

Fear finds its niche

Sandhya Rani

Failed experiment, bad day at office or a displeased boss, in spite of their malevolence, these unkind memories fade away and life eventually bounces back to normal. But for individuals subjected to violent crimes, the traumatic memories persist and eclipse their life and future. This scenario in laboratory conditions is called as fear conditioning. Fear conditioning (associative memory) is learning to associate emotion to some perceptual experience, it is an adaptive process that allows an individual to predict and minimize exposure to danger¹. Under pathological conditions fear conditioning transforms to fear generalization which occurs when non-threatening situation is inappropriately treated as harmful, based on similarity to a known threat².

Fear is instantaneous, yet carefully orchestrated sequence of hormonal and physiological changes controlled by the brain, hence referred to as an unspoken memory. Charles Darwin was one of the first scientists to suggest that 'fear' has a biological basis, when he noted that nearly all animals exhibit fear in the same manner. Fear lives in a small almond-shaped pair of brain structures, called the amygdalae. It is referred to as the emotional hub of the brain and plays a role in acquisition, storage, retrieval and regulation of fear memory. When the amygdala is damaged the ability to learn and respond to threats becomes impaired. In response to fear amygdala neurons undergo specific chemical and structural changes that form an imprint of the sensory image that accompanies a particular threat³. When this cue-specific response to fear is generalized, it manifests in to pathological conditions such as anxiety and post-traumatic stress disorders (PTSD) and studies indicate amygdala to play a central role in this generalization. However, little is known about how neurons in the amygdala encode the transition from specific to generalized fear.

This lacuna, in the field of 'neurobiology of fear' is addressed in the study published in *Nature Neuroscience* by Chatterji and Ghosh⁴. This study gives new insight into the role of amygdala in the formation of generalized fear by using discriminative fear conditioning

protocol. It provides understanding of how information is processed in individual neurons of the amygdala and how it maintains the delicate balance between whether individuals should or should not be afraid in a given situation.

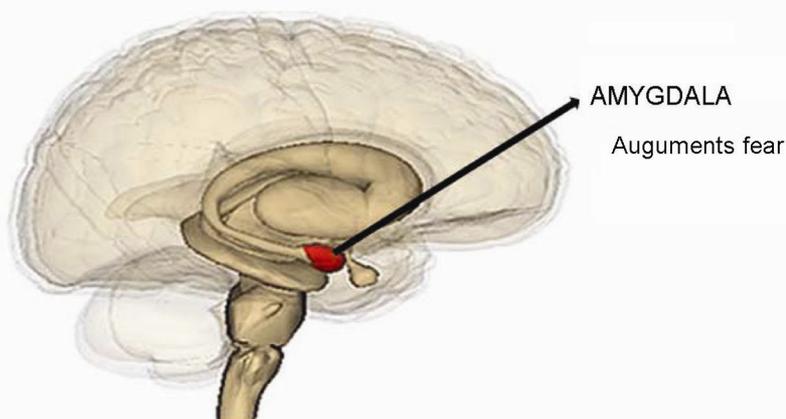
Classical fear conditioning represents the process by which neutral/conditioned stimulus (CS) comes to evoke fear following its repeated pairing with an aversive/unconditioned stimulus (US). Thus provides behavioural measure of the learned association, but does not explain the concept of fear generalization. In an attempt to circumvent this limitation, authors adopted discriminative fear conditioning procedure.

In an elegant experiment the authors trained rats to discriminate between two auditory tones of different frequencies, one (CS+) was paired with mild foot shock (US) and the other was not (CS-). The CS+–US pairings were interceded with CS- presentations during conditioning. Using this protocol the rats were successfully trained to distinguish the two auditory tones CS+ versus CS-. During testing of fear memory recall, the researchers recorded electrical signals from individual neurons in the amygdala. This analysis revealed three distinct classes of neurons, one group (42%) that responds exclusively to CS+, the other group (51%) that does not fire in response to either CS+ or CS- and the last group which indiscriminately responds to both CS+ and CS- (6%). This initial experi-

ment shows that in the context of perception of a mild threat only, a small number of neurons (6%) in the amygdala are responsible for the phenomenon of generalization.

