The Influence of Stress Hormones on Fear Circuitry

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Key Words
fear conditioning, glucocorticoids, norepinephrine

Abstract
Fear arousal, initiated by an environmental threat, leads to activation of the stress response, a state of alarm that promotes an array of autonomic and endocrine changes designed to aid self-preservation. The stress response includes the release of glucocorticoids from the adrenal cortex and catecholamines from the adrenal medulla and sympathetic nerves. These stress hormones, in turn, provide feedback to the brain and influence neural structures that control emotion and cognition. To illustrate this influence, we focus on how it impacts fear conditioning, a behavioral paradigm widely used to study the neural mechanisms underlying the acquisition, expression, consolidation, reconsolidation, and extinction of emotional memories. We also discuss how stress and the endocrine mediators of the stress response influence the morphological and electrophysiological properties of neurons in brain areas that are crucial for fear-conditioning processes, including the amygdala, hippocampus, and prefrontal cortex. The information in this review illuminates the behavioral and cellular events that underlie the feedforward and feedback networks that mediate states of fear and stress and their interaction in the brain.
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INTRODUCTION

Evolution has endowed each species with an array of instinctual defense mechanisms to help organisms cope with environmental threats and other challenges to safety and well-being. Skunks spray offensive odors, blowfish inflate to look bigger than they actually are, and roaches scurry away into crevices for safety. Although humans in modern societies usually have the luxury of not having to worry about escaping from predators (except for the odd shark, bear, or axe murderer), the emotion of fear and the defensive behaviors connected with it help to save us from peril both in everyday situations as well as under rare but hazardous conditions: We duck for cover, slam on the brakes, run for the hills, or scream for help.

Fear arousal is one of the most reliable routes for activating the stress response (LaBar & LeDoux 2001), an array of peripheral autonomic and neuroendocrine changes that aid survival (McEwen 2003, Sapolsky et al. 2000). At the same time, the peripheral changes induced by fear impact the brain, altering the way emotional and cognitive systems process information. Although such responses enhance survival in the short run, chronic activation of stress responses contributes to the development of pathological states such as anxiety, phobias, depression, and post-traumatic stress disorder (PTSD), as well as a host of physical ailments, including compromised immune function, hypertension, and insulin resistance (McEwen 2003, Sapolsky et al. 2000).

The aim of this article is to explore how fear arousal, manifested on the neurobiological level, leads to activation of peripheral stress responses and how stress responses, in turn, alter brain systems that control emotion and cognition. In the interest of space, we cannot cover some important topics, including how early-life experiences, genetics, or gender might interact with stress in altering fear (DeRijk & de Kloet 2005, Kalin & Shelton 2003, Luine 2002, Lyons & Parker 2007, Meaney 2001, Weinstock 2007).

WHAT IS FEAR, AND HOW IS IT ORGANIZED IN THE BRAIN?

The term fear refers to both a psychological state and a set of bodily responses that occur in response to threat (LeDoux 1996). Much progress has been made in understanding how fear is organized in the brain through studies of Pavlovian fear conditioning, which we focus on here (Fanselow & Poulos 2005; Lang & Davis 2006; LeDoux 1995, 1996, 2007; Maren 2001, 2005; Paré et al. 2004; Rodrigues et al. 2004). There are a variety of ways to assess learned fear besides measuring Pavlovian conditioned response effects. For example, a number of studies have examined the role of brain areas in passive and active avoidance conditioning (Gabriel et al. 2003, McGaugh 2003, Sarter & Markowitsch 1985). In such studies, fear is measured indirectly in terms of instrumental behaviors that avoid danger. Because studies of avoidance conditioning have not led to a coherent view of the neural system that mediates avoidance (Cain & LeDoux 2008), we emphasize the effects of stress on fear conditioning in this review. However, we mention some findings from other procedures where relevant.

Studying Fear in the Laboratory with Fear Conditioning

Pavlovian fear conditioning is a behavioral procedure in which an emotionally neutral conditioned stimulus (CS), such as an auditory tone, is paired with an aversive conditioned stimulus (US), typically a foot shock. After one or several pairings, the CS comes to elicit defensive behaviors, including freezing behavior, as well as increased arousal in the brain and secretion of norepinephrine (NE) and glucocorticoids (GCs) peripherally. These conditioned responses (CRs) are hard-wired and occur in response to both innate and learned threats. Thus, fear learning does not create conditioned fear responses but allows environmental stimuli to come to elicit such responses.
Phases of Fear Conditioning

In studies of fear conditioning, responses elicited by the CS are often measured during acquisition and retrieval tests. Acquisition is initial learning of the association between the CS and US during the training portion of fear conditioning, when the animal first learns to pair the two. Retrieval involves a test of the CS-US association in which the CS is presented alone. Retrieval tests that occur within a few hours after acquisition measure short-term memory (STM), whereas those that occur later (typically 24 hours after learning) measure long-term memory (LTM). STM and LTM tests are used to study memory consolidation, the process through which an unstable STM is converted into a more stable and enduring LTM trace (Dudai 2004, McGaugh 2000, Rodrigues et al. 2004).

