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Redundant Neuromodulatory Mechanisms That Control Fear Memory Consolidation

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Abstract
The ability to reliably and powerfully store memories for frightening experiences is crucial to survival in an ever-changing and potentially dangerous environment. Consolidation is the process by which long-term memories are stored in the brain, and much is understood about the processes that occur within a neuron in the hours after a learning event that stabilize learning-induced changes. However, the specific mechanisms through which fear exacerbates those processes remains unclear. Neuromodulators are a prime object of research to understand the consolidation of fear memory given that their release is a hallmark of the fear response. While several neuromodulatory systems are known to facilitate consolidation, no individual system yet appears to be essential. This dissertation explores the hypothesis that several neuromodulators work together to ensure proper consolidation of fear memory. The research contained in this dissertation employs pharmacologic and genetic manipulation of individual neuromodulatory receptor systems and associated intracellular signaling pathways to determine the essential neurobiology for consolidating of Pavlovian fear conditioning in mice. The results of this investigation reveal that β2-adrenergic, D5-dopaminergic and M1-muscarinic receptors in the basolateral amygdala (BLA) are essential to fear conditioning in a redundant manner, wherein two or more receptor types must be blocked in order to prevent consolidation. Furthermore, these three receptors are observed to redundantly activate phospholipase C (PLC), which this dissertation shows is necessary for consolidation in the BLA. Finally, evidence is provided to suggest that PLC promotes fear memory consolidation by inhibiting a voltage-dependent potassium channel (KCNQ/M) that regulates neuronal excitability and also appears to control consolidation. Together, this dissertation proposes that fear-induced neuromodulatory release promotes consolidation through redundant neuromodulatory activation of PLC, which puts the BLA in an excitable state that does not persist into the consolidation window after emotionally neutral experiences.

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Matthew Brandon Young

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ABSTRACT

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The ability to reliably and powerfully store memories for frightening experiences is crucial to survival in an ever-changing and potentially dangerous environment. Consolidation is the process by which long-term memories are stored in the brain, and much is understood about the processes that occur within a neuron in the hours after a learning event that stabilize learning-induced changes. However, the specific mechanisms through which fear exacerbates those processes remains unclear. Neuromodulators are a prime object of research to understand the consolidation of fear memory given that their release is a hallmark of the fear response. While several neuromodulatory systems are known to facilitate consolidation, no individual system yet appears to be essential. This dissertation explores the hypothesis that several neuromodulators work together to ensure proper consolidation of fear memory. The research contained in this dissertation employs pharmacologic and genetic manipulation of individual neuromodulatory receptor systems and associated intracellular signaling pathways to determine the essential neurobiology for consolidating of Pavlovian fear conditioning in mice. The results of this investigation reveal that β_2-adrenergic, D_5-dopaminergic and M_1-muscarinic receptors in the basolateral amygdala (BLA) are essential to fear conditioning in a redundant manner, wherein two or more receptor types must be blocked in order to prevent consolidation. Furthermore, these three receptors are observed to redundantly activate

phospholipase C (PLC), which this dissertation shows is necessary for consolidation in the BLA. Finally, evidence is provided to suggest that PLC promotes fear memory consolidation by inhibiting a voltage-dependent potassium channel (KCNQ/M) that regulates neuronal excitability and also appears to control consolidation. Together, this dissertation proposes that fear-induced neuromodulatory release promotes consolidation through redundant neuromodulatory activation of PLC, which puts the BLA in an excitable state that does not persist into the consolidation window after emotionally neutral experiences.

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CHAPTER 1: A GENERAL INTRODUCTION TO FEAR MEMORY CONSOLIDATION

"We are the inheritors of a million years of striving for the unspeakable."

- Terence McKenna

From a subjective perspective, memories associated with an emotion seem to have privilege over others. Without having made any conscious attempt to stow those memories away, a ghostly sense of joy, sadness, or fear seems to make a memory more vivid, more persistent, more readily accessible, more real. Indeed, memory for aversive experiences is very well stored, not only in humans (Burke et al, 1992; Heuer and Reisberg, 1990), but also in insects and sea slugs (Abramson, 1986; Carew et al., 1983), rodents and birds (Blanchard and Blanchard, 1972; Macphail, 1968), and in non-human primates (Kalin et al., 2001). The power of a fear memory is perhaps no more apparent than in the case of post-traumatic stress disorder (PTSD), in which memory for an extremely frightening event recurrently intrudes upon an individual (McDonald and Calhoun, 2010). This dissertation explores the neurobiological systems that promote the changes in the brain necessary for the powerful storage of fear memory.

Learning and Memory. All cognition and behaviors are merely probabilities that any number of neurons will successfully communicate along the given pathway that carries out a particular action or thought. For example, the pathway that travels from the nerves of a hand that feels a hot stovetop, through the spinal cord, and back into the muscles that quickly pull the hand away is very reliable, and results in a very reliable behavior. Safety and survival depend on reliable communication between every neuron in that pathway, and most biologically relevant pathways have been refined over countless generations. Yet, in a wildly complex and ever-changing world where many different

kinds of “stovetops” arise, responses that ensure safety and survival depend on efficient adaptability of cognition and behavior. Altering the strength of a pathway, or forming a new pathway altogether, changes the probability that a given situation will elicit a particular thought or behavior. Therefore, in general terms, memory formation can be understood as an evolutionary adaptation wherein the cognitive or behavioral response to a given stimulus is updated by changing the strength of neural pathways.

In the brain, changes in the strength of neural pathways that underlie a memory occur at the synaptic connection between neurons in that pathway. Bliss and Lomo first observed that high frequency stimulation of a synapse could induce long-term increases in the reliability of that synapse (Bliss and Lomo, 1973) – the likelihood that stimulation of the presynaptic neuron will elicit a response in the postsynaptic neuron – suggesting that synaptic activity itself could change the dynamics of its own responsiveness and the fidelity of the larger neuronal pathway. The functional changes at the synapse are driven by physiological changes that enhance the signaling power of the presynaptic neuron (Rogan and LeDoux, 1995; Schroeder and Schinnick-Gallagher, 2005; Overeem et al., 2010) and the capacity of the postsynaptic neuron to receive and transmit that signal (Rumpel et al., 2005; Ostroff et al., 2010). While speculation remains as to whether or not the *in vitro* observations of long-term potentiation (LTP) actually reflect what happens in the brain during learning, several manipulations that disrupt LTP in brain slices also disrupt learning and memory *in vivo* (Hernandez and Abel, 2008). Therefore, LTP is generally assumed to be a proof of the concept of synaptic plasticity induced by activity at the point of the synapse.

Fear Memory in the Brain. The fear response and the ability to form memories about frightening experiences both require the amygdala (Blanchard and Blanchard, 1972; Campeau and Davis, 1995; Goosens and Maren, 2001; LeDoux et al, 1990; Nader at al, 2001; Slotnick, 1973). The amygdala complex consists of several distinct and interconnected nuclei, including the lateral nucleus, the basal nucleus, and the central nucleus. Multimodal sensory inputs from both the cortex and thalamus converge in the lateral and basal amygdala (BLA) (LeDoux et al, 1990; McDonald, 1998Romanaski and LeDoux, 1993; Turner and Herkenham, 1991), which then projects to the central nucleus for further projection to areas of the nervous system that regulate avoidance behaviors (LeDoux et al, 1988; Petrovich et al, 1996). The BLA also projects to brain regions important for processing higher order sensory information, such as the hippocampus, which is required for processing and remembering spatial and contextual information about an environment (Holland and Bouton, 1999).

LTP has been observed in BLA neurons (Sigurdsson et al., 2007), and the ability to form fear memories is sensitive to manipulations targeted there (Johansen et al., 2011). The plastic changes in neuronal pathways occur at the synapse between cortical/thalamic projections activated by sensory stimuli and BLA neurons that project to the central amygdala (Royer et al., 1999). Therefore, while fear memory and the neural pathways that support it rely on many neurons and brain regions working together, the BLA is believed to be the site where neural pathways connecting a stimulus and the fear response are modified and formed (Maren et al., 2003).

To understand learning and memory behavior, science relies on empirical methods of experimentation that recreate the experience of learning and memory and also permit their quantification. Most of the neuroanatomical structures that mediate fear in humans are found serving the same purpose in animals (Aggleton, 1995), and much has been

learned about fear memory from rodents by using simple Pavlovian conditioning. In cued Pavlovian fear conditioning, an animal is exposed to an auditory tone (conditioned stimulus; CS) that elicits very little defensive behavior. The pathway that leads from the neurons that sense the sound to the neurons that execute the defense response is weak. However, when the tone is made to co-terminate with a footshock (unconditioned stimulus; US), which is a biologically-relevant pathway to fear, the formerly weak pathway becomes more reliable. Memory for the CS-US association is assessed by re-exposing the animal to the training tone and measuring the amount of time the mouse spends "freezing" – the instinctual fear response of a rodent (Blanchard and Blanchard, 1969). Another form of Pavlovian fear conditioning uses the training context as the CS, and memory for the context-shock association is assessed by re-exposing the animals to the training context. Contextual fear memory depends on both the amygdala and the hippocampus, and is often susceptible to disruptive interventions that fail to inhibit strictly amygdala-dependent memory (Anagnostaras et al., 2003; Murchison et al., 2004). Therefore, while several other fear learning paradigms are also used, this dissertation focuses on cued conditioning because its effect resembles the persistence and strength characteristic of PTSD.

The strengthening of a weak neuronal pathway by a nearby strong pathway that signals at the same time is called associative or Hebbian synaptic plasticity (Hebb, 1949). Hebbian plasticity is an appealing model for cued fear conditioning because the BLA connects incoming aversive information with fear-expressing neurons (Pitkanen, 2000). In fact, individual populations of BLA neurons receive convergent auditory and somatosensory projections (Li et al., 1996; Romanksi et al., 1993), a relationship in connectivity that supports the Hebbian model of learning in fear conditioning. After fear conditioning to an auditory tone, subsequent auditory or electrical stimulation elicits a

greater signal from the thalamo-cortical neuron (Rogan and LeDoux, 1995), and response from the amygdalar neuron (Maren, 2000; Quirk et al., 1995; Repa et al., 2001). Therefore, it is believed that strengthening of connections, or synapses, within the BLA underlie fear memory formation.

Fear Memory Consolidation. The ability to retrieve long-term memories after learning requires that molecular changes to the strength of a synaptic connection in the BLA be stabilized or "consolidated" at the site of plasticity in the hours after initial learning (Goddard; 1964; Gold et al., 1974; LeDoux, 2000). Several biological and physiological changes occur within and at the surface of neurons at a synapse soon after learning, and these changes facilitate synaptic strength and the corresponding behavior (Johansen et al., 2011). These changes are generally associated with the initial acquisition and short-term phase of a memory. Subsequent, consolidation is a compendium of processes that promote a persisting facilitation of a synapse and behavior, and it is a process characteristic of neurons in brain regions where memories are stored. To specifically explore consolidation processes, manipulations are introduced immediately after learning, and the effects of those manipulations on memory are measured by subsequently testing the ability to remember the learned behavior when the manipulation is absent. Manipulations that are not reversible, such as lesions or congenital genetic deletion, are often interpreted as affecting only consolidation when short-term memory remains intact. Similar short-term vs. long-term distinctions are made when manipulations are introduced before the learning event. In general, initial consolidation is believed to take place over the course of just a few hours. Manipulations that affect memory when administered immediately after training become decreasingly effective to the point of complete ineffectiveness as the latency to administer increases (McGaugh, 1966).

