

# Molecular Mechanisms of Fear Learning and Memory

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Pavlovian fear conditioning is a particularly useful behavioral paradigm for exploring the molecular mechanisms of learning and memory because a well-defined response to a specific environmental stimulus is produced through associative learning processes. Synaptic plasticity in the lateral nucleus of the amygdala (LA) underlies this form of associative learning. Here, we summarize the molecular mechanisms that contribute to this synaptic plasticity in the context of auditory fear conditioning, the form of fear conditioning best understood at the molecular level. We discuss the neurotransmitter systems and signaling cascades that contribute to three phases of auditory fear conditioning: acquisition, consolidation, and reconsolidation. These studies suggest that multiple intracellular signaling pathways, including those triggered by activation of Hebbian processes and neuromodulatory receptors, interact to produce neural plasticity in the LA and behavioral fear conditioning. Collectively, this body of research illustrates the power of fear conditioning as a model system for characterizing the mechanisms of learning and memory in mammals and potentially for understanding fear-related disorders, such as PTSD and phobias.

Fear is the emotion that is best understood in terms of brain mechanisms. Because fear plays a prominent role, either directly or indirectly, in a variety of psychiatric conditions, understanding its neural basis is of great importance. The term “fear” refers to a subjective feeling state and to the behavioral and physiological responses that occur in response to threatening environmental situations. The objectively measurable behavioral and physiological responses are the subject of scientific investigations of fear in laboratory animals.

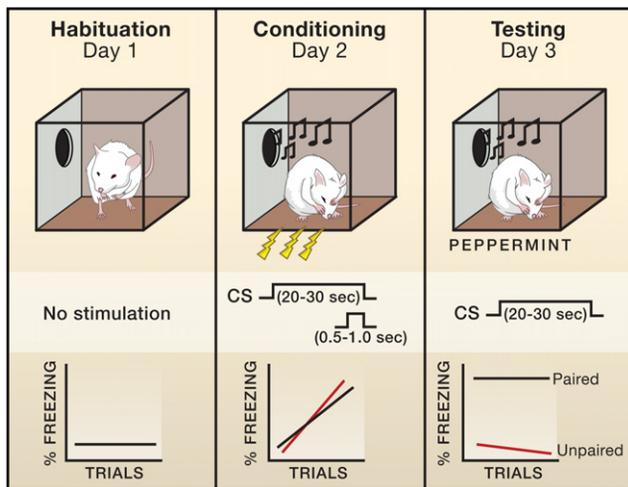
Research on fear has been successful in large part because of a behavioral paradigm, which is well suited for neurobiological analysis: Pavlovian fear conditioning. Fear conditioning is valuable as a neurobiological tool because it involves a specific stimulus, under the control of the experimenter, that reliably elicits a measurable set of behavioral and physiological responses once learning has occurred.

In fear conditioning, an emotionally neutral conditioned stimulus, such as a tone, is paired with an emotionally potent, innately aversive unconditioned stimulus, (e.g., an electric shock) during a conditioning or acquisition phase (Figure 1). This procedure is referred to as auditory fear conditioning. The assessment of conditioning then involves measuring conditioned responses elicited by the auditory conditioned stimulus independent of the unconditioned stimulus during a memory test phase. This somewhat artificial procedure mimics real-life experiences in which the unconditioned stimulus causes pain or other harm and the conditioned stimulus occurs in connection with the harmful one. For example, a rat that is wounded by a cat

but escapes may well form a memory of the sound of rustling leaves as the cat was about to attack.

Pavlovian conditioning is believed to take place by the convergence of pathways transmitting the conditioned stimulus and unconditioned stimulus. In fear conditioning, the key circuits involve sensory areas that process the conditioned stimulus and unconditioned stimulus, regions of the amygdala that undergo plasticity during learning, and regions that control the expression of specific conditioned responses (Figure 2; LeDoux, 2000; Fanselow and Poulos, 2005; Maren 2005; Davis and Whalen, 2001; Kim and Jung, 2006). These pathways converge in the LA, where synaptic plasticity that enhances the response of LA neurons to the conditioned stimulus occurs. As a result, the conditioned stimulus is then able to flow from the LA to the central nucleus of the amygdala (CE). The LA connects with the CE directly and indirectly by way of the basal (B) and intercalated masses of the amygdala. Pathways from CE to downstream areas then control defensive behavior (freezing) and autonomic and endocrine responses. Recent studies implicate the prelimbic cortex in fear expression as well, possibly by way of its connections to B and, from there, to CE (Sotres-Bayon and Quirk, 2010).

In this Review, we examine recent research on cellular and molecular mechanisms in LA that contribute to auditory fear conditioning. We focus on the LA because molecular changes in this area have been shown to make essential contributions to the formation, storage, and expression of the memory of the experience (see Rodrigues et al., 2004b; Pape and Pare, 2010; Sah et al., 2008). Although molecular changes occur in other



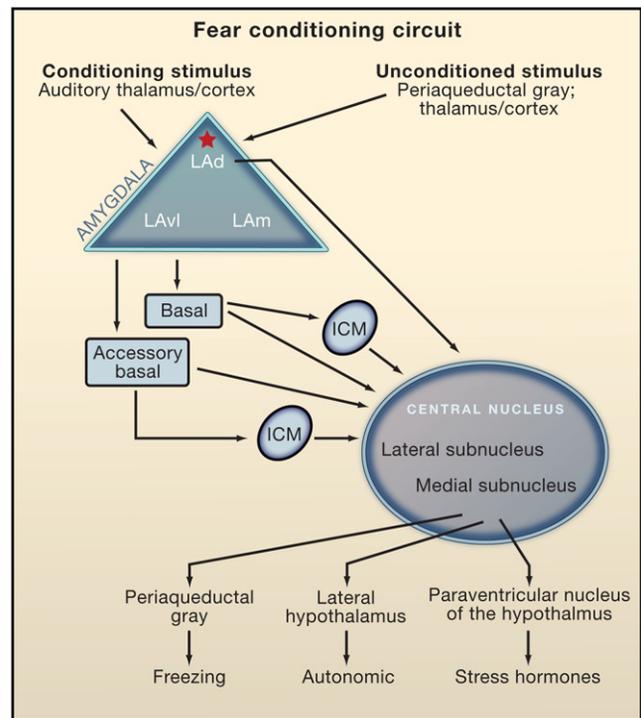
**Figure 1. Auditory Fear Conditioning in Rats**

In a typical auditory fear conditioning procedure, rats are habituated to the conditioning chamber but given no stimuli. During the conditioning session, the electric shock unconditioned stimulus (US) is paired with the auditory-conditioned stimulus (CS) several times (usually 1–5). The effects of conditioning are then assessed in a test session during which the conditioned stimulus is presented alone. Most studies measure “freezing” behavior, which is an innate defensive response elicited by the conditioned stimulus after conditioning. An unpaired control group in which the conditioned stimulus and unconditioned stimulus are presented in a nonoverlapping manner is often used. The conditioned stimulus elicits little or no freezing prior to conditioning in either the paired or unpaired group (not shown). Both the paired and unpaired group freeze during the training session due to the shock presentation. In the test session, the paired group exhibits considerably more conditioned stimulus-elicited freezing than the unpaired. Differences between the paired and unpaired group reflect the association that is learned as a result of conditioned-unconditioned stimulus pairing.

areas of the amygdala and in other areas of the brain, the molecular contributions to LA plasticity as it relates to fear learning are understood in the greatest detail.

We restrict the discussion to molecular mechanisms that have been linked directly to the behavioral expression of conditioning, as opposed to mechanisms that underlie long-term potentiation (LTP), in which synaptic plasticity is induced by electrical or chemical stimulation of LA circuits. LTP has provided a rich array of candidate mechanisms for the plasticity processes that could occur during actual fear learning but will not be the focus of this Review (see Pape and Pare, 2010, Sah et al., 2008, Sigurdsson et al., 2007, and Dityatev and Bolshakov, 2005 for reviews on this topic). LTP will only be mentioned when findings are directly relevant to auditory fear conditioning.