Retrieval tests are also used to study reconsolidation. Reconsolidation occurs when fear memories are retrieved and enter a new labile state that requires stabilization via protein synthesis to persist as an enduring LTM trace (Dudai 2006, Nader 2003, Nader et al. 2000). In addition, retrieval tests are utilized to measure extinction, which is the gradual reduction in the ability of the CS to elicit fear CRs that occur when the CS is presented repeatedly in the absence of the US (Quirk & Mueller 2008, Sotres-Bayon et al. 2004). Unlike the disruption of reconsolidation, which is robust and long lasting, extinction involves a new learning process. As a result, after extinction training, the CS can spontaneously produce CRs again after the animal is reexposed to the US or is placed in a novel context (Bouton et al. 2006, Ji & Maren 2007).

Neural Circuitry Underlying Fear Conditioning

Fear conditioning is especially useful for studying the neural basis of fear because the circuit can be described in terms of pathways processing the CS and controlling the CRs. Indeed, the pathways have been mapped from the CS sensory processing to the CR motor control systems. A key link in this circuitry is the amygdala, which sits between the sensory input systems on one hand and the motor output systems on the other (Fanselow & Poulos 2005; Lang & Davis 2006; LeDoux 1995, 1996, 2007; Maren 2001, 2005; Paré et al. 2004; Rodrigues et al. 2004). However, other brain structures also contribute to fear conditioning.


The lateral nucleus (LA) is typically viewed as the sensory gateway to the amygdala because it receives auditory, visual, gustatory, olfactory, and somatosensory (including pain) information from the thalamus and the cortex (olfactory and taste information is transmitted to other nuclei, as well). The LA receives sensory information about the CS from thalamic and cortical projections. The thalamoamygdala pathway is a shortcut of sorts, transmitting rapid and crude information about the fear-eliciting stimulus without the opportunity for filtering by conscious control (LeDoux 1995, 1996). The cortico-amygdala pathway, in contrast, provides slower, but more detailed and sophisticated sensory information. Neurons in the LA respond to both the CS and the US, and damage to, or a disruption of, the LA prevents fear conditioning. It appears that the convergence takes place in the dorsal half of the LA (Romanski et al. 1993), and indeed single-unit recordings show that cellular plasticity occurs in the dorsal LA during fear conditioning (LeDoux 2007, Maren & Quirk 2004). Moreover, molecular changes in the LA consolidate the fear memory, converting STM into LTM traces (Dudai 2004, Rodrigues et al. 2004).

The central nucleus (CE), in contrast, is viewed as the major output region of the amygdala. The CE controls the expression of the fear reaction, including behavioral,

The LA communicates with the CE directly, but the connections between these two nuclei are somewhat modest and other amygdaloid nuclei also help mediate between these two. For instance, the LA projects to the basal nucleus (B), which in turn projects to CE (LeDoux 2000, Pitkanen et al. 1997). The LA and B also project to the intercalated region (ITC), an inhibitory network that connects with the CE (Likhitik et al. 2008, Paré et al. 2004). Projection neurons in the CE tend to be inhibitory, thus LA and B projections to the ITC may stimulate the ITC’s inhibition of CE neurons to allow the expression of fear responses (LeDoux 2007). In addition, the B projects to the striatum (McDonald 1991) to orchestrate instrumental behaviors, such as escape to safety (LeDoux 2007). Furthermore, CE neurons project to the medial peri-locus coeruleus dendritic region, resulting in increased NE release (Aston-Jones 2004), as well as to other monoamine systems in the brainstem and forebrain (Fudge & Emiliano 2003, Gray 1993, Paré 2003).

Information about the context of a fearful situation is conveyed to the LA and B by the hippocampus (Ji & Maren 2007, Kim & Fanselow 1992, Phillips & LeDoux 1992, Pitkanen et al. 2000). Thus, although fear conditioning to discrete sensory cues, such as a tone or a light, are hippocampal independent, conditioning to contextual information, including details about the environment, are hippocampal dependent. The LA and B are also interconnected to a variety of cortical areas, such as the prefrontal and polymodal association cortices (Amaral & Insausti 1992, McDonald 1998, McDonald et al. 1996, Pitkanen et al. 2000, Price 2003, Stefanacci & Amaral 2002). These connections allow higher-order cognitive processes, such as emotion regulation, complex associations, imagination, and reactions to the news of an abstract state, to influence amygdala functions.

The LA, B, and accessory basal nuclei are sometimes grouped together as the BLA (basolateral amygdala). It is important to keep these nuclei separate when possible.

Although the LA is required for fear conditioning under standard training conditions, it appears that, with overtraining, fear conditioning can be mediated by circuits that do not depend on the LA (Lee et al. 2005, Maren 1999). With overtraining, weak connections to the CE appear to induce fear conditioning (Zimmerman et al. 2007), but such pathways are not normally used. Moreover, although the B is not required for conditioning, it nevertheless appears to contribute to the appearance of the fear response (Anglada-Figueroa & Quirk 2005). Some authors suggest that under some conditions, especially those involving the use of instrumental responses to assess Pavlovian conditioning, the CE can mediate conditioning independent of LA (Balleine & Killcross 2006, Cardinal et al. 2002). However, this conclusion is extrapolated largely on the basis of appetitive conditioning findings and needs to be studied more systematically in aversive conditioning.

