**Embargoed until November 18, 1:30 p.m. ET**
**Press Room, November 15–19: (202) 249-4125**

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**BUILDING THE BRAIN: WHAT CAN GO RIGHT?**

*Scientists are learning a lot about how the brain develops normally; findings increase ability to identify and treat dysfunction and enhance performance*

**Washington, DC —** New studies released today show remarkable new understanding of how the normal brain and nervous system develops and wires itself early in development, including two new discoveries about 'critical periods' — limited time windows for acquiring a particular skill — and a new method to map brain circuits, which will help researchers understand how brain cells connect and communicate to produce action. The studies were released at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. The findings may ultimately lead to treatments that can reactivate critical periods in adults to rewire injured or diseased brains.

The human brain has 100 billion neurons, and each neuron communicates with other neurons — using both electrical and chemical signals — to form circuits and share information. Proper nervous system function involves coordinated action of neuronal circuits in many brain regions.

The new findings show that:

- Two key proteins involved in development and spinal cord regeneration play important roles in regulating a critical period in vision. Properly timed visual experience is necessary to wire the brain connections that are vital for binocular vision. Disrupting the proteins reactivated the brain plasticity in the visual system of mice (Hirofumi Morishita, abstract 28.5, and Sayaka Sugiyama, abstract 28.4, see attached summary).
- Scientists have developed a new technique that allows researchers to trace neural circuits in the living brain. How neurons function as a unit to perform a variety of tasks is largely unknown and the scarcity of ways to study them has been a major roadblock until now (James Marshel, abstract 884.4, see attached summary).

Other recent discoveries discussed at the meeting demonstrate that:

- Birdsong is teaching us a lot about the amazing capacity of the human brain to learn language. Like humans, birds must hear the sound of adults during a critical period and then hear their own voice while learning to sing or chirp. Researchers have found that, like humans, they have a network of brain regions that connect to allow for communication. Researchers are zeroing in on a circuit that is crucial for learning and controlling movement (see attached speaker's summary).
- In the motor system, which controls all muscles in our bodies, scientists have made significant progress in understanding how neurons connect correctly to ultimately create movement. They have found new evidence of genetic switches, which likely help determine how millions of nerve cells are connected into precisely wired neural networks throughout the body (see attached speaker's summary).

"If we want to know how to repair brains that develop incorrectly in a few unfortunate individuals, we need to know how they develop correctly in most of us," said press conference moderator Joshua Sanes, PhD,

– more –

of Harvard University. “Today’s findings offer significant new understanding of just what goes *right* in brain development. Neuroscientists are now obtaining crucial insights on which other scientists and medical professionals can build to develop new ways to diagnose dysfunction and increase performance.”

**Related Presentations:**

Presidential Special Lecture: **What Songbirds Can Teach Us About Learning and the Brain**
Saturday, November 15, 5:15–6:15 p.m., Washington Convention Center, Hall D

Symposium: **Genetic Determinants Specifying Neuronal Connections**
Wednesday, November 19, 8:30–11 a.m., Washington Convention Center, Ballroom A

Albert and Ellen Grass Lecture: **The Right Synapse in the Right Place**
Monday, November 17, 3:15–5 p.m., Washington Convention Center: Hall D

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**Abstracts 28.5 and 28.4 Summary**

**Takao Hensch, PhD** **(617) 919-4650**
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**Boston, Mass.**

## Research Identifies Proteins that Regulate Critical Periods in Brain Development

*Findings may help develop drugs to rewire the injured or diseased brain*

New research identifies factors that control critical periods, which are limited windows of time when experience permanently shapes brain development and, ultimately, brain function. Two new studies show that proteins that stimulate embryonic head development and block spinal cord regeneration have key roles in regulating a critical period in the visual system. The findings may ultimately lead to treatments that extend or reactivate critical periods in adults to rewire the injured or diseased brain. They were presented at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"Much of our adult behavior reflects the neural circuits sculpted by experience in infancy and early childhood. At no other time in life does the surrounding environment so potently shape brain function," said Takao Hensch, PhD, from Harvard University, who led both studies. "An understanding of how this plasticity waxes and wanes with age carries an impact far beyond bench science, including education policy, therapeutic approaches to developmental disorders, or strategies for recovery from brain injury in adulthood."

