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Rubbing out Fearful Memories

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Abstract:

This report discuss 3 different studies about erasing fears by many strategies using drugs, optogenetics & using Protein kinase M zeta.

Introduction:

A hurricane, a car accident, a roadside bomb, a rape — extreme stress is more common than you might think, with an estimated 50 to 60 percent of people experiencing it at some point in their lives. About 8 percent of that group will be diagnosed with post-traumatic stress disorder, or PTSD. They will have flashbacks and nightmares. they will feel amped up, with nerves on a permanent state of high alert. they won't be able to forget.

So that's why it is so important to erase fears from our heads.

Study 1 (Drug Tweaks Epigenome to Erase Fear Memories)

A mouse study published in *Cell* throws the spotlight on a drug that acts in concert with exposure therapy to help extinguish fear memories. The drug works by changing the epigenome, the chemical markers that attach to DNA and can turn genes on and off.

“It's remarkable,” says Li-Huei Tsai, a neuroscientist at the Massachusetts Institute of Technology who led the work. “If we inject a single dose of this drug it actually is sufficient to reactivate neuroplasticity.”

The drug works by changing the way DNA is expressed in the brain.

In order to fit into the nucleus of each cell, DNA wraps tightly around spherical proteins called histones. Histones are littered with chemical groups, such as methyl and acetyl, that influence how nearby genes get turned on and off.

For many years, Tsai has been studying enzymes called histone deacetylases, or HDACs, which switch off genes by removing acetyl groups from histones. In 2012, she showed that one such enzyme, dubbed HDAC2, is overactive in a mouse model of Alzheimer's disease and shuts down genes related to learning. In that study she also showed that blocking HDAC2 led to dramatic gains in the animals' memory.

“HDAC2 is a master regulator of the expression of neuroplasticity genes,” Tsai says. “And HDAC inhibitors seem to be very beneficial for memory formation.”

In the new study, Tsai's team investigated whether this enzyme is also involved in the way that fear memories cement themselves into brain circuits.

Mice don't get PTSD, but they can acquire fear memories. Using so-called Pavlovian fear conditioning, researchers train the animals to fear a particular cue, such as a sound or smell, by pairing it with a mild shock to the foot. After a few trials, the animal freezes at the cue alone.

There's also a mouse version of exposure therapy. After a mouse learns to fear, say, a certain tone, researchers can extinguish that fear by repeatedly playing the tone without a shock. Gradually the animal learns to associate the tone with the safer context.

But in mice (and, importantly, in some people with severe PTSD), this extinction therapy only works for recently acquired fear memories. If a fear memory is old, then no amount of retraining will erase the animal's fear. “One of the major challenges in developing treatments for PTSD is that traumatic memories can persist for a lifetime,” notes Matt Lattal, a neuroscientist at Oregon Health and Science University who was not involved in the new study. “It is therefore critical that laboratory models of PTSD include this long interval between traumatic experience and testing.”

Tsai and her colleagues trained mice to fear a tone and then gave them extinction therapy either a day later or 30 days later. When extinction training happened a day later, the HDAC2 enzyme was inactivated in brain cells, the study found. With

HDAC2 quiet, acetyl groups stayed latched on to histones and various memory genes stayed on. Presumably, this window of plasticity allowed the mice to un-learn the fear memory. In contrast, when extinction training happened 30 days later, the HDAC2 enzyme was active. It removed those acetyl groups, effectively shutting off neuroplasticity genes.

But here's the exciting part. The animals were able to un-learn the fear memory 30 days after it was formed when the researchers paired extinction therapy with a drug that inhibits HDAC2, dubbed "CI-994." It only took one dose, and the researchers saw no side effects, Tsai says. "We did a lot of control experiments to show that this mechanism doesn't wipe out other memories. It really is very specific to the training condition."

HDAC inhibitors are becoming a hot class of drugs. In 2012, Yossef Itzhak and his colleagues at the University of Miami reported that giving a different HDAC inhibitor to mice before they acquire the fear memory accelerates the extinction of the memory weeks later.¹

Study 2

Published in the journal *Neuron* by Cho and his colleague Woong Bin Kim, the research reveals how the team used genetically modified mice to examine the pathways between the area of the brain involved in processing a particular sound and the area involved in emotional memories, known as the amygdala.