The nature of the processes that drive consolidation specifically within the BLA escapes full comprehension due to the complexity of the BLA itself. Its heterogeneous composition of excitatory pyramidal neurons (McDonald, 1982; Muller et al., 2006; Rainnie et al., 1993) and non-pyramidal inhibitory neurons (Kempainnen and Pitkanen, 2000; McDonald and Mascagni, 2002; Rainne et al., 2006), as well as the variety of its afferent and efferent connections, have given way to observations underscoring the potential importance of a number of state-changes during consolidation (Daoudal and Debanne, 2003; Ehrlich, 2011; McGaugh, 2000; LeDoux, 2000; Pelletier and Pare, 2004). However, one thing remains certain in all sites of memory storage: consolidation consists of a byzantine array of molecular signaling cascades within neurons that result in the expression of genes and the synthesis of new proteins that promote synaptic growth and synaptic strength (Hernandez and Abel, 2008; Ploski et al, 2008; Ostroff et al, 2010; Stork and Welzl, 1999).

While the importance of individual consolidation molecules can be debated, in general, molecules within these consolidation cascades include transcription factors (Han et al., 2007; Josselyn et al., 2001; Zhou et al., 2009), growth factors (Ou and Gean, 2007), kinases (Goosens et al., 2000; Miller et al., 2002; Schafe et al., 1999; Schafe and LeDoux, 2000; Weeber et al., 2000), mRNA (Duvarci et al., 2008), and calcium (Dolmetsch et al., 2001; Bauer et al., 2002). Activation of these molecules during consolidation leads, in some manner, to the expression of genes and synthesis of proteins. Most neurobiological fear memory research has focused on pathways driven by cyclic adenosine monophosphate (cAMP) that lead to the activation of the cAMP response element-binding (CREB) transcription factor, because manipulating these molecules reliably inhibits memory consolidation and LTP (Bernabeu et al., 1997; Huang et al., 2000; Schafe et al., 1999). However, other signaling pathways may contribute to

consolidation as well (Brambilla et al., 1997). Manipulating pathways important to consolidation soon after initial learning regulates the strength of long-term memory: inhibition of consolidation pathways impairs consolidation and enhancement supports it. Importantly, the effects of manipulations diminish as the time between learning and treatment increases, indicating that consolidation is a prolonged, but finite process (McGaugh, 1968).

Regulation of Consolidation Pathways. Pharmacologic and genetic technologies permit the manipulation of specific signaling molecules within a neuron, but, under natural conditions, the activity of consolidation molecules is regulated by receptors at the surface of a neuron that are activated by binding respective neurochemicals released by presynaptic neurons. Neurochemical receptors at the surface of neurons transmit activity from the outside of a neuron (i.e. binding a neurochemical) to the inside of a neuron by anchoring within and across the neuronal membrane.

In general, there are two classes of transmembrane receptors: ionotropic and metabotropic. Ionotropic receptors conduct ionic currents when activated, which either promote or inhibit action potential firing. Small glutamate-dependent depolarizations that lead to an action potential promote the activation of consolidation pathways primarily by allowing calcium to enter the neuron through NMDA receptors (NMDA-R) and L-type voltage-gated calcium channels (L-VGCC) (Dolmetsch et al., 2001; Bauer et al., 2002). Calcium activates several molecules that are important for increasing gene expression and protein synthesis (Bading, 2000; West et al., 2001). Conversely, metabotropic receptors, including receptor tyrosine kinases and G protein-coupled receptors, do not conduct ionic currents. Instead, the binding of a ligand to the receptors induces changes in the receptor on its intracellular side, which promote molecular signaling cascades within a neuron. In the case of G protein-coupled receptors, the receptor's protein

interacts directly and/or indirectly with intracellular consolidation pathways (Simon et al., 1991), and can also contribute to the regulation of action potential firing by regulating the conductance of ionotropic receptors and other ion-conducting channels (Dascal, 2001). G proteins consist of an α subunit and a βγ complex, both of which can initiate signaling processes within a neuron when activated. Four classes of G proteins are distinguished by various signaling properties, and are classified as $G\alpha_s$ proteins that stimulate molecules in cAMP/CREB pathways, $G\alpha_{i/o}$ proteins that inhibit them, $G\alpha_{q/11}$ proteins that affect those pathways indirectly by mobilizing calcium from intracellular stores, and $G\alpha_{12/13}$ that regulate different intracellular signaling pathways (Simon et al., 1991). Therefore, investigation of neuromodulatory receptors that couple to $G\alpha_s$ proteins has dominated research into the mechanisms supporting the powerful consolidation of fear memory because of their association with cAMP/CREB pathways.

While there are two main classes of transmembrane neurochemical receptors, a wide variety of neurochemicals can be released by a neuron when it fires. The neurotransmitters glutamate and GABA are primarily responsible for regulating neuronal membrane potential and electrical communication between neurons by binding to ionotropic receptors. Glutamate is released from excitatory neurons and initiates synaptic plasticity, while GABA is released from inhibitory neurons. Several different classes of neuromodulators tune the effects of glutamate/GABA and also regulate their effects on non-electrical properties of a neuron. Neuromodulators are typically released from specific neurons that originate from distinct loci in the brain. The overwhelming majority of receptors for these types of neuromodulators are G protein-coupled metabotropic receptors. During fear, the release of several neurochemicals increases in the amygdala (Acquas, 1996; Inglis and Moghaddam, 1999; Pezze and Feldon, 2004; Yokoyama et al., 2005), and, given the broad range of effects exacted by surface

neurotransmitter/neuromodulator receptors on a neuron, the powerful storage of fear memory is thought to be facilitated by physiological changes that occur during and after fear itself.

The Neurobiology of Fear and Fear Memory. Fear consists of a number of neurological and physiological responses that maximize the perception and avoidance of threatening stimuli in an environment rich with sensory information (Ohman and Mineka, 2001; Ohman et al, 2001). As previously mentioned, the amygdala sits at the center of a discrete and evolutionarily conserved fear neuroanatomy that connects sensory information to defensive psychological and physiological responses. In addition to the excitatory sensory afferents that stimulate it, several different neuromodulators are released in the BLA during fear in order to prepare the body for defensive behavior, and the mind for alertness (Aston-Jones et al., 1994, 1998; Herrero et al, 2008; Nieoullon, 2002). Individual neurons in the BLA can receive input from several presynaptic neurons, which permits a wide range of modulation after a learning event. These neuromodulatory influences driven by fear provide a number of candidate mechanisms upon which to build a model for the consolidation of fear memory.

Glutamate. Given that the amygdala becomes active in response to even unperceived aversive stimuli (Morris et al., 2001; Vuilleumier et al., 2002), and remains active after the stimulus has passed (Pelletier et al., 2005), glutamate is an attractive candidate for facilitating fear memory consolidation in the hours after learning. Glutamate is the primary excitatory neurotransmitter, and it facilitates the firing of neuronal action potentials that permit communication between neurons and LTP by binding to NMDA-Rs and AMPA receptors (AMPA-R). In fact, elevation of the number of AMPA-Rs in post-synaptic membranes facilitates long-term memory (Hu et al., 2007; Rumpel et al., 2005). In addition, glutamate binds to eight different metabotropic glutamate receptors that

couple to different G proteins (Ferraguti and Shigamoto, 2006). The BLA is composed primarily of excitatory neurons (McDonald, 1985), and it also receives extensive glutamatergic sensory inputs (Li et al., 1996; McDonald, 1998; Romanski and LeDoux, 1993), which can fire well after initial aversive learning (Guzman-Ramos et al., 2012). However, most evidence suggests that glutamatergic signaling does not play a crucial role in the consolidation of cued fear memory.

Fear induces only modest increases in BLA activity (Pare and Collins, 2000) and extracellular glutamate levels (Yokoyama et al., 2005), and, while increasing NMDA receptor activity can promote consolidation (Kalisch et al., 2009), drugs that have the opposite effect appear to inhibit initial acquisition and not consolidation (Rodrigues et al., 2001; Bauer et al., 2002). In addition, much evidence suggests that the quality of glutamatergic signaling during consolidation, regulated by GABA, contributes more meaningfully than its quantity (Ehrlich et al., 2011). Therefore, while glutamatergic signaling is important for the initial acquisition of cued fear memory, and it may contribute to a very early phase of consolidation (Jerusalinsky et al., 1992), other non-glutamatergic inputs to the BLA are likely responsible for driving the intracellular signaling pathways that consolidate long-term memory. While the neurobiology of fear consists of many neurochemicals working together, the remainder of this document will focus on just three of those neuromodulators that densely innervate the BLA and have been well-studied there.

Norepinephrine (NE). The release of NE from neurons that originate in the locus coeruleus (LC) increases in response to aversive stimuli (Abercrombie and Jacobs, 1987; Galvez et al, 1996), and can persist well into the consolidation window after an emotionally arousing event (Tronel et al., 2004). Indeed, levels of NE in the amygdala after learning correlate with subsequent retention of the memory (McGaugh et al., 2002;

McIntyre et al., 2002), and pharmacologically increasing NE after learning can enhance long-term fear memory (Frankland et al., 2004; Hu et al., 2007; Liang et al, 1990) and LTP in the BLA (Tully et al., 2007).

NE primarily targets excitatory pyramidal neurons in the BLA (Zhang et al., 2013) and binds to three different classes of G protein-coupled adrenergic receptors (AR) – α_1-AR, α_2-AR, and β-AR. While all three classes of receptors can regulate plasticity (Marzo et al., 2009), the β-AR class of receptors (composed of β_1-AR, β_2-AR, β_3-AR) has been the most attractive candidate for promoting fear memory consolidation, given that they promote the cAMP/PKA signaling cascades known to be important for LTP in the hippocampus (Frey et al., 1993; Gelinas and Nguyen, 2005; Hernandez and Abel, 2008; Huang and Kandel,1996; Huang et al., 2000). While the majority of experiments exploring the effects of β-AR on LTP have been performed in hippocampal slices, β_1- and β_2-AR are both widely expressed in the amygdala (Farb et al., 2010; Qu et al., 2008), and are important for LTP there (Johnson et al., 2006). Recent studies suggest that β_2-AR can couple to $G_{i/o}$ and influence fear memory retrieval in the hippocampus, presumably through cAMP/PKA inhibition (Schutsky et al., 2011). However, a role for $G_{i/o}$ signaling by β_2-AR has not been explored in cued fear memory consolidation.

Behaviorally, stimulating β-ARs in the BLA after learning facilitates the consolidation of several forms of fear memory. While one study failed to observe enhancing effects of epinephrine on fear memory consolidation (Lee et al., 2001), its use of systemic epinephrine precludes any effect of the compound in the brain, as epinephrine does not cross the blood-brain barrier. However, many other studies have observed consolidation-enhancing effects of adrenergic drugs that do enter the BLA in a number of different fear learning paradigms (Ferry and McGaugh, 1999; Frankland et al., 2004;

Hu et al., 2007; LaLumiere et al., 2003). In humans, administration of drugs that elevate NE levels after learning enhanced recall for learned information in the future (Soeter and Kindt, 2011; Southwick et al., 2002). Enhancements of memory by post-training β-AR activation extends also into non-emotional forms of memory (Roozendaal et al., 2008), suggesting that β-ARs drive processes fundamental to the consolidation of memory.