Unique molecular mechanisms are known to underlie different stages of memory formation. Therefore, we have organized the Review by these stages—specifically, the acquisition, consolidation, and reconsolidation of fear memories. Molecular mechanisms of fear extinction will not be discussed here (for reviews, see Myers and Davis, 2007, Quirk and Mueller, 2008, Sotres-Bayon et al., 2006, Herry et al., 2010, and Pape and Pare, 2010). The stages of memory formation and storage are distinguished by measuring the effects of various manipulations—often pharmacological—on fear-conditioned responses at different times with respect to training or testing (see Ro-



**Figure 2. Fear Conditioning Circuit**

Convergence of the auditory-conditioned stimulus and nociceptive unconditioned stimulus in the amygdala is essential for fear conditioning. Convergence of conditioned and unconditioned stimuli occurs in lateral nucleus of the amygdala (LA), especially in the dorsal subnucleus (LAAd), leading to synaptic plasticity in LA. Plasticity may also occur in the central nucleus (CE) and in the auditory thalamus. LA connects with CE directly and indirectly by way of connections in the basal (B), accessory basal (AB), and intercalated cell masses (ICM). CE connects with hypothalamic and brainstem areas that control the expression of conditioned fear responses, including freezing and autonomic (ANS) and hormonal responses. CeL, lateral nucleus of CE; CeM, medial nucleus of CE; PAG, periaqueductal gray; LH, lateral hypothalamus; PVN, paraventricular nucleus of the hypothalamus.

drigues et al., 2004b). Table 1 summarizes the phases and how drug manipulations are used to assess effects on a particular phase. We limit our discussion to studies that manipulated molecular processes directly in the LA. Other studies that target multiple brain structures (such as genetic knockout and systemic drug studies) are discussed in Rodrigues et al. (2004b), Pape and Pare (2010), Sah et al. (2008), Silva (2003), Mayford and Kandel (1999), and Sweatt (2003).

#### **Acquisition: Cellular and Molecular Processes in LA that Underlie Learning**

Learning is the basis of memory. If there is no learning, there can be no memory later. This is true in a psychological description of memory and also appears to be true when looking at the molecules that initiate memory formation. Disruption of molecular mechanisms that mediate memory acquisition invariably affect long-term memories as well. We thus start our exploration of the cellular and molecular mechanisms of fear memory formation by considering the acquisition/training phase of fear conditioning during which learning occurs.

**Table 1. Relation of Time Course of Drug Administration to Different Aspects of Fear Conditioning**

Acquisition	If a drug given before, but not after, training affects STM and LTM, it is said to disrupt acquisition.
Consolidation	If drug given before or after training has no effect on STM but impairs LTM, it is said to disrupt consolidation.
Reconsolidation	If drug given after retrieval of a consolidated memory has no effect within several (2–4) hr after but impairs memory later (usually 24 hr or longer), it is said to disrupt reconsolidation.

### Hebbian Mechanisms in LA: Contributions to Fear Learning

A common view in neuroscience is that learning involves so-called Hebbian synaptic plasticity. This view is based on Donald Hebb's influential proposal, which can be paraphrased as follows: a synaptic input can be strengthened when activity in the presynaptic neuron co-occurs with activity (membrane depolarization, especially depolarizations that produce action potentials) in the postsynaptic neuron (Hebb, 1949; Brown et al., 1990; Sejnowski, 1999). Implicit in Hebb's original formulation was the idea that associative plasticity can be implemented if a strong presynaptic input produces activity in the postsynaptic neuron at the same time that another presynaptic input weakly stimulates the neuron. As a result, the weak input is strengthened by its temporal relationship with the strong input. The Hebbian hypothesis is especially appealing as an explanation for how simple associative learning, such as that taking place in fear conditioning, might occur. In a Hebbian model of fear conditioning, strong depolarization of LA pyramidal cells evoked by the aversive unconditioned stimulus leads to strengthening of coactive conditioned stimulus inputs onto the same neurons (Blair et al., 2001; LeDoux, 2000; Paré, 2002; Sah et al., 2008).

Existing data support the idea that LA associative plasticity and fear memory formation are triggered by unconditioned stimulus-induced activation of LA neurons. Thus, unconditioned stimulus-evoked depolarization is necessary for the enhancement of conditioned stimulus-elicited neural responses in LA after conditioned-unconditioned stimuli pairing (Rosenkranz and Grace, 2002a), and pairing a conditioned stimulus with direct depolarization of LA pyramidal neurons as an unconditioned stimulus supports fear conditioning (Johansen et al., 2010b). Though there is evidence that Hebbian plasticity in LA may not entirely explain fear conditioning (see below), it is clear that synaptic plasticity at conditioned stimulus input pathways to the LA does occur with fear conditioning. Supporting this, *in vivo* studies demonstrate an enhancement of auditory stimulus-evoked responses in LA neurons after fear conditioning, (see Maren and Quirk, 2004, LeDoux, 2000, Blair et al., 2001, and Pape and Pare, 2010 for review). Further, *in vitro* experiments find a strengthening of putative auditory thalamo-LA and cortico-LA synapses following the pairing of a conditioned stimulus with an unconditioned stimulus (Clem and Haganir, 2010; McKernan and Shinnick-Gallagher, 1997; Rumpel et al., 2005;

Schroeder and Shinnick-Gallagher, 2005). Also supporting the idea that enhancement of synaptic strength in the LA is important for fear learning, LTP is occluded in amygdala slices from fear-conditioned animals (Schroeder and Shinnick-Gallagher, 2004; Schroeder and Shinnick-Gallagher, 2005; Tsvetkov et al., 2002), suggesting that LTP-induced changes in synaptic strength occur in LA during fear learning.

In the remainder of this section, we will examine the mechanisms mediating possible Hebbian forms of synaptic plasticity in LA during the acquisition of fear conditioning. Hebbian mechanisms can be defined as those that are directly engaged by or regulate coordinated pre- and postsynaptic activity. We will then consider how other mechanisms, specifically monoamine transmitters, could modulate Hebbian plasticity in LA.

**NMDA-Type Ionotropic Glutamate Receptors.** Hebbian plasticity is believed to involve N-methyl-D-aspartate receptors (NMDARs) located on postsynaptic neurons in LA. NMDARs are known to be coincidence detectors of presynaptic activity (for example, in conditioned stimulus input synapses) and postsynaptic depolarization (evoked by the unconditioned stimulus, for example) (Malenka and Nicoll, 1999). As a result of correlated pre- and postsynaptic activity, NMDARs pass calcium, and this is thought to be important for synaptic plasticity and possibly memory formation (Malenka and Nicoll, 1999). Auditory inputs are indeed presynaptic to glutamate receptors, including NMDARs, in LA and use glutamate as a transmitter (see LeDoux, 2000 for review). Further, LA cells that receive auditory inputs also receive unconditioned stimulus inputs (Romanski et al., 1993; Johansen et al., 2010b), and broad spectrum NMDAR antagonists (such as APV) microinjected into the LA and basal amygdala disrupt the acquisition of fear learning (Gewirtz and Davis, 1997; Maren et al., 1996; Miserendino et al., 1990; Rodrigues et al., 2001). APV also disrupts normal synaptic transmission in the LA (Li et al., 1996) and interferes with the expression of previously acquired fear memories (Rodrigues et al., 2001). This finding raises the possibility that APV reduces the acquisition of fear conditioning by inhibiting synaptic transmission instead of blocking second messenger signaling downstream of NMDARs. However, microinjections in LA of an antagonist that targets the GluN2b (formerly called NR2B) subunit of the NMDAR reduce the acquisition of fear conditioning without affecting expression of fear memories or normal synaptic transmission (Rodrigues et al., 2001; Bauer et al., 2002). Further, viral-mediated knockdown of the cell adhesion molecule neuroligin-1 in LA attenuates fear memory formation, possibly by reducing NMDAR number (Kim et al., 2008). Thus, in spite of the effects of APV on synaptic transmission and fear expression, NMDARs at conditioned stimulus input synapses in LA may indeed serve as coincidence detectors of pre- and postsynaptic activity to initiate associative plasticity during fear conditioning.