The expression of fear responses can be regulated by the medial prefrontal cortex (mPFC) via projections to the LA, B, and ITC. The infralimbic subregion (IL) of the mPFC is especially important for fear extinction (Quirk et al. 2006, Rosenkranz et al. 2003, Sotres-Bayon et al. 2004). Because it inhibits amygdala output (Vidal-Gonzalez et al. 2006). However, investigators have debated the nature of the role of prefrontal amygdala interactions in extinction (Quirk et al. 2006, Rosenkranz et al. 2003, Sotres-Bayon et al. 2004). The various sensory and higher-order influences on the amygdala are shown in Figure 1.
FEAR AROUSAL AND THE STRESS RESPONSE: OVERVIEW OF THIS FEEDFORWARD AND FEEDBACK NETWORK

The presence of an environmental threat, in effect a stressor, leads to the activation of the brain’s fear system, thus initiating the stress response in both the brain and the body. A key structure in the fear system, as we have just reviewed, is the amygdala. It is responsible for the detection of threat and the orchestration of stress responses in the brain and the body (see Figure 2).

Once the amygdala detects a threat, its outputs lead to the activation of a variety of target areas that control both behavioral and physiological responses designed to address the threat (Lang & Davis 2006; LeDoux 1996, 2002). In addition to the expression of defensive behaviors, such as freezing (Blanchard & Blanchard 1969, Fendt & Fanselow 1999), amygdala activation leads to responses in the
brain and the body that support the fear reaction. In the brain, monoaminergic systems are activated, resulting in the release of neurotransmitters such as NE, acetylcholine, serotonin, and dopamine throughout the brain. These neurotransmitters lead to an increase in arousal and vigilance and, in general, an enhancement in the processing of external cues (Aston-Jones & Cohen 2005, LeDoux 2007, Ramos & Arnsten 2007, Talarovicova et al. 2007). Detection of threat by the amygdala also has endocrine consequences. Amygdaloid signaling causes secretion of corticotropin-releasing hormone (CRH) from the periventricular nucleus of the hypothalamus. CRH, along with other hypothalamic secretagogs, causes the release of adrenocorticotropic hormone from the pituitary, which in turn stimulates the secretion of GCs from the adrenal cortex. Circulating GCs bind to the high-affinity mineralocorticoid receptor (MR) and the low-affinity glucocorticoid receptor (GR) in tissues throughout the body and the brain (de Kloet 2004, Korte 2001).

Finally, detection of threat by the amygdala has autonomic consequences. Connections from the amygdala to the brainstem lead to the activation of the sympathetic nervous system, involving the release of epinephrine and NE from the adrenal medulla and NE from the terminals of sympathetic nerves throughout the body. Adrenal medullary hormones and sympathetic nerves produce an array of effects, including increasing blood pressure and heart rate, diverting stored energy to exercising muscle, and inhibiting digestion (Goldstein 2003, McCarty & Gold 1996, Sapolsky 2004). Coming full circle, fear circuitry is profoundly influenced by these endocrine and autonomic stress responses. Thus, GCs travel in the circulation to the brain and affect the amygdala and a variety of other structures. Although NE does not cross the blood-brain barrier, it may affect the brain indirectly by binding to visceral afferent nerves, which transmit to the brain (McGaugh 2003). Thus, the detection of threat and the consequent activation of the fear system elicit a variety of effects that feed back onto the system that initiates and modulates emotional processing (McEwen 2003, Paré 2003, Sapolsky 2003).

How do stress, norepinephrine, and glucocorticoids influence different phases of fear conditioning?

In the remainder of this review we examine how stress and its biochemical concomitants (Charmandari et al. 2005, Sapolsky 2004) influence the acquisition, consolidation, reconsolidation, and extinction of conditioned fear, as well as the underlying neural mechanisms responsible for different elements of fear processing. Throughout, we compare animal and human studies and their implications for stress-induced psychopathology. Instead of an exhaustive survey of all pertinent studies, we highlight overarching trends in the literature. To distinguish effects on acquisition from consolidation, it is necessary to compare pretraining manipulations with immediate posttraining manipulations (Rodrigues et al. 2004). This comparison is necessary because the effects of stress or drugs tend to last beyond the short training session in such studies (sometimes only a single training trial). If pretraining manipulations disrupt STM but postraining manipulations do not, the effect is said to be on acquisition (though an effect on STM itself cannot be ruled out). If postraining manipulations leave STM intact but disrupt LTM, then the effect is said to be on LTM consolidation. Another important manipulation involves treatments given immediately before testing LTM. This treatment is called an expression test. However, many studies examine pretraining or postraining effects only on LTM but not on STM or on acquisition. Similarly, relatively few studies observe effects on expression. Therefore, more investigations are needed to fully determine how stress, NE, and GCs influence different phases of fear learning. 

GR: glucocorticoid receptor
and its expression in memory. Below we discuss what has been discovered so far. Although variability in protocols makes it difficult to compare stress manipulations directly across studies, we make distinctions between acute and chronic stress throughout.