While β-ARs promote the consolidation of several forms of fear memory, their role as requisite mediators of fear memory consolidation is less clear. Hippocampus-dependent fear memory has some sensitivity to blockade of β-AR or NE signaling (Grillon et al., 2004; Ji et al., 2003), but the degree to which consolidation can be impaired by these manipulations is inconsistent (Schutsky et al., 2011) and may be due to interactions with other stress hormones (Quirarte et al., 1997). This inconsistency may be due in part to the resistance of strictly amygdala-dependent consolidation to the inhibition of β-adrenergic and noradrenergic signaling. Consolidation of cued fear memory in particular is unaffected by pharmacologic and genetic inhibition of individual β-ARs or NE altogether (Bush et al., 2010; Debiec and LeDoux, 2004; Murchison et al., 2004; Schutsky et al., 2011). Moreover, β-AR antagonists fail to inhibit consolidation of fear memory in humans (Grillon et al., 2004). Therefore, while β-ARs are widely expressed in the BLA and can promote fear memory there, it is likely that they are merely a component of a larger set of neuromodulatory changes that occur in the BLA after an emotionally arousing event and during consolidation.

Dopamine (DA). DA release increases in the brain as part of the physiological response to stress (Inglis and Moghaddam, 1999). While classically associated with reward-learning (Wise, 2004), growing evidence suggests that populations of DA neurons fire in response to a salient, rather than just rewarding, stimuli, and may play a role in general motivational learning (Bromberg-Martin et al., 2010). DA's role in aversive responses

and learning remains cloudy. For example, a single aversive stimulus may increase or decrease the firing of different individual populations of DA neurons (Chiodo et al., 1980; Mantz et al., 1989). Regardless, neuroanatomical and biological relationships between the mesolimbic DA system and the BLA strongly implicate a role for DA in consolidation.

The ventral tegmental area sends dense and widespread projections to the BLA (Asan 1997, 1998; Muller et al., 2009; Pinard et al., 2008), and aversive stimuli induce prolonged increases in DA levels there that could affect processes during the consolidation window (Inglis and Moghaddam, 1999; Yokoyama et al., 2005). DA binds to both the D_1 and D_5 subtypes of the D1 class of G protein-coupled dopaminergic receptors, which are both expressed in the BLA (Mansour et al.,1991; Muly et al, 2009). D_1 and D_5 receptors couple to G_s and $G_{q/11}$ respectively, but in the BLA D1 class agonists initiate the phospholipase C pathway associated with $G_{q/11}$ (Sahu et al., 2009) rather than the adenylyl cyclase-cAMP pathway characteristic of G_s (Jin et al., 2001; Leonard et al., 2003). Therefore, while G_s-coupled D1 receptors contribute to LTP in the hippocampus (Frey et al., 1990; Huang and Kandel 1995), amygdalar LTP may rely on alternative intracellular signaling cascades.

Behaviorally, DA appears to contribute to fear learning primarily through the D1 class of receptors. The firing of some DA neurons is required for some forms of fear learning (Fadok et al., 2009; Zweifel et al., 2011). In humans, binding to D1 receptors increases following emotional arousal (Takahashi et al., 2010), and pharmacological manipulation of D1 receptors can influence the acquisition and retrieval of fear memory (Guarraci et al., 1999; Lamont and Kokkinidis, 1998; Nader and LeDoux, 1999). However, while pharmacological elevation of DA levels in the BLA can enhance fear memory consolidation, blocking DA receptors does not impair consolidation (LaLumiere et al., 2005). Conversely, in mice congenitally lacking DA, restoring DA signaling

immediately after training rescues learning, suggesting that DA is required for consolidation (Fadok et al., 2009). Conclusions are further clouded by the use of multiple training paradigms that often recruit brain regions outside of the amygdala. Therefore, the role of DA and DA receptors in BLA-dependent fear memory consolidation remains unclear.

Acetylcholine (ACh). ACh is also released in response to stressful or frightening experiences (Acquas et al., 1996) and is generally important to the processes of learning and memory (Robinson et al., 2011). A potential role for ACh in fear memory consolidation is underscored by the dense cholinergic projections from the basal forebrain to the BLA (Carlsen et al., 1985; Kordower et al., 1989) and high levels of the enzymes that synthesize and catabolize ACh there (Girgis, 1980; Hellendall et al., 1986). ACh binds to two types of cholinergic receptor: the ionotropic nicotinic receptors and the metabotropic muscarinic receptors. However, nicotinic receptors in the BLA do not appear to influence cued fear memory consoldiation, while muscarinic receptors have a variety of effects on a broad scale of fear learning paradigms (Robinson et al., 2011). Two classes of muscarinic receptors are found in the brain: the M1 class (m1, m3, m5) and the M2 class (m2, m4) (Ehlert et al., 1995). The m1 subtype (M_1R) is the primary and most robustly expressed muscarinic receptor in the BLA (Levey, 1991; McDonald and Mascagni, 2010).

The expression pattern of muscarinic receptors in the BLA underlies an important role in fear learning. Pharmacologically activating muscarinic receptors in the BLA enhances the consolidation of fear learning (Barros et al., 2002; Vazdarjanova and McGaugh, 1999). These effects are believed to be mediated via the M1 class of muscarinic receptors, which couples to $G_{q/11}$ and activates the second messenger phospholipase C (PLC) (Caulfield, 1993). Muscarinic activation of PLC initiates an

intracellular rise in calcium in the BLA (Power and Sah, 2007), generates a number of intracellular signaling molecules (Suh et al., 2008), and also affects a variety of ion channels on the surface of BLA neurons that alter their responsiveness to excitatory inputs (Washburn and Moises, 1992; Womble and Moises, 1993; Yajeya et al., 1999). While these changes in the BLA may promote consolidation, the contribution of muscarinic receptors does not appear to be essential, as muscarinic antagonists and receptor knockouts fail to impair consolidation of cued fear memory in the BLA (Anagnostaras et al., 2003; Fornari et al., 2000). Therefore, other neuromodulatory mechanisms might either contribute to the same processes induced by muscarinic receptors, or initiate separate processes that support consolidation.

Other Neuromodulators. The fear/stress response induces the release of several other neuromodulators, including serotonin (Yokoyama et al., 2005) and stress hormones (Ulrich-Lai and Herman, 2009). These neuromodulators have also been observed to contribute to fear learning to some extent (Homberg, 2012; Roozendaal et al., 2009), and they must be accounted for in completely understanding the neural processes that control fear memory. However, given the scope of the following dissertation, these and other neuromodulators will not be discussed in detail.

Motivation for this Research. This dissertation sets out to explore the neuromodulatory systems required to consolidate cued fear memory. As described previously, fear memory consolidation is understood through the lens of general consolidation principles that reside within (i.e. intracellular signaling cascades) and between (i.e. glutamate) neurons at a synapse. Yet these principles are not sufficient to explain how long-term memory for the association between fear and a distinct sensory stimulus – *vis a vis* cued fear conditioning – is so well stored. The key to understanding how fear memory is so powerfully stored is to determine the neurobiological mechanisms initiated by fear that

increase gene expression and protein synthesis in the BLA. If it is well-understood that consolidation at a synapse relies fundamentally on neurobiological processes within neurons, a complete understanding of how frightening experiences enhance those processes is far from reach. This is no more apparent than in the low efficacy of pharmacological treatments for PTSD (Steckler and Risbrough, 2012), where the primary avenues of therapy are neuromodulatory interventions, as pharmacologically targeting intracellular consolidation pathways is potentially fatal.

The value of developing a broad and reliable neuromodulatory model of fear memory consolidation extends beyond potential translational benefits to fundamental contributions to a scientific corpus for which such a model has remained elusive. The pursuit of a single neuromodulatory system underlying cued fear memory consolidation has yet to bear fruit despite research into many different candidate targets. Multiple individual systems have been observed to contribute to fear memory consolidation, but none appear to constitute it alone. Given the importance of the BLA to the neurophysiology of fear and fear memory, the release of several neuromodulators in the BLA as a characteristic of the fear response, and the observed importance of some of those neuromodulators to consolidation, this dissertation explores the hypothesis that neuromodulatory systems work in concert to constitute fear memory consolidation in the BLA.

Potential relationships between neuromodulatory systems in fear memory consolidation have been previously explored (LaLumiere and McGaugh, 2005; Roozendaal et al., 2006), but those studies investigated relationships wherein multiple receptor systems interact with one another to alter the strength of their individual signaling mechanisms. Here, neuromodulatory systems are investigated as partners working in parallel to one another to redundantly activate intracellular consolidation

pathways. In such a model, blocking a single system does not prevent consolidation, but instead requires that two or more systems be blocked to overcome the redundant relationship. The pharmacological and genetic techniques used herein, combined with the robustness of Pavlovian fear conditioning, provide powerful resolution for understanding individual receptor systems, and how they might cooperate with one another to store fear memory. Successfully identifying such a model will provide insight into how signaling pathways are upregulated such that normal fear memory is driven to the devastatingly persistent and powerful memories formed in individuals suffering from PTSD.

Early interventions soon after a traumatic experience have been previously explored, but most treatments have aimed to minimize emotional responses rather than directly target the mechanisms responsible for the consolidation of the memory for the trauma (Parsons and Ressler, 2013). This dissertation is guided largely by pharmacological and biological principles of memory, rather than of emotion, and will likely contribute to understanding how memory is consolidated in general, and not only in the case of fear memory. Moreover, because many processes known to promote fear memory consolidation also facilitate the reconsolidation of a memory after it is retrieved (Alberini, 2005), findings in regard to consolidation will likely also contribute to understanding reconsolidation and reconsolidation-based treatments for PTSD.

CHAPTER 2: REDUNDANT CATECHOLAMINE SIGNALING CONSOLIDATES FEAR MEMORY VIA PHOSPHOLIPASE C

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PREFACE

While the first author of the following publication is Ming Ouyang, PhD, I have included it in this dissertation due to the contributions that I made to the labor and design of the study, as well as to the writing of the manuscript. More importantly, the experiments that I conducted in the following study represent the commencement of my thesis work, and are essential to the whole of my contribution to the field of learning and memory. My contributions to the research conducted in this publication comprise all of Figures 1.5 and 1.7.

ABSTRACT

Memories for emotionally arousing experiences are typically vivid and persistent. The recurrent, intrusive memories of traumatic events in post-traumatic stress disorder (PTSD) are an extreme example. Stress-responsive neurotransmitters released during emotional arousal are proposed to enhance the consolidation of fear memory. These transmitters may include norepinephrine and epinephrine (NE/E) because stimulating β-adrenergic receptors shortly after training can enhance memory consolidation. However, mice lacking NE/E acquire and consolidate fear memory normally. Here, we show by using pharmacologic and genetic manipulations in mice and rats that NE/E are not essential for classical fear memory consolidation because signaling by the β(2)-adrenergic receptor is redundant with signaling by dopamine at the D(5)-dopaminergic receptor. The intracellular signaling that is stimulated by these receptors to promote consolidation uses distinct G proteins to redundantly activate phospholipase C. The results support recent evidence indicating that blocking β-adrenergic receptors alone shortly after trauma may not be sufficient to prevent PTSD.

INTRODUCTION

The basolateral nuclei of the amygdala (BLA) is a brain region critical to the consolidation of fear memory (Pape and Pare, 2010). Changes in neuromodulatory signaling in the BLA during emotional arousal are believed to underlie the powerful and persistent consolidation of long-term fear memory. Neuromodulators such as norepinephrine and epinephrine (NE/E) that are released during fearful or traumatic events can promote memory consolidation for such experiences. For example, stimulating β-adrenergic receptors by infusing NE or β-adrenergic selective agonists into the BLA shortly after instrumental fear conditioning enhances long-term memory (McGaugh and Roozendaal, 2002).