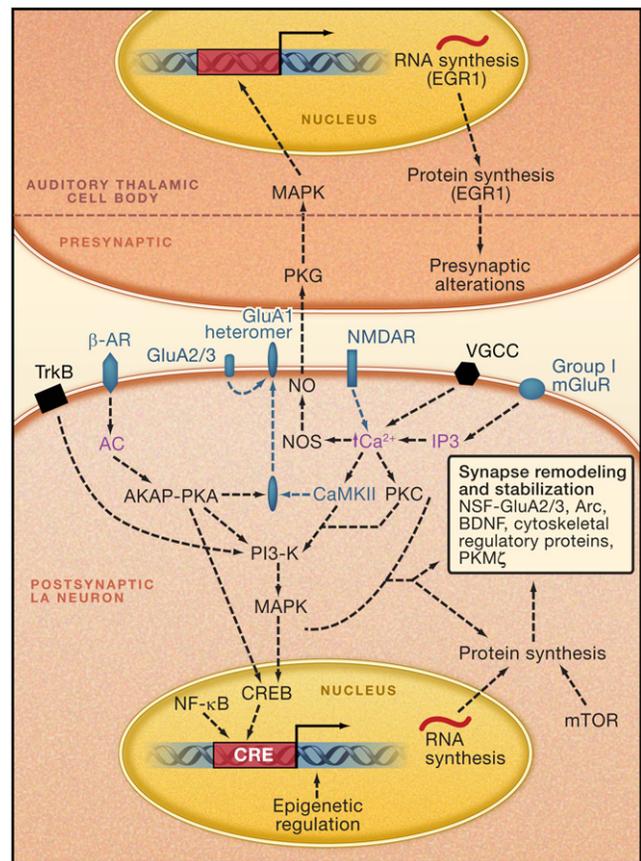
Although postsynaptic NMDARs may contribute to Hebbian synaptic plasticity by facilitating an LTP-like processes involving calcium influx, there is a form of NMDAR-dependent LTP that is induced and expressed presynaptically in LA (Fourcaudot et al., 2009; Humeau et al., 2003). This provides a potential alternate mechanism for NMDAR activation during fear learning that is non-Hebbian in nature (i.e., does not require postsynaptic depolarization) and should be taken into consideration when

discussing the effects of pharmacological manipulations of the NMDAR in the LA. When a pairing protocol is used, LTP can become independent of NMDAR activation and instead depend on calcium entry through voltage-gated calcium channels (Weiskopf et al., 1999) in LA. However, in real learning in whole animals, calcium influx through both NMDARs and VGCCs may be required, though VGCCs appear to be involved in consolidation and not acquisition of fear conditioning (Bauer et al., 2002; McKinney and Murphy, 2006; Shinnick-Gallagher et al., 2003).

**Ca<sup>2+</sup>/Calmodulin-Dependent Protein Kinase II.** As mentioned, Hebbian processes can activate NMDA receptors, leading to calcium elevation in postsynaptic cells. An increase in intracellular calcium levels is known to lead to autophosphorylation of Ca<sup>2+</sup>/Calmodulin (Cam)-dependent protein kinase II (CaMKII), and this process is integral to memory formation in a variety of learning paradigms (Silva, 2003). CaMKII phosphorylation has been shown to increase in dendritic spines in the LA following fear learning, and prevention of CaMKII activation blocks the acquisition of fear (Rodrigues et al., 2004a). CaMKII autophosphorylation can then engage a number of intracellular events in LA neurons, which may result directly or indirectly in fear memories (see working model of molecular processes in LA mediating fear conditioning in Figure 3).

**Non-NMDA-Type Ionotropic Glutamate Receptors.** Autophosphorylated CaMKII can directly influence STM formation by phosphorylating the serine 831 (ser831) site on the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid type glutamate receptor (AMPA) GluA1 subunit, which produces GluA1 insertion, resulting in an increase in synaptic strength at the activated synapse (Malinow and Malenka, 2002). Supporting this as a mechanism for fear learning, GluA1 is increased in the LA following fear conditioning and is required for fear memory formation (Humeau et al., 2007; Nedelescu et al., 2010; Rumpel et al., 2005; Yeh et al., 2006). Interestingly, one study found that fear conditioning produces GluA1 insertion at putative thalamic-LA pyramidal cell synapses and that LA GluA1 insertion is necessary for the acquisition of fear learning (Rumpel et al., 2005).

**Inhibitory GABAergic Neurotransmission.** Inhibitory neurotransmission through  $\gamma$ -aminobutyric acid (GABA) synthesizing interneurons in the LA is important for fear conditioning and potentially for regulating Hebbian processes during fear learning (for review, see Paré et al., 2003 and Ehrlich et al., 2009). Local amygdala GABAergic interneurons strongly modulate neural activity in LA projection neurons and LTP induction (Marowsky et al., 2005; Bissière et al., 2003; Tully et al., 2007; Morozov et al., 2011), and this may serve as a mechanism to regulate Hebbian processes and gate fear learning and/or memory consolidation. The regulation of LTP induction may be mediated through feedforward inhibition activated by auditory and thalamic inputs onto GABAergic neurons in LA, which could control depolarization of LA pyramidal cells undergoing plasticity. In fact, reduction in inhibition by neuromodulators has been shown to enhance LTP (Bissière et al., 2003; Tully et al., 2007; see below). Supporting a functional role for inhibitory transmission in fear conditioning, GABA<sub>A</sub> receptor activation reduces the acquisition of fear learning (Muller et al., 1997; Wilensky et al., 1999). In addition, blocking the  $\alpha$ 1 subunit of the GABA receptor



**Figure 3. Working Model of Molecular Processes in the LA Mediating Acquisition and Consolidation of Fear Memories**

All dotted lines denote hypothetical pathways. Molecules and processes in blue are known to be involved in the acquisition of fear conditioning. Molecules and process in black are known to be involved specifically in the consolidation or maintenance of fear conditioning. Purple labels denote molecules or elements whose role is not established for fear conditioning but are part of an established intracellular signaling pathway. Abbreviations: AC, adenylyl cyclase; AKAP, A-kinase anchoring protein;  $\beta$ -AR,  $\beta$ -adrenergic receptor; BDNF, brain-derived neurotrophic factor; Ca<sup>2+</sup>, calcium; CaMKII, Ca<sup>2+</sup>/calmodulin (Cam)-dependent protein kinase II; CREB, cAMP response element (CRE) binding protein; EGR-1, early growth response gene 1; GluA1, glutamate AMPA receptor subunit 1; GluA2/3, glutamate AMPA receptor subunit 2 and 3 heteromer; IP3, inositol 1,4,5-triphosphate; MAPK, mitogen-activated protein kinase; mGluR, metabotropic glutamate receptor; mTOR, mammalian target of rapamycin; NF- $\kappa$ B, nuclear factor  $\kappa$  light-chain enhancer of activated B cells; NMDAR, N-methyl-D-aspartate glutamate receptor; NO, nitric oxide; NOS, nitric oxide synthase; NSF, N-ethylmaleimide-sensitive factor; PI3-K, phosphatidylinositol-3 kinase; PKA, protein kinase A; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PKM $\zeta$ , protein kinase M  $\zeta$ ; RNA, ribonucleic acid; TrkB, tyrosine kinase B; VGCC, voltage-gated calcium channel.

in the LA impairs fear learning, possibly by increasing ligand-initiated GABA receptor conductance in GABA heteromers that do not express this subunit (Wiltgen et al., 2009). Furthermore, GABA and the GABA-synthesizing enzyme GAD65 levels, as well as LTP of inhibitory transmission, are reduced transiently following fear conditioning (Stork et al., 2002; Szinyei et al., 2007; Bergado-Acosta et al., 2008). Though the specific role of LA GABAergic transmission in fear learning is not entirely clear,

one function may be to reduce generalization, possibly through actions on the metabotropic GABA<sub>B</sub> receptor (Shaban et al., 2006; Bergado-Acosta et al., 2008).

### **Monoamine Neuromodulatory-Dependent Mechanisms Involved in Fear Learning**

Though Hebbian plasticity may indeed occur during learning, it does not fully explain learning—especially learning in highly charged emotional situations. It is generally thought that monoamine transmitters such as norepinephrine (NE) and dopamine (DA) that are released in emotional situations regulate glutamatergic transmission and Hebbian plasticity (for review, see Bailey et al., 2000, McGaugh, 2000, and Tully and Bolshakov, 2010). The modulation of Hebbian (or activity-dependent) plasticity by neuromodulators (such as monoamines) or plasticity that is entirely independent of postsynaptic activity is called heterosynaptic plasticity. This is in contrast with purely activity-dependent Hebbian plasticity, which is referred to as homosynaptic plasticity (Bailey et al., 2000). Indeed, in a variety of model systems, it has been shown that monoamines modulate plasticity underlying memory formation (Carew et al., 1984, Bailey et al., 2000, and Glanzman, 2010 for review). Neuromodulators also contribute to fear conditioning.