Hensch and colleagues study a critical period in the visual system. Properly timed visual experience is necessary to wire the brain connections vital for binocular vision. During a brief period following birth, if one eye is closed or deviant (lazy eye), most animals will never develop clear vision (acuity) through that eye. This condition is called amblyopia and affects 2–5 percent of the human population.

In the first of two studies presented at the meeting, the researchers found that disrupting Otx2 — a protein involved in embryonic head development — reactivated developmental brain plasticity in the visual system of adult mice, well after the critical period had ended. In previous work, the researchers found that Otx2 acts on the maturation of brain cells in the visual cortex (at the back of the brain), but is made in the eye. Visual experience causes Otx2 to be transported from the eye to the visual cortex. In the current study, the researchers investigated how Otx2 targets and accumulates in these visual cortex cells. They reactivated plasticity in the adult brain by physically blocking the transfer of Otx2 into visual cortex neurons.

In the second study, the researchers found that the Nogo receptor, which inhibits spinal cord regeneration after injury, may also block plasticity in the adult visual system. Unlike other mice experiencing one closed eye following birth, mice lacking the Nogo receptor gene did not develop permanent amblyopia. Once their deprived eye was opened, adult mice without the Nogo receptor recovered full vision. The findings suggest that the Nogo receptor normally inhibits plasticity and regeneration throughout the adult nervous system and may help end critical periods for brain development.

Hensch speculates that some neurological and psychiatric disorders may represent errors in critical period brain development. The ability to reactivate critical periods might offer an exciting therapeutic approach to address a variety of disorders.

This research was supported by the Human Frontiers Science Program; the Japanese Ministry of Education, Culture, Sports, Science, and Technology; the Japan Science and Technology Agency; and the U.S. National Institutes of Health.

28.5, Recovery from amblyopia in Nogo Receptor knockout mice
*H. MORISHITA, M. FAGIOLINI, T. K. HENSCH; Neurol, Div. Neurosci, Children's Hosp Boston, Harvard Univ., Boston, MA
TECHNICAL ABSTRACT: Understanding mechanisms that limit adult plasticity carries a broad impact for functional recovery in adulthood. Here, we examined whether Nogo receptor signaling, a major inhibitor of CNS regeneration following axonal injury, also contributes to the inability to recover from amblyopia in the adult primary visual cortex (V1).
First, we confirmed that short-term (4d) monocular deprivation (STMD) in adult wild- type mice yields no reduction of acuity through the deprived-eye (0.47+/- 0.03 cyc/deg), as measured by VEP in the contralateral V1. Instead, the Contra/Ipsi ratio of VEP amplitude decreased for low spatial frequency stimuli (0.05 cyc/deg), consistent with recent reports. Simply reopening the deprived eye after an initial long-term (P19-33) MD spanning the critical period also revealed no recovery of acuity in adulthood.
Next, we examined the magnitude of plasticity in Nogo Receptor1 (NgR1) knockout (KO) mice. Long-term MD spaning the critical period resulted in significant acuity reduction through the deprived eye similar to wild-type mice (0.25+/-0.04 cyc/deg). In contrast, after reopening the deprived eye, acuity recovered significantly (0.44+/-0.07 cyc/deg), reaching that of non-deprived animals. Consistent with this recovery of vision, single-unit recordings from NgR1KO mice exhibited a shift in favor of the originally closed contralateral eye (CBI=0.65).
These results establish the NgR1KO mouse as the first animal model which spontaneously recovers from amblyopia simply by reopening the deprived eye. This mouse will be a useful tool for further understanding the mechanisms underlying recovery of visual function in adulthood.