In contrast, a requirement for β-adrenergic signaling in fear memory consolidation is less clear. In one study, BLA infusion of β-adrenergic receptor antagonists impaired consolidation of instrumental fear (Gallagher et al., 1977). However, results from other studies suggest that β signaling is not required for consolidation of instrumental fear (Izquierdo and Dias, 1983; Izquierdo et al., 1992). Negative results have also been reported for classical fear. Infusing a β-adrenergic receptor antagonist into the BLA shortly before or immediately after classical fear conditioning does not impair consolidation (Miserendino et al., 1990; Debiec and LeDoux, 2004). Furthermore, instrumental and classical fear memory consolidation are normal in mice completely lacking NE/E (Thomas and Palmiter, 1997; Murchison et al., 2004) or harboring a targeted disruption of either the β_1- or β_2-adrenergic receptor gene (Schutsky et al., 2011).

A potential resolution to the observation that the adrenergic system can influence, but is not required for, consolidation is that there is redundancy between the adrenergic system and another neurotransmitter system. In redundancy, the loss of a single system

will have no effect on consolidation. However, interfering with two relevant but redundant systems simultaneously could have considerable effect. Classically, β-adrenergic receptors couple to G_s proteins that act to stimulate adenylyl cyclase (AC) and elevate intracellular levels of cAMP, a second messenger required in the BLA for fear memory consolidation (Schafe and LeDoux, 2000). It is through G_s and cAMP that β receptors are currently proposed to enhance synaptic plasticity and memory consolidation (Sara, 2009; Tully and Bolshakov, 2010).

For this study, we considered whether DA might serve as a stress-responsive system that acts in a redundant manner with NE/E in promoting consolidation. Like NE/E, extracellular levels of DA are elevated for minutes following fear conditioning (Inglis and Moghaddam, 1999; Anstrom and Woodward, 2005). Like β receptors, the D_1 class of DA receptors, consisting of the D_1 and D_5 receptors ($D_{1,5}$), can couple to the G_s class of G proteins and elevate cAMP (Sibley and Monsma, 1992), providing an opportunity for redundant signaling between these two classes of catecholamine receptors. Here, we use genetic and pharmacologic approaches in mice and rats to demonstrate that DA and NE/E have redundant roles in the BLA for the consolidation of classical fear memory. Surprisingly, we found that the signaling by these neuromodulators that is critical for consolidation converges on the activation of phospholipase C.

MATERIALS AND METHODS

Animals

Wild-type, *Dbh*$^{+/-}$, *Dbh*$^{-/-}$, *Adrb1*$^{-/-}$ (β_1 KO), *Adrb2*$^{-/-}$ (β_2 KO), and *Drd1*$^{-/-}$ (D_1 KO) mice were on a hybrid 129/Sv × C57BL/6 background, while *Drd5*$^{-/-}$ (D_5 KO) mice were on a C57BL/6 background (Xu et al., 1994; Thomas et al., 1995; Rohrer et al., 1996;

Chruscinski et al., 1999; Holmes et al., 2001). Mice were generated by mating either heterozygotes or homozygotes, and genotype was determined by PCR. The prenatal loss of *Dbh*$^{-/-}$ mice was rescued as previously described (Ouyang et al., 2004). No significant differences were found by gender or parental genotype so data were combined. Female Fischer 344 rats (Harlan) were 3–4 weeks old upon arrival. Animals were maintained on *ad libitum* food and water and a 12 h light/dark cycle, with lights on beginning at 7:00 A.M. Animals were housed in small, quiet rooms for at least 3 weeks before studies began. Mice were 3–6 months old and rats were 8–11 weeks old when tested. Studies were performed during the light phase, with most experiments taking place between 9:00 A.M. and 5:00 P.M. Studies were in accordance with NIH guidelines and had the approval of the Institutional Animal Care and Use Committee at the University of Pennsylvania.

Classical fear conditioning

Adjacent to the training room, animals were placed in pairs (mice) or singly (rats) into opaque plastic holding buckets (12 cm diameter) with bedding and lids for 30–60 min before being manipulated further. Animals were given two 3 min preconditioning handling sessions over 2 d in the training room. Saline was injected at the end of handling each day. For conditioning, animals were placed in the training apparatus (ENV-010MC with ENV-414S, Med Associates) for 2 min, after which an 84 dB, 4.5 kHz tone was activated for 30 s. Two seconds before the end of the tone, a 2 s footshock was delivered (1 mA for mice, 1.7 mA for rats). The animal was removed from the apparatus and injected 30 s after shock, and then returned to its home cage. The apparatus was cleaned with Versa-Clean (Thermo Fisher Scientific) between subjects. Experiments examining enhancement of consolidation in mice were conducted identically except that a 0.4 mA footshock was used. Pseudoconditioning was similar to

conditioning except that animals received the 2 s, 1 mA footshock immediately after being placed in the conditioning apparatus, while activation of the 30 s tone occurred at the normal time 2 min later. Contextual fear was tested for 5 min in the conditioning apparatus in the absence of the tone. Cued fear was tested in a Plexiglas cylinder (21 cm diameter, 24 cm tall) with green wire grid floor and vertical green and white wall stripes 240° around, and was cleaned with lemon-scented Ajax between subjects. After 2 min, the training tone was turned on for 3 min. Percentage freezing was estimated by scoring the presence or absence of nonrespiratory movement every 5 s. Tests were conducted 1 d after training.

Instrumental fear conditioning

Animals were handled as described above. Training consisted of placing an animal in the lighted chamber of the apparatus used for classical conditioning and timing its latency to fully enter (except for the tail) the dark chamber. Once the animal entered the dark chamber, the retractable partition separating the two chambers was lowered and a footshock was delivered for 2 s (0.35 mA). The animal was removed from the apparatus and injected 15 s after shock, and then returned to its home cage. Animals that did not enter the dark chamber after 100 s during conditioning were excluded (<4% of mice, independent of genotype). Testing was identical to training except that no shock was delivered and the partition remained up. Latencies to enter the dark chamber were recorded. If an animal did not enter the dark chamber within 10 min, it was returned to its cage and assigned a latency of 10 min. Tests were conducted 1 d after training.

Drugs

SCH 23390 HCl, ecopipam (SCH 39166 HBr), SKF 38393 HBr, SKF 83959 HBr, SKF 83822 HBr, (−)-propranolol HCl, CGP 20172A HCl, ICI 118,551 HCl, procaterol

HCl, pertussis toxin, edelfosine (all Tocris Bioscience) and m-3M3FBS (Sigma) were administered intraperitoneally or infused into the BLA immediately after training. The phospholipase C (PLC) inhibitor U73122 could not be used in these studies because its delivery requires high percentage organic vehicles that disrupt memory consolidation on their own. Xamoterol hemifumarate (Tocris Bioscience) was administered intraperitoneally 60 min before testing contextual fear in *Dbh*$^{-/-}$ mice to rescue memory retrieval (Murchison et al., 2004). Drugs were dissolved in 0.9% saline (SKF compounds and procaterol also contained 0.1 mg/ml ascorbic acid, pH 7.4, Sigma). Vehicle was saline with or without 0.1 mg/ml ascorbic acid. Systemic injection volumes were 10 µl/g body weight.

CNS infusion

Two guide cannulae mounted on a base plate (C315GS system, Plastics One) were implanted under pentobarbital anesthesia (72.5 mg/kg) using a stereotax (SAS75/EM40M, Cartesian Research). The guides were placed 1.25 mm posterior to bregma and 3.5 mm bilateral for BLA infusions. The guide and dummy cannulae projected 3 mm below the base plate. Habituation of the animals to the investigator and the infusion procedure began a couple of days later with a 3 min handling session followed by 3 min of immobilization (gently holding the nape of the neck and body) that mimicked infusion. Five handling sessions were given, with two of them being on the 2 d immediately preceding training and the final one being 1 h before training. Immediately after training, mice were infused bilaterally using injection cannulae that extended 2.8 mm below the tip of the guide cannulae. Infusion was 0.2 µl/side at 0.08 µl/min, with the injection cannulae being left in place for 30 s before the mouse was returned to its home cage. Because studies indicate that the effects of PTx are best evaluated 3 d after infusion, PTx was infused into the BLA 3 d before training (Goh and Pennefather, 1989;

Stratton et al., 1989). For sites adjacent to the BLA, infusions were displaced 0.75 mm from the BLA coordinates in the direction indicated. As a result, dorsal was in the posterior striatum, ventral was near the ventral piriform cortex, medial was at the central/medial amygdala border, lateral was in the dorsal piriform cortex, rostral was in the extreme anterior amygdala, and caudal was in the extreme posterior amygdala.

IP_3 levels

Mice were anesthetized with CO_2, killed by cervical dislocation and brains were rapidly removed, frozen in isopentane on dry ice and stored at −80°C. Two frozen coronal sections (400 µm) that contained the BLA were cut by cryostat (HM505E, Microm) from each mouse, and a 0.5 mm diameter punch of BLA tissue was collected bilaterally from each slice. The four punches per mouse were pooled and homogenized on ice with three 2 s pulses (5 s interval) in 125 µl of 4% perchloric acid using a Sonic Dismembrator 100 set on level 3 (Thermo Fisher Scientific). After 15 min on ice, samples were stored overnight at −80°C. The next day samples were centrifuged at 4°C and 2000 × *g* for 15 min, and the pellet was stored at −80°C for subsequent Bradford assay to determine total protein. Supernatants were neutralized on ice with 10 m KOH (to precipitate the perchloric acid) and centrifuged at 4°C and 2000 × *g* for 15 min. Supernatant (100 µl) was then used in the [^{3}H]-IP_3 radioreceptor assay (PerkinElmer) according to instructions. Pilot experiments indicated that IP_3 levels were elevated 30 min after systemic agonist injection, but not at 15, 22, or 35 min.

Statistics

Data were analyzed with Statistica 9.1 (StatSoft) using one- or two-way ANOVA with $\alpha = 0.05$. The Bartlett *Chi*-square test was used to analyze homogeneity of variances. *Post hoc* comparisons were made using Duncan's range test. In Figures 1–9,

data are presented as mean ±SE. Comparisons marked as significant are to the reference group except where indicated.

RESULTS

Redundancy between $D_{1/5}$-dopaminergic and β_2-adrenergic signaling

To determine whether signaling by DA might be redundant with that for NE/E, we first examined the effect of the $D_{1,5}$ antagonist SCH 23390 (SCH) on memory consolidation when administered immediately after classical fear conditioning in wild-type mice and mice completely lacking NE/E (Iorio et al., 1983). Mice that lacked NE/E were homozygous for targeted disruption of the dopamine β-hydroxylase gene (*Dbh*$^{-/-}$) (Thomas et al., 1995). The same general treatment protocol was used for this and subsequent experiments (Fig. 1.1*A*). One day after fear conditioning, *Dbh*$^{-/-}$ mice treated immediately after training with the $D_{1,5}$ antagonist SCH at 10-30 µg/kg body weight exhibited low freezing in response to the training cue (a tone that immediately preceded shock) compared to *Dbh*$^{-/-}$ mice treated with vehicle or to wild-type mice treated with either SCH or vehicle (Fig. 1.1*B*).

In a separate group of mice tested for their contextual fear of the training apparatus (no tone), SCH also impaired consolidation selectively in *Dbh*$^{-/-}$ mice relative to wild-type mice (Fig. 1.1*C*). Mice lacking NE/E exhibit impaired retrieval of contextual fear one day after conditioning due to lack of β_1 signaling (Murchison et al., 2004). Thus, to examine potential effects of NE/E deficiency on consolidation, retrieval was rescued in the contextual fear experiment by administering the β_1-selective agonist xamoterol shortly before testing (Hicks et al., 1987; Murchison et al., 2004). For simplicity, subsequent experiments using classical fear conditioning focused on cued fear, for which retrieval is independent of NE/E.