**Figure a**

**Functional connectivity**

- **Sensory thalamus**
- **Sensory cortex**
- **PFC**
- **Amygdala**
- **Hippocampus**

**Figure b**

**Processing threat stimulus**

- **Environmental threat**
- **Sensory thalamus**
- **Sensory cortex**

**Figure c**

**Release of stress hormones**

- **PVN**
- **Pituitary**
- **Adrenal gland**
- **Cortex**
- **Medulla**
- **Glucocorticoids**
- **Epinephrine and norepinephrine**

- Increase in cardiovascular tone
- Increase in blood pressure
- Mobilization of stored energy to muscle
- Transient enhancement of immunity
- Inhibition of costly, long-term processes such as growth and reproduction

**Figure d**

**Feedback loop**

- **MRs and GRs**
- **ARs**
- **Epinephrine and norepinephrine**
ACQUISITION OF FEAR CONDITIONING

The Effects of Stress on the Acquisition of Fear Conditioning

Although many studies have looked at the effects of pretraining stress on the LTM of fear conditioning (see below), influences of pretraining stress on acquisition, as tested in STM, have not been formally assessed. However, postshock freezing levels during fear conditioning are greater in chronically stressed rats that are classified as highly reactive, as indexed by their locomotion in a novel environment (Cordero et al. 2003a). This classification suggests that at least in some conditions, stress may influence behavior during the learning process. However, this possibility needs further study because postshock freezing during acquisition reflects contextual conditioning as much as cued.

The Effects of NE on the Acquisition of Fear Conditioning

Locus coeruleus lesions or pretraining infusion of propranolol, a beta adrenergic receptor antagonist, into the amygdala (LA/B) blocks STM, and consequently LTM (Bush et al. 2006; Neophytou et al. 2001). This finding is consistent with an effect on acquisition and its expression in STM.

The Effects of GCs on the Acquisition of Fear Conditioning

The contribution of GCs to fear acquisition has been studied by either removing their effects via adrenalectomy, blocking GRs in the amygdala with an antagonist, or infusing a viral vector into the amygdala prior to training. Although more STM evaluations are needed to determine the precise time course of GCs on fear, adrenalectomized rats show reduced contextual LTM but no immediate memory impairments (Pugh et al. 1997b). Likewise, GR blockade in the amygdala (LA/B) or hippocampus disrupts contextual LTM but leaves postshock levels of freezing intact (Donley et al. 2005). A similar pattern emerges from pretraining intra-amygdala (LA/B) administration of a viral vector expressing a gene to blunt GR signaling, which effectively disrupts both cued and contextual LTM without affecting acquisition or postshock levels of freezing (Rodrigues & Sapolsky 2009). These studies suggest that GCs may not be involved in acquisition so much as in the consolidation of fear LTM. We discuss additional results that support this claim in the following section.

CONSOLIDATION OF LONG-TERM MEMORY

Studies assessing memory consolidation typically administer manipulations immediately after training (Dudai 2004, McGaugh 2000, Rodrigues et al. 2004). If such manipulations fail to disrupt STM but do disrupt LTM, consolidation (conversion of STM to LTM) is said to be affected.

The Effects of Stress on the Consolidation of LTM of Fear Conditioning

Pretraining exposure to both acute and chronic stressors can enhance LTM of both cued and contextual fear conditioning for at least

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Figure 2
Overview of fear arousal and the stress response. **Upper left**: Black arrows indicate the functional connectivity of brain structures involved in fear conditioning. **Upper right**: Red arrows represent the activation of brain areas recruited during the processing of threatening stimuli. **Bottom left**: The stress response leads to the release of stress hormones. **Bottom right**: Dashed lines indicate feedback of the stress response to neural structures involved in fear conditioning. ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing factor; GRs, glucocorticoid receptors; MRs, mineralocorticoid receptors; NTS: nucleus of the solitary tract; PVN, periventricular nucleus of the hypothalamus; SNS, sympathetic nervous system.
3 months (Conrad et al. 1999; Cordero et al. 2003a,b; Kohda et al. 2007; Rau et al. 2005; Rau & Fanselow 2008). Acute posttraining stress also enhances LTM for cued fear conditioning (Hui et al. 2006). Furthermore, preexposure to stress sensitizes fear-conditioning responses such that less-intense stressors can produce robust fear behaviors, which may help explain why individuals with PTSD react strongly to mild stimuli and quickly form new fears (Rau et al. 2005). Because measurements of pretraining stress on acquisition and STM are lacking in the literature, more research is needed to address the temporal dynamics of stress on fear conditioning.

The Effects of NE on the Consolidation of LTM of Fear Conditioning

Central NE depletion or pretraining infusions of propranolol into the LA/B prior to conditioning impair auditory fear LTM (Bush et al. 2006, Selden et al. 1990). In addition, systemic injections of epinephrine, which leads to the release of NE in the brain (McGaugh 2003), enhance contextual fear LTM (Frankland et al. 2004, Hu et al. 2007) via a facilitation of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate) receptor insertion in the hippocampus (Hu et al. 2007). However, posttraining administration of propranolol systemically or into the LA/B does not affect LTM consolidation of auditory or contextual fear conditioning (Bush et al. 2006, Debiec & Ledoux 2004, Lee et al. 2001). Thus, NE in the amygdala does not seem critical for the consolidation of fear conditioning, but it does seem to interact with GCs to fortify memories (Roozendaal et al. 2006).

