This Week in The Journal

Amygdala Projections to Lateral Hypothalamus Elicit Avoidance

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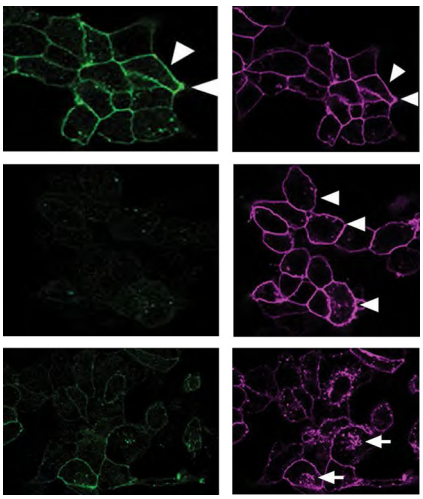
(see pages 61–72)

If an individual feels threatened in a particular situation, they might avoid that situation in the future. For example, if rats smell bobcat urine when placed in one chamber of a cage, some will continue to avoid that chamber even after the smell is removed. Notably, some rats do not show such avoidance behavior. Therefore, comparing avoiders with nonavoiders might provide insight into the neural mechanisms that lead some individuals to maintain cautious behavior when no threat is present.

One difference between avoider and nonavoider rats is that stress activates more central amygdala (CeA) neurons in avoiders. The targets of these neurons is unclear. But because neurons in the lateral hypothalamus (LH) of mice respond to predator odor and activation of subpopulations of these neurons can induce place avoidance or place preference (Giardino et al., 2018, Nat Neurosci 21:1084), Weera et al. hypothesized that differences in activation of LH-projecting CeA neurons underlies differences in avoidance behavior in rats.

The authors first confirmed that a population of CeA neurons projects to the LH. Activation of these projections elicited monosynaptic IPSCs in LH neurons. Notably, more LH-projecting CeA neurons were activated by exposure to predator odor in avoider rats than in nonavoider or naive rats. Moreover, inhibiting LH-projecting CeA neurons via designer receptors reduced predator-associated place avoidance in avoider rats without affecting behavior in nonavoiders. Conversely, activating LH-projecting CeA neurons induced place avoidance in naive rats. Additional studies showed that the resting membrane potential was more hyperpolarized in LH-projecting CeA neurons of nonavoider rats than in those of avoiders. Furthermore, LH-projecting CeA neurons in avoider rats showed a greater depolarizing sag current (I_h) during hyperpolarization steps than neurons in nonavoiders. These differences in intrinsic properties may make LH-projecting CeA neurons in avoiders more excitable than those in nonavoiders.

These results suggest that enhanced activation of CeA neurons that send inhibitory projections to the LH may lead to persistent avoidance of places previously associated with threat. What leads to the different excitability of CeA neurons in avoiders and nonavoiders and what neurons they target in the LH should be investigated in future studies.



When PAR_2 is intact (top) the extracellular N terminal (green) and intracellular C terminal (magenta) are present at the plasma membrane of transfected HEK cells. After cleavage by protease, the N terminal diffuses away from cells and the C terminal either remains associated with the plasma membrane (after legumain treatment, middle) or is internalized into endosomes (after trypsin treatment, bottom). See Tu, Jensen, et al. for details.

The Protease Legumain Causes Oral Cancer Pain

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(see pages 193–210)

Cancer is often painful. In some cases, pain occurs only after a tumor grows large enough to compress a nerve; but in other cases, tumors induce pain by secreting factors that activate nociceptors. This happens, for example, in oral squamous cell carcinoma (OSCC). Proteases secreted by OSCC cells cleave protease-activated receptor-2 (PAR_2) on nociceptors, thus activating G-protein-dependent signaling pathways that lead to heightened nociceptor sensitivity. Which proteases activate PAR_2 in OSCC has been unclear, but Tu, Jensen, et al. hypothesized that legumain is involved.

Legumain is a widely expressed pH-dependent protease that normally acts in acidic compartments, particularly lysosomes. Nevertheless, legumain is secreted by many types of tumor cells, and it has been shown to promote metastasis. Tu, Jensen, et al. found that legumain activity was greater in human OSCC tissue than in normal oral-cavity tissue from the same patients, and OSCC-derived cells secreted more legumain than cells from a noncancerous cell line. Furthermore, when OSCC was induced in mice, animals lacking legumain or PAR_2 exhibited less mechanical sensitivity than wild-type mice. In addition, injection of legumain into the foot or cheek lowered pain-response thresholds in wild-type mice, but not in mice with PAR_2-deficient nociceptors. Finally, treating cultured trigeminal ganglion sensory neurons with legumain reduced the amount of current required to evoke action potentials, and this effect was absent in PAR_2-deficient neurons.

Additional experiments showed that legumain cleaved PAR_2 at an asparagine residue in the N terminus, freeing the receptor's extracellular domain. Like other proteases, legumain triggered PAR_2-dependent release of calcium from internal stores and increased cAMP levels in HEK cells. Notably, inhibitors of protein kinases A and C attenuated legumain-induced increases in nociceptor excitability in culture.

These results suggest that oral cancer cells evoke pain by secreting legumain, which activates nociceptor PAR_2 receptors. This leads to activation of cAMP- and calcium-dependent protein kinases. How this signaling increases the sensitivity of nociceptors is unclear, but it might involve phosphorylation of TRPV channels. Future work should test this hypothesis and investigate whether blocking legumain secretion or protease activity can relieve pain in OSCC patients.

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