preventing its expression in undifferentiated embryonic stem cells (Navarro et al., 2008; Donohoe et al., 2009). RLIM might regulate the stability of such factors or their binding to regulatory elements of *Xist*. Alternatively, RLIM may regulate *Xist* via other, unknown factors. Importantly, *Rnf12* itself may be regulated by pluripotency factors. Jonkers et al. show that a 10 kilobase region 5′ to *Rnf12*, containing multiple strong binding sites for pluripotency factors (Marson et al., 2008), is essential for its expression. This raises the interesting possibility that *Xist* may in fact be regulated both directly and indirectly by pluripotency factors.

Another important question concerns the location of the *Xist cis*-regulatory elements that are targeted by RLIM and its partners. The fact that *Xist* cannot be activated in the context of a large single-copy transgene, even in female embryonic stem cells where a double dose of Rnf12/RLIM is present and *Xist* is activated on one of the two X chromosomes (Heard et al., 1999), suggests that sequence targets of Rnf12/RLIM-mediated *Xist* activation may lie several hundred kilobases away from *Xist*. Candidate regions for such long-range regulatory sequences include *Xpr*, which lies 5′ to *Xist* and has been proposed to influence *Xist* expression in *trans*, or the genetically defined *Xce* locus, which lies 3′ to *Xist* (Heard and Avner, 2001, for review). Importantly, Jonkers et al. also show that the human RLIM protein can *trans*-activate the mouse *Xist* gene, arguing that RLIM could act on *Xist* independently of its regulatory partner *Tsix*, which is not well conserved in humans.

Finally, Jonkers et al. also demonstrate that Rnf12/RLIM is not the only dose-dependent trigger of XCI. Female embryonic stem cells missing one *Rnf12* allele are still able to initiate XCI, albeit with reduced efficiency. This finding suggests the existence of further X-linked *Xist trans*-activators. The study by Jonkers and coworkers has far-reaching implications for X chromosome inactivation. Not only do these investigators identify the first dosage-sensitive protein involved in sensing or counting the number of X chromosomes, they also prompt re-evaluation of the extent and content of the *Xic*. Indeed, these exciting observations suggest that other X-linked genes might be involved in *Xist trans*-activation either within or maybe even beyond the *Xic* interval.

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An Acid-Sensing Channel Sows Fear and Panic

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The amygdala is a brain region that coordinates fear responses to a variety of threats. Ziemann et al. (2009) now show that acid-sensing channels in the amygdala mediate fear responses that accompany inhalation of carbon dioxide, suggesting that aberrant chemosensation may underlie anxiety disorders associated with a fear of suffocation.

The primary function of the nervous system is to generate flexible behavior in a changing environment. Behaviors devoted to securing food, water, and shelter are, of course, essential to survival. Yet even more important to survival is defending against immediate threat, inasmuch as failing to do so often makes the difference between life and death. Not surprisingly, the brain's defensive system is highly adapted to generate rapid autonomic and behavioral responses to threatening stimuli, such as a predator in the forest, a bully at the office, or an aversive stimulus in the laboratory. Extensive

work over the last several decades has shown that the amygdala, a collection of nuclei buried in the temporal lobe of the brain, is essential for both innate and learned fear in rodents and humans. In this issue, Ziemann et al. (2009) reveal that an acid-sensing ion channel (acid sensing ion channel-1a or ASIC1a) in the amygdala contributes to the production of fear behavior by detecting a decrease in extracellular pH. This finding provides new insight into the mechanisms by which amygdala neurons detect threat and suggests a new role for amygdala chemosensation in learned fear.

Established neuroanatomical models of the circuitry underlying the brain mechanisms of fear have emphasized the convergence of multimodal sensory information in the amygdala (Maren, 2001; LeDoux, 2000). This circuitry has largely been derived from the study of Pavlovian fear conditioning, a form of learning in which a neutral conditional stimulus, such as an acoustic tone, signals the delivery of a noxious unconditioned stimulus, such as electric shock. In this case, sensory information about both the conditioned and unconditioned stimuli converge in the basolateral complex of the amygdala (BLA, including the lateral, basolateral, and basomedial nuclei). It is widely believed that coincident activity among sensory afferents in the BLA leads to potentiation of glutamatergic synapses in the conditioned stimulus pathway that permits the production of a learned fear response. These fear responses are mediated by direct connections between the BLA and the central nucleus of the amygdala (CEA), which in turn projects to hypothalamic, midbrain, and medullary centers that regulate heart rate, freezing behavior, and respiration.