Posttraining intrahippocampal propranolol infusions given immediately, but not six hours after training, block LTM, but not STM, of contextual fear conditioning (Ji et al. 2003). Furthermore, systemic or intrahippocampal infusions given before testing one day after conditioning impair the retrieval of contextual memories (Murchison et al. 2004).

In humans, systemic NE blockade disrupts the consolidation of contextual, but not cued, fear conditioning LTM (Grillon et al. 2004). Studies involving avoidance conditioning, an indirect means of measuring fear, show that posttraining systemic and intra-amygdala (LA/B) pharmacological manipulations show that NE is crucial to consolidate avoidance learning (Ferry & McGaugh 1999, Gallagher et al. 1977, Liang et al. 1986, Quirarte et al. 1997, Roozendaal & McGaugh 1997). Moreover, NE levels following avoidance training correlate with subsequent retention (McGaugh et al. 2002, McIntyre et al. 2002). Thus, direct measurement of fear responses with fear conditioning gives a different pattern of results than do indirect measures with avoidance conditioning. More work systematically examining these effects is needed.

The Effects of GCs on the Consolidation of LTM of Fear Conditioning

Pretraining systemic, intrahippocampal, or intra-amygdala (LA/B) manipulation of GCs influences the LTM of fear conditioning; contextual memories are more vulnerable (Conrad et al. 2004; Cordero et al. 2002; Donley et al. 2005; Pugh et al. 1997a,b) likely because contextual fear memory is generally weaker (i.e., harder to learn and easier to disrupt) than cued fear memory (Phillips & LeDoux 1992).

Posttraining systemic manipulations of GCs also impact both auditory and contextual fear memory consolidation (Corodimas et al. 1994, Hui et al. 2004, Pugh et al. 1997a, Zorawski & Killcross 2002). Moreover, posttraining GR blockade in the LA/B affects auditory fear LTM but not STM (Jin et al. 2007), providing more evidence that GCs are most important for consolidation processes. Furthermore, posttraining GCs administered immediately, but not 3 hours, after auditory fear conditioning facilitates freezing to a tone previously paired with a US but does not alter responses to unpaired tones or to tone or shock alone, suggesting a selective and
time-dependent role for GC-facilitated memory of the tone-shock association (Hui et al. 2004). In addition, elevation of GCs systemically or in the LA/B enhances the retrieval of fear memories in avoidance paradigms (Izquierdo et al. 2002, Roozendaal & McGaugh 1997), and this enhancement can be effective for memories acquired from one day to many months before (Izquierdo et al. 2002). Finally, in humans, high GC levels positively correlate with fear memory consolidation (Zorawski et al. 2006).

RECONSIDERATION

The Effects of Stress on the Reconsolidation of Fear Conditioning

Preliminary evidence suggests that acute stress before, but not after, memory reactivation disrupts auditory fear reconsolidation LTM (Bush et al. 2004). It would be interesting to see how chronic stress or intra-amygdala manipulations influence this phenomenon and the time course of reconsolidation processes.

The Effects of NE Manipulation on the Reconsolidation of Fear Conditioning

Although the effects of prereactivation NE manipulations on reconsolidation are unknown, systemic or intra-amygdala (LA/B) administration of propranolol after reactivation disrupts reconsolidation of auditory and contextual fear LTM in rats (Abrari et al. 2008, Debiec & Ledoux 2004). The same trend holds true for avoidance conditioning (Przybyslawski et al. 1999). These findings are consonant with reports that propranolol can effectively treat PTSD when administered after aversive memory activation (Brunet et al. 2008, Orr et al. 2006, Pitman & Delahanty 2005, Pitman et al. 2002).

The Effects of GC Manipulation on the Reconsolidation of Fear Conditioning

How alterations of GC activity before reactivation might influence reconsolidation has yet to be investigated. After reactivation, GR antagonism in the LA/B disrupts LTM but not STM of auditory fear reconsolidation; this effect occurs if blockade is immediate, but not 6 hours, after reactivation. Furthermore, this immediate postreactivation blockade is effective both 1 day and 10 days after conditioning (Jin et al. 2007). Likewise, GR blockade in the LA/B immediately after retrieval blocks reconsolidation in an avoidance paradigm (Tronel & Alberini 2007).

EXTINCTION

The Effects of Stress on the Extinction of Fear Conditioning

Chronic stress prior to conditioning impairs the LTM of auditory and contextual extinction LTM (Garcia et al. 2008, Miracle et al. 2006); similarly, patients with PTSD show impaired cued fear extinction (Blechert et al. 2007, Wessa & Flor 2007). However, reports conflict regarding the influence of stress on the learning of extinction (Izquierdo et al. 2006, Miracle et al. 2006), perhaps because of differences in stress types or protocols or species (mice versus rats). Stress induction after fear conditioning but before extinction does not influence extinction LTM (G. Quirk, unpublished results). If a stressor is controllable, the mPFC inhibits activation of brainstem nuclei (Amat et al. 2005). Moreover, stress and environmental enrichment produce opposite effects on fear renewal in a new context; the influence of enrichment outweighs that of stress (Mitra & Sapolsky 2009).

