erant species to outcompete human-sensitive ones. Additionally, lethal activities may drive rapid evolutionary changes in seed size through the functional extinction of large seed dispersers in tropical forests (*9*), with concomitant shifts in ecosystem composition and structure. Finally, there is growing evidence of microevolutionary changes associated with adaptive responses to urban environments, including changes in bird song frequency (in response to anthropogenic noise) and increased sedentariness in urban blackbirds (*10*).

Wildlife responses are not limited to spatial or temporal avoidance of human disturbance. Although not included in their analysis, Gaynor *et al.* highlight other behavioral adjustments to cope with human activities, including an increase in vigilance rates and reduction in foraging activity, which may be detrimental for individual condition and reproductive success. However, there are many other responses that organisms use to cope with increased disturbance. Indeed, some animals may not display any visible behavioral adjustment in response to humans, giving the impression that human disturbance has no remarkable effect in this species. Yet, at the physiological level, animals may increase the heart rate and the rate of glucocorticoid production (a physiological indicator of stress in wildlife) through the activation of stress responses (*11*, *12*). The short-term release of glucocorticoids is an adaptive response that redirects energy from nonvital activities toward survival, but prolonged exposure to stressors and the cumulative effects of maintaining elevated glucocorticoid levels may impair immune and reproduction functions (*13*). Eventually, long-term disturbances may result in lower fitness, lower juvenile survival, or lower reproduction rates (*14*), with negative consequences at population level.

Given the continuing increase of the global human footprint (*1*), Gaynor *et al.*'s study is timely and of paramount importance for understanding the influence that humans may have on the behavior of diurnal, twilight-active, and nocturnal wildlife. Similar studies are needed to assemble the multiple responses to human disturbance of nonmammalian taxa. It is also important to keep in mind that the modulation of activity patterns is just one of the multiple responses of wildlife to anthropogenic activities. Holistic approaches that take into account behavioral, physiological, population, and evolutionary responses to human disturbance across taxa are urgently needed to fully understand the consequences of human encroachment for the persistence of wildlife populations. This knowledge will be crucial to develop new tools in conservation planning that address temporal human-wildlife interactions, similar to the way in which spatial ecology is currently incorporated for land planning or spatial conservation prioritization. ■

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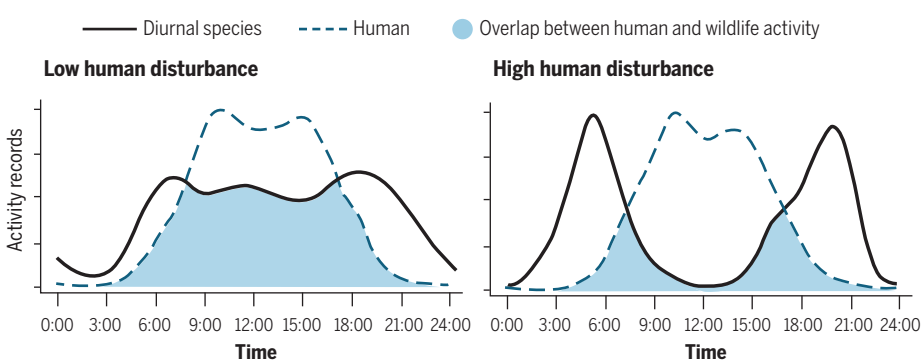
Human disturbance shifts animal activity into the night

Animals that are active during the day in areas with low human disturbance (left) change their activity patterns in areas with high human disturbance (right). Instead of a broad distribution of activity throughout the day, their activity peaks in the early morning and again in the early evening.



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NEUROSCIENCE

Facing your fears

Activation of remote fear memory promotes fear attenuation

By **Paul W. Frankland** *and* **Sheena A. Josselyn**

Remembering traumatic fearful events is adaptive. However, treating no-longer-threatening situations as dangerous may be maladaptive and lead to anxiety disorders, including phobias and posttraumatic stress disorder. Central to many forms of therapy designed to tackle these anxiety disorders is the idea that to overcome fear, one needs to face it. For instance, cognitive behavioral therapy and exposure therapy allow patients to confront the objects or situations that provoke their anxiety in the controlled environment of the therapist's office. With repeated exposures, the patients' anxiety levels gradually decline, and the objects or situations that they once feared no longer trouble them. On page 1239 of this issue Khalaf *et al.* (*1*) provide a neural mechanism in mice for "facing one's fears." These findings may inform the development of more effective forms of treatment for anxiety disorders.

The therapeutic strategy of facing your fear has its roots in Ivan Pavlov's studies of classical fear conditioning in dogs. In his experiments, Pavlov paired an otherwise innocuous stimulus (such as a buzzer) with an aversive stimulus (such as an electric shock). Subsequent presentation of the buzzer (the now-conditioned stimulus), by virtue of its association with the electric shock (the unconditioned stimulus), evoked fearlike behaviors (conditioned responses) in the dogs. However, similar to patients in therapy, repeated presentations of the conditioned stimulus alone (in the absence of the unconditioned stimulus) eventually led to a decline in conditioned fearful responding (called behavioral extinction) (*2*).

Khalaf *et al.* provide fundamental insights into the neural mechanisms underlying behavioral extinction in mice. During memory formation, populations of neurons (neuronal ensembles) become co-

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active. Almost 70 years ago, Donald Hebb proposed that memory formation involves the strengthening of synaptic connections between neurons in these ensembles (*3*). This synaptic strengthening was postulated to ensure that, given an appropriate retrieval cue, the pattern of activation in the ensemble during memory formation would be recapitulated, promoting memory recall. These neuronal ensembles are thought to correspond to engrams—the neural representation of an encoded event or experience (*4*). Engrams exist in one of two states. Most of the time, engrams are dormant, with only the potential for memory retrieval. However, an appropriate retrieval cue reactivates the engram to promote memory recall (*5*).

