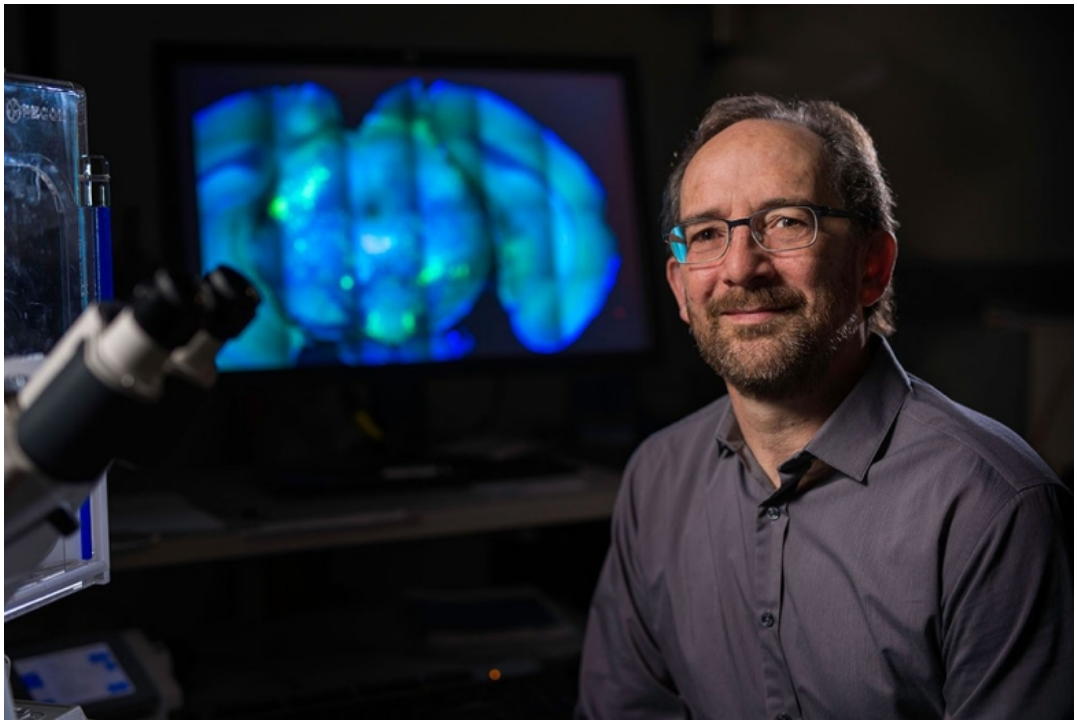
Study shows how slow breathing induces tranquility

Stanford scientists have identified a small group of neurons that communicates goings-on in the brain's respiratory control center to the structure responsible for generating arousal throughout the brain.

March 30, 2017 - By Bruce Goldman



Mark Krasnow and his colleagues have identified a tiny cluster of neurons that link breathing to relaxation, attention, excitement and anxiety.
Steve Fisch

Try it. Breathe slowly and smoothly. A pervasive sense of calm descends. Now breathe rapidly and frenetically. Tension mounts. Why?

It's a question that has never been answered by science, until now.

In a new study, researchers at the Stanford University School of Medicine and their colleagues have identified a handful of nerve cells in the brainstem that connect breathing to states of mind.

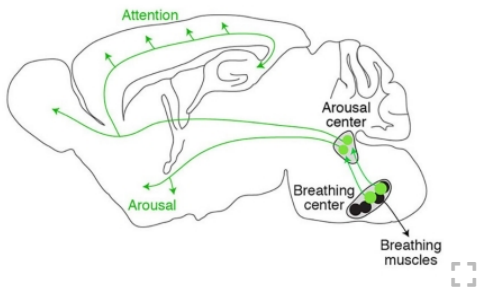
A paper describing the findings was published March 31 in *Science*. Mark Krasnow, MD, PhD, professor of biochemistry, is the senior author. The lead author is former Stanford graduate student Kevin Yackle, MD, PhD, now a faculty fellow at the University of California-San Francisco.

Medical practitioners sometimes prescribe breathing-control exercises for people with stress disorders. Similarly, the practice of pranayama — controlling breath in order to shift one's consciousness from an aroused or even frantic state to a more meditative one — is a core component of virtually all varieties of yoga.

"This study is intriguing because it provides a cellular and molecular understanding of how that might work," Krasnow said.

Tiny cluster of neurons

The tiny cluster of neurons linking respiration to relaxation, attention, excitement and anxiety is located deep in the brainstem. This cluster, located in an area Krasnow calls the pacemaker for breathing, was discovered in mice by study co-author Jack Feldman, PhD, a professor of neurobiology at UCLA, who published his findings in 1991. An equivalent structure has since been identified in humans.