Involvement of NE in the Extinction of Fear Conditioning

NE in the LA/B after extinction training enhances the consolidation of extinction memories of contextual fear LTM (Berlau & McGaugh 2006). Furthermore, NE efflux increases in the PFC during presentation of a CS previously paired with a US (Feenstra et al. 2001). Similarly, administration of propranolol in the IL subregion of the mPFC before, but
not after, extinction training impairs retrieval of extinction the following day while leaving within-session extinction intact. Furthermore, NE signaling in the IL strengthens extinction memory (Mueller et al. 2008).

**Involvement of GCs in the Extinction of Fear Conditioning**

Chronic GC exposure, which decreases endogenous GC secretion, prior to fear conditioning impairs contextual extinction LTM but not STM and results in a decrease of NMDA (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate) excitatory receptor subunits in the mPFC (Gourley et al. 2008). In addition, systemic and intra-amygdala (LA/B) GC manipulations prior to extinction training suggest that GR activation is key for extinction learning in a fear-potentiated startle paradigm (Yang et al. 2006). Although systemic GC administration before extinction training does not produce extinction learning or LTM impairments (G. Quirk, unpublished results), metyrapone, a GC synthesis inhibitor, affects extinction LTM while leaving learning intact (Barrett & Gonzalez-Lima 2004). Moreover, adrenergic receptor (AR) blockade or GR activation after retrieval disrupts recall of contextual fear conditioning, but only the effects of GR activation are reversed by a reminder shock (Abrari et al. 2008). Along these same lines, GR-mediated disruptions of fear memories show spontaneous recovery and renewal of CRs (Barrett & Gonzalez-Lima 2004, Cai et al. 2006). However, GR inactivation impairs CRs in an avoidance task, and these do not reemerge after strong reminders (Tronel & Alberini 2007), possibly because of the involvement of other processes in this paradigm. Nonetheless, the results from animal studies that show GC activity may promote extinction (Barrett & Gonzalez-Lima 2004, Cai et al. 2006) parallel findings that PTSD and phobia symptoms, but not general anxiety, can improve with GC treatment (Aerni et al. 2004, de Quervain 2008, Schelling et al. 2004, Soravia et al. 2006).

**SUMMARY OF THE EFFECTS ON FEAR CONDITIONING**

Table 1 summarizes results from animal studies presented in this review. This information comes with a number of caveats: First, data were often gathered from only a very small number of papers for each topic; second, few studies examined both contextual and cued fear conditioning; third, it was rarely the case that both STM and LTM were examined; fourth, little work has examined effects of stress, NE, or GCs on the expression of fear; and fifth, the important modulatory effects of age, genetics, and gender have not been discussed throughout this review in the interest of space.

**HOW STRESS AFFECTS FEAR CIRCUITRY ON A CELLULAR LEVEL**

**The Influence of Stress on the Morphology of Neurons in Fear Circuitry**

Stress can alter the morphology of pyramidal neurons in structures involved in fear circuitry (see Figure 3). To begin, chronic stress causes an increase in the dendritic arborization, spine formation, and synaptic connectivity in principal neurons in the LA/B (Vyas et al. 2002, 2006) but not in the CE (Vyas et al. 2003). Moreover, chronic immobilization stress, but not chronic unpredictable stress, induces this dendritic remodeling in the LA/B (Vyas & Chattarji 2004). In addition, the duration of stress directly impacts the spatiotemporal patterns of spine formation such that both acute and chronic stressors increase spines in LA/B neurons, but only the latter has an effect on dendritic arborization and hypertrophy of LA/B neurons (Mitra et al. 2005). Furthermore, a single, acute dose of GCs is sufficient to produce anxiety and hypertrophy of LA/B neurons (Mitra & Sapolsky 2008). Contrasting with these effects, stress decreases dendritic branching and spine count in the hippocampus (for review, see McEwen & Magarinos 2001). Neurons in the mPFC endure morphological changes due to stress and GCs, including...
Table 1 Localization of manipulations: systemic/brain-wide (black), LA/B (red), hippocampus (blue), and mPFC (green).a Postshock freezing findings included in contextual STM results

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pretrain-</th>
<th>Posttrain-</th>
<th>Pretest-</th>
<th>Prereactivation</th>
<th>Postreactivation</th>
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aAbbreviations: LA/B, lateral/basal nucleus of the amygdala; GCs, glucocorticoids; LTM, long-term memory; mPFC, medial prefrontal cortex; NE, norepinephrine; STM, short-term memory.

bKey:
↑, Stress, NE, or GCs facilitate fear memory here.
↓, Stress or GCs impair fear memory here.
×, Stress, NE, or GCs manipulations produce no influences at this time.
?, Effects unknown.
∗, Conflicting reports.
§, Effectively disrupts consolidation of avoidance learning, but not for conditioning.
atrophies and spine loss (Brown et al. 2005; Cook & Wellman 2004; Czeh et al. 2008; Izquierdo et al. 2006; Radley et al. 2004, 2006, 2008; Wellman 2001). Furthermore, atrophy in IL neurons through brief uncontrollable stress is accompanied by a resistance to fear extinction (Izquierdo et al. 2006). Stress-induced atrophy of hippocampal (Conrad et al. 1999, McEwen 1999, Sousa et al. 2000) and mPFC neurons (Radley et al. 2005) reverses after a stress-free period. However, the same recovery period does not rescue stress-induced hypertrophy of amygdala neurons (Vyas et al. 2004).

