

Fear: It's All in Your Line of Sight

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The brain circuits that create our sense of fear rely on ancient 'hard-wired' components of the limbic system, but also use sensory processing to determine what we become afraid of. A new study shows that, when viewing of simple oriented line stimuli is coupled with aversive experiences, neurons in primary visual cortex rapidly alter their responses in a manner that indicates the line stimuli become a source of fear.

Fear is an emotional state of anxiety that occurs in response to a perceived threat and that can trigger a variety of defensive reactions [1–3]. Because fear can be attached to stimuli of various kinds, it can serve either adaptive or maladaptive roles. For example, it can promote avoidance and escape from genuinely threatening situations or, when attached to innocuous stimuli, can promote unnecessary stress. Given that maladaptive fear is a central component of many debilitating psychiatric and mental conditions, such as post-traumatic stress disorder (PTSD) and major depression, understanding how the brain labels particular sensory events as 'fearful' is of paramount importance. The study of the brain circuits controlling fear has largely centered on the role of the limbic system: a collection of ancient neural circuits that reside deep beneath the neocortex and that include the amygdala complex, the stria terminalis and various hypothalamic nuclei [4]. A hallmark feature of these circuits is their ability to activate the sympathetic branch of the autonomic nervous system, which relies on neural circuits and hormonal pathways such as adrenalin secretion to increase overall alertness and bias us for action. What has been less clear is how the brain decides which particular sensory stimuli to become fearful of and when that process occurs during the fear-learning process. As they report in this issue of *Current Biology*, Li *et al.* [5] have discovered that the neurons in primary visual cortex (V1) that fire action potentials to simple oriented line stimuli alter their responses when

those stimuli are paired with aversive experiences. Given that V1 resides well outside the limbic system, but early in the hierarchical processing stream of objects and their locations, these new findings have important implications for understanding how generic fear states created in the limbic pathways are attached to different types of life events, and the stability of those associations over time.

To assess how aversive experiences impact sensory processing of particular stimuli, Li *et al.* [5] focused on the visual system — vision being the sensory modality humans rely on most to navigate the world and survive. The authors recorded from V1 neurons in adult macaque monkeys using implanted microelectrode arrays. Macaque monkeys are an Old-World primate species that has a visual system very similar to that of humans, including trichromatic color vision, high acuity and goal-directed eye movements. A hallmark of V1 neurons is their orientation selectivity: many V1 neurons fire best when the animal is viewing particular angles of light or dark 'line stimuli' [6]. After they obtained stable responses from a V1 cell and recorded its 'preferred orientation', the authors introduced a fear-conditioning paradigm in which oriented gratings tilted to one side (rightward or leftward from the vertical) were paired with an aversive air-puff delivered to the face of the monkey. In this configuration the gratings served as the conditioned stimulus (CS) and the air puff served as the unconditioned stimulus (US). Gratings tilted to the opposite side of the conditioned stimulus served as

the non-conditioned stimulus (NS) and were paired with a juice reward. Like humans, monkeys can be stubborn about performing tasks, so the juice reward was needed to motivate the monkeys to stay focused on the stimuli. Eye-blinking is a well-established response that monkeys and humans show when anticipating an aversive air-puff. The authors measured the eye-blink responses during the interval between the stimulus delivery and the air-puff or juice delivery as the main assessment of fear-associated learning. The conditioning lasted for 4–6 days. At that point the authors switched the CS and NS orientations and repeated conditioning with the reversed orientations to control for any orientation-specific effects. They recorded V1 responses to the gratings in the absence of the air puff before and after conditioning sessions every day to assess the effect of conditioning in neural responses and fear extinction, respectively.

