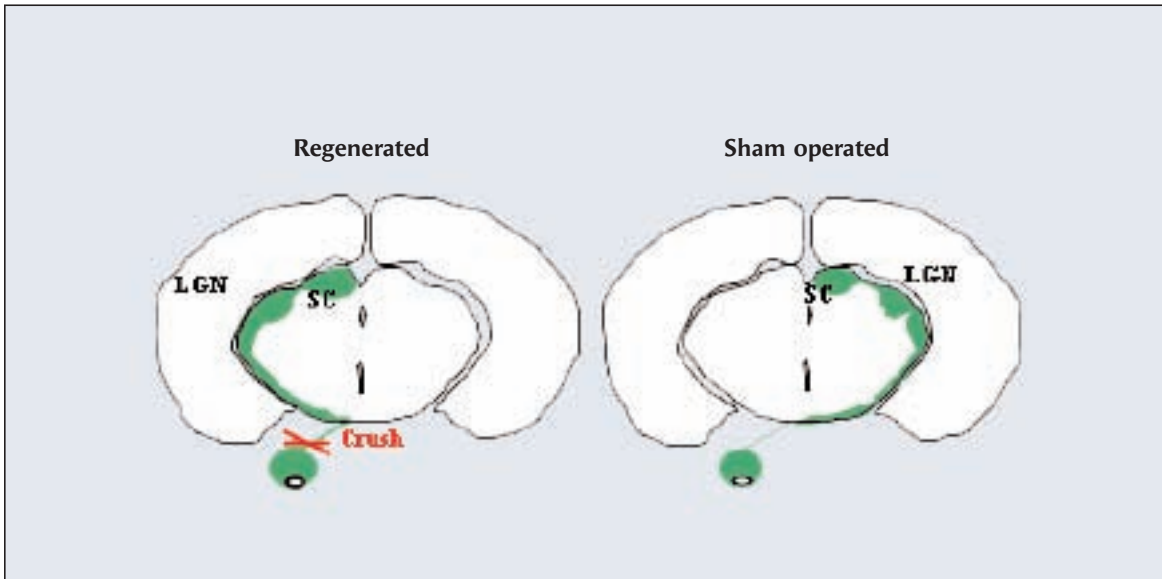
Chen’s team showed that Bcl-2 gives neurons the intrinsic properties they need to grow. “By turning on the Bcl-2 gene,” she says, “optic nerve growth resumes after injury in the early neonatal stage, before the brain environment becomes growth inhibitory.”

Recent studies, however, indicate that Bcl-2 alone is not sufficient for neuronal regeneration; in fact, one study showed that adult engineered mice with an overexpression of Bcl-2 could not regrow nerve cells after injury.

Knowing that the damaged CNS forms glial scars (dense tissue at the injury site formed by small, star-shaped cells called astrocytes that surround and support neurons in the brain and spinal cord), Chen and her colleagues sought to determine if changing this physical environment had any effect



Dr. Dong Feng Chen (left) and Dr. Kin-Sang Cho. (Photo by Steve Gilbert)



Right target, wrong side. Up to two weeks after birth, mice genetically engineered by Dong Feng Chen and her colleagues were able to regenerate the optic nerve into the brain (left) after the nerve was injured. Though the pattern of regeneration mimicked normal development (right), the axons did not cross over to the opposite side of the brain. (Image courtesy of Dong Feng Chen)

on nerve regrowth. They found that optic nerves grow if an astrocyte-specific toxin is applied to the injured area. They then created mice that overexpressed Bcl-2 and lacked cytoskeleton proteins that help form glial scars. This combination restored optic nerve regeneration in just a few days, with at least 40 percent of the optic nerve restored.

“Basically,” Chen says, “we’re reversing the nerves back to their embryonic stage, when they could regenerate.”

While Chen’s research has shown how nerves can be made to regenerate, she says the key is to have them form functional connections. The optic nerve in her engineered mice developed nerves that grew to the same side of the brain, rather than crossing to the opposite side as they do in a normal brain. She says a not-yet-identified signal may be required to perform this crossover.

Practical Applications

Consisting of millions of cells, the optic nerve transmits electrical signals from the retina to the brain. Because the optic nerve loses its ability to regenerate so early in its development, injuries or diseases

that occur after this milestone can cause permanent blindness.

Many common eye diseases affect the optic nerve, including glaucoma, in which the optic nerve is damaged by high intraocular pressure; optic neuritis, swelling or inflammation of the optic nerve; optic neuropathy, in which the optic nerve is damaged due to a blockage of its blood flow or by nutritional deficiencies or toxins; macular degeneration, progressive damage to the macula, the part of the eye responsible for central vision; and retinitis pigmentosa, progressive degeneration of the retina.

“This research will not only be useful in restoring sight where diseases have robbed people of their vision,” she says, “but it could also be used to reverse paralysis or treat other brain and spinal cord degenerative diseases.”

Imaging the Visual Circuit at the Level of Individual Neurons

BY COMBINING two advanced imaging techniques, researchers at Harvard Medical School have done what neuroscientists have always wanted, but have never been able, to do: view the activity of single neurons in the brain.

The imaging technique combines 2-photon microscopy, a type of laser microscopy that has been used for years, with calcium imaging in which dye is introduced into neurons. When the neurons fire, calcium in the neurons rises and the dye glows brighter. High-powered laser microscopes then make time-lapse images of the neurons turning on and off as laboratory animals view black and white bars moving in various directions on a computer screen.

Principal investigator R. Clay Reid, M.D., Ph.D., a professor of neurobiology at HMS and a member of Harvard's systems neuroscience initiative, says this technique "allows us to see cortical function at a level of detail never before imagined."