The Influence of Stress on the Electrophysiological Properties of Neurons in Fear Circuitry

Stress and its accompanying biological effects influence the electrophysiological activity of neurons in fear circuitry (see Figure 4). Electrophysiological studies often explore neuronal excitability, as well as long-term potentiation (LTP), an artificial means of inducing synaptic connectivity between two brain areas, and thus a physiological model of fear learning and memory (Blair et al. 2001, Rodrigues et al. 2004, Schafe et al. 2001). Stress has differential effects on the amygdala, hippocampus, and prefrontal cortex, which we now review.

LA/B neurons from stressed animals display increased firing rates and greater responsiveness (Garcia et al. 1998, Kavushansky et al. 2006, Kavushansky & Richter-Levin 2006, Rodriguez Manzanares et al. 2005). Although some stress paradigms, especially acute inductions, enhance LTP in the LA/B (Rodriguez Manzanares et al. 2005, Vouimba et al. 2004, 2006), other stress protocols suppress it (Kavushansky & Richter-Levin 2006, Kavushansky et al. 2006, Kohda et al. 2007). Along these same lines, the type of stress is also a critical factor in the influence of stress on hippocampal plasticity such that chronic, but not acute, stress suppresses hippocampal LTP (Pavlides et al. 2002).

Temporal qualities of stressors have opposite influences on the hippocampus, boosting LTP when the tetanizing stimulation is in close proximity to emotional processing and disrupting LTP when there is a substantial delay between the stimulation and initiation of stress.
mimics this emotion-induced influence on hippocampal plasticity (LA/B) (Diamond et al. 2007, Richter-Levin 2004, Richter-Levin & Akirav 2003). Hippocampal stimulation after fear extinction training disrupts the recall of extinction (Garcia et al. 2008). Furthermore, uncontrollable stress drastically impairs hippocampal LTP relative to the influence of the same amount of controllable stress (Shors et al. 1989). The mPFC seems to play a role in determining the controllability of stress (Amat et al. 2005), and neurons here display unique patterns of firing rates that encode fast and slow responses to stress. Whereas some mPFC neurons respond phasically during the duration of a stressor, others show activity that persists after the stress ceases, and yet others respond during the initiation and termination of stress (Jackson & Moghaddam 2006). Furthermore, stress reduces LTP induction in projections from both the LA/B (Maroun & Richter-Levin 2003) and hippocampus (Cerqueira et al. 2007, Rocher et al. 2004) to the PFC.

Figure 4
Influence of stress, norepinephrine (NE), and glucocorticoids (GCs) on the electrophysiological properties of lateral (LA)/basal nucleus (B) neurons of the amygdala. Representative traces and long-term potentiation (LTP) data reproduced with permission from Tully et al. (2007).
The Influence of NE on the Electrophysiological Properties of Neurons in Fear Circuitry

NE increases the spiking of neurons in the LA and facilitates the induction of LTP (Tully et al. 2007). What's more, fear conditioning increases depolarization-evoked NE release (Liu et al. 2007). Moreover, ARs seem particularly pivotal for a late phase of LTP in the LA, which persists for at least three hours and requires the synthesis of new mRNA and protein (Huang et al. 2000). The reactivity of the hippocampal neurons is enhanced by NE (Harley 2007, Segal et al. 1991), and suppression of GC secretion increases NE transmission here (Mizoguchi et al. 2008); the relevance of this to fear learning and memory processes is unclear. In the IL of the mPFC, fear conditioning decreases, and extinction increases, neuronal excitability (Santini et al. 2008). Pertinent to this, NE signaling in this region increases neuron excitability (Barth et al. 2007, Mueller et al. 2008) and strengthens the memory for extinction (Mueller et al. 2008).

The Influence of GCs on the Electrophysiological Properties of Neurons in Fear Circuitry

Both high and low GC concentrations enhance the excitability of LA/B neurons (Duvarci & Paré 2007, Kavushansky & Richter-Levin 2006). In addition, GCs depolarize the resting potential, increase input resistance, and significantly decrease spike-frequency adaptation of LA/B neurons (Duvarci & Paré 2007). The influences of GCs are different on the hippocampus; there low concentrations (via MR activation) are excitatory, whereas high concentrations (via MR plus GR activation) are inhibitory (de Kloet et al. 1999, Joels & de Kloet 1992). A similar biphasic relationship of MR and GR activation exists for enhancing and suppressing effects of GCs on hippocampal LTP, respectively (Pavlides et al. 1996, Pavlides & McEwen 1999). We do not know how GCs directly influence the electrophysiological profiles of mPFC neurons. Therefore, more data are needed to illustrate how GCs interact with other factors to influence plasticity in structures that modulate fear conditioning.