Several lines of evidence support the idea that neuromodulatory regulation of Hebbian mechanisms contributes to plasticity in the LA and fear learning. USs and CSs activate neurons in the LC and substantia nigra/VTA, which are thought to provide NE and DA input, respectively, to the LA (Brischoux et al., 2009; Chen and Sara, 2007; Ennis and Aston-Jones, 1988). Further, NE and DA are increased in the amygdala following presentation of aversive stimuli (Galvez et al., 1996; Quirarte et al., 1998; Yokoyama et al., 2005; Young and Rees, 1998). These findings suggest that NE and DA acting through their respective receptors may modulate the acquisition of fear learning, possibly by synergizing with Hebbian processes to promote associative neural plasticity in the LA. Below, we focus on the modulation of learning-related plasticity in LA by NE and DA. Whereas other modulators (serotonin, acetylcholine, and endocannabinoids) and various peptides (such as gastrin-releasing peptide, NPY, opiates, and oxytocin) have been studied, the role of these modulators and peptides in the LA during auditory fear conditioning has not been examined in detail.

**Norepinephrine.** Supporting a role for NE involvement in fear memory formation, recent work demonstrates that blockade of NE  $\beta$ -adrenergic receptors ( $\beta$ -ARs) in the LA interferes with the acquisition of fear learning when given pretraining but has no effect when applied posttraining or before memory retrieval (Bush et al., 2010). In contrast to effects of  $\beta$ -AR receptor blockade on other forms of learning (McGaugh, 2000), this effect is thus specific to acquisition, as opposed to the posttraining consolidation process or to the expression of fear memory (Bush et al., 2010; Lee et al., 2001). Furthermore, preliminary data suggest that activation of  $\beta$ -ARs in the LA synergistically regulates Hebbian processes to trigger LA associative plasticity and fear learning (J.P. Johansen et al., 2010, Soc. Neurosci., abstract). Although the mechanism of  $\beta$ -AR involvement in the acquisition of fear learning is unclear, one possibility is that they act on GABAergic interneurons to suppress feed-forward inhibition and enhance Hebbian plasticity mechanisms (Tully

and Bolshakov, 2010; Tully et al., 2007). Though  $\beta$ -ARs are found on GABAergic interneurons, they are also expressed abundantly in LA pyramidal cells (Farb et al., 2010) and might also function synergistically with Hebbian mechanisms (see working model in Figure 3) in these cells to promote plasticity and fear learning (Bailey et al., 2000; Hu et al., 2007; Tully and Bolshakov, 2010; and J.P. Johansen et al., 2010, Soc. Neurosci., abstract).  $\beta$ -ARs are coupled to G<sub>s</sub> signaling cascades, which activate protein kinase A (PKA).  $\beta$ -AR-dependent PKA activation can produce phosphorylation of NMDARs as well as ser845 site on GluA1, which could facilitate AMPAR insertion at the synapse (Hu et al., 2007; Raman et al., 1996). This raises the possibility that  $\beta$ -AR activation could modulate LA Hebbian plasticity mechanisms and the acquisition of fear learning through regulation of glutamate receptor function. In addition, activation of  $\beta$ -AR and PKA reduces calcium-activated potassium (SK) channel activity, leading to increased excitability of LA neurons and enhanced LTP, and this could also occur during learning (Faber et al., 2008).  $\beta$ -AR activation could also regulate Hebbian plasticity-induced fear memory consolidation processes through PKA-dependent cAMP response element binding protein (CREB) phosphorylation (which is also phosphorylated by Hebbian processes; see below) (see Alberini, 2009 for review) or activation of phosphatidylinositol-3 kinase (PI3-K; Figure 3).

In contrast to  $\beta$ -AR effects in LA, blockade of  $\alpha$ 1 adrenergic receptors enhances fear acquisition, but not consolidation (Lazzaro et al., 2010). This effect may be mediated via presynaptic receptors on inhibitory interneurons in LA, as activation of LA  $\alpha$ 1 receptors is known to enhance feed-forward inhibition (Braga et al., 2004) and blockade of these receptors impairs LA IPSCs, enhances EPSCs, and facilitates tetanic LTP of field responses *in vitro* (Lazzaro et al., 2010). However, a role in excitatory pyramidal neurons to modulate Hebbian processes cannot currently be ruled out.  $\alpha$ 1 adrenergic receptors are typically coupled to the Gq pathway, stimulating PLC, IP3, and DAG, and may result in mobilization of Ca<sup>2+</sup> from intracellular stores or influx (Braga et al., 2004; Chen and Minneman, 2005). Thus, NE release may also inhibit plasticity in fear circuits via activation of  $\alpha$ 1 adrenoceptors.

**Dopamine.** Other studies suggest that dopamine receptor activation (both D1 and D2 receptor subtypes) in the amygdala contributes to the acquisition of fear conditioning (Greba et al., 2001; Guarraci et al., 1999, 2000; Nader and LeDoux, 1999). However, it is not clear from these studies whether DA receptor activity is required specifically in the LA (as the injection sites in most of these studies were centered between LA and CE) or whether activation of these receptors is necessary for acquisition and/or consolidation processes. D1 and D2 receptors are G protein coupled, and traditionally, D2 receptors are thought to inhibit adenylate cyclase (G<sub>i</sub>-coupled) and D1 receptors to stimulate adenylate cyclase (G<sub>s</sub>-coupled). However, in the amygdala, it appears that D1 receptors are not G<sub>s</sub> coupled but may instead stimulate phospholipase C (PLC) and IP3 production (Leonard et al., 2003). Like  $\beta$ -ARs, dopamine receptors may modulate Hebbian processes directly by reducing feed-forward inhibition (Bissière et al., 2003; Marowsky et al., 2005; Rosenkranz and Grace, 2002b). Additionally, again similar to  $\beta$ -ARs, DA receptors are expressed on LA pyramidal neurons (Muly et al., 2009),

suggesting that they may also act in a parallel fashion with Hebbian mechanisms to implement LA plasticity and fear learning through their respective signaling pathways.

### **Metabotropic Glutamate Receptor-Mediated Neuromodulation during Fear Learning**

Plasticity and learning can also be modulated by metabotropic glutamate receptors (mGluRs) (Nakanishi, 1994). Although they are activated by glutamate, mGluRs likely serve a modulatory function and do not participate directly in Hebbian processes. This is because: (1) these receptors do not, in most cases, contribute strongly to depolarization and synaptic transmission, (2) they are not activated by receptors that participate in Hebbian processes, and (3) they do not detect coincident pre- and postsynaptic neural activity. Several behavioral pharmacology studies provide evidence that mGluRs are important for fear learning. Group I mGluR (mGluR5) activity in the LA is required for the acquisition, but not consolidation or expression, of fear learning (Rodrigues et al., 2002). Furthermore, activation of group I mGluRs in the LA and basal nucleus enhances the acquisition of fear conditioning (Rudy and Matus-Amat, 2009). In addition, activation of group III mGluRs (mGluR7 and 8) in the LA reduces the acquisition of fear conditioning (Siegl et al., 2008; Schmid and Fendt, 2006). Activation of mGluR5 during conditioning may enhance NMDAR function as well as further increase  $Ca^{2+}$  levels through inositol 1,4,5-triphosphate (IP3)-mediated release of  $Ca^{2+}$  from intracellular stores and thereby activate CaMKII and protein kinase C (PKC, which is involved in fear memory consolidation; see below).