If the neurons in the amygdala are indeed involved in discriminating CS+ and CS- and encoding their representative behavioural differentiation, then behavioural manipulations that modulate fear generalization should also cause predictable changes in the activity of these neurons. Thus the small population of neurons (6%) that represented fear generalization in previous experiment should change with respect to change in the behavioural protocol. With this hypothesis, the authors conducted the next set of experiments, wherein the same rats were trained using the same protocol, but the only variable was the US, viz. shock associated with the auditory tone, was made stronger. In this scenario, it was observed that strong US conditioning tilted the balance towards a greater proportion of generalizing (6% → 30%) over cue-specific cells (42% → 32%) than that seen after weak US conditioning. The behavioural representation of this upheaval was that the animal showed increased freezing behaviour even to the safe tone, although the tone was never paired with a shock.

In real life, say sitting on a cosy couch of a multiplex, you are witnessing a train accident, you see disaster everywhere. For many people in the multiplex the



Structure of human brain. Courtesy: Life Science Database.

crackling sound of the derailed train is just a clipping in the movie which some times call for accolades to the cinematographer and nothing threatening at all. But for someone with severe anxiety disorder, such as PTSD, the accident may be associated with terrible memories, loss of loved ones or first hand experience of the distress causing them horrible agony and terrible anxiety. This individual will forget the environ of the multiplex and start reliving the traumatic episode. A movie clipping of a train accident and the horrible things endured in real life are entirely discriminative, but in individuals with severe anxiety disorders, this makes no difference at all. This generalization of fear and entailed freezing behaviour would not have occurred if the individual's loss due to train accident was not a calamity. Thus the intensity of the incident has a direct effect on the response to the same or similar situation.

The authors observed that the shift in balance between cue-specific and generalizing neurons was due to transition, that occurred at three levels, namely the conversion of cue-specific to generalizing cells, neural cells that did not respond to either tones after weak US conditioning, yet showed a robust increase to both tones after strong US conditioning and became generalizing cells and the third transition involved conversion of non-conditioned cells into cue-specific cells after strong US conditioning. The cumulative effect of these three transitions resulted in larger proportion of amygdala neurons to lose their

ability to discriminate between the safe and dangerous stimuli causing the increased fear generalization in rats.

Next, the authors conducted an interesting experiment to distinguish the effects of increased intensity and repetitive conditioning with the same intensity in the paradigm of fear generalization. A separate group of rats were conditioned twice with the same weak US, this training failed to cause generalization at both the behavioural and neuronal levels, thereby ruling out the possibility that simply reconditioning increases fear generalization. The authors hypothesize that, in situations involving highly aversive outcomes, efficient discrimination of stimuli may be less important than engaging neural mechanisms in the amygdala that respond quickly to danger. Thus the intensity and the extent of devastation of the incident decides if the experience should be extrapolated to generalization. This study proposes that aberrant electrical signalling in individual neurons can add up to give rise to amygdala hyperactivity and generalization of fear in panic disorders such as PTSD. PTSD is a psychological condition where the patient in addition to repeated flashbacks; respond with intense fear and hyperarousal, similar to that experienced during the original traumatic event, to sensory stimuli that by themselves pose no threat. These results explain as to why people who succumb to violent crimes are prone to anxiety disorders and not those lucky individuals who escape the dreaded acrimony.

Next the NCBS team found that activation of cAMP-PKA signalling in amygdala using a chemical called forskolin, leads to an enhancement of fear generalization comparable in magnitude to that elicited by strong US conditioning alone. Thus the authors propose cAMP-PKA signalling to be the central amygdaloid signalling pathway that is likely at work in inducing generalized anxiety commonly seen in PTSD patients. This pathway can be targeted to develop new therapeutic regimes.

Treatment protocols are easier to be administered if the ecosystem and the etiology of the disease is threadbare. In the purview of anxiety disorders Chatterji and his student Ghosh have shown where and how the pathological facet of fear is hidden, thus providing a framework to counter-claim the burden of anxiety disorders.

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3. Díaz-Mataix, L., Tallot, L. and Doyère, V., *Behav. Process.*, 2014, **101**, 112–122.
4. Ghosh, S. and Chatterji, S., *Nature Neurosci.*, 2015, **18**(1), 112–120.

Sandhya Rani (S. Ramaseshan Fellow).
e-mail: bsandhya163@gmail.com