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Learning How Not to Be Afraid

Why do some people have the ability to remain calm and relaxed even in the most stressful situations? New experiments in mice by Howard Hughes Medical Institute (HHMI) researchers are providing insight into how the brain changes when the animals learn to feel safe and secure in situations that would normally make them anxious.

HHMI investigator Eric R. Kandel and Daniela D. Pollak conducted experiments in which they conditioned mice to feel safe in stressful situations. Their experiments showed that the mice developed a conditioned inhibition of fear, which Kandel calls learned safety.

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The behavioral changes observed in the mice squelched anxiety as effectively as antidepressant drugs such as Prozac, said Kandel, who is at Columbia University. It's a little bit like psychotherapy, he noted. This shows that behavioral intervention works.

The research is reported on October 9, 2008, in the journal *Neuron*. Kandel conducted the study with Pollak, who will soon leave Kandel's lab to assume a position at the Medical University of Vienna.

The new study is noteworthy because it reveals in elegant detail how behavioral conditioning can affect the brain. According to Kandel, knowing how behavioral intervention works at the molecular and cellular levels may prove to be an interesting route to identifying new ways to treat depression and anxiety disorders.

Kandel, who trained as a psychiatrist, is intrigued by the new discoveries. I've always been interested in how psychoanalysis works, he said. Since it is a learning experience, there must be a biological basis in the brain.

Two types of fear, instinctive and learned, have deep evolutionary roots and are essential for survival. But in some people, pathological forms of learned

fear can lead to debilitating anxiety disorders, post-traumatic stress syndrome, or depression. Learned safety, on the other hand, reduces chronic stress, one of the hallmarks of depression and other psychopathologies. The ability to identify, develop, and exploit conditions of safety and security is central to survival and mental health, said Kandel, but little is known of the neurobiology of these processes.

In previous research, Kandel's group taught mice to associate a specific audible tone with protection from an impending aversive event. Over time, the mice became conditioned to take advantage of sources of safety and security in their environments. In the new *Neuron* study, Pollak and Kandel sought to tease out the behavioral and molecular characteristics of learned safety in mice.

In their experiments, mice were trained to associate safety or fear with specific auditory stimuli (tones). For fear conditioning, the auditory stimulus was paired with a mild shock to the mouse's foot. For safety conditioning, the auditory stimulus was not followed by a shock. The experiments showed that the safety-conditioned mice learned to associate the tone with the absence of danger and displayed less anxiety in the presence of this safety signal.

Moving to a stress test, Kandel's team placed the safety-conditioned mice into a pool of water for a swim test. The forced-swim test is commonly used by researchers to measure how antidepressant drugs affect the behavior of mice. In this seemingly desperate situation - where the mice have no option to escape from the water — they start to show signs of behavioral despair that are ameliorated by antidepressant medications. We found that the mice trained for safety could overcome their sense of hopelessness in the swim test, Kandel explained. The antidepressant effect in the safety-conditioned mice was similar and comparable in magnitude to treatment with the drug fluoxetine (Prozac), Kandel noted.

Pollak and Kandel then looked at how learned safety influenced the development of newborn cells in the dentate gyrus, a structure located in a region of the brain called the hippocampus. The dentate gyrus is notable because it is one of the few structures in the brain that spawns new neurons - even in adult animals.

The researchers found that mice that had been conditioned for safety had a greater number of newborn cells in the dentate gyrus. When Kandel's team used radiation to blunt the birth of new cells in the dentate gyrus, they discovered that their interventions both slowed safety learning and stunted the antidepressant effects of learned safety.

Pollak and Kandel also found that safety learning ramped up expression of brain-derived neurotrophic factor or BDNF in the dentate gyrus. BDNF is a growth factor that promotes the growth and differentiation of new neurons and their connections.

Intriguingly, genetic analyses revealed that in the amygdala, the brain's fear center, learned safety tunes the expression of key components of the

dopamine neurotransmitter system and the neuropeptide system. Both systems are thought to influence learning, mood, and cognition.

Kandel said his group was intrigued to find that learned safety did not influence serotonin, the neurotransmitter typically targeted by antidepressant drugs. Learned safety appears to influence levels of both dopamine and neuropeptide neurotransmitters, suggesting new avenues for antidepressant drug development, he said.

This has given us several interesting insights and led us to a number of potential targets for new drugs, Kandel explained, noting there are already agents in development that influence the dopamine and neuropeptide pathways.