28.4, Reactivation of postnatal plasticity by Otx2 disruption in adult visual cortex
*S. SUGIYAMA, T. K. HENSCH; Dept Neurol, Children's Hosp Boston, Harvard Univ., Boston, MA
TECHNICAL ABSTRACT: Binocular vision is established in the primary visual cortex (V1) through an activity-dependent competition during early postnatal life. Experience-dependent circuit rewiring is triggered by a balanced excitation-inhibition via distinct GABAergic connections within V1. We previously found that maturation of this parvalbumin (PV)-cell network and hence plasticity onset is regulated by a non-cell autonomous accumulation of the Otx2 homeoprotein (Sugiyama et al., Cell 2008), which persists into adulthood (>P60).
Here, we show that peri-neuronal nets (PNNs) may be a plausible mechanism for Otx2 accumulation in PV-cells. PNNs are composed of a core protein, glycosaminoglycan chains and other extracellular matrix factors, including known direct and indirect targets of Otx2. Visual experience enhanced both Otx2 localization and PNNs surrounding mature PV-cells. Indeed, PNN formation was induced by Otx2 infusion (even in complete darkness) and was weakened in conditional Otx2 knockout (KO) mice.
Disruption of PNNs by chondroitinase ABC (4 days) conversely removed Otx2 from adult V1, resetting PV-cells to an immature state. Importantly, chondroitinase treatment is known to reactivate plasticity in the adult (Pizzorusso et al. 2002, 2006). We found that cortical infusion (7d) of inhibitory antibody to prevent Otx2 transfer into PV-cells also reactivated plasticity in adult wild-type mice (CBI=0.59+/-0.01). Cortical plasticity in conditional Otx2 KO mice was restored at any age by enhancing GABA function with diazepam. Once triggered by diazepam treatment (P24-28), the plastic state in the absence of Otx2 was strikingly sustained even one month later (>P60).
Thus, the persistence of Otx2 within PV-cells may serve as a positive feedback loop to promote PNN maturation and further Otx2 uptake, which ultimately maintains an inflexible cortical milieu in the adult.

**Abstract 884.4 Summary**

**Lead author: James Marshel** **(858) 453-4100 x1073**
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**The Salk Institute for Biological Studies**
**La Jolla, Calif.**

## New Technique Maps Brain Circuits

*Findings help researchers understand the development and function of brain cell connections*

A new technique allows researchers to trace neural circuits in the living brain. The method exploits a naturally occurring virus to determine the connections to a single brain cell. The findings may help address the function and development of complex brain circuits. It was presented at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"Ensembles of brain cells called neurons connect to each other to form interwoven circuits. How these complex networks of neurons develop and how they function as a unit to perform diverse computations is largely unknown," said James Marshel from the University of California, San Diego, and the Salk Institute for Biological Studies, who is the first author of the study led by Ed Callaway. "A major roadblock has been the lack of technology to study individual circuits directly."

The rabies virus infects neurons and is transmitted from one neuron to the neurons that contact it backwards through brain connections called synapses. In a previous publication from the Callaway lab, the rabies virus was modified so that it could enter and spread only to certain neurons and would leave behind a fluorescent marker.

In the new abstract, Marshel and colleagues describe progress extending this method for use in the intact brain. They used electrical impulses to inject single neurons in the rodent brain with genes that allow the modified rabies virus to enter and spread.

This method allows researchers to map out brain circuits in the living brain. Two additional studies presented at the meeting describe other aspects of the technique (abstracts 496.1 and 884.21). "The new technology we discuss progress on here may offer us the ability to probe and understand the brain and its development based on the genetics, structure, and function of targeted neural circuits," Marshel said.

The research was supported by the Kavli Institute for Brain and Mind and the Institute for Neural Computations at the University of California, San Diego, the U.S. National Institutes of Health, and the Defense Advanced Research Projects Agency.

Scientific Presentation: Wednesday, November 19, 4–5 p.m., Washington Convention Center, Hall A-C