### **Summary of Acquisition**

Current evidence suggests that Hebbian plasticity mechanisms are necessary for associative plasticity in the LA and for the acquisition of fear learning. Supporting this, a number of receptors and intracellular signaling molecules that are known to respond to or regulate coordinated pre- and postsynaptic activity as well as *in vitro* forms of Hebbian synaptic plasticity are also involved in fear memory formation. In addition, emerging data suggest that neuromodulators, such as NE and DA, and mGluRs may regulate these Hebbian processes during fear learning. This is an intriguing possibility, as it provides another mechanism by which the acquisition of fear memories can be regulated and controlled. It will be important in future work to utilize new optical and genetic tools to more carefully test the plasticity rules (i.e., the temporal, spatial, and molecular mechanisms) in the amygdala that are involved in instantiating fear learning. In addition, it will be vital in future studies to define how individual receptors and molecules are recruited during fear conditioning and how they contribute to intracellular processes mediating fear learning, particularly in defined LA cell types.

### **Consolidation: Molecular Processes that Stabilize and Maintain Fear Memory**

Consolidation is the process by which temporary STM is stabilized into a persistent LTM. Plasticity important for immediate learning, and STM is mediated by covalent modification of existing synaptic proteins (for example, by phosphorylating glutamate receptors), whereas consolidation of this plasticity is generally thought to occur via activation of second messengers

that initiate gene transcription and translation of new proteins (Kandel, 2001; Bailey et al., 2000; Alberini, 2009; Hernandez and Abel, 2008). Hebbian and neuromodulatory mechanisms likely implement the initial intracellular processes during acquisition, but they may also trigger the second messengers that lead to gene transcription and protein translation, processes that stabilize and consolidate LTM. As noted, molecules that are necessary for LTM, but not STM, are said to be involved in memory consolidation (McGaugh, 2000; Rodrigues et al., 2004b).

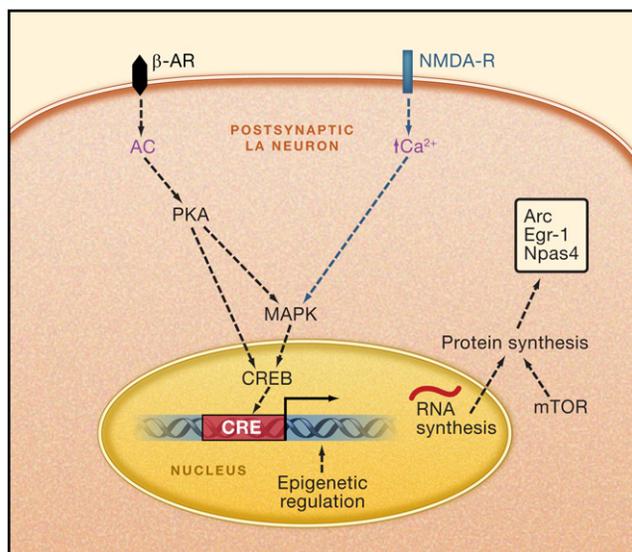
Most of what we will describe below involves plasticity that is assumed to occur within postsynaptic neurons. Presynaptic plasticity also occurs and will be mentioned as well. As with acquisition, the emphasis will be on the molecules in LA that contribute to memory consolidation.

### **Gene Transcription and Protein Translation**

Activation of genes by transcription factors leads to RNA and eventually protein synthesis. Protein synthesis has been implicated in memory consolidation in many systems (Hernandez and Abel, 2008; Davis and Squire, 1984; McGaugh, 2000). Fewer studies have examined RNA synthesis, but it too has been implicated in memory consolidation. Once LA was identified as a key area required for memory formation in fear conditioning, it was natural to ask whether RNA and protein synthesis in LA underlies the consolidation of fear conditioning memories.

Indeed, direct infusion of anisomycin, an inhibitor of protein synthesis, into the LA before or after training has no effect on subsequent STM but impairs the conversion of STM to LTM (Schafe and LeDoux, 2000; Duvarci et al., 2008). Consolidation of STM into LTM is also disrupted by infusion of broad spectrum inhibitors of RNA synthesis or an inhibitor of cap-dependent (a specific form of protein synthesis) RNA synthesis into the LA after fear conditioning (Bailey et al., 1999; Duvarci et al., 2008; Hoeffler et al., 2011). Thus, both gene transcription and protein translation in LA are required for fear memory consolidation. Interestingly, recent work suggests that, in addition to new protein synthesis, protein degradation through the ubiquitin-proteasome system may also play a role in fear memory consolidation (Jarome et al., 2011).

New protein synthesis is required for consolidation of fear conditioning, and an overall increase in translation has been observed after training (Hoeffler et al., 2011). Translation occurs in both the soma and dendrites, and dendritic translation appears to have a role in synaptic plasticity (Sutton and Schuman, 2006; Helmstetter et al., 2008). Supporting a role for local dendritic protein synthesis in LA following fear conditioning, a recent study found that the number of polyribosomes increases in LA postsynaptic dendritic spines following fear conditioning (Ostroff et al., 2010). Activity-regulated cytoskeletal-associated protein (Arc/Arg3.1) is known to be locally translated at synapses following learning (Guzowski et al., 2000), is upregulated in the LA following fear learning, and is required for fear memory consolidation (Ploski et al., 2008). Microtubule-dependent transport of RNA and proteins from the nucleus to the synapse following fear conditioning may also be important in consolidation of fear memories, as one study found that knockout of Stathmin, a regulator of microtubule dynamics, reduces fear memory formation (Shumyatsky et al., 2005). Translational regulation may modulate



**Figure 4. Working Model of Molecular Mechanisms Mediating Fear Memory Reconsolidation**

All lines are hypothetical. Molecules and processes in blue are known to be involved in the initiation of reconsolidation. Molecules and processes in black are known to be involved in reconsolidation of fear conditioning. Purple labels denote molecules or elements whose role is not established for fear conditioning but are part of an established intracellular signaling pathway. AC, adenylyl cyclase; AKAP, A-kinase anchoring protein; Arc, activity-regulated cytoskeletal-associated protein;  $\beta$ -AR,  $\beta$  adrenergic receptor; BDNF, brain-derived neurotrophic factor;  $Ca^{2+}$ , calcium; CREB, cAMP response element (CRE) binding protein; Egr-1, early growth response protein 1; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NMDA-R, N-methyl-d-aspartate glutamate receptor; Npas4, neuronal PAS domain protein 4; RNA, ribonucleic acid.

protein synthesis, and activation of an important regulator of protein synthesis, the mammalian target of rapamycin (mTOR), in the LA is required for auditory fear memory consolidation (Parsons et al., 2006b). Thus, spine-associated and/or cell body-initiated protein synthesis in LA neurons may be important for fear memory consolidation. Molecules such as mTOR could provide dynamic regulation of these processes during fear conditioning.

Transcription and thus protein synthesis depend on transcription factors. A major transcription factor implicated in memory formation in a variety of systems is CREB (for review, see Alberini, 2009). CREB's importance in fear memory consolidation has been demonstrated in studies of genetically altered mice (Bourtchuladze et al., 1994; Kida et al., 2002), and viral overexpression of CREB, specifically in the LA, facilitates LTM for fear (Josselyn et al., 2001; Han et al., 2007, 2009; Zhou et al., 2009). CREB also appears to be important for determining which neurons in the LA circuit are recruited into the memory representation (Han et al., 2007, 2009; Zhou et al., 2009). This work shows that CREB overexpression in a subset of LA neurons increases the excitability of these neurons and increases the likelihood that they will be incorporated into the fear memory trace.

Once CREB is phosphorylated, it can, in combination with several cofactors, promote gene transcription of cAMP response element (CRE)-dependent genes (see Alberini, 2009, Deisseroth

and Tsien, 2002, and Yin and Tully, 1996 for review). One of these cofactors is the transcription factor nuclear factor  $\kappa$  light-chain enhancer of activated B cells (NF- $\kappa$ B), which is activated following fear conditioning, and this activation in the LA is required for fear LTM (though STM was not examined) (Lin et al., 2001).

Supporting the involvement of CRE-mediated gene transcription during fear memory consolidation, one study showed that this form of transcription is increased in the LA following associative fear conditioning (though this may also occur nonassociatively) (Impey et al., 1998b). In addition, another study found that blocking CRE-mediated transcription of the neurotrophin brain-derived neurotrophic factor (BDNF) reduces LTM for fear (Ou and Gean, 2007).