In addition to classical fear, we examined whether consolidation of instrumental fear requires NE/E and/or $D_{1,5}$ signaling. Wild-type and *Dbh*$^{-/-}$ mice were treated with either vehicle or SCH immediately after conditioning. Neither the absence of NE/E by itself nor treatment of wild-type mice with SCH impaired consolidation of instrumental fear (Fig. 1.1*D*), confirming previous observations with *Dbh*$^{-/-}$ mice (Thomas and Palmiter, 1997). Further, and in contrast to the results for classical fear, treatment of *Dbh*$^{-/-}$ mice with SCH also had no effect on the consolidation of instrumental fear. Differences in signaling mechanisms between instrumental and classical fear conditioning may parallel differences in their reliance on the amygdala (Wilensky et al., 2000).

A potential confound of the above results for classical fear conditioning is that SCH is also an agonist at serotonin 5-HT_{2C} receptors (Ramos et al., 2005). To further examine a role for $D_{1,5}$ receptors, we employed a less potent but more selective $D_{1,5}$ antagonist, ecopipam, that lacks serotonin receptor activity (Chipkin et al., 1988). Similar to SCH, ecopipam impaired cued fear memory consolidation selectively in *Dbh*$^{-/-}$ mice (Fig. 1.1*E*).

The results to this point are consistent with two distinct potential roles for DA in classical fear memory consolidation. First, DA might not normally play a role in consolidation, but instead might compensate for the chronic lack of NE/E in *Dbh*$^{-/-}$ mice. Alternatively, NE/E and DA might both contribute to consolidation, but their roles would be redundant. In the latter case, stimulation of either system might facilitate consolidation, but only impairments in signaling by both systems would cause deficits in consolidation. To evaluate these possibilities, wild-type mice were treated with SCH, the β-adrenergic receptor antagonist propranolol, or the combination. Mice treated with SCH or propranolol alone exhibited normal cued fear memory, while mice treated with the combination of SCH plus propranolol exhibited impaired memory (Fig. 1.1*F*).

Further, a selective β_2 antagonist (ICI 118,551 = ICI) but not a selective β_1 antagonist (CGP 20712A) impaired consolidation when combined with SCH, while ICI alone had no effect (O'Donnell and Wanstall, 1980; Dooley et al., 1986). Dose-response data indicated that ICI was fully effective at 30 µg/kg when it was combined with SCH (Fig. 1.1*G*). The results provide strong initial support for the idea that DA and NE/E act in a redundant manner to consolidate classical fear memory.

Results from the antagonist studies suggest that β_2 signaling is required for the role of NE/E in consolidation. To determine whether β_2 signaling is sufficient for the role of NE/E, *Dbh*$^{-/-}$ mice were treated with both SCH (to impair consolidation) and various doses of the selective β_2 agonist procaterol (Waelbroeck et al., 1983). Procaterol provided a dose-dependent rescue of cued fear memory consolidation, suggesting that β_2 signaling is sufficient for mediating the role of NE/E in consolidation in the absence of $D_{1,5}$ signaling (Fig. 1.1*H*).

Redundancy also occurs in rats, is localized to the BLA, and is mediated by D_5 receptors

Many prior studies examining the roles of catecholamines in fear memory have employed rats rather than mice. To determine whether redundancy between catecholamines generalizes across species, rats were fear conditioned and treated with SCH and/or ICI. Consistent with the results obtained from mice, only concurrent administration of SCH and ICI impaired cued fear memory consolidation in rats (Fig. 1.2).

Because the BLA is critical for fear memory consolidation, we asked whether redundant catecholamine signaling occurs in this brain region. One week before conditioning, mice were cannulated to permit infusions into the BLA. When SCH and/or ICI were infused bilaterally immediately after training, only the combination of drugs

impaired memory consolidation (Fig. 1.3*A*). To determine whether drug infusions into the BLA impair consolidation by affecting adjacent brain regions instead of the BLA, the SCH/ICI combination was infused into each of six locations surrounding the BLA (Fig. 1.3*C*). These infusions did not impair consolidation, indicating that the BLA is the site of drug action (Fig. 1.3*B*).

To genetically test a role for β receptors, mice with a targeted disruption of the gene for either the β_1- or β_2-adrenergic receptor were treated with SCH or vehicle. While SCH had no effect on consolidation in β_1 knockout (KO) mice (Fig. 1.4*A*), SCH impaired consolidation in β_2 KO mice (Fig. 1.4*B*). As a genetic complement to non-selective β receptor blockade, cued fear memory was also examined in $\beta_{1,2}$ double KO mice, and no deficit was observed (Fig. 1.4*C*). Importantly, the pharmacologic studies to this point were unable to distinguish between the potential roles of D_1 and D_5 receptors in consolidation due to a lack of receptor subtype selectivity of the drugs. For this purpose, gene-targeted mice were employed. While the β_2 antagonist ICI had no effect on consolidation in D_1 KO mice (Fig. 1.4*D*), ICI impaired consolidation in D_5 KO mice (Fig. 1.4*E*), indicating that the role of DA in consolidation is mediated by the D_5 receptor.

Agonists of β_2 or D_5 receptors enhance fear memory consolidation

Given the roles for β_2 and D_5 receptors defined above, we asked whether activating these specific receptors shortly after conditioning would enhance fear memory consolidation in mice when trained with a lower shock intensity. For NE/E, enhancement of consolidation by β receptor stimulation has not been demonstrated for classical fear, although this has been demonstrated for instrumental fear (McGaugh and Roozendaal, 2002). For DA, results from BLA infusion of a $D_{1,5}$ receptor agonist prior to classical fear conditioning suggest that stimulation of these receptors enhances either acquisition or consolidation (Guarraci et al., 1999).

When wild-type mice were systemically injected with the β_2 agonist procaterol immediately after conditioning, cued fear memory was significantly enhanced (Fig. 1.5*A*). When mice were injected with the $D_{1,5}$ agonist SKF 38393 immediately after conditioning, cued fear memory was also significantly enhanced (Fig. 1.5*B*). Of note, $D_{1,5}$ receptors can activate various downstream effectors, including AC and phospholipase C (PLC), and certain $D_{1,5}$ agonists can induce the selective activation of either AC or PLC (Undie et al., 1994; Jin et al., 2003). To gain insight into the initial mechanism by which $D_{1,5}$ receptors might enhance consolidation, effector-selective $D_{1,5}$ agonists were employed. While an AC-selective $D_{1,5}$ agonist did not significantly alter consolidation, a PLC-selective $D_{1,5}$ agonist enhanced consolidation to an extent similar to that for the non-selective $D_{1,5}$ agonist employed initially (Fig. 1.5*B* - *D*). Because $D_{1,5}$ agonists do not distinguish between these two receptors, receptor KO and wild-type littermate control mice were utilized. The ability of the non-selective and PLC-selective $D_{1,5}$ agonists to enhance consolidation was absent in D_5 KO mice, although the β_2 agonist procaterol remained effective (Fig. 1.5*E*). Similarly, the ability of procaterol to enhance consolidation was absent in β_2 KO mice, although a $D_{1,5}$ agonist remained effective (Fig. 1.5*F*).

β_2 and D_5 signaling in consolidation converge on the activation of PLC

Given that D_5 may activate PLC to enhance fear memory consolidation, we asked whether directly stimulating PLC activity with the agonist m-3M3FBS enhances consolidation (Bae et al., 2003). In support of a role for PLC, infusion into the BLA of this PLC agonist also enhanced consolidation (Fig. 1.6*A*). We next asked whether inhibiting PLC activity would impair consolidation by infusing the PLC inhibitor edelfosine into the BLA immediately after training (Powis et al., 1992). Edelfosine dose-dependently impaired consolidation (Fig. 1.6*B*), suggesting that activation of PLC may

be required for fear memory consolidation, and that this could be a site of convergence for D_5 and β_2 signaling. Infusion of edelfosine either one day before or 4 h after conditioning had no effect on cued fear memory, indicating that edelfosine does not lesion the BLA or impair expression (Fig. 1.6*C*). Although prior studies support the possibility that D_5 may signal via G_q and PLC in the amygdala (Friedman et al., 1997; Leonard et al., 2003; Sahu et al., 2009), the only data indicating that β_2 could signal via PLC come from heterologous expression of β_2 receptors in HEK-293 cells *in vitro* (Keiper et al., 2004).

To determine whether D_5, β_2 or both receptors might signal via PLC to promote consolidation, we employed edelfosine in two complementary experiments. The first experiment was based on the idea that PLC activity could be reduced to a point where it becomes rate-limiting for consolidation. To achieve this, edelfosine was infused into the BLA at the highest dose (0.2 ng) that did not impair consolidation. We then examined the combinatorial effects of PLC and receptor blockade. Edelfosine at 0.2 ng was administered with a dose (50 ng) of either SCH or ICI that impairs consolidation when combined with each other but not when given alone (Fig. 1.3*A*). The combination of edelfosine plus SCH and the combination of edelfosine plus ICI each impaired consolidation (Fig. 1.6*B*).

The second experiment examined the ability of agonists to enhance consolidation when PLC was inhibited. Edelfosine, at the smallest dose (2 ng) that impaired consolidation of high-shock intensity training, was infused into the BLA while administering a $D_{1,5}$ or β_2 agonist systemically immediately after training with lower shock intensity. Edelfosine blocked the enhancements of consolidation induced by the $D_{1,5}$ agonist and by the β_2 agonist (Fig. 1.6*D*).

Results from the above two experiments suggested that D_5 and β_2 receptors may both signal via PLC to promote consolidation. To further test this possibility, inositol-1,4,5-trisphosphate (IP_3), a second messenger molecule generated by PLC activity, was measured in the BLA following systemic administration of a $D_{1,5}$ or β_2 agonist. Significant increases in IP_3 levels in the BLA were observed *ex vivo* with each agonist in wild-type mice (Fig. 1.7*A*). Because this is a novel finding for β_2 stimulation, β_2 KO mice were also examined. In these mice, a $D_{1,5}$ agonist but not a β_2 agonist caused BLA IP_3 levels to increase (Fig. 1.7*B*). For D_5, others have shown that the ability of $D_{1,5}$ agonists to augment IP_3 levels in various brain regions is absent in D_5 KO mice (Sahu et al., 2009).

Finally, we asked whether learning-specific activation of PLC occurs in the BLA by measuring IP_3 levels *ex vivo* following fear conditioning. IP_3 levels were elevated in the BLA 30 min after fear conditioning when compared to pseudoconditioning or no conditioning (Fig. 1.7*C*). The elevation in IP_3 was selective for this time point, as several earlier and later time points from 3 - 60 min did not show elevation (Fig. 1.7*D*). Systemic administration of SCH or ICI immediately after conditioning had no effect on IP_3 levels 30 min after conditioning (Fig. 1.7*E*). In contrast, administration of SCH and ICI combined significantly reduced IP_3 levels relative to vehicle administration, demonstrating that redundancy in receptor function extends to the learning-induced production of IP_3 in the BLA.