While these methods had been available to scientists for some time, this is the first time they have been used in conjunction to track the responses of individual neurons in the visual cortex. With previous imaging techniques, Reid says, specific neurons at a certain depth of the brain were blurred by hundreds of other neurons that were in higher layers of brain tissue, preventing scientists from studying the activity of specific neurons.

Reid's research builds on the work of Torsten Wiesel and David Hubel, Harvard neurobiologists who received the Nobel Prize for Physiology or Medicine in 1981 for their studies of the visual cortex. Their research showed that cells in the visual cortex are arranged in columns and that cells within each column perform similar functions in interpreting impulses messages from the eyes. With knowledge gleaned from Wiesel and Hubel's work, scientists know that, in the visual cortex, neurons are good at signaling the orientation of a stimulus – usually, vertical or horizontal bars – and where, in approximation, they live in the cortex.

Reid's study, which was published in *Nature* in January, sought to determine the actions of specific neurons in the visual circuit of cats and rats. The results were surprising. In the cat brain, neurons that share similar functions (such as sensitivity to movement in the same direction) were more segregated

than expected, bunching tightly together. The relationship between where neurons are and what they do, he says, are "extraordinarily precise," with sharp boundaries, only one or two cells wide, where neurons respond to opposite-direction motion.

In rats, however, these neurons were mixed with neurons that perform other functions. Neighboring neurons in the rat brain bear no relationship between the location of the neuron and the orientation of a stimulus that makes it fire.

Reid says one practical application of his research is that it will allow scientists to perform "circuit cracking," which involves placing an electrode on a single neuron to determine to what it is connected. "We can listen to one neuron with an electrode attached and image hundreds of other neurons and ask what that single neuron is connected to. This is the beginning of a circuit diagram that will allow us to develop a roadmap of a part of the brain," he adds.

While Reid and his team (neurobiology research fellows Kenichi Ohki, Sooyoung Chung and Yeang Ch'ng and neurobiology instructor Prakash Kara) are studying the visual cortex, he says the imaging technique can be applied to other regions of the brain, including those responsible for motor function and higher cognitive processes, to determine what neurons are doing in both healthy and diseased brains.

In the January 28, 2005 issue of HMS's *Focus* magazine, Bradley Hyman, an Alzheimer's researcher at Massachusetts General Hospital and the John B. Penney Jr. professor of neurology at HMS, said Reid's single-neuron imaging technique might have multiple applications. "This is basically a very advanced method for studying neuronal function, and any disease process where neuronal function is involved can be studied with this technology," he told *Focus*. "We have rodent models of Alzheimer's disease, Huntington's disease and Parkinson's disease, and this imaging will be a powerful tool to dissect the cellular basis for the cognitive problems we see in these diseases."

Of the technique he used in his research, Reid says, "This is a tool that works. We can look at both the anatomical and physiological pathology in a living brain. It's an entirely new way of looking at brain function."



Dr. R. Clay Reid.
(Photo by Yeang Ch'ng)

Harvard Scientists Explore Brain Research in Interactive Videoconference

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“Almost everything we’re going to talk about today,” said Summers, could not have been done 25 years ago because our knowledge of how the brain works was so primitive. “Of all the questions that can be asked, few are more fundamental than how it is that a bunch of chemical reactions make you feel you are you. Those were not questions amenable to scientific inquiry 20 years ago. They are questions that are amenable to scientific inquiry today.”

Exploring the Brain

In her laboratory, Shatz conducts research on brain development and function that helps scientists better understand neurological disorders such as cerebral palsy, epilepsy and dyslexia, as well as learning and memory. She studies how neuronal signaling sculpts and reinforces proper brain circuits as they form between nerve cells in the eye and brain.

She told the conference attendees that recent research has shown that, while humans are all drawn from the same genetic blueprint, we are shaped by our individual experiences. She added that research has determined that individual experience and physical conditions affect how our brains are wired.

Pinker is an experimental psychologist whose work focuses on language development in children. Language processing, he told the audience, is an extremely complex process that many of us take for granted. “Language comes so naturally to us,” he said, “that we often don’t understand how miraculous it is.”

Pinker described how his research using functional magnetic resonance imaging has pinpointed the regions of the brain used in specific parts of language processing.

Sanes, a neurobiologist who pioneered the study of synaptic development, as well as the mechanisms that regulate the formation of these structures, spoke about recent advances that use a fluorescent protein to watch single nerve cells grow and contact target cells in laboratory mice. He said researchers at the Center for Brain Science are hoping to use a similar technique to understand the millions of neurons and the trillions of

connections they make to allow our brains to work as they do.

Brain Science of the Future

The work of these three brain research pioneers, as well as many other scientists, forms the backbone of Harvard’s new Center for Brain Science, which will be housed in a new 500,000-square-foot building at Harvard. The Center will be one of the largest groups housed in the Northwest building.

More than 400 people participated in the brain-science videoconference.

Blocking Protein in Inner Ear May Be Key to Restoring Hearing

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hair cells are still susceptible to damage. For example, if someone’s hearing loss is due to sudden trauma, such as exposure to extremely intense noise, he will need to be more careful about protecting himself after hearing restoration through hair cell regeneration. If a child was deafened by aminoglycoside antibiotics and regained his hearing after hair cell regeneration, doctors would have to be careful about overuse of the same or similar drugs in the future.

Chen says much work remains to be done to prove that hearing can indeed be restored through hair cell regeneration. In his laboratory, he is investigating the use of RNAi, both in vitro and in vivo, as a primary means of blocking pRb function.

Because the ears of mice and humans develop almost identically, Chen says promising results from mouse studies may lead to trials in humans to restore hearing by blocking the function of the retinoblastoma protein.