It is clear that neurochemical and cellular events that occur during fear learning can signal the nucleus to trigger gene transcription likely through a CREB-dependent mechanism (Figure 4). Though there is an increasing amount known about the molecular signaling pathways leading to gene transcription and memory consolidation during fear conditioning, the specific genes that are regulated during fear memory consolidation are relatively unknown. The studies that have examined this question found that a number of genes are upregulated following fear conditioning, and some of them contain a CRE site within their promoter region or directly bind CREB (Lamprecht et al., 2009; Ou and Gean, 2007; Ploski et al., 2008, 2010; Rattiner et al., 2004b; Ressler et al., 2002; Stork et al., 2001, Keeley et al., 2006). Though there is inconsistency between the different studies with regard to the specific genes that are upregulated, this work provides a first glimpse into the learning-induced regulation of gene expression following fear conditioning. Another way to study which genes and proteins are important for fear conditioning is to examine the effects of knocking down specific gene products that are regulated by fear conditioning. For example, early growth response gene 1 (Egr-1) and the neuronal PAS domain protein 4 (Npas4) are upregulated in the LA after fear conditioning, and reducing the levels of these proteins in the LA attenuates fear memory consolidation (Maddox et al., 2011; Ploski et al., 2011).

Gene transcription can also be controlled by epigenetic mechanisms in which alterations in chromatin structure and DNA methylation can influence gene expression (Day and Sweatt, 2011). Recent work has implicated learning-induced changes in chromatin structure and DNA methylation in fear memory consolidation (Monsey et al., 2011; Bredy and Barad, 2008).

#### Protein Kinases

Hebbian mechanisms engaged during learning may activate specific signaling cascades that are dependent on autophosphorylation of CaMKII. As discussed above, CaMKII is required for the acquisition of fear conditioning (Rodrigues et al., 2004a). Though CaMKII activation triggers processes that are involved in STM and LTM, other kinases such as PKA, mitogen-activated protein kinase (MAPK), and PKC may also participate (independently or through CaMKII activation) in the consolidation of LTM for fear and different forms of learning (Abel et al., 1997; Adams and Sweatt, 2002). These kinases all phosphorylate CREB, and convergent activation of CREB by these different pathways could gate the transcription of numerous plasticity-related genes

through dimerized binding to CRE during fear memory consolidation (Figure 4, as discussed above).

**Protein Kinase A.** Protein kinase A (PKA) is activated following fear learning, but in contrast to CaMKII, it is required in the LA for fear memory consolidation (Goosens et al., 2000; Schafe and LeDoux, 2000; Weeber et al., 2000; Figure 3). Although the effect of specific PKA inhibitors on acquisition of fear conditioning has not been tested, one study did find that acquisition was not affected by pretraining disruption of the A kinase anchoring protein (AKAP150), which localizes PKA to the synapse (Moita et al., 2002). However, like posttraining PKA inhibitors, disruption of AKAP after learning reduces fear LTM (Moita et al., 2002). PKA may be activated by NE binding to  $\beta$ -ARs and stimulation of  $G_s$  and cAMP during fear conditioning or by increases in intracellular  $Ca^{2+}$  (Impey et al., 1994, 1998a; Wayman et al., 1994). Activation of PKA can then lead to phosphorylation of multiple proteins involved in nuclear signaling and fear memory consolidation (such as CREB and other kinases), and PKA itself can translocate to the nucleus and regulate RNA synthesis.

**Protein Kinase C.** Protein kinase C (PKC) activation in the LA is also required for fear memory consolidation (Goosens et al., 2000; Weeber et al., 2000; Figure 3). PKC may be activated by increases in intracellular  $Ca^{2+}$  and/or indirectly through mGluRs (as discussed above). PKC may also be activated directly by  $Ca^{2+}$  influx through voltage-gated calcium channels (VGCCs), and VGCCs also contribute to fear memory consolidation (Bauer et al., 2002; McKinney and Murphy, 2006; Shinnick-Gallagher et al., 2003). PKC activation can then lead to activation of signaling cascades that regulate gene transcription and could mediate PKC's role in fear memory consolidation.

**Mitogen-Activated Protein Kinase.** CaMKII, PKA, and PKC can directly or indirectly phosphorylate mitogen-activated protein kinase (MAPK), which is known to play an integral role in memory consolidation in many neural systems (Kandel, 2001; Sweatt, 2004). The MAPK-signaling pathway in the LA has also been implicated in auditory fear conditioning, as MAPK is upregulated in the LA following fear learning, and blockade of MAPK activation in the LA attenuates fear memory consolidation (Schafe et al., 2000). Furthermore, intra-LA blockade of MAPK phosphorylation also abolishes consolidation of fear-conditioned enhancement of auditory-conditioned stimulus inputs to the LA (but not auditory inputs to MGm/PIN). This study demonstrates that blocking consolidation of fear conditioning-induced synaptic plasticity in LA reduces fear learning, providing a direct link between synaptic changes occurring in LA and behavior. Further supporting the involvement of MAPK in fear conditioning, PI3-K also activates MAPK and is necessary in the LA specifically for fear LTM (Lin et al., 2001, 2003). Finally, protein tyrosine phosphatase, a protein that is known to be convergently activated by G protein-coupled and Hebbian processes in other neural systems (Valjent et al., 2005), may also regulate MAPK signaling in the LA during fear memory consolidation (Paul et al., 2007).

### Neurotrophin Signaling

Neurotrophin signaling has also been implicated in fear memory consolidation (Cowansage et al., 2010; Rattiner et al., 2005). BDNF mRNA and protein levels are increased after fear conditioning, and phosphorylation of the BDNF receptor (tyrosine kinase receptor B [TrkB]), which occurs upon ligand binding, is

also enhanced following training. Furthermore, blocking activation of LA TrkB receptors through pharmacological or genetic means reduces fear memory consolidation (Ou and Gean, 2006; Ou et al., 2010; Rattiner et al., 2004a; Rattiner et al., 2004b). TrkB stimulation by BDNF activates PI3-K and MAPK, and the effects of TrkB activation on fear memory consolidation may be mediated through these signaling cascades (Lin et al., 2001, 2003; Schafe et al., 2000). This neurotrophin-signaling pathway appears to be specifically involved in fear memory consolidation and not in acquisition. Neurotrophin-signaling pathways may also converge with parallel Hebbian and neuromodulatory-triggered intracellular processes to dynamically regulate experience-dependent alterations in LA synaptic efficacy and fear memory consolidation (Figure 3).

### Consolidation within Presynaptic Neurons

Much research to date has focused on postsynaptic modifications that occur in LA neurons during fear learning. However, evidence also suggests that presynaptic molecular alterations are important as well for the consolidation of fear learning (Apergis-Schoute et al., 2005; Huang and Kandel, 1998; McKernan and Shinnick-Gallagher, 1997; Ota et al., 2010a; Pan et al., 2008; Tsvetkov et al., 2002).

Recent studies have implicated nitric oxide synthase (NOS) and nitric oxide (NO) in fear memory consolidation (Schafe et al., 2005). NO is a soluble gas that is produced in postsynaptic neurons and can retrogradely signal presynaptic terminals. There, it can act through guanylyl cyclase and cGMP-dependent protein kinase (PKG) to influence intracellular processes and nuclear signaling in the presynaptic neuron and influence memory consolidation (Brenman and Bredt, 1997; Prast and Philippu, 2001).

Some of the presynaptic terminals that carry auditory information to the LA originate in cell bodies in the MGm/PIN. Fear conditioning produces an increase in the expression of the immediate early gene early growth response gene 1 (EGR-1) in the MGm/PIN neurons as well as an increase in the expression of markers of presynaptic terminals (synaptophysin and synapsin) in the LA (Overeem et al., 2010). Consistent with the idea that NO in LA produces biochemical changes in the auditory thalamus, fear conditioning-induced increases in EGR-1 in MGm/PIN neurons require NMDAR activation, NO signaling, and PKG activation in the LA, as well as MAPK activation in the MG/PIN (Ota et al., 2010b; Overeem et al., 2010). This suggests that NO acting on MGm/PIN presynaptic terminals in the LA drives changes in gene expression in the thalamus. In addition, the increase in LA synaptophysin and synapsin (both of which are regulated by EGR-1) is dependent on EGR-1 expression in the thalamus, as well as NMDAR activation, NO signaling, and PKG in LA (Ota et al., 2010a; Overeem et al., 2010). Together, these data support the hypothesis that, during fear conditioning, NO mediates a retrograde signal that activates PKG signaling in presynaptic thalamic input terminals, which then activates MAPK-dependent increases in EGR-1 expression in the MGm/PIN cells, resulting in an increase in the number of presynaptic terminals in the LA (Figure 3).