Using this approach, Li *et al.* [5] found that V1 neurons had enhancements in their firing rate in response to the orientation that had been paired with the air-puff (CS) during the conditioning sessions, compared to the pre-conditioning sessions. This was specific to the CS and did not occur in trials with NS–juice pairings (Figure 1). In other words, the air puff/oriented line stimulus pairing potentiated the neuron's response to the CS orientation. This enhancement, referred to by the authors as the 'fear-related' signal, emerged only after a few trials of pairings during the conditioning session and

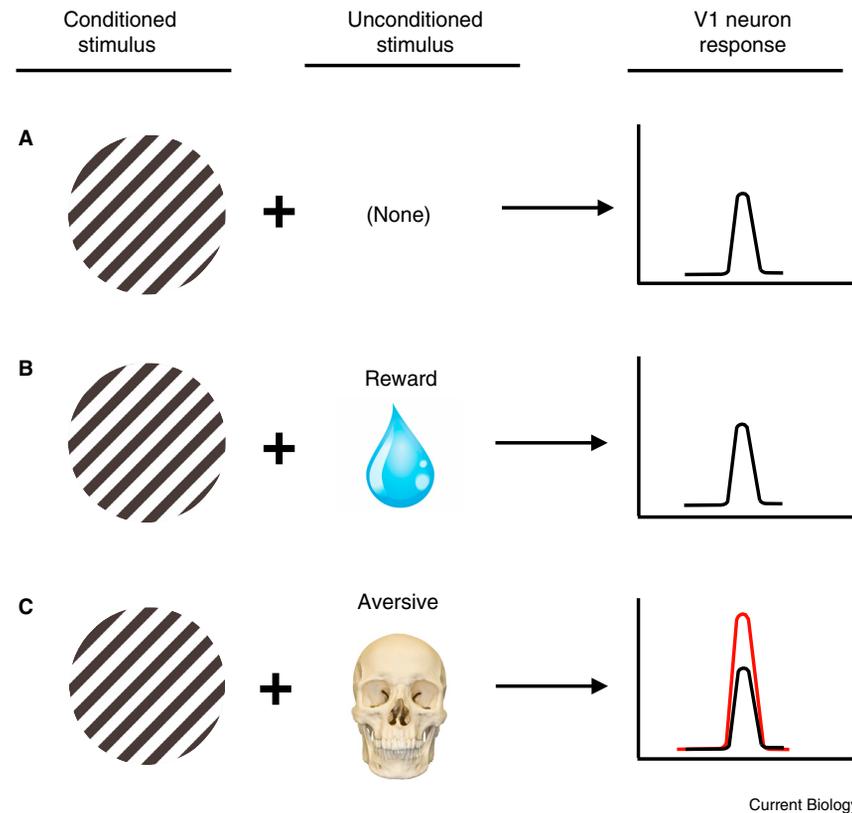


remained for a full ~20 presentations of the CS gratings when the air puff was removed after conditioning. The signals diminished quickly after that.

Interestingly the responses to the CS were potentiated regardless of whether or not it was the neuron's preferred orientation. This also impacted the neuron's orientation-tuning properties. For example, if the CS orientation was the neuron's preferred orientation, the neuron became even more responsive to it — that is, made it more sharply tuned. If the CS was the un-preferred orientation, however, the neuron still became more responsive to that orientation compared to the pre-test condition, and in doing so, made the neuron relatively *less* well tuned to its preferred orientation. This is remarkable as it means that fear-related signals can be attached to any stimulus, regardless of a neuron's pre-fear tuning properties.

The fear-related enhancement observed by Li *et al.* [5] was surprisingly fast, appearing as early as 30 milliseconds after the stimulus onset. This raises important questions about the possible sources of V1 neuron modulation in this context. The amygdala, a central component of subcortical limbic circuitry involved in threat detection, projects to and synapses in V1 [7–9]. V1 also receives feedback projections from higher visual cortical areas too [10]. However, the activation of these inputs to V1 occurs with latencies longer than the mere 30–70 milliseconds it took to see V1 neuron enhancement [10,11]. Neither amygdala-feedforward nor cortical-feedback signals, therefore, are likely to be the source of the V1 enhancement reported in this paper. Instead, the authors hypothesized that the enhancement is likely due to 'bottom-up processing' carried out in V1 itself. If they are correct, that means that primary sensory areas can acquire fear-associated responses independently of the classical fear pathways of the limbic system.

To test that hypothesis, Li *et al.* [5] examined whether the enhancement of signals in V1 occurred even when



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Figure 1. Preferential enhancement of V1 responses to oriented gratings when coupled with aversive but not rewarding experiences.