The new work by Ziemann and colleagues suggests that changes in extracellular pH in the amygdala trigger cationic currents mediated by ASIC1a channels. They report that inhalation of carbon dioxide (CO_2) decreases the pH in the amygdala and yields freezing behavior in mice. Genetic deletion or pharmacological disruption of ASIC1a channels reduces fear associated with CO_2 inhalation, and viral-mediated expression of ASIC1a in the BLA of ASIC1a-deficient mice restores CO_2-induced fear. Interestingly, mice deficient in ASIC1a also show impaired fear to a predator odor and impairments in contextual fear conditioning. This suggests that a variety of aversive stimuli in addition to CO_2 inhalation may influence the activity of the amygdala through changes in extracellular pH. Indeed, ASIC1a channels may be involved in forms of synaptic plasticity thought to underlie the acquisition of learned fear (Wemmie et al., 2002). Nonetheless, it remains to be determined whether predator odors or aversive foot shocks, for example, produce changes in synaptic pH sufficient to activate ASIC1a channels in the amygdala.

Where in the amygdala does ASIC1a modulate fear behavior? Ziemann et al. demonstrate that acidifying the BLA in wild-type mice or overexpressing ASIC1a channels in the BLA of mice lacking these channels increases freezing behavior to inhaled CO_2. But are ASIC1a channels in the BLA also involved in the acquisition of contextual fear conditioning or innate fear to a predator odor? Interestingly, viral-mediated rescue of ASIC1a function in the BLA of ASIC1a-deficient mice restores contextual fear conditioning but does not influence freezing evoked by predator odor (Coryell et al., 2008). Hence, BLA ASIC1a channels appear to have a role in both CO_2-induced fear and contextual fear conditioning but not in freezing evoked by exposure to a predator odor. This suggests that ASIC1a channels in other brain areas, whether in the amygdala or elsewhere, are responsible for the loss of fear to a predator odor that is apparent in ASIC1a-deficient mice. Indeed, the medial nucleus of the amygdala is essential for freezing to predator odors, and it is possible that ASIC1a channels in this region contribute to odor-evoked fear.

Another open question concerns the contribution of ASIC1a channels in the CEA to fear behavior. It has long been known that neuronal activity in the CEA is coupled to respiration, and its stimulation modulates respiration rate (Pascoe and Kapp, 1985). The CEA has robust projections to the midbrain parabrachial complex, which is involved in respiratory control. Hence, one function of ASIC1a channels in the CEA may be to regulate changes in respiration that accompany the expression of fear. That said, the role of the ASIC1a channels in respiratory regulation may be quite limited. Homeostatic increases in respiration during a CO_2 challenge do not involve ASIC1a channels (Ziemann et al., 2009) but are mediated instead by medullary serotonergic neurons (Richerson, 2004).

Although much of the work concerning the role of the amygdala has centered on threats coming from outside the body (such as predator odors, electric shocks, and startling sounds), the sensitivity of the amygdala to CO_2 inhalation suggests that it also processes internal threats. Indeed, respiratory acidosis and hypercarbia, which result from CO_2 inhalation, are associated with suffocation and, ultimately, death. Because oxygen-breathing organisms are under a constant threat of asphyxiation, it could be argued that the threat of suffocation has had a primary influence on shaping the brain's defensive systems. The present discovery that chemosensors in the amygdala are involved in generating fear responses to a variety of aversive stimuli suggests that a system that evolved to generate behavior to defend against suffocation was subsequently adapted to deal with both innate and learned threats in the external environment. In some regards, this is not surprising. In the grasp of a predator, suffocation is the ultimate fear—it signals imminent death.

Intriguingly, fear of suffocation is associated with anxiety disorders including claustrophobia and panic disorder. Inhalation of CO_2 triggers panic and has recently been reported to induce fear in otherwise normal adults (Griez et al., 2007). For these reasons, it has been argued that panic disorder might arise from the brain's inability to suppress biological alarms that signal suffocation (Klein, 1993). The localization of acid sensors in the amygdala that respond to CO_2 inhalation provides a possible neural substrate for the suffocation alarm. Indeed, the role for amygdala acid-sensing channels in conditioned fear suggests that they may mediate conditioning to internal cues that precede panic attacks (Bouton et al., 2001). Dysregulation of amygdala chemosensation might predispose individuals to panic disorder, as well as other anxiety disorders that involve fear conditioning. Ultimately, the discovery of chemosensors for fear in the amygdala comes as a breath of fresh air, opening up new avenues of research into the neurobiology of anxiety and panic.

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