### Maintenance of Fear Memories

Though there has not been much work examining how consolidated memories are maintained over time, evidence suggests

that both structural and molecular changes may play a role in this process.

**Neuronal Structural Alterations Resulting from Fear Learning.** As in other systems (Bailey and Kandel, 1993), structural modifications at synapses may be induced by fear learning, and transcriptional and translational processes may serve to stabilize these changes and maintain fear memories (Lamprecht and LeDoux, 2004; Figure 3). These learning-induced synaptic alterations may involve rearrangement of the actin cytoskeleton and/or a change in synapse number.

Some work suggests that fear conditioning produces an increase in synapse number (Ostroff et al., 2010; Radley et al., 2006). In addition, a recent study lends support to the idea that synapse structure is altered following fear conditioning by demonstrating that fear learning produces an increase in synapse size (Ostroff et al., 2010). This may be partly mediated by GluA1 containing AMPA receptor insertion (Humeau et al., 2007; Nedelescu et al., 2010; Rumpel et al., 2005; Yeh et al., 2006) and then replacement by GluA2/3-containing AMPARs (Malinow and Malenka, 2002). This mechanism could function to produce long-term increases in spine size (Kasai et al., 2010). Supporting this idea, recent work shows that disrupting N-ethylmaleimide-sensitive factor (NSF) interactions with GluA2, which is necessary to maintain GluA2/3 receptors in the synapse, impairs fear memory consolidation (Joels and Lamprecht, 2010). In addition, fear conditioning drives profilin, a protein that regulates actin dynamics, into spines, and profilin-positive spines exhibit larger synapses (Lamprecht et al., 2006a). Further supporting a role for cytoskeleton rearrangement during fear memory consolidation, disrupting actin polymerization or the Rho-GAP-signaling pathway (which has been implicated in cytoskeletal alterations) disrupts consolidation of fear memories (Lamprecht et al., 2002, 2006b; Mantzur et al., 2009). In addition, myosin light-chain kinase, a cytoskeleton regulatory protein, appears to normally inhibit fear learning, suggesting that modulation of cytoskeletal dynamics can influence fear memory formation (Lamprecht et al., 2006b). Furthermore,  $\beta$ -catenin, which is involved in structural processes at the synapse and gene transcription (Takeichi and Abe, 2005), is stabilized in the LA following fear learning and is necessary in the LA for memory consolidation (Maguschak and Ressler, 2008).

**A Possible Molecular Mechanism Underlying Memory Maintenance.** Recent studies demonstrate kinase involvement in memory maintenance (Sacktor, 2008; Dudai, 2009). Specifically, this work indicates that an atypical isoform of PKC called protein kinase M $\zeta$  (PKM $\zeta$ ) is particularly important in the maintenance of fear memories after they are formed. Inhibition of PKM $\zeta$  in the LA following fear learning abolishes fear memories (Kwapis et al., 2009; Miguez et al., 2010; Serrano et al., 2008; but see also Parsons and Davis, 2011). Though the mechanisms for this are not completely understood, it appears that PKM $\zeta$  maintains fear memories by reducing GluA2 AMPAR subunit removal from synapses and thereby sustaining the synaptic strengthening originally induced by fear learning (Miguez et al., 2010). Clearly, more work is necessary to elucidate how PKM $\zeta$  interacts with transcriptional and translational mechanisms to support retention processes over the life of a memory, but these findings open a new area of research that holds great promise.

### **Fear Memory Consolidation Conclusions**

Together, these data show that multiple interacting intracellular signaling cascades regulate gene transcription in LA neurons to mediate fear memory consolidation (Figure 3 for working model of molecular processes mediating fear conditioning). There are numerous studies examining these interactions in the context of LTP in reduced preparations, and this work has been highly beneficial in defining candidate processes that could occur during actual learning. Because of the complexity of these systems, however, it is important to assess these consolidation mechanisms during actual memory formation, and the fear memory system is ideal for these types of studies. It will be important in future work to examine how these signaling cascades are regulated by activation of specific receptor subtypes and processes and to define the flow of information in these signaling pathways. This will be important because it appears that external sensory events during fear learning activate multiple processes, including Hebbian, neuromodulatory, and neurotrophinergic (as discussed above), which together lead to fear memory formation and consolidation. In addition, it will be important in future studies to examine which second messenger pathways are linked to neuromodulatory receptors and how activation of these receptors works synergistically and/or in parallel with Hebbian mechanisms to promote fear learning. To answer these questions, it will be necessary to take advantage of molecular genetic techniques to target manipulations of cell surface receptors and their associated signaling cascades to specific cell types (for example, interneuron versus pyramidal cells) and to specific synaptic domains (for example, pre- versus postsynaptic).

### **Reconsolidation: Molecular Mechanisms through which Fear Memory Is Altered after Retrieval**

Although fear memories are consolidated and stored following learning, memories can become labile when they are recalled through a process that has been termed "reconsolidation." During reconsolidation, memories become labile when they are reactivated by presentation of a memory-associated environmental cue (such as a fear conditioned CS). Pharmacological or behavioral manipulations following memory reactivation can, like posttraining manipulations, transform the newly labile memory (Alberini et al., 2006; Dudai, 2004; Monfils et al., 2009; Nader and Hardt, 2009; Sara, 2000; Schiller et al., 2010; Tronson and Taylor, 2007). This process is thought to facilitate incorporation of new information into the memory trace.

Historically, reconsolidation has been studied via systemic pharmacological manipulations in a number of learning systems (see Sara, 2000 for review). The finding that blockade of protein synthesis in the LA disrupts the consolidation of STM into LTM of fear conditioning (Schafe et al., 2001) led the way to the discovery that blockade of protein synthesis in the LA also disrupted reconsolidation after retrieval of a fully consolidated fear memory (Nader et al., 2000). This latter finding sparked a new wave of interest in reconsolidation.

Our understanding of the cellular and molecular mechanisms of memory reconsolidation is at an early stage. Auditory fear conditioning is well suited for examining these processes because we understand a great deal about fear memory consolidation in LA. Indeed, blockade of reconsolidation reduces auditory

conditioned stimulus-evoked neural responses in the LA (Doyère et al., 2007). This suggests that auditory thalamic (and possibly cortical) inputs to LA neurons are depotentiated when reconsolidation is blocked, and physiological evidence supports this idea (Kim et al., 2010). Thus, we will focus specifically on the molecular mechanisms of auditory fear memory reconsolidation in LA neurons.

### **Neurotransmitter Systems**

Two neurotransmitter systems have been studied most extensively in relation to reconsolidation: glutamate and norepinephrine.

*Glutamate Receptors and the Triggering of Reconsolidation.* Blockade of NMDARs in the LA before memory reactivation blocks the initiation of reconsolidation, as it renders the reactivated memory insensitive to subsequent reduction by intra-LA protein synthesis inhibitors (Ben Mamou et al., 2006). In addition, microinjection of a partial agonist of the NMDAR into the LA before reactivation enhances subsequent fear memories. This same study found that intra-LA microinjection of an AMPAR antagonist had no effect on fear memory reconsolidation. Thus, reconsolidation might be best thought of as a new learning experience, one in which plasticity is initiated via activation of NMDARs. This is somewhat surprising, given that AMPAR activation is typically thought of as being necessary to depolarize neurons to the point of allowing calcium to pass through NMDARs. However, in LA, NMDARs can contribute to spike generation independent of AMPARs (Li et al., 1995).