884.4, Targeting single monosynaptic neuronal networks for gene expression and cell labeling in vivo
*J. H. MARSHEL1,2, T. MORI2, K. J. NIELSEN2, E. M. CALLAWAY2,1; 1Neurosciences Grad. Program, Univ. California San Diego, La Jolla, CA; 2Systems Neurobio. Labs., The Salk Inst. for Biol. Studies, La Jolla, CA
TECHNICAL ABSTRACT: Understanding information processing by neuronal networks requires new technology to study neuronal networks directly in vivo. Here, we demonstrate an efficient way to target the monosynaptic inputs to a single neuron both in vitro and in vivo, for gene expression and cell labeling. The method employs a recently published modified rabies virus strategy that targets the monosynaptic inputs to genetically transfected host neurons (Wickersham et al., Neuron 2007). In the current study, we target infection of the virus to a single neuron in the cerebral cortex. A single neuron is selected for gene transfection under two-photon visualization in vivo. A loose patch is formed with the cell body, and a series of negative voltage steps is applied to the micropipette containing intracellular solution and plasmid DNA. The voltage pulses drive negatively charged DNA into the cell for gene expression--a procedure known as single cell electroporation (Kitamura et al., Nature Methods 2008). The DNA encodes for three genes: a marker fluorescent protein (e.g., GFP), a bird virus receptor (TVA), and rabies glycoprotein. Two days after electroporation, the animal is reimaged under two-photon microscopy to verify marker expression. Then, modified rabies virus is injected near the location of the cell. The virus is pseudotyped with envelope protein (EnvA) from a bird virus that interacts specifically with the TVA receptor and effectively targets infection of the rabies virus to the electroporated cell alone. The rabies glycoprotein gene is also deleted

the cerebral cortex. A single neuron is selected for gene transfection under two-photon visualization in vivo. A loose patch is formed with the cell body, and a series of negative voltage steps is applied to the micropipette containing intracellular solution and plasmid DNA. The voltage pulses drive negatively charged DNA into the cell for gene expression--a procedure known as single cell electroporation (Kitamura et al., Nature Methods 2008). The DNA encodes for three genes: a marker fluorescent protein (e.g., GFP), a bird virus receptor (TVA), and rabies glycoprotein. Two days after electroporation, the animal is reimaged under two-photon microscopy to verify marker expression. Then, modified rabies virus is injected near the location of the cell. The virus is pseudotyped with envelope protein (EnvA) from a bird virus that interacts specifically with the TVA receptor and effectively targets infection of the rabies virus to the electroporated cell alone. The rabies glycoprotein gene is also deleted from the viral genome and replaced with a gene for a fluorescent marker (e.g. RFP) to label infected cells. Within several days, the virus replicates in the electroporated neuron, assembles particles containing the rabies glycoprotein expressed from electroporation, and spreads retrogradely to infect and label presynaptic neurons to the host cell. The virus stops spreading from this point because both the presynaptic neurons and the virus itself lack the gene for the rabies glycoprotein that is essential for secondary infection by the virus. The end result is a single host cell labeled by the initial transfection step and the virus, and its monosynaptic, presynaptic partners labeled only by the virus. We demonstrate the technique is effective and characterize the cell-type specific inputs to targeted layer 2/3 pyramidal neurons in rodent visual cortex. Results are consistent with known projections to layer 2/3. For example, layer 5 pyramidal cell input is comprised of slender tufted cells.

**Speaker's Summary**

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**San Francisco, Calif.**

Presidential Special Lecture: **What Songbirds Can Teach Us About Learning and the Brain**
Saturday, November 15, 5:15–6:15 p.m., Washington Convention Center, Hall D

One of the most amazing capacities of humans is our ability to learn to speak. Young children listen to speech and then gradually learn to produce sounds like those of adults with very little specific instruction — they simply arrive in the world well-equipped to learn, and will learn whatever language (or languages) they happen to hear. Hearing is critical to the process: children must be able to hear the sounds of others accurately, and deficits in hearing lead to speech difficulties. Importantly, children (and adults) must also be able to hear themselves: they need accurate 'feedback' about what their own vocal production sounds like, and disruptions of this feedback (as when the sound of one's voice is delayed during a phone call) are disruptive to speech. In this regard, it is useful to remember that learning to produce sounds is a motor skill similar to learning to play tennis or piano, but with sensory feedback required not from vision or touch, but from hearing. It is also increasingly recognized that kids are greatly aided by the social signals and reinforcement that normally accompany speaking, a highly social skill.

