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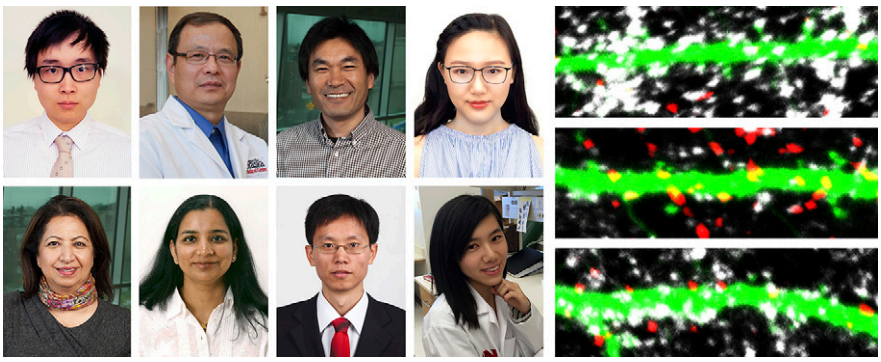
How morphine tips the synaptic balance

Study identifies pathway that alters hippocampal synapses after exposure to morphine.

FOCAL POINT



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Yu Cai (top left), Shilpa Buch (bottom left), and colleagues investigate how long-term morphine use impairs learning and memory, and show that the opioid alters the balance of synapses in hippocampal neurons. Compared with control neurons (green, top), morphine treatment (middle) decreases the density of excitatory synapses (white) while increasing the density of inhibitory synapses (red). The researchers reveal that these changes are induced by a pathway that involves the production of ROS by NADPH oxidase, followed by the sequential activation of ER stress and autophagy. Treatment with PDGF-BB (bottom) inhibits morphine-induced oxidative stress, thereby preventing the changes in synaptic density, and suggesting a potential new therapy for long-term morphine users.

Though morphine is a highly effective painkiller, long-term use can lead to tolerance, addiction, and cognitive impairment, including defects in learning and memory. Precisely how morphine alters learning and memory remains unclear, but Cai et al. now show that the opioid alters the balance of excitatory and inhibitory synapses in the hippocampus through a pathway involving the generation of reactive oxygen species (ROS) and the subsequent activation of ER stress and autophagy (1). Moreover, the researchers find that the growth factor PDGF-BB can counteract these effects, suggesting new ways to improve the cognition of morphine users.

The hippocampus is a key center for learning and memory, and hippocampal neurons express high levels of the μ opioid receptor (MOR), one of the main receptors for morphine (2). The cognitive defects associated with morphine use are therefore likely to be caused by structural and functional alterations at hippocampal synapses; experiments in rats, for example, have shown that morphine decreases the density of excitatory synapses in this region of the brain (3). "The underlying mechanisms regulating this process, however, have not been explored before," explains Shilpa Buch, from the University of Nebraska Medical Center in Omaha. "In order to develop therapeutic approaches that can restore normal cognitive function in morphine users, it is imperative that we understand the pathways by which morphine mediates its effects."

Buch and colleagues, led by graduate student Yu Cai, first examined the hippocampi of mice treated with morphine and found that the opioid decreased the density of excitatory synapses, while enhancing the density of inhibitory synapses (1). These alterations are accompanied by corresponding changes in synaptic function. Other regions of the brain, such as the cerebral cortex, showed no such changes in synapse density, suggesting that hippocampal synapses are particularly vulnerable to morphine.

Morphine also altered the balance of excitatory and inhibitory synapses in primary cultures of rat hippocampal neurons, but the MOR antagonist naltrexone blocked this effect, indicating that morphine alters synaptic density by binding and activating MOR. The researchers then examined the pathway downstream of MOR and found that morphine stimulated the production of ROS by the enzyme NADPH oxidase. Inhibiting this enzyme prevented the morphine-induced decrease in excitatory synapses and increase in inhibitory synapses.

The oxidative stress caused by increased ROS production led, in turn, to ER stress, as indicated by the morphine-induced upregulation of several proteins involved in the unfolded protein response, including the transcription factor ATF6 and the ER-localized chaperone BIP. Blocking the ER stress response prevented morphine from altering the balance of excitatory and inhibitory synapses in hippocampal neurons.

Finally, Cai et al. found that, in response to morphine, ROS production and ER stress induced the formation of autophagosomes, double-membrane organelles that engulf cytoplasmic content and deliver it to lysosomes for degradation. Similar to NADPH oxidase or ER stress inhibitors, blocking the formation of autophagosomes prevented morphine's effects on hippocampal synapses.

It remains to be seen precisely how the ER stress response and autophagy pathways mediate the synaptic changes induced by morphine. "It is likely that these signaling processes could control synapse elimination, stabilization, or generation, leading to an imbalance in density and function of excitatory and inhibitory synapses," Buch says.

Nevertheless, Cai et al.'s findings suggest several ways in which morphine's effects on synapses, and thus, perhaps, its long-term effects on learning and memory, can be prevented or reversed. The growth factor PDGF-BB, for example, is known to protect hippocampal neurons from oxidative stress (4). Cai et al. found that treating cultured hippocampal neurons with PDGF-BB ameliorated morphine-mediated generation of ROS and inhibited the activation of ER stress and autophagy, thereby preventing the subsequent changes in synaptic densities.

"It may be possible to develop mimetics that can specifically activate the PDGF pathway in hippocampal neurons," Buch says. "We're currently doing proof-of-concept studies using targeted delivery of recombinant PDGF. Of course, additional behavioral and electrophysiological studies are warranted to further validate the effect of PDGF on learning and memory."

1. Cai, Y., et al. 2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201605065
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3. Robinson, T.E., et al. 2002. *Synapse*. 46:271–279.
4. Cheng, B., and M.P. Mattson. 1995. *J. Neurosci.* 15:7095–7104.