*Norepinephrine Modulates Reconsolidation.* Norepinephrine transmission is also involved in reconsolidation, as postreactivation systemic or intra-LA microinjection of a  $\beta$ -AR antagonist reduces fear memories assayed drug free at later LTM time points (Debiec and Ledoux, 2004; Muravieva and Alberini, 2010). The effects of  $\beta$ -AR manipulation of consolidation and reconsolidation differ because posttraining blockade of  $\beta$ -ARs has no effect on the consolidation of the initial memory (Bush et al., 2010; Lee et al., 2001; Debiec and Ledoux, 2004), but postreactivation  $\beta$ -AR manipulation does. This work suggests that  $\beta$ -AR blockers given after retrieval of traumatic memories may alleviate some anxiety disorders such as PTSD. In fact, systemic  $\beta$ -AR blockers disrupt memory reconsolidation in healthy humans (Kindt et al., 2009; Soeter and Kindt, 2010). Furthermore, a recent study found promising results in PTSD patients (Brunet et al., 2008), although the lack of certain controls limits its conclusions.

### **Intracellular Signaling Cascades: Kinases, Transcription Factors, and Protein Synthesis**

A number of studies have examined the intracellular mechanisms mediating fear memory reconsolidation. As with fear memory consolidation, these intracellular processes may be triggered convergently by activation of glutamate and norepinephrine receptors on the cell membrane. Similar to fear memory consolidation, blockade of PKA (Tronson et al., 2006) and MAPK (Duvarci et al., 2005) in the LA following memory reactivation reduces LTM, but not STM. This shows that activation of these molecules, which are downstream of both NMDARs and  $\beta$ -ARs, is required for memory reconsolidation to occur. PKA and MAPK can induce gene transcription through phosphorylation of CREB (as discussed above). Indeed, this pathway may be involved in reconsolidation of fear memories. Thus, CREB is

phosphorylated in the amygdala following fear memory reactivation (Hall et al., 2001b), and forebrain expression of a CREB repressor reduces fear memory reconsolidation (Kida et al., 2002). As mentioned previously, CREB phosphorylation can lead to CRE-mediated gene transcription and synthesis of new proteins. As noted, protein synthesis is essential to reconsolidation, just as it is to consolidation, as protein synthesis blockade in the LA after reactivation blocks fear reconsolidation (Nader et al., 2000; Parsons et al., 2006a; Parsons et al., 2006b). In addition, like fear memory consolidation, reducing levels of Arc/Arg3.1, Npas4, or Egr-1 in the LA reduces memory reconsolidation (Maddox and Schafe, 2011a; Maddox et al., 2011; Ploski et al., 2011). Furthermore, mTOR is also required for fear memory reconsolidation, suggesting that translational regulation is also important in this process (Parsons et al., 2006a). In addition to translation of new proteins, degradation of existing proteins also appears to be important for fear memory reconsolidation (Jarome et al., 2011). Two proteins that contain CRE sites in their genes are c-Fos and Egr-1, and both are upregulated in the amygdala following fear memory reactivation (Hall et al., 2001a, 2001b). Thus, CRE-mediated gene transcription may be important in restabilizing fear memories following reactivation. In support of this hypothesis, blockade of gene transcription in the LA after memory retrieval blocks auditory fear memory reconsolidation (Duvarci et al., 2008; see also Parsons et al., 2006a). As with fear memory consolidation, gene transcription during reconsolidation may be controlled by epigenetic mechanisms (Maddox and Schafe, 2011b).

Though this work is just beginning, existing studies reveal that similar (though probably not entirely overlapping: Lee et al., 2004 and Tronson and Taylor, 2007) molecular mechanisms are engaged during memory consolidation and reconsolidation. For example, in a contextual fear conditioning paradigm, hippocampal BDNF is involved in consolidation, but not reconsolidation, and the transcription factor zif-268 is required for reconsolidation, but not consolidation (Lee et al., 2004). Future work will surely delineate molecules that are differentially involved in fear memory consolidation and reconsolidation.

### **Combining Extinction and Reconsolidation**

Though manipulating intracellular processes can interfere with reconsolidation following memory retrieval, behavioral manipulations following reactivation can also alter fear memory. For example, extinction training, which weakens fear memory by repeated presentation of the conditioned stimulus without the US, is usually temporary, as the memory can be revived by exposure to a new context, to the US, or by the simple passage of time (Bouton et al., 2006). However, when extinction training follows a single conditioned stimulus reactivation trial, fear memories do not seem to be recovered by these procedures (Monfils et al., 2009). Thus, extinction following a single retrieval trial appears to abolish or alter the previously acquired fear memory. An important question is what the difference is between a reactivation trial and an extinction trial, as extinction training is a series of conditioned stimulus presentations. The key factor is that a temporal gap must be placed between the first and second conditioned stimulus presentation in order for the effect to occur. The gap should be between 10 min and 4 hr in order to be effective. This finding has been replicated (Schiller et al., 2010; Clem

and Huganir, 2010), but it does not occur under all conditions (Clem and Huganir, 2010; Chan et al., 2010; Soeter and Kindt, 2011).

The molecular mechanisms by which behavioral manipulations such as this affect memory reconsolidation are only beginning to be studied. Initial work showed that the ser845 PKA site on the AMPAR subunit GluA1 is phosphorylated following memory reactivation (Monfils et al., 2009), suggesting that GluA1 phosphorylation engages reconsolidation processes. Supporting a functional role of GluA1 phosphorylation at the ser845 site in this process, another recent study showed that extinction training after memory reactivation is no longer effective at abolishing or altering the original fear memory in mutant mice with mutations at the ser845 GluA1 phosphorylation site (Clem and Huganir, 2010). Instead, in mutant mice, this training procedure produces normal extinction learning, exhibiting both renewal and spontaneous recovery. Thus, reactivation produces phosphorylation at ser845 on GluA1, and this process may be essential in producing lability of LA synapses and triggering reconsolidation rather than extinction. Though only beginning, this is an exciting line of research because it demonstrates the possibility of modulating fear memories with behavioral instead of pharmacological interventions and may have some applicability to treatment of fear-related disorders such as PTSD and phobias. However, there are boundary conditions that may limit the effectiveness of this approach (Chan et al., 2010; Clem and Huganir, 2010), and they need to be fully understood before these interventions are used for treatment of anxiety related disorders.

### Fear Memory Reconsolidation Conclusions

In summary, researchers are beginning to understand the cellular and molecular processes mediating reconsolidation (Figure 4 for working model of molecular processes mediating fear memory reconsolidation). Reconsolidation is an intriguing phenomenon because it provides a mechanism by which previously acquired memories can be altered through subsequent experience—something that is a clear feature of human memory. Understanding the mechanisms of this process is appealing both for understanding how memories are encoded in the brain and because it could provide new treatment avenues for debilitating memory-related disorders such as PTSD. Mechanistically, fear memory reconsolidation and fear memory formation and consolidation share some key features in terms of the neurotransmitters that trigger them as well as in the intracellular signaling cascades that are recruited. However, there are also key differences between initial fear memory consolidation and reconsolidation both in terms of the molecules involved and the timing of their involvement. It will be important in future work to parse these differences.

### Overall Conclusions

Research on Pavlovian fear conditioning has revealed in great detail the cellular and molecular mechanisms involved in the acquisition, storage (consolidation), and reconsolidation of memories about harmful events. This paradigm is especially useful for three major reasons. First, it involves specific stimuli that are under the experimenter's control. Second, the conditioned responses are stereotyped and innate responses that,

through learning, are elicited by the conditioned stimulus. Finally, the behavior can be studied similarly in animal models and humans.

By following the conditioned stimulus through the brain, many of the pathways involved in Pavlovian fear conditioning have been mapped. Importantly, a key brain region for the learning and memory in all mammals, including humans, is the amygdala. Studies in rodents have shown that plasticity occurs in the LA of the amygdala during learning, and this is also where memories are consolidated. Synaptic changes take place in the LA following conditioned-unconditioned stimulus pairings, such that the conditioned stimulus is able to flow through downstream circuitry and elicit fear responses after (but not before) conditioning.

The molecular mechanisms underlying fear learning and memory in the LA involve both Hebbian and neuromodulatory processes. These mechanisms have been implicated in fear conditioning through behavioral studies in which fear conditioning is chemically or genetically manipulated in LA and through studies of learning-induced synaptic plasticity, using *in vivo* and *in vitro* electrophysiological approaches. These studies suggest that a number of molecular mechanisms, including neurotransmitters (e.g., glutamate and monoamines and their receptors) and intracellular signaling events, contribute to these processes. This work demonstrates the power of using the fear conditioning system to decipher molecular processes underlying memory formation and learned changes in neural processing within a specific, behaviorally defined circuit.

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