CONCLUSIONS

An extensive literature has demonstrated the ways in which stress, and the endocrine mediators of the stress response, can impact explicit, declarative memory processes (McEwen 1999). Though based on a smaller literature, this review highlights the ability of stress and arousal-triggered fear to impact emotional learning and memory, as assessed by fear conditioning. Indeed, stressful experiences, NE, and GCs alter the morphological and electrophysiological characteristics of neurons in areas that play a vital role in fear processing, including the amygdala (LA/b), hippocampus, and PFC. As a result, they can have a powerful influence on fear conditioning.

In the laboratory, investigators use a number of models to induce stress, including exposure to predator odor, restraint, foot shock, forced swimming, saline injections, and cold temperatures. Furthermore, the duration of stress manipulations can be acute, moderate, or chronic. Although these differences make direct comparisons between studies difficult, stereotypical responses to these various stressors do occur and activate the brain’s fear system, thus initiating the stress response in both the body and the brain. Nonetheless, because stress magnitude and duration differentially impact the morphological and electrophysiological properties of fear circuitry neurons, it would be important for future studies to compare and contrast the influence of different stress protocols.

Existing studies show that stress before or after training boosts LTM of fear conditioning. In addition, stress before, but not after, reactivation enhances reconsolidation. Finally, stress before fear conditioning, but not before...
extinction training, interferes with extinction LTM. Altogether, these findings suggest that during initial learning, stress activates events that can influence later consolidation and extinction processes. Whether effects exist on the acquisition and expression of fear is less clear owing to the relative paucity of studies.

In regard to NE, cumulative evidence suggests that NE is important for fear conditioning, reconsolidation, and extinction, but not for consolidation. NE increases neuronal excitability and LTP in the LA, and its activity there is needed for both STM and LTM of fear conditioning. NE also increases neuronal excitability in the IL of the mPFC and is important for the LTM of extinction. Because pretraining but not posttraining intra-amygdala (LA/B) or intramPFC infusions of propranolol block LTM of fear conditioning and extinction, respectively, it seems that NE must be tonically active during learning to promote rapid postlearning cascades in the LA and IL to facilitate STM retrieval and to promote the conversion of STM to LTM.

Next, GCs appear to be particularly crucial for LTM, but not the acquisition, of fear conditioning. Therefore, the overall trend of systemic or intra-amygdala (LA/B) GC disrupting LTM is consonant with electrophysiological findings that GCs have long-term, but not short-term, enhancing effects on neuronal excitability in LA/B (Duvarci & Paré 2007). GRs are located both at synaptic and at nuclear sites in the LA. The classic actions of GR, as a steroid receptor, involve binding GCs in the cytoplasm and acting as a transcription factor upon translocating to the nucleus, whereas less traditional GR actions include rapid, nongenomic effects. The fact that GRs seem more important for fear consolidation than for learning, and for enduring but not immediate influences on the firing of LA/B neurons, suggests that more traditional genomic effects underlie these behavioral and electrophysiological findings.

Stress-induced enhancement of LTP in auditory inputs to the LA alongside disruption of LTP in the projections to the PFC from the hippocampus and the LA/B is congruent with the notion that powerful fear inputs can facilitate strong emotional pathways at the cost of activating more regulatory pathways. Indeed, an abundant literature showing that NE is important particularly for hippocampal and PFC function, in addition to LA/B activation, is in line with NE's role in arousal and cognition.

Given that hippocampal function is disrupted by major stressors or exposure to elevated levels of GCs, how might it play a pivotal role in forming fear memories, particularly contextual ones? This answer might be explained by the model of the hippocampus shifting from a cognitive mode to a flashbulb memory mode under intense stress, which explains why the contextual memories of traumatic experiences are vivid and endure for many years but tend to be disjointed and fragmented (Diamond et al. 2007).

In summary, stress, NE, and GCs appear to be potent modulators of fear memory formation. Furthermore, the ability of stress, NE, and GCs to decrease GABA (γ-aminobutyric acid)-A receptor-mediated inhibition (Duvarci & Paré 2007, Rodriguez Manzanares et al. 2005, Tully et al. 2007), thereby allowing for increased excitability in LA/B, is harmonious with the fact that benzodiazepines act on this receptor to decrease anxiety.

During difficult times, we are often advised to “use our heads” or “follow our hearts” or “go with our gut.” As this review highlights, our bodies and our minds really are not separate, but instead mutually inform each other on how to process the emotional events of our lives.

**DISCLOSURE STATEMENT**

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.
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LITERATURE CITED


McDonald AJ. 1991. Topographical organization of amygdaloid projections to the caudatoputamen, nucleus accumbens, and related striatal-like areas of the rat brain. *Neuroscience* 44:15–33
Orr SP, Milad MR, Metzger LJ, Lasko NB, Gilbertson MW, Pitman RK. 2006. Effects of beta blockade, PTSD diagnosis, and explicit threat on the extinction and retention of an averively conditioned response. Biol. Psychol. 73:262–71


Rau V, Fanselow MS. 2008. Exposure to a stressor produces a long lasting enhancement of fear learning in rats. *Stress* 18:1


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