The diagram depicts the pathway (in green) that directly connects the brain's breathing center to the arousal center and the rest of the brain.
Courtesy of the Krasnow lab

"The respiratory pacemaker has, in some respects, a tougher job than its counterpart in the heart," said Krasnow, who is also a Howard Hughes Medical Institute investigator. "Unlike the heart's one-dimensional, slow-to-fast continuum, there are many distinct types of breaths: regular, excited, sighing, yawning, gasping, sleeping, laughing, sobbing. We wondered if different subtypes of neurons within the respiratory control center might be in charge of generating these different types of breath."

On that hunch, Yackle searched through public databases to assemble a list of genes that are preferentially activated in the part of the mouse brainstem where the breathing-control center resides. This center's technical term is the pre-Bötzinger complex, or preBötC.

He pinpointed a number of such genes, allowing the investigators to identify more than 60 separate neuronal subtypes, physically differentiated from one another by their gene-activation signatures but comingling in the preBötC like well-stirred spaghetti strands. The scientists were able to use these genes, and the protein products for which they are recipes, as markers allowing them to zero in on the different neuronal subtypes.

Knocking out neurons

Now the scientists could systematically assess the role of each neuronal subpopulation in laboratory mice. With advanced technologies, they could selectively destroy any one of these neuronal subtypes — and only that subtype — based on its unique signature of active genes. Then they could observe how this particular subtype's loss affected the animals' breathing. In 2016, in collaboration with Feldman, they succeeded in isolating a subpopulation of neurons in the preBötC that explicitly controls one type of breathing: sighing. Knocking out these neurons eliminated sighing but left other modes of breathing unaffected. The discovery was published in *Nature* in 2016.

Krasnow and Yackle then set out to discover the respiratory role of another subpopulation of about 175 preBötC neurons distinguished by their shared expression of two genetic markers called Cdh9 and Dbx1. They bioengineered mice in which they could wipe out, at will, the neurons bearing both of these markers.

Kevin Yackle

But once these rodents had their Cdh9/Dbx1 neurons eliminated, they seemed to take the loss in stride. Unlike their sigh-deprived brethren, there was no lacuna in these mice's portfolio of breathing variations.

"I was initially disappointed," said Yackle.

But a few days afterward, he noticed something: For mice, the animals were extraordinarily calm. "If you put them in a novel environment, which normally stimulates lots of sniffing and exploration," Yackle said, "they would just sit around grooming themselves" — evidence of what passes for mellowness when you're a mouse.

Chilling out

Further analysis showed that while these mice still displayed the full palette of breathing varieties from sighs to sniffs, the relative proportions of those varieties had changed. There were fewer fast "active" and faster "sniffing" breaths, and more slow breaths associated with chilling out.

The investigators surmised that rather than regulating breathing, these neurons were spying on it instead and reporting their finding to another structure in the brainstem. This structure, the locus coeruleus, sends projections to practically every part of the brain and drives arousal: waking us from sleep, maintaining our alertness and, if excessive, triggering anxiety and distress. It's known that neurons in the locus coeruleus exhibit rhythmic behavior whose timing is correlated with that of breathing. In a series of experiments, the Stanford researchers proved that the preBötC neurons that express Cadh9 and Dbx1 not only project to the locus coeruleus — a new finding — but activate its long-distance-projections, promoting brainwide arousal.

"If something's impairing or accelerating your breathing, you need to know right away," said Krasnow. "These 175 neurons, which tell the rest of the brain what's going on, are absolutely critical."

"The preBötC now appears to play a key role in the effects of breathing on arousal and emotion, such as seen during meditation," said Feldman. "We're hopeful that understanding this center's function will lead to therapies for stress, depression and other negative emotions."

Other Stanford co-authors are John Huguenard, PhD, professor of neurology and neurological sciences; Liqun Luo, PhD, professor of biology and an HHMI investigator; former postdoctoral scholar Lindsay Schwarz, PhD; and graduate student Jordan Sorkin.

A researcher at the Chicago Medical School also co-authored the study.

Krasnow is also executive director of the Wall Center for Pulmonary Vascular Disease, a member of the Stanford's Neurosciences Institute, Cardiovascular Institute, Cancer Institute and Bio-X.

The study was funded by the National Institutes of Health (grants HL70029 and HL40959) and HHMI.

Stanford's Department of Biochemistry also supported the work.

Bruce Goldman

Bruce Goldman is a science writer in the Office of Communications. Email him at goldmanb@stanford.edu.

Media Contacts

Becky Bach
Tel 530-415-0507
retrout@stanford.edu

Margarita Gallardo
Tel 650-723-7897
mjgallardo@stanford.edu

Stanford Medicine integrates research, medical education and health care at its three institutions - Stanford School of Medicine, Stanford Health Care, and Stanford Children's Health. For more information, please visit the Office of Communications website at http://mednews.stanford.edu.