(A) Illustration showing typical V1 responses to an oriented grating stimulus. (B) V1 neuron response to the same grating is unchanged when coupled with a reward. (C) V1 neuron responses are enhanced when coupled with an aversive stimulus (illustrated with the skull).

the CS and NS orientations were masked. They did that by adding horizontally oriented drifting gratings on top of them, making the CS appear as a coherently moving 'plaid' rather than as a set of overlapping gratings. The presentation of these plaids was randomly interspersed with the CS and NS trials during conditioning and did not lead to eye-blink responses. This stimulus did, however, preferentially enhance the neural responses to the plaids containing the CS orientation. This is noteworthy because it means that a given stimulus is 'tagged' or 'learned' as fearful even if the observer (in this case, the monkey) is unaware of the presence of a stimulus inside a masking stimulus. Put differently, neurons in V1 change their responses to visual stimuli that are paired with a bad experience, even if the animals do not consciously perceive

what they saw at the time the learning occurred.

Neurons in V1 respond to stimuli presented within specific regions of visual space and thereby exhibit a 'visuotopic receptive field'. Li *et al.* [5] asked whether the response enhancement they observed was specific to the visuotopic location in which the CS (oriented grating) was presented. In other words, once a V1 neuron adopts a threat response to a given stimulus — in this case an oriented line (CS) — does the brain care about where a threat is in the world or merely that it is present *someplace*? They trained the monkeys with a CS shown at a different location than the receptive field of the neuron they were recording from (that is, outside the neurons so-called 'classical receptive field'). After training was complete, they recorded responses of the V1

neuron to the CS orientation that was presented *within* their receptive fields. The fear-related enhancement was not observed and animals did not show eye-blink responses to the CS presented in a different location, indicating that fear learning is specific to the location in which the CS is presented and reinforcing the idea that individual neurons, rather than entire maps of neurons or regions of V1, change their firing properties in response to the CS (lines)–US (air puff) pairings. A stimulus is only tagged in V1 if it appears in the same visual location as the location where it had previously been paired with an aversive outcome. This suggests that the plasticity mechanisms underlying this form of fear learning involve synaptic specificity and/or intrinsic properties of neurons rather than, say, broad scale neuromodulation.

Arguably one of the most surprising findings of Li *et al.* [5] is the extremely short latency with which fear-associated response modulation is observed in V1 neurons. While these latencies argue against the involvement of feedback signals from the cortex or the amygdala [12–14], the data presented were not sufficient to rule those influences out. More definitive conclusions about the source of this fast-non-visual modulation of V1 will likely be available when the current paradigm is combined with simultaneous recordings from cells in V1, amygdala and other cortical areas. Another mechanism brought up by the authors as a potential source of this fast-non-visual modulation is feed-forward signals from the pulvinar, a thalamic structure known to modulate cortical function and to incorporate contextual cues such as the overall state of the animal, speed of locomotion and so on [15,16]. Previous studies showed that excitation of pulvinar neurons causes a fast enhancement of V1 signals in the early phase of their responses [17]. The pulvinar has also been implicated in fast, innate defensive behaviors such as freezing in response to a looming stimulus in rodents [18]. Future studies should focus on manipulating

the activities of pulvinar during fear conditioning to test its role in the fear-related signals observed by Li *et al.* [5].

Our behavioral repertoire is dependent on integration of external sensory events with our internal states. Where and when this integration occurs in the brain remains largely unknown. The new paper from Li *et al.* [5] suggests that such integration occurs earlier than previously thought along the hierarchy of visual processing circuits, and that it can impact neural processing of features as simple as oriented lines. In other words, sensory events can arrive in our awareness already pre-labeled as ‘potential threats’ or ‘neutral’. Given that fast-fear-tagging of what we see, the question arises: can we ever truly separate our sensory perceptions from emotions? Studies of emotional and sensory processing have traditionally come from non-overlapping fields of neuroscience. As a consequence, we now understand a great deal about how physical stimulus features like direction and orientation are encoded, but still know quite little about how our internal states shape those responses. The current study adds strong evidence to the idea that emotional context impacts sensory perception very fast, even before that information reaches our awareness. This has major implications for how we approach the study of emotion and neural circuits involved in mental disorders involving fear and anxiety. They also suggest it may be time to shift our efforts away from studying emotions and sensory perception as separate processes and instead establish more paradigms that resemble events that trigger fear states in real life.

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