# Method reveals new view of human nerve cells, opening door to potential drug targets

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Scientists at The Scripps Research Institute and University of Pennsylvania have found a way to uncover potential drug targets that have so far remained hidden from researchers' view.

By applying the new method to a type of nerve cell critical to regulating body temperature, the authors found more than 400 "receptors" (structures that bind other molecules, triggering some effect on the cell) responding to neurotransmitters, hormones, and other chemical signals. This represents 20 to 30 times more receptors than previous studies had identified.

The technique, described in detail in a review article in the March 11, 2011 issue of the journal *Pharmacology and Therapeutics*, may be applied to finding "hidden" receptors in other types of nerve cells, expanding the repertoire of potential drug targets for diseases ranging from schizophrenia to Parkinson's disease.

"This technique will enable people to uncover many more drug targets," said Tamas Bartfai, chair of the Department of Molecular and Integrative Neuroscience at Scripps Research. "That may be a game changer for some diseases."

## Uncovering Rare Receptors

Receptors found on cells are among the most important targets for the

development of drugs because of the key roles they play in the communication circuits regulating various body functions. So far scientists have identified only a few of the receptors present on different types of nerve cells.

Bartfai's group has long been interested in a class of nerve cells in the brain called "warm sensitive neurons." These cells sense and respond to changes in body temperature, acting like a thermometer inside the brain. As body temperature increases, warm sensitive neurons become more active, telling the body to bring its temperature down. Without this regulation, body temperature could reach dangerous levels, even leading to death.

In the past 60 years, scientists had identified about a dozen receptors on warm sensitive neurons that regulate these nerve cells' activity. But Bartfai wanted to find additional receptors to better understand how the cells function.

To do so, he turned to long-time collaborator University of Pennsylvania Professor James Eberwine. Eberwine had pioneered a number of techniques to identify genes active in individual cells.

## Sequencing Single Neurons

Bartfai and Eberwine took a unique approach to indentifying gene activity.

Scientists know a gene is "on" in a cell if its messenger RNA (which carries information from genes to sites of protein synthesis) is present. To study gene activity in warm sensitive cells, Eberwine and Bartfai isolated single cells and extracted their RNA. They then made cDNA copies of the messenger RNAs and determined the sequence of the nucleotide bases (adenine, guanine, cytosine, and thymine) in each

cDNA molecule.

By matching the DNA sequences obtained to published sequences, the scientists were able to identify the corresponding genes, and thus which genes are turned "on" in the nerve cells.

The technique differs from commonly used methods for studying gene activity. Typically researchers "pool" neurons of one type and examine them as a group, rather than studying single cells. In addition, current techniques generally rely on searching for active genes using microarrays—a technique that relies on the preferential binding of sequences in the messenger RNAs /cDNAs to matching DNA sequences "spotted" on the microarray. However, these methods only detect RNAs for which "probes are present on the microarray," in other words, those that are expected. Also, because of the lower sensitivity of this technique than sequencing, only the cDNAs cells produce in relatively large amounts are detected.

"Using single cells, rather than pooling, and sequencing, rather than microarrays, uncovers many more receptors active in neurons," says Bartfai. "With other methods you miss receptors present in only a few copies. But that does not mean that they are not important."

## Revealing Neurons' Complexity

Using their new method Bartfai and Eberwine identified more than 400 receptors active in warm sensitive neurons. About one-third of the receptors are so-called "orphan" receptors, meaning the chemicals they bind to are unknown. The rest were receptors whose ligands (substances they bind to) are known—among them, the authors found a few surprises.

For example, Bartfai and Eberwine discovered that the receptor

responsible for binding insulin is active on warm sensitive neurons—something no one had previously suspected.

The insulin receptor is known to be involved in regulating a person's metabolism. Follow-up studies by Bartfai's group have now shown that insulin binds to receptors on warm sensitive neurons to decrease their activity, causing an increase in body temperature, or hyperthermia. Thus, insulin is a key regulator for both body metabolism and temperature.

"This study highlights the complexity of these cells by showing us the large number of different RNAs that are present," said Eberwine.

## Game-Changing Research

In addition to providing important insights into the complexity of nerve cells, the study has implications for identifying potential drug targets for diseases that currently have few or no treatments.

"We would like to repeat similar studies for key neurons involved in Parkinson's disease and schizophrenia," explained Bartfai. "If we again discover 400 receptors, we could then ask which ones are reasonably selectively expressed in these neurons." Any receptor active primarily in one class of neurons involved in a particular disease process represents a possible target for developing drugs to affect the course of that disease.

**More information:** dx.doi.org/10.1016/j.pharmthera.2010.09.010

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