



### New Biosensors Shed Light on Brain Cell Cross-Talk: October 29, 2010

Communication between brain cells is crucial for brain function. Brain cells communicate with one another by exchanging signaling molecules known as neurotransmitters and neuromodulators. These chemical messages only latch onto receiving cells that carry appropriate “mailboxes” or receptors.

Altered chemical signaling in the brain (brain chemistry) underlies many psychiatric, cognitive, and mood disorders. For example, a person may have too much or too little of a particular signaling molecule and/or receptor. For instance, a low level of dopamine transmission is linked to depression and attention deficit disorder, while a high level is linked to schizophrenia. Typically, such conditions are treated with medications. Most classes of psychiatric drugs in use today were discovered by chance; how they work is largely a mystery, in part due to lack of technology to monitor brain chemistry on an ongoing basis.

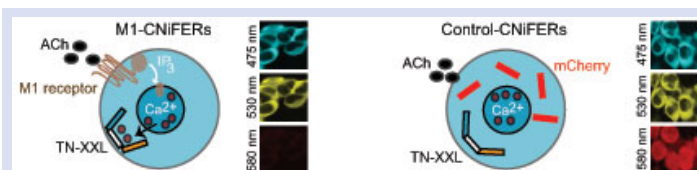
#### A Window Into the Inner Workings of the Brain

This technology gap motivated a University of California San Diego (UCSD) research team to devise a customizable system to monitor different types of receptors and chemical signals in the brain. Their cell-based neurotransmitter fluorescent engineered reporters (CNiFERS) consist of cultured cells that have been engineered to make a specific receptor of interest, along with a protein, called TN-XXL, that acts to report the intracellular concentration of calcium.

The implanted biosensor cells last in the brain for many days so that researchers can follow processes occurring over time. This allows scientists to use the same animal over again, an important feature for studying processes such as neuroplasticity (the ability of the brain to change in response to experience) and sleep.

The team first used CNiFERS to follow the activity of a

receptor for acetylcholine (ACh), a chemical involved in learning, attention, and neuroplasticity. ACh is thought to play a role in schizophrenia. “The pharmacology of schizophrenia is pretty sketchy. No one is truly sure what’s going on,” says the study’s principal investigator, David Kleinfeld, a professor of physics at UCSD. Although there are drugs that manage schizophrenia’s symptoms – hallucinations, delusions, and apathy – only one drug on the market, clozapine, has some beneficial effect on the cognitive aspects of the disease, such as learning and verbal memory. “The cognitive symptoms of schizophrenia correlate most with the ability to interact socially, keep a job, and function with activities of daily living,” points out Lee Schroeder, Ph.D., a former doctoral student in Kleinfeld’s lab. One hypothesis has been that clozapine affects cognitive function by causing a surge in ACh in the brain. On the other hand, the drug also appears to block the receptors for ACh. That leads to the question, which of these two modes of action predominates?



Calcium release triggered by activation of the acetylcholine receptor in M1-CNIFERs brings the yellow and blue “arms” of TN-XXL closer together, changing its color. In control CNiFERS, which lack the acetylcholine receptor, TN-XXL keeps an open shape and stable color. (Adapted by permission from Macmillan Publishers Ltd: Nature Neuroscience, Volume 13, 127-132, 2010.)

To answer this question, the research team implanted CNiFERs bearing ACh receptors into an animal's brain. A small part of the skull was removed to create a window through which the fluorescent cells could be viewed using a sophisticated instrument called a two-photon laser scanning microscope.

When ACh latches onto its receptor, it triggers a chain of events inside the cell: calcium is released and attaches to TN-XXL, causing it to change its shape slightly. This shape shift brings together the blue and yellow fluorescent proteins on two arms of TN-XXL, which in turn changes the color of the molecule. Essentially, the more yellow the molecule, the greater the receptor activation.

Researchers monitored the cells over a 6-day period. Using CNiFERs, the team discovered that, in spite of its ability to stimulate ACh release, clozapine's main mode of action is to block ACh receptors in the brain. This surprising finding may inform design of similar drugs with fewer side effects.