Until recently, discussions about how engrams were engaged in memory formation and extinction were largely theoretical. However, the development of new tools has provided more direct evidence that neuronal ensembles active during memory encoding correspond to the engram. In mice, researchers are now able to permanently "tag" active neuronal ensembles at, for example, the time of learning with genetically encoded fluorescent reporters or actuators of neuronal activity [optogenetic opsins or chemogenetic DREADDs (designer receptors exclusively activated by designer drugs)]. This tagging process allows ensembles to be visualized or manipulated (inhibited or activated) at later time points (*6*). Consistent with Hebb's proposal, neuronal populations engaged during learning are reengaged at above-chance levels during memory recall (*7*). Moreover, tagged ensembles appear to be both necessary and sufficient for memory recall, because inhibiting the activity of these ensembles prevents memory recall (*8*), whereas activation of these populations leads to the artificial (and involuntary) expression of the memory in the absence of retrieval cues (*9*).

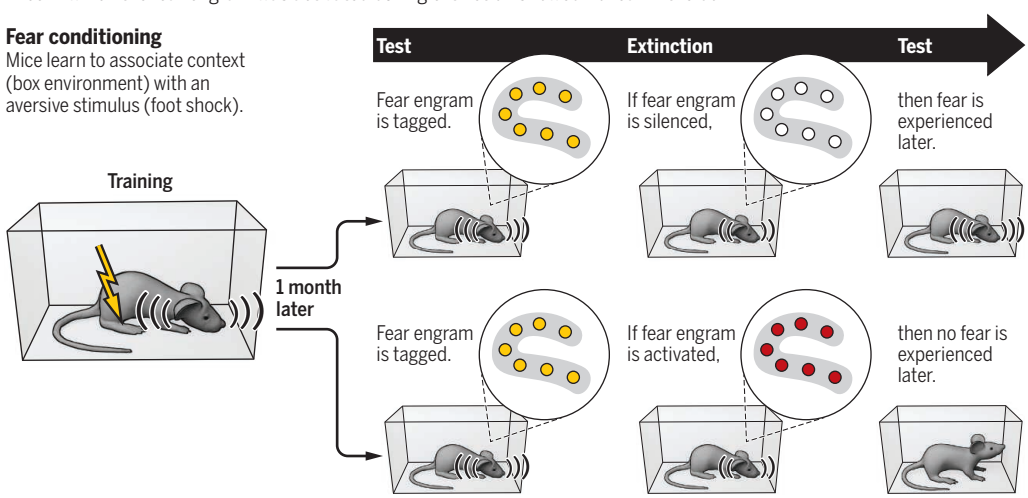
Khalaf *et al.* take advantage of engram-tagging techniques to examine how fear memories are extinguished. They used a contextual fear-conditioning task, in which mice learned an association between a conditioning box (or contextual conditioned stimulus) and a mild foot shock (the unconditioned stimulus) (see the figure). Mice froze when placed in the box 1 month later, indicating that they recognized and feared this context. Active neuronal ensembles were permanently tagged at this remote memory test. Mice were then repeatedly reexposed to the conditioning box in the absence of foot shock to induce extinction, allowing the authors to ask whether the tagged neurons were selectively reactivated during extinction learning. "Engram neurons" in the dentate gyrus, a structure within the hippocampus, were preferentially reactivated during extinction. Moreover, the degree of reactivation correlated with levels of fear reduction, suggesting that engram reactivation may drive extinction.

Khalaf *et al.* directly tested whether engram reactivation and fear attenuation are causally related. Inactivating tagged neuronal ensembles in the dentate gyrus slowed behavioral extinction, suggesting that engram reactivation plays a necessary role in fear extinction. Conversely, when the activity of engram neurons in the dentate gyrus was artificially increased during extinction training, fear attenuation was facilitated. No effects on extinction were noted if the activity of a similarly sized population of neurons that were activated by placement in a new context (not the feared context) was manipulated. These results are consistent with the idea that directly activating the engram supporting the fearful memory (akin to facing your fears) is necessary for fear extinction. In many respects, these findings confirm what any accomplished therapist already knows—that, to a large degree, patients with anxiety disorders must relive their trauma to overcome it. Importantly, however, they also identify the neural mechanism underlying this effect. Without understanding the neural basis of fear extinction, it may be impossible to develop pharmacological agents to facilitate behavioral therapy for anxiety disorders or to optimize behavioral interventions.

After extinction training, fear frequently returns. In the clinic, relapse represents a major hurdle in therapies designed to reduce anxiety and fear. In the lab, extinguished conditioned fear responses may return with the passage of time (spontaneous recovery), after an aversive event (reinstatement) or in a new unextinguished context (renewal), suggesting that extinction produces new learning, rather than erasing the original memory engram (*10*–*12*). Whether direct activation of the engram decreases the return of fear after extinction training is an important unanswered question that may have direct clinical relevance and answer long-standing questions as to whether extinction changes or erases the original fear memory. ■

Facing fear is key for extinction

One month after fear conditioning in a box, mice exhibit engram activation in the dentate gyrus and fear when placed back into the same box. Mice in which the fear engram was silenced during extinction continued to exhibit fear in the box, whereas mice in which the fear engram was activated during extinction showed no fear in the box.



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