β_2 activation of PLC in the BLA is mediated by $G_{i/o}$

While there is considerable evidence indicating that D_5 couples to G_q to activate PLC, evidence for the coupling of β_2 to G_q is lacking. A study in HEK-293 cells suggested that by coupling to G_s, β_2 receptors can sequentially activate AC, Epac, Rap2 and PLCβ. However, recent results from our lab suggested an alternate potential

mechanism for coupling β_2 to PLC in the brain. Retrieval of contextual fear memory requires NE, β_1, G_s and AC signaling in the hippocampus (Ouyang et al., 2008). β_2 signaling impairs retrieval by stimulating the inhibitory G_i class of G proteins, causing cAMP levels to decrease in the hippocampus (Schutsky et al., 2011). Of potential relevance, G_i signaling can also result in the activation of PLC by releasing βγ subunits that are capable of stimulating PLCβ isoforms. Therefore, we asked whether such a mechanism might apply to β_2 signaling in the BLA for consolidation.

Pertussis toxin (PTx) inactivates $G_{i/o}$ proteins through ADP ribosylation, uncoupling them from their receptors. Because it takes several days to observe optimal efficacy when PTx is administered *in vivo*, PTx was infused into the BLA three days prior to conditioning (Goh and Pennefather, 1989; Stratton et al., 1989). BLA pretreatment with PTx had no effect on consolidation when saline was infused immediately after training, suggesting that $G_{i/o}$ signaling is not essential for consolidation (Fig. 1.8*A*). That outcome was expected if only one of the redundant pathways utilizes $G_{i/o}$. Interestingly, pretreatment with PTx impaired consolidation when SCH was infused immediately after training. This outcome suggested that PTx pretreatment might be mimicking β_2 blockade. In support of this idea, pretreatment with PTx did not affect consolidation when ICI was infused immediately after training. Taken together, these results suggest that β_2 but not D_5 signaling in the BLA is mediated by $G_{i/o}$. To further test this possibility, PTx was infused into the BLA prior to treating with receptor agonists to enhance consolidation. PTx pretreatment blocked the enhancement of consolidation normally observed following systemic treatment with a β_2 agonist, but had no effect on the ability of a $D_{1,5}$ agonist to enhance consolidation (Fig. 1.8*B*).

DISCUSSION

In summary, our experiments identify an important but redundant role in classical fear memory consolidation for adrenergic signaling by β_2 receptors and dopaminergic signaling by D_5 receptors (Fig. 1.9). It is well recognized that there are multiple stress-response mediators with distinct but overlapping temporal and mechanistic attributes (Joels and Baram, 2009). Redundancy in systems responsible for potentially life-preserving processes such as long-term fear memory consolidation could be advantageous for survival. While we have found that NE and DA act in a redundant manner to consolidate classical fear memory, it is also possible that each has additional unique roles during (but not essential to) consolidation that may be identified in future studies.

While it has been widely hypothesized that endogenous NE/E and β-adrenergic signaling play critical roles in amygdala-dependent emotional memory consolidation, there is a paucity of evidence indicating that such signaling is uniquely required for this. In one study suggesting a unique role, various β receptor antagonists (including ICI) were infused into the BLA of rats immediately after cued fear conditioning, and impairment of cued fear was reported one day later (Qu et al., 2008). However, the doses of antagonist used were 100-fold higher than those found to be effective for ICI (when combined with SCH) in the current study, and are considerably higher than what should be necessary for the size difference between rats and mice. Another study suggested that β receptors contribute to the acquisition but not consolidation of cued fear memory (Bush et al., 2010). However, it is difficult to reconcile the above observations with results from mouse genetic models, which do not support a unique role for β receptors in the acquisition, consolidation or retrieval of cued fear memory: neither *Dbh*$^{-/-}$, β_1 KO, β_2 KO, nor β_1/β_2 double KO mice exhibit cued fear deficits.

Further, our pharmacologic data from the current study and a previous study using mice and rats support the genetic findings (Murchison et al., 2004).

With respect to the role for DA, results from some studies suggest that $D_{1,5}$ signaling might be required for fear memory acquisition or consolidation (Guarraci et al., 1999; Greba and Kokkinidis, 2000). However, doses of SCH used in those studies (systemic or intra-BLA) were 10- to 100-fold higher than those found to be effective here, potentially lacking specificity for $D_{1,5}$ signaling. Relevant to this, we did not observe a fear conditioning deficit in D_1 or D_5 KO mice, confirming previous results (El-Ghundi et al., 1999; Holmes et al., 2001). These and our results contrast with a recent study reporting a deficit in either the acquisition or consolidation of fear-potentiated startle in D_1 KO mice (Fadok et al., 2009). In that study, conditioning utilized 30 trials reinforced by mild footshocks (0.2 mA for 0.5 s), while the current study examined the consolidation of more intense fear resulting from a single, strongly aversive footshock (1 mA for 2 s). It is possible that the mechanisms underlying the consolidation of multiple weakly reinforced training trials are different from those for a single, strongly reinforced event.

Given that β_2 and D_5 receptors can couple to G_s, and that cAMP signaling is required for classical fear memory consolidation (Schafe and LeDoux, 2000), it is interesting that neither β_2 nor D_5 signaling in the amygdala may increase cAMP levels. DA and $D_{1,5}$ agonists fail to elevate cAMP in the BLA (Leonard et al., 2003), and stimulation of β_2 receptors in hippocampal slices causes a decrease in cAMP (Schutsky et al., 2011). However, our results are consistent with observations indicating that $D_{1,5}$ agonists activate PLC rather than AC in the BLA, and that activation of PLC by DA is greatly diminished in D_5 but not D_1 KO mice (Friedman et al., 1997; Leonard et al., 2003; Sahu et al., 2009). Our results are also consistent with observations indicating that

signaling by β_2 receptors in the heart and hippocampus depends predominantly on $G_{i/o}$ rather than G_s (Rockman et al., 2002; Schutsky et al., 2011).

Results from the current study indicate that β_2 and D_5 receptor signaling converge and become redundant by activating PLC. Remarkably, there is little evidence that canonical neurotransmitter signaling pathways that activate G_q/PLC are required for fear memory. Gene-targeted mice lacking expression of either metabotropic glutamate receptor mGluR1 or mGluR5, muscarinic acetylcholine receptor M_1 or M_3, serotonergic receptor 5-HT_{2a} or 5-HT_{2c}, adrenergic receptor α_{1d}, or histaminergic receptor H_1 all exhibit intact cued fear memory (Aiba et al., 1994; Lu et al., 1997; Tecott et al., 1998; Anagnostaras et al., 2003; Sadalge et al., 2003; Weisstaub et al., 2006; Dai et al., 2007; Poulin et al., 2010). Pharmacologically, there is evidence for and against mGluR5 signaling being required for cued fear memory (Nielsen et al., 1997; Rodrigues et al., 2002; Gravius et al., 2006), although stimulating mGluR1/5 receptors can enhance fear memory (Rudy and Matus-Amat, 2009). For NE/E, antagonist treatment suggests that β_1-adrenergic signaling is not required for fear memory (Lazzaro et al., 2010). On the other hand, mice with a targeted disruption of the gene for PLC-β1 exhibit greatly reduced contextual fear, although this could be due to a deficit in hippocampus-dependent memory rather than BLA-dependent fear memory per se (McOmish et al., 2008b; McOmish et al., 2008a).

Stimulation of PLC is likely to activate Ca^{2+}- and diacylglycerol-dependent signaling such as that mediated by protein kinase C (PKC) and calmodulin-dependent kinases (CaMKs). Genetic and pharmacologic data support a role for these kinases in fear memory. Genetic disruption of the PKCβ gene or the CaMKIV gene results in impaired cued and contextual fear (Weeber et al., 2000; Wei et al., 2002), and inhibitors of PKC infused into the BLA shortly after conditioning impairs consolidation of instrumental

fear (Bonini et al., 2005). In addition to the generation of IP_3, it will be valuable in future studies to identify the signaling events that are altered when β_2 and D_5 receptors are antagonized. It will also be valuable to determine in what cell type(s) these receptors act to promote consolidation, given that the potential expression and physiological effects of these receptors in the BLA are broad and diverse (Ciliax et al., 2000; Qu et al., 2008; Farb et al., 2010).

In humans, some studies indicate that blocking β receptors eliminates enhanced memory for emotionally arousing items, although other studies have not corroborated these findings (Cahill et al., 1994; O'Carroll et al., 1999). If β blockers have this effect, it would suggest a lack of redundancy under these conditions. This could be due to differences in arousal systems engaged by viewing aversive material versus experiencing a potentially life-threatening event, such as may occur with fear conditioning or events that can lead to the development of PTSD. Results from recent clinical trials suggest that β blockers are of limited efficacy in the prevention of PTSD (Stein et al., 2007; McGhee et al., 2009; Nugent et al., 2010). Our results suggest that combined D_5/β_2 blockade might be more efficacious.

FIGURE LEGENDS

Figure 1.1. $D_{1/5}$-dopaminergic signaling is redundant with β_2-adrenergic signaling for fear memory consolidation. (***A***) General time line for fear memory experiments, indicating that drugs were administered immediately after conditioning and testing was performed the next day. More extensive pretraining handling was performed for CNS infusion experiments. For this figure, conditioning was with intense shock (1 mA). (***B - E***) A $D_{1/5}$ receptor antagonist (SCH 23390 or ecopipam) was injected intraperitoneally. (***B***) Cued fear test. $P = 0.0001$ for the main effect of dose; $P < 0.0001$ for the main effect of genotype; and $P = 0.0004$ for the interaction of dose and genotype (6/group). (***C***) Contextual fear test. The β_1-adrenergic receptor agonist xamoterol (3 mg/kg) was administered 60 min before testing contextual fear in *Dbh*$^{-/-}$ mice to rescue their contextual memory retrieval deficit (Murchison et al., 2004). $P = 0.027$ for the main effect of treatment; $P = 0.005$ for the main effect of genotype; and $P = 0.039$ for the interaction of treatment and genotype (6/group). (***D***) Cued fear test. $P = 0.0006$ for the main effect of dose; $P < 0.0001$ for the main effect of genotype; and $P = 0.0003$ for the interaction of dose and genotype (5-8/group). (***E***) Instrumental fear test. Main effects and their interaction were not significant (7-8/group). (***F, G***) Cued fear test. The $D_{1/5}$ antagonist SCH 23390 (30 µg/kg) was administered to wild-type mice either in saline (- or 0) or in combination with a β-adrenergic receptor antagonist. (***F***) The β antagonists employed were either the non-selective β blocker (-)-propranolol (β), the β_1-selective blocker CGP 20712A (β_1), or the β_2-selective blocker ICI 118,551 (β_2), each at 1 mg/kg. $P = 0.0005$ for the main effect of treatment (6-9/group). (***G***) ICI tested at lower doses in combination with SCH (30 µg/kg). $P = 0.003$ for the main effect of dose (6-12/group). (***H***) SCH (30 µg/kg) was administered with the β_2 agonist procaterol. $P = 0.0008$ for the main effect of dose (6/group).

Figure 1.2. Redundancy for fear memory consolidation also occurs in rats. Experimental design was as depicted in Fig. 1*A* using intense shock (1.7 mA for rats). Rats were treated with either saline (0), SCH, ICI or the combination. $P < 0.0001$ for the main effect of treatment (4-8/group).

Figure 1.3. BLA is the locus of redundant signaling in fear memory consolidation. Experimental design was as depicted in Fig. 1*A* using intense shock (1 mA for mice). (***A***) Drugs were infused bilaterally into the basolateral amygdala (BLA) of wild-type mice. $P < 0.0001$ for the main effect of treatment (5-8/group). (***B***) The combination of SCH plus ICI (each 50 ng) was infused bilaterally into the BLA or into adjacent brain regions displaced 0.75 mm in the direction indicated. $P < 0.0001$ for the main effect of treatment (5-6/group). (***C***) Injection cannula tips were located within spheres marked by the circles on the atlas drawings (Frankland and Paxinos, 1997).