In order to understand how the brain lets us learn to speak, and to be able to fix it if learning goes awry, it is extremely helpful to have animal models. However, there are relatively few examples of vocal learning in non-human animals. Thus far humans appear to be the only primates so endowed, and amongst mammals the only other major examples are whales and dolphins, and some bats. This is where the many species of songbirds provide an additional and important animal model: like humans, they must hear the sounds of adults during a sensitive period, and then must hear their own voice while learning to vocalize. They also possess networks of brain regions required for song learning, with many similarities to mammalian brains, making it straightforward to study the nerve cells actually involved in learning and producing song. These networks include areas devoted to hearing sounds, areas responsible for producing sounds, and a circuit known as the anterior forebrain pathway (AFP). The AFP is a 'basal ganglia' pathway, very much like those found in humans (and all vertebrates); such circuits are critical for learning and controlling movements, as well as for most learning that is driven by rewards, and are an important site of diseases such as Parkinson's disease and addictions. The study of songbirds is now going on in numerous highly skilled labs around the world, and there has been recent progress in many areas.

However, this talk will focus primarily on the AFP and its function in song motor learning and production. There is wide interest in the possibility that the AFP, because it is a specialized basal ganglia circuit for a simple behavior, can be a very useful model for how such circuits work in all animals. From the earliest studies of the song system, the AFP was found to be critical for vocal learning, with striking deficits caused by early damage to this pathway. However it was thought that it played no role in adult song maintenance. It has since become clear that this circuit is essential for any change in song, even in adulthood, and that one of its functions may be to allow the bird to vary its song as it practices, so that it can continue to optimize its song in a process of trial-and-error. It has long been known that the ability to vary one's output was a key feature of motor learning, but the source of this variability was not clear. Songbirds have raised the possibility that this is one of the important jobs of the basal ganglia. The AFP has also proved to be one of the brain areas heavily influenced by the social signals that we know enhance learning in many animals, including humans. For example, depending on the social setting, the AFP markedly changes its activity: when males sing alone, AFP neurons are characterized by rapid volleys of neural activity known as 'bursts', and the neurons' activity during singing is also subtly different during each rendition of the bird's song, so-called 'trial-by-trial variability'. In contrast, when males sing courtship song to females, the same neurons rapidly switch to reliable firing of single spikes precisely

timed to the song. These changes in neural variability are accompanied by corresponding changes in the variability of the song: when males sing to females, their song is more stereotyped than when they sing alone, and this song is preferred by females over the variable song. Moreover, silencing the activity of the AFP eliminates the socially-driven modulation of song variability, and all songs instead become stereotyped. Thus, this circuit seems to actively contribute to a switch from a ‘performance’ state, in which the bird sings its best, stereotyped song version, but may not be able to learn new things, to a variable, ‘exploratory’ mode that may be important for learning. By tracking down how the songbird brain generates these two states, we should learn a great deal about how motor learning is enabled by basal ganglia circuits, and how social signals influence this learning. Ultimately this should also lead to better understanding of what happens in the many disorders based in these pathways.

**Speaker's Summary**

**Speaker: Silvia Arber, PhD** **(41) 61-267-2057**
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**Basel, Switzerland**

**Genetic Control of Morphological and Functional Specificity in the Spinal Cord (**697.3)
Symposium: Genetic Determinants Specifying Neuronal Connections
Wednesday, November 19, 9:10–9:45 a.m., Washington Convention Center, Ballroom A

One of the most stunning features of the nervous system is its enormous complexity. Millions of neurons are interconnected into highly complex networks responsible for the control of animal behavior. Neuronal networks arise during development, when the many functionally distinct neuronal classes are generated. This process is guided by precise genetic programs steering the emergence of neuronal identity and the formation of neuronal connections. Our research is focused on the development of neuronal networks controlling motor behavior, the ultimate output of all nervous system activity. Motor neurons in the spinal cord are the key output for motor behavior, controlling the contraction of all muscles in our bodies. Whether or not a motor neuron can initiate the contraction of a muscle fiber or not depends on its activation by thousands of contact points on motor neurons in the spinal cord. These contact points between input and motor neurons — so-called synapses — form during development with high precision and steer the activation of motor neurons. However, mechanisms guiding the placement of precise synaptic contacts are poorly understood. In our recent work, we have found that specific tags made by motor neuron groups can instruct whether or not inputs derived from particular sources can be established. These results provide evidence for the existence of genetic switches involved in the formation of correct contact sites on motor neurons in the spinal cord. We expect that similar mechanisms controlling the formation of appropriate networks also act elsewhere in the nervous system to determine how the millions of nerve cells are connected into precisely wired neuronal networks.