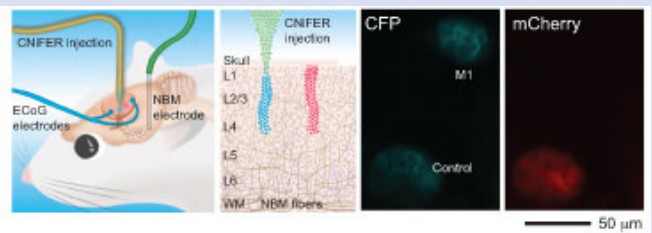
#### Building a CNiFER Library

CNiFERs can be used to measure receptor activity as well as the level of signaling molecules in response to pharmacological manipulation or electric stimulation. Encouraged by the success of the first application of CNiFERs, Kleinfeld's lab is moving toward testing other molecules. The system's modular design enables creation of new CNiFERs that combine cells containing TN-XXL with various receptors. At present, the team is focusing on G-protein-coupled receptors, which are targeted by roughly 50% of neuropsychiatric medicines in use today.

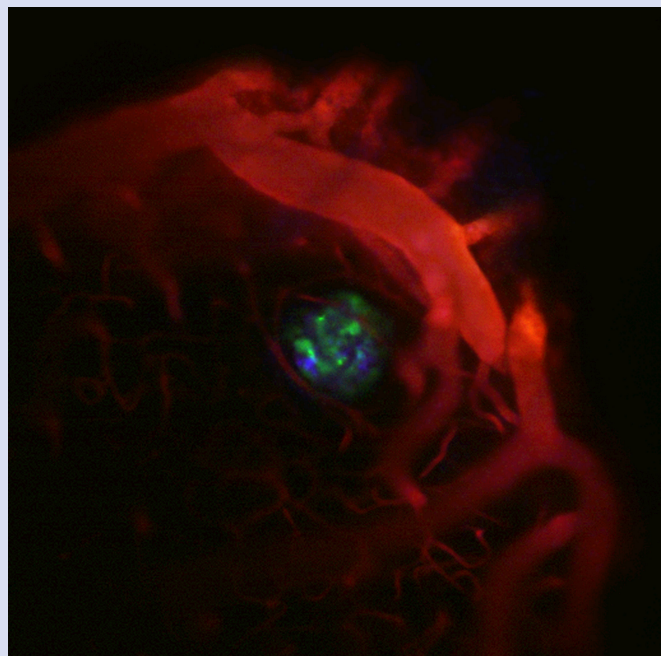
The group is exploring signaling molecules that affect blood vessels in the brain. Because all brain activity is accompanied by changes in local blood flow, the team hopes "to make sensors to look at the spectrum of molecules that change the constriction of blood vessels and to use those sensors in the brain," indicates Kleinfeld.

Paul Slesinger, an associate professor at the Salk Institute's Clayton Foundation Laboratories for Peptide Biology, is collaborating with Kleinfeld's team to build a library of CNiFERs that will respond to specific neurotransmitters, including neuropeptide transmitters. Slesinger's lab is interested in using CNiFERs to study the molecular mechanisms of drug addiction. "We would like to monitor levels of dopamine in specific animal models of addiction," indicates Slesinger, "and one of the obstacles we are trying to overcome is measuring activity in deep areas of the brain." Deep imaging is difficult, though physically possible, and it is here that the collaboration of Kleinfeld and Slesinger may well shine.

CNiFERs offer an open-ended opportunity to gain insight into the basic biology of the brain as well as evaluate new drugs. "The ability to measure how the brain chemistry is changing from moment to moment is



Testing CNiFER sensitivity *in vivo*: M1-CNiFERs (blue) and control CNiFERs (red) were implanted at separate places in the rat brain, and electrodes were implanted to stimulate acetylcholine release. (Adapted by permission from Macmillan Publishers Ltd: Nature Neuroscience, Volume 13, 127-132, 2010.)



CNiFER cells chronically embedded into frontal cortex of rat. Two-photon laser microscopy shows acetylcholine-sensitive CNiFERs (green) and control cells (blue) within a nest of vasculature (red). (Adapted by permission from Macmillan Publishers Ltd: Nature Neuroscience, Volume 13, 127-132, 2010.)

fundamental to neuroscience,” states Schroeder. If successful, CNiFERs would be the first tool that can successfully measure neuropeptides *in vivo*.

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#### References

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