Figure 1.4. The role of DA in fear memory consolidation is mediated by D_5 receptors. Experimental design was as depicted in Fig. 1*A* using intense shock (1 mA). (***A***, ***B***) Either saline (Sal) or a $D_{1/5}$ antagonist (SCH, 30 μg/kg) was administered to wild-type (WT) and β receptor knockout (KO) mice. For β_2 mice, $P = 0.016$ for the main effect of treatment; $P = 0.0044$ for the main effect of genotype; and $P = 0.0006$ for the interaction of treatment and genotype (5/group). (***C***) Sal was administered to WT and $\beta_{1,2}$ double KO mice. No significant difference was observed (5/group). (***D***, ***E***) Either Sal or a β_2 antagonist (ICI, 30 μg/kg) was administered to WT and DA receptor KO mice. For D_5 mice, $P = 0.004$ for the main effect of treatment; $P = 0.0006$ for the main effect of genotype; and $P = 0.029$ for the interaction of treatment and genotype (5-8/group).

Figure 1.5. Agonists of β_2 or D_5 receptors enhance fear memory consolidation. Experimental design was as depicted in Fig. 1*A* using moderate shock (0.4 mA). (***A***) Various doses of the β_2 agonist procaterol were injected immediately after conditioning. $P = 0.043$ for the main effect of dose (6-11/group). (***B - D***) Various doses of either a non-selective $D_{1/5}$ agonist (SKF 38393), a $D_{1/5}$ agonist that selectively activates AC (SKF 83822), or a $D_{1/5}$ agonist that selectively activates PLC (SKF 83959) were tested. $P = 0.017$ and $P = 0.0038$ for the main effect of dose for SKF 38393 and SKF 83959, respectively (8-13/group). (***E, F***) Procaterol (50 µg/kg) or $D_{1/5}$ agonist (5 mg/kg for SKF 38393 or 2 mg/kg for SKF 83959) was administered to β_2 KO and D_5 KO mice and their wild-type littermate controls. (***E***) $P = 0.0001$ for the main effect of treatment; $P = 0.013$ for the main effect of genotype; and $P = 0.015$ for the interaction of treatment and genotype (8-31/group). (***F***) $P < 0.0001$ for the main effect of treatment; $P = 0.0009$ for the main effect of genotype; and $P = 0.009$ for the interaction of treatment and genotype (5-13/group).

Figure 1.6. PLC is a critical regulator of consolidation. Experimental design was similar to that depicted in Fig. 1*A* using moderate or intense shock (0.4 or 1 mA). (***A***) The PLC agonist m-3M3FBS was infused into the BLA of wild-type mice after training with 0.4 mA. $P = 0.001$ for the main effect of dose (5/group). (***B***) The PLC inhibitor edelfosine alone or in combination with either ICI (50 ng) or SCH (50 ng) was infused into the BLA after training with 1 mA. $P < 0.0001$ for the main effect of treatment (5-7/group). (***C***) Infusion time is relative to conditioning. Cued fear testing was performed one day after training. The main effects of treatment and of time, as well as their interaction, were not significant (5/group). (***D***) Mice were fear conditioned using 0.4 mA. Saline (Sal) or edelfosine (2 ng) was then infused into the BLA, and immediately afterward either vehicle, SKF 83959 (2 mg/kg) or procaterol (50 ng/kg) was injected intraperitoneally. *P*

= 0.001 for the main effect of agonist; $P < 0.0001$ for the main effect of antagonist; and $P = 0.0027$ for the interaction of agonist and antagonist (5/group).

Figure 1.7. Redundancy between β_2 and D_5 signaling occurs via activation of PLC. Experimental design was similar to that depicted in Fig. 1*A* using moderate or intense shock (0.4 or 1 mA). (***A***) Wild-type mice were injected intraperitoneally with either vehicle, SKF 83959 (2 mg/kg) or procaterol (50 ng/kg) and sacrificed 30 min later. Punches from the BLA were assayed for IP_3 (pmol/mg protein). $P = 0.045$ for the main effect of agonist (10/group). (***B***) □$_2$ KO mice were treated as described in panel A. $P = 0.044$ for the main effect of agonist (9-10/group). (***C***) Mice were treated as indicated and sacrificed 30 min later. Shock intensity was 1 mA for the pseudo- and classical-conditioned groups. The classical-conditioned group exhibited significantly higher IP_3 levels in the BLA relative to the pseudo-conditioned ($P = 0.033$) and naïve ($P = 0.016$) groups. $P = 0.021$ for the main effect of conditioning (6-10/group). (***D***) Mice were handled as in (*C*) and sacrificed at the times indicated. Main effects and their interaction were not significant (5-7/group). (***E***) Mice were fear conditioned using 1 mA and then injected intraperitoneally with either Sal, SCH (30 □g/kg), ICI (30 □g/kg) or the combination of SCH and ICI, and sacrificed 30 min later. Only combined treatment caused a significant reduction in conditioning-induced IP_3 levels in the BLA. $P = 0.039$ for the main effect of treatment (21/group).

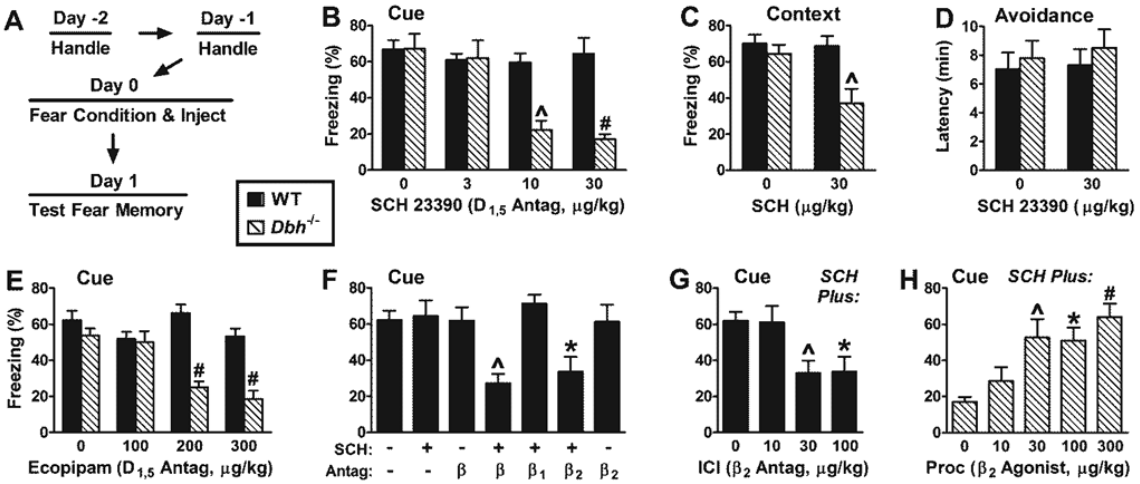
Figure 1.8. β_2 activation of PLC in the BLA is mediated by $G_{i/o}$. Experimental design was similar to that depicted in Fig. 1*A* using moderate or intense shock (0.4 or 1 mA). (***A***) Pertussis toxin (PTx, 1 ng) or Sal was infused into the BLA 3 days before training. Immediately after training with 1 mA, either Sal, SCH (50 ng) or ICI (50 ng) was infused into the BLA. $P = 0.0002$ for the main effect of pretreatment; $P < 0.0001$ for the main effect of treatment; and $P < 0.0001$ for the interaction of pretreatment and treatment

(5/group). (***B***) Sal or PTx (1 ng) was infused into the BLA 3 days before training. Immediately after training with 1 mA, either Veh, SKF 83959 (2 mg/kg) or procaterol (50 μg/kg) was injected intraperitoneally. $P = 0.018$ for the main effect of pretreatment; $P = 0.0009$ for the main effect of treatment; and $P = 0.014$ for the interaction of pretreatment and treatment (5-10/group).

Figure 1.9. Schematic diagram of functional redundancy. DA and NE are functionally redundant for fear memory consolidation in the BLA due to activation of PLC by D_5-$G_q\alpha$ and β_2-$G_{i/o}\beta\gamma$ receptor signaling. Whether downstream signaling activated by the second messengers IP_3/Ca^{2+}, diacylglycerol (DAG) or both is required is currently unclear, but both are likely required for activating a conventional isozyme of protein kinase C that is implicated in fear memory consolidation (Weeber et al., 2000). The cell type(s) in which this signaling occurs has yet to be defined. Phosphatidylinositol 4,5-bisphosphate (PIP_2) is a substrate of PLC. The endoplasmic reticulum (ER) stores Ca^{2+} for cytosolic release that is induced by IP_3.

LIST OF FIGURES

Figure 1.1



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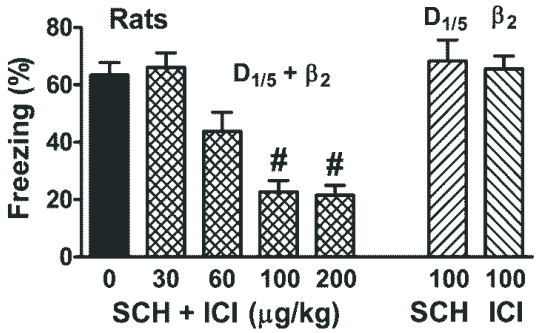

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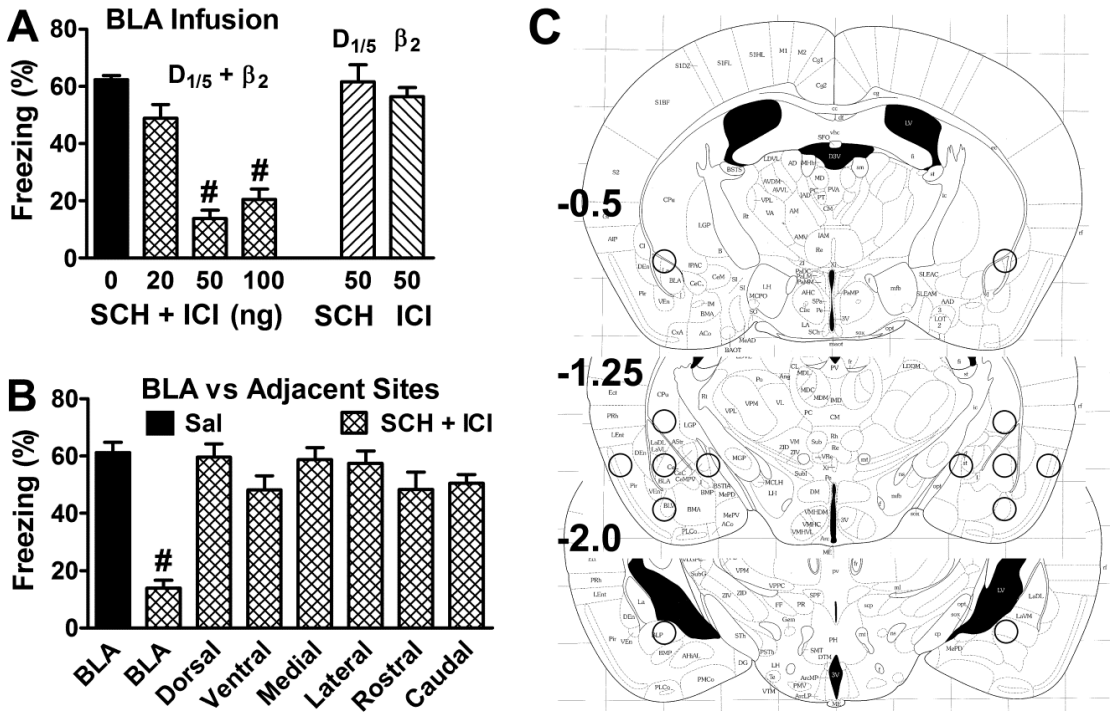

