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## Controlling Brain Wiring With the Flick of a Chemical Switch

With the flick of a chemical switch, researchers can now exert unprecedented control over the activity of molecules that help wire the developing brains of mice.

The new technique permits researchers to use drugs to switch the molecules on and off as precisely and reversibly as a light switch controls a lamp. Current genetic and chemical manipulation techniques are more akin to eliminating entire electrical circuits or breaking the light bulbs in the lamps.

The researchers said the technique will enable them to explore how the molecules, which are called neurotrophins, regulate the growth and survival of neurons in newborn and adult animals. Studying the regulation of neurotrophins is important because it will help researchers understand how the brain develops and functions in both normal and diseased states, they said. For example, neurotrophins play a role in supporting survival of neurons that are lost in neurodegenerative diseases such as Alzheimer's disease.

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The researchers described their chemical-genetic approach to controlling neurotrophin signaling in the April 7, 2005, issue of the journal *Neuron*. They were led by Howard Hughes Medical Institute investigator David D. Ginty at the Johns Hopkins University School of Medicine, and Pamela England and Kevan Shokat at the University of California, San Francisco. Shokat was among the 43 scientists recently selected in a nationwide competition to become HHMI investigators.

In their studies, the scientists sought to control neuronal growth more precisely by employing nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). These neurotrophins regulate neuronal growth by activating specific tyrosine kinase (Trk) receptors on the surface of neurons. The Trk receptors translate the neurotrophin signals to regulate neuronal growth and survival machinery.

Traditionally, when researchers wanted to study neurotrophin function in mice, they either knocked out those genes completely or attempted to switch them off after birth using genetic manipulation, drugs, or antibodies. According to Ginty, all these techniques have significant drawbacks.

“All the neurotrophin knockouts are lethal around birth,” said Ginty. “And other techniques of conditionally knocking out the genes after birth are limited in their application and are irreversible.” Drugs and antibodies that target neurotrophins are not specific enough, Ginty said. Antibodies are further limited because they trigger a general immune response and cannot cross the blood-brain barrier to infuse into brain tissues.

Shokat and his colleagues, however, had developed a technique for mutating a single amino acid in protein kinases that rendered these enzymes susceptible to kinase-inhibiting drugs that target only a specific type of kinase. The mutations have no other effect on the kinases' functions.

Ginty and his colleagues applied the chemical-genetic technique, which had been developed in cultured cells, to animals. They found that they could specifically deactivate the function of mutated receptors for either NGF, BDNF or NT-3 with the Trk-inhibiting drugs. When the drug was removed, the receptors would reactivate to their normal function. Thus, said Ginty, the new approach represents a powerful technique for exploring the neurotrophins by switching them off and on at will.

“A major advantage of this approach is that one has an ideal experimental control animal in the wild-type mouse, which has a normal amino acid instead of the mutation at the key position,” said Ginty. “Such an ideal control animal is something that one rarely, if ever, has in such experiments. Also critical is that since this is a pharmacological approach to controlling neurotrophin activity it is rapidly acting and reversible.”

Such a chemical-genetic approach in mice should be widely applicable, given that cells use a vast array of kinase switches, Ginty said. “I am so impressed with how well these in vivo experiments have gone that there is a good argument that a large number of protein kinases should be targeted in this way. There are over five hundred protein kinases in the genome and we have good inhibitors for only a very small number of them.”

The chemical-genetic approach could offer a far more comprehensive picture of neurotrophin function, said Ginty. “The null mutations reveal a phenotype,

but if it's a lethal phenotype then you never understand the full range of functions of that molecule," said Ginty. "Often times in the animal with a null mutation, one only sees the first function, and so you miss all the others," he said.

Ginty said that he and his colleagues plan to use the technique to explore how neurotrophins and their receptors control development of the forebrain immediately after birth. "A lot of the developmental events that control patterning of the forebrain occur postnatally, and until this technique, the roles of neurotrophins have been very difficult to study," he said. In addition, the technique can be used to explore where in the extensive geography of the neuron a particular neurotrophin receptor functions to regulate a particular developmental event, said Ginty.

"I am also hoping that other laboratories will use these engineered mice to study the role of neurotrophins in adult animals—for example in learning, memory, and neural plasticity. With this technique, people can ask questions about windows of time during which these molecules contribute to a given function, because it's possible to inhibit with fine temporal control and reversibility," he said.