"These mice are special in that we can label or tag specific pathways that convey certain signals to the amygdala, so that we can identify which pathways are really modified as the mice learn to fear a particular sound," said Cho. "It is like a bundle of phone lines," he added. "Each phone line conveys certain auditory information to the amygdala."

In the first part of the experiment the team played both a high pitched and low-pitched tone to mice. But, when the high-pitched sound was played, the researchers also gave the mice a small electric shock to their feet.

When the high-pitched tone was subsequently played on its own, the mice froze in fear; no such response was seen when the alternative, low-pitched, tone was played. The team then looked to see if there were differences between the high-pitch and low-pitch tone pathways in the brains of the mice, revealing that, among the mice exposed electric shocks, the connections within the "high-pitched" pathway had become stronger, while the other pathway remained unchanged.

The team found that when mice were subsequently repeatedly exposed to high-pitched sounds without the shocks they lost their fear – a process known as fear extinction.

"Fear extinction is the psychological basis of exposure therapy used in [treating] post-traumatic stress disorder," said Cho. But, he said, "after exposure therapy, for example two weeks, the fear relapses or recurs spontaneously."

The new research, he adds, offers an explanation: even after fear extinction, the team found the neural pathway for the high-pitched tone remained strengthened in the mice.

"Fear extinction is not an eraser of fear memory," said Cho. "It just hides the fear memory transiently."

But the team discovered that using a technique called optogenetics, it was possible to truly erase the unpleasant memories.

This technique involved the researchers using a virus to introduce genes into particular neurons in the brains of the mice that were involved in the "high-pitch" pathways.

Once inside the cells, the genes result in the production of proteins which respond to light, allowing researchers to control the activity of the neurons.

Taking mice with the fearful memories, the team exposed the neurons involved in the “high-pitch” pathway to low-frequency light – an approach which weakens the connections between the neurons.

The upshot was that the mice no longer appeared fearful when they heard the high-pitched tone.

“It permanently erases the fear memory,” said Cho. “We no longer see the relapse of fear.”

Peter Giese, professor of neurobiology of mental health at King’s College London, said it was too soon to think of using the research to help those with psychopathologies, saying it would be unethical to use optogenetic techniques on people. “Exactly how this can be applied to humans is a little bit unclear to me,” he said.²

Study 3

Background

Protein kinase M zeta (PKM), a constitutively active isoform of protein kinase C, has been implicated in protein synthesis-dependent maintenance of long-term potentiation and memory storage in the brain. Recent studies reported that local application of ZIP, a membrane-permeant PKM inhibitor, into the insular cortex (IC) of behaving rats abolished long-term memory of taste associations.

Method

This study assessed the long-term effects of local applications of ZIP microinjected immediately (1 h) or 10 days after predator scent stress exposure, in a controlled prospectively designed animal model for PTSD. Four brain structures known to be involved in memory processes and in anxiety were investigated: lateral ventricle (LV), dorsal hippocampus (DH), basolateral amygdala and IC. The outcome measures included behavior in an elevated plus maze and acoustic startle response 7 days after microinjection, and freezing behavior upon exposure to trauma-related cue 8 days after microinjection. Previously acquired/encoded memories associated with the IC were also assessed.

Results in

Inactivation of PKM in the LV or DH within 1h of exposure effectively reduced PTSD-like behavioral disruption and trauma cue response 8 days later. Inactivation of PKM 10 days after exposure had equivalent effects only when administered in the IC. The effect was demonstrated to be specific for trauma memories, whereas previously acquired data were unaffected by the procedure.³

Conclusions:

1. Giving a different HDAC inhibitor to mice before they acquire the fear memory accelerates the extinction of the memory weeks later.
2. It would be unethical to use optogenetic techniques
3. Predator scent related memories are located in different brain areas at different times beginning with an initial hippocampus-dependent consolidation process, and are eventually stored in the IC. These bring the IC to the forefront as a potential region of significance in processes related to traumatic stress-induced disorders.

References:

- (1). <http://phenomena.nationalgeographic.com/2014/01/22/drug-tweaks-epigenome-to-erase-fear-memories/>
- (2). <https://www.theguardian.com/science/2017/aug/17/memories-of-fear-could-be-permanently-erased-study-shows>
- (3). [https://www.europeanneuropsychopharmacology.com/article/S0924-977X\(10\)00002-7/abstract](https://www.europeanneuropsychopharmacology.com/article/S0924-977X(10)00002-7/abstract)