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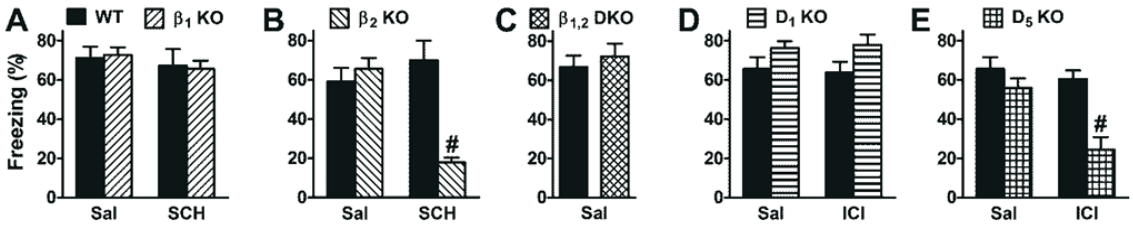

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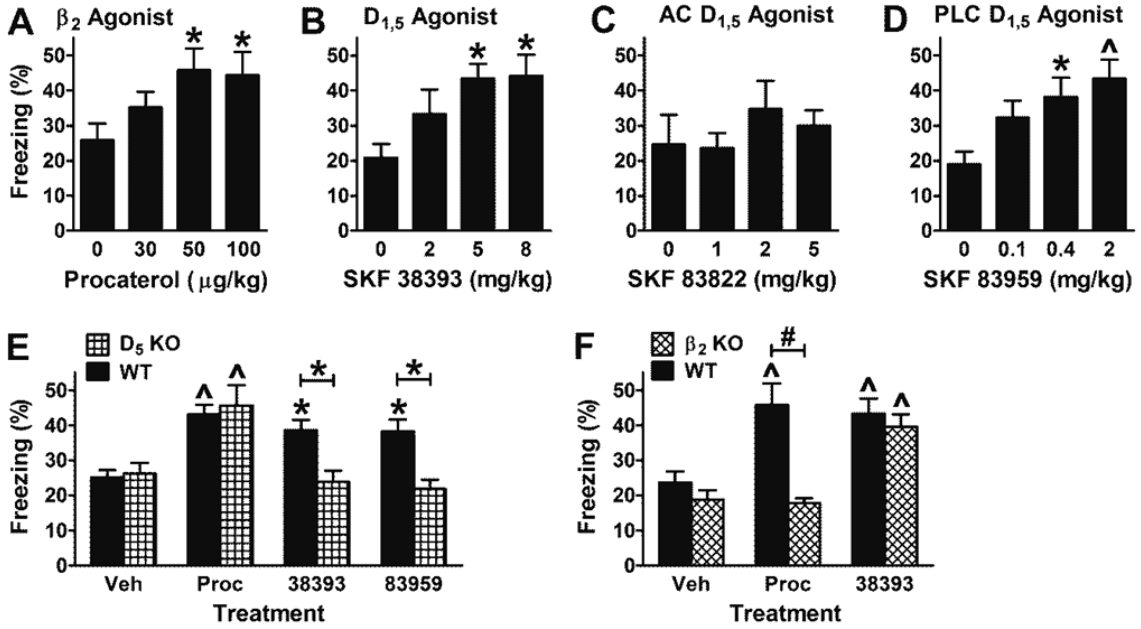
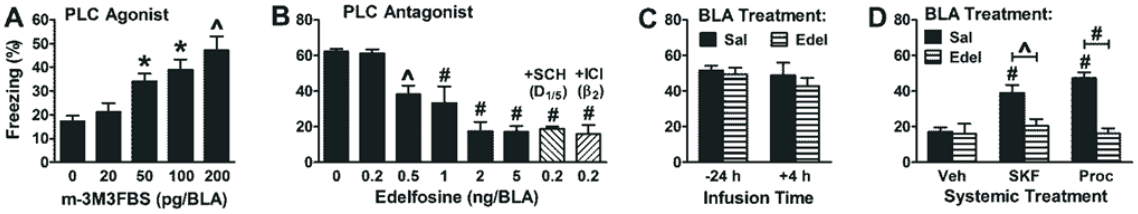


Figure 1.6



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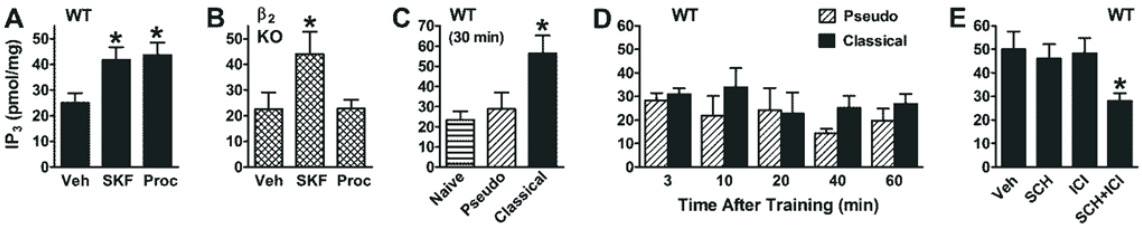

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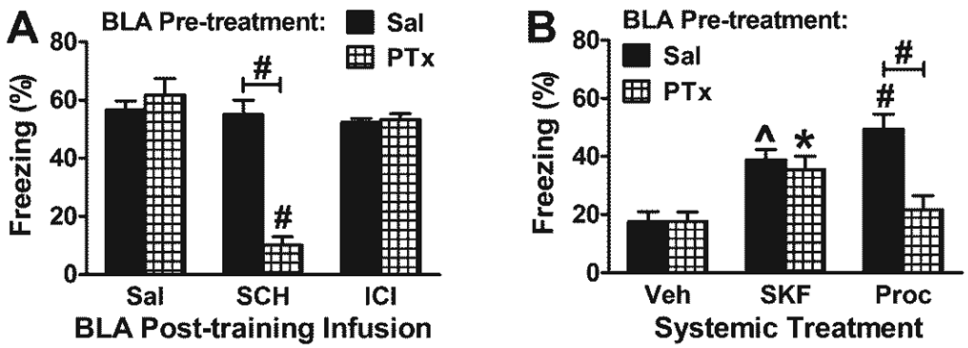
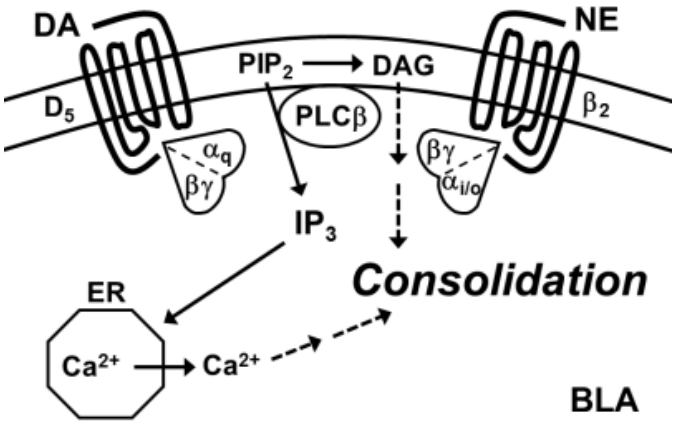


Figure 1.9



CHAPTER 3: M_1-MUSCARINIC RECEPTORS PROMOTE FEAR MEMORY CONSOLIDATION VIA PHOSPHOLIPASE C AND THE M-CURRENT

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ABSTRACT

Neuromodulators released during and after a fearful experience promote the consolidation of long-term memory for that experience. Because over-consolidation may contribute to the recurrent and intrusive memories of post-traumatic stress disorder, neuromodulatory receptors provide a potential pharmacological target for prevention. Stimulation of muscarinic receptors promotes memory consolidation in several conditioning paradigms, an effect primarily associated with the M_1 receptor (M_1R). Conversely, neither inhibiting nor genetically disrupting M_1R impairs the consolidation of cued fear memory. Using the M_1R agonist cevimeline and antagonist telenzepine, as well as M_1R knockout mice, we show here that M_1R, along with β_2-adrenergic (β_2AR) and D_5-dopaminergic (D_5R) receptors, regulates the consolidation of cued fear memory by redundantly activating phospholipase C (PLC) in the basolateral amygdala (BLA). We also demonstrate that fear memory consolidation in the BLA is mediated in part by neuromodulatory inhibition of the M-current, which is conducted by KCNQ channels and is known to be inhibited by muscarinic receptors. Manipulating the M-current by administering the KCNQ channel blocker XE991 or the KCNQ channel opener retigabine reverses the effects on consolidation caused by manipulating β_2AR, D_5R, M_1R and PLC. Finally, we show that cyclic AMP and protein kinase A (cAMP/PKA) signaling relevant to this stage of consolidation is upstream of these neuromodulators and PLC, suggesting an important presynaptic role for cAMP/PKA in consolidation. These results support the idea that neuromodulatory regulation of ion channel activity and neuronal excitability is a critical mechanism for promoting consolidation well after acquisition has occurred.

INTRODUCTION

Long-term consolidation of fear memory in the basolateral amygdala (BLA) depends on the activity-dependent induction of intracellular signaling pathways that promote gene expression and protein synthesis (Johansen et al., 2011). Regulation of these signaling pathways by G protein-coupled neuromodulatory receptors can affect the strength of fear memory consolidation, a process that may underlie the recurrent and intrusive memories of post-traumatic stress disorder (PTSD). Traditionally, these effects on consolidation have been attributed to signaling activated by G_s proteins (Sara, 2009; Tully and Bolshakov, 2010; Johansen et al., 2011). However, while consolidation of fear memory can be enhanced by pharmacological activation of neuromodulatory receptors in the BLA, it is not reliably blocked by antagonism of individual receptors.

Recently it has been proposed that, rather than a single neuromodulatory system, a combination of neuromodulatory systems coupled to phospholipase C (PLC) redundantly mediates consolidation in the BLA (Ouyang et al., 2012). Activation of either the $G_{i/o}$-coupled β_2-adrenergic receptor (β_2AR) or the $G_{q/11}$-coupled D_5-dopaminergic receptor (D_5R) enhances consolidation; however, both receptors must be blocked to impair consolidation. Further, β_2AR and D_5R in the BLA redundantly activate PLC as an initial signaling mechanism that is necessary for consolidation.

Based on the observation that PLC, and neuromodulatory receptors that control its activity, can regulate fear memory consolidation, we asked whether other $G_{q/11}$-coupled receptors contribute to the redundant relationship of β_2AR and D_5R. In this study, we investigated a potential role for $G_{q/11}$ protein-coupled muscarinic receptors. Release of acetylcholine (ACh) increases following the presentation of fearful stimuli (Acquas et al., 1996), and cholinergic neurons in the basal forebrain project to the BLA (Rao et al., 1987; Kordower et al., 1989), where muscarinic agonists initiate PLC-dependent

intracellular calcium release (Power and Sah, 2007), increase the excitability of neurons (Womble and Moises, 1992, 1993), and enhance fear memory consolidation (Vazdarjanova and McGaugh, 1999; Barros et al., 2002).

Both M_1- and M_3-muscarinic receptors couple to $G_{q/11}$ and are expressed in the BLA; however, in situ hybridization suggests that the M_1 receptor (M_1R) is most abundant (Buckley et al., 1988). Activation of M_1R in the BLA enhances consolidation of inhibitory avoidance and contextual fear memory (Vazdarjanova and McGaugh, