Optical technique reveals unnexpected complexity in mammalian olfactory coding

October 18 2010

A team co-led by neuroscientists at Cold Spring Harbor Laboratory (CSHL) has shed light -- literally -- on circuitry underlying the olfactory system in mammals, giving us a new view of how that system may pull off some of its most amazing feats.

It has long been known from behavioral experiments that rodents, for instance, can tell the difference between two quite similar odors in a single sniff. But in such instances, what precisely happens in the "wiring" leading from sensory neurons in the nose to specialized cells in the olfactory bulb that gather the signals and transmit them to the brain? How can this occur within the brief span of a single respiratory cycle -- one inhalation and one exhalation?

Using a new method of exploring this question, CSHL scientists, in collaboration with researchers at Harvard University and the National Centre for Biological Science in Bangalore, India, have assembled evidence suggesting that the olfactory bulb in mice is not merely a relay station between the nose and brain, as many have supposed. Their data, published today in Nature Neuroscience, indicates that "there are many more information output channels leaving the olfactory bulb [en route to the cortex] than the number of information types entering it," from sensory receptors in the nose.

This complexity in sensory coding, which the team speculates may help the brain rapidly make highly accurate odor distinctions, became evident when the team used beams of light to activate highly specialized cells

within the olfactory bulb, as prelude to measuring their electrical activity during single respiratory cycles.

Using beams of light to trace the circuit

The first step of the investigation involved using genetic engineering to generate a line of mice whose sensory neurons expressed a gene borrowed from a kind of algae that make them fire when beams of light are focused upon them. The algal gene expresses a photoreceptor protein called channelrhodopsin-2 (ChR2).

"We did this, when I was still at Harvard, in Venki Murthy's group, to overcome a problem that has limited olfactory circuit measurement in the past," says Florin Albeanu, Ph.D., a newly appointed assistant professor at CSHL who co-led the investigation. "Previously, we would have tried to insert electrodes into the olfactory bulb of the mouse. This is a bit like flying blind -- you can record the electrical activity of any cells you happen to hit, but you had no way of controlling which cells you would be recording from. Ashesh Dhawale, whom I met at a CSHL course on advanced imaging methods three years ago, and I decided to use light stimulation of the sensory neurons coupled with electrical recordings from the output neurons of the bulb to solve this problem."

The cells Albeanu, Dhawale and colleagues wanted to monitor are called mitral cells. These specialized cells are the final "broadcasting" stations for messages to the mouse's cortex that start out when neurons in the nasal epithelium sense an odor. Each sensory neuron is specialized to express a single molecular odor receptor type, from among some 1500 in a mouse's olfactory palette. Cells with the same sensitivity report their signals to a single specialized spherical unit -- literally a ball of synapses -- in the mouse's olfactory bulb. These collecting stations are called glomeruli.

The glomeruli each have about 15,000 axons feeding into them, and each, in turn, signals via primary dendrites to mitral cells. One glomerulus may be connected to as many as 30 mitral cells, all of which are also located in the olfactory bulb. Mitral cells also sport secondary dendrites, fibers which connect them laterally with one another via reciprocal synapses that link them to inhibitory local interneurons. By directly activating glomeruli in the olfactory bulb with tiny beams of light, the team was able to identify mitral cells with great specificity.

Explaining 'sister' cells in and out of synch

They were able to target -- and record electrical signals (action potentials) -- from mitral cells that they call "sister cells," whose responses on average are quite similar. This stands to reason because sister cells, by definition, receive information via primary dendrites from the same glomerulus. The scientists were also able to identify and monitor activity of "non-sister" mitral cells, ones whose firing activity is fundamentally dissimilar because of the comparatively wide contrast in the odors that their glomeruli report.

Perhaps the most interesting and surprising finding of the electrical recordings obtained in this fashion is that sister mitral cells, although they are connected to the same glomerulus, do not respond redundantly when a mouse is presented with an odor to which the glomerulus is responsive. In fact, says Albeanu, "we observed that odors de-synchronize pairs of sister mitral cells. This is something we did not expect."

How to explain this? The team formed a hypothesis based on comparisons of recordings made of the activity of many pairs of sister and non-sister mitral cells. According to Albeanu, "We thought of two metrics of activity with which to compare sisters and non-sisters. One kind compares the number of times cells fire in a single respiratory

cycle; the other compares the intervals between their firing 'spikes,' and more specifically the points at which they occur relative to the start of a respiratory cycle.

"In other words, we looked at the rate of their firing, and compared it, in the same cells, both sisters and non-sisters, to the timing of their firing," says Albeanu. The team found that in sister cells, when the rate of firing changed in one sister, it changed in a similar manner in the other sister. But the timing of their firing differed -- instead of firing at the same moment, they fired asynchronously.

"This leads us to believe that two types of code are being utilized -- it's as if mitral cells are capable of conveying two different kinds of information to the cortex, and that they do so by multiplexing rate and time codes in the same message," according to Albeanu.

More information = more accurate odor representations?

These independent channels, the team speculates, might convey to the brain two different things. Changes in the rate of firing, they say, reflect the information coming from a glomerulus that sisters share. This very specific information is accompanied by an independent message that may reflect the average of the outputs of glomeruli in the general region, or "surround," of the specific glomerulus that the sisters share. These would be the product of information exchanged via the lateral connections between mitral cells with different lag times in firing, which the scientists call latencies. Broadcasting to the cortex on these two channels "would give the cortex more information with which to discriminate the odor that has been sensed," Albeanu says.

In future studies the scientists plan to systematically explore the effect of

changing odor concentration on the rate and timing properties they have already observed. They have also already started to use the mouse line they created with light-sensitive olfactory sensory neurons in behavioral studies that may shed light onto what spatio-temporal rules govern odor coding at the level of the olfactory bulb.

More information: "Non-redundant odor coding by sister mitral cells revealed by light-addressable glomeruli in the mouse" appears in *Nature Neuroscience* on October 17. www.nature.com/neuro/index.html

Provided by Cold Spring Harbor Laboratory