Researchers develop mouse with 'off switch' in key brain cell population

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NIH-funded scientists have developed a strain of mice with a built-in off switch that can selectively shut down the animals' serotonin-producing cells, which make up a brain network controlling breathing, temperature regulation, and mood. The switch controls only the serotonin-producing cells, and does not affect any other cells in the animal's brains or bodies.

When the researchers powered down the animals' serotonin cells, the animals failed to sufficiently step up their breathing to compensate for an increase of carbon dioxide in the air, and their body temperatures dropped to match the surrounding temperature.

The finding has implications for understanding sudden infant death syndrome, or SIDS, which has been linked to low serotonin levels, and is thought to involve breathing abnormalities and problems with temperature control. The finding may also provide insight into depressive disorders, which also involve serotonin metabolism.

The study results appear in the current issue of the journal *Science*.

SIDS is the death of an infant before his or her first birthday that cannot be explained after a complete autopsy, an investigation of the scene and circumstances of the death, and a review of the medical history of the infant and of his or her family. According to the National Center for Health Statistics, SIDS is the third leading cause of infant death.

"The single most effective way to reduce the risk of SIDS is to always

place infants on their backs for sleep," said Marian Willinger, Ph.D., special assistant for SIDS at the NIH's Eunice Kennedy Shriver National Institute of Child Health and Human Development, which provided major funding for the study. "This new animal model of the serotonin-producing system holds the promise of helping us to understand the biological processes contributing to SIDS, which is critical for the development of tests and interventions to prevent these deaths." Additional NIH support was provided by the National Institute of Mental Health, National Institute on Drug Abuse, and National Center for Research Resources.

To conduct the study, the researchers developed mice with a unique molecule, or receptor, on the surface of their serotonin-producing brain cells, or neurons. Typically, cells communicate via chemicals that bind to receptors on their surfaces, with the molecules binding to their receptors in much the same way a key fits into a lock.

The researchers added this special receptor to the animal's serotonin-producing neurons using a genetic manipulation technique they developed called intersectional genetics. The special receptor was developed by NIH-funded researcher Bryan Roth. The approach consists of manipulating the animals' genetic material so that it manufacturers an additional receptor on the surface of its neurons. In this case, the animals' serotonin-producing cells began making a receptor that is not found in nature. Rather than binding to a naturally occurring brain chemical, the receptor binds to a chemical compound manufactured in a laboratory, clozapine-N-oxide (CNO).

"CNO was identified for its ability to bind specifically to this foreign receptor that we placed into the serotonin cells, and because it does not react with other cells or tissues in the animal's body," Dr. Dymecki explained.

When CNO binds with the receptor, it deactivates only the serotonin cells, effectively switching off all communications in the serotonin network. CNO does not affect any other cells in the animals' brains or bodies.

"By selectively switching off the serotonin-producing cells, we can get a definite idea of what bodily functions the serotonin cells specifically control" she said.

The researchers exposed genetically normal mice and mice with the receptor for CNO to elevated levels of carbon dioxide. Carbon dioxide is the waste product given off when a breath is exhaled. If carbon dioxide builds up in the body, due to insufficient breathing, it can be toxic, leading to loss of consciousness and death. The response to high carbon dioxide accumulation is increased breathing and a faster breathing rate, which releases carbon dioxide through the lungs.

When the normal mice were exposed to carbon dioxide, they almost immediately began to breathe faster and more deeply. In contrast, after their serotonin-producing neurons were switched off, mice with the receptor to CNO had a smaller response to carbon dioxide and did not increase their breathing as much.

"This finding shows that the breathing response to carbon dioxide is regulated by serotonin neurons," Dr. Dymecki said.

The researchers next tested the ability of the CNO-responsive mice to regulate their body temperatures. When the room temperature was set at 74 degrees Fahrenheit, the body temperature of normal mice remained at about 98.6 degrees—the normal temperature for mice. Normal mice can maintain a normal body temperature even when the room temperature is cool and below that of body temperature, Dr. Dymecki added. However, after their serotonin neurons were switched off with

CNO, the body temperatures of the CNO mice soon plunged. Like reptiles faced with a sudden temperature drop, the body temperatures of the mice soon dropped to the 74 degree room temperature.

"Their body temperatures were equilibrating with the room temperature," Dr. Dymecki said. "Our finding affirms that temperature is regulated by the serotonergic system."

Dr. Dymecki explained that the researchers added the CNO receptor to all the animals' serotonergic neurons. In future studies, she and her colleagues plan to selectively add the receptor to subsets of serotonergic receptors, to better understand their functioning in health, and in disorders such as SIDS and depression.

The finding provides support for previous autopsy studies by NIH grantees implicating abnormalities in serotonin metabolism in the brainstem as playing a role in SIDS.

Researchers theorize that infants who die of SIDS may have been unable to respond to breathing challenges, such as low levels of oxygen or high levels of carbon dioxide. High levels of carbon dioxide may accumulate around the face of an infant sleeping face down, when the infant's exhaled breath accumulates in a pocket formed by bedding materials.

The ability to regulate body temperature is also thought to play a role in SIDS deaths. The NICHD's Back to Sleep campaign advises parents and caregivers to avoid letting infants overheat during sleep, to dress them in light sleep clothes, avoid blankets or coverings, and to keep the room at a temperature that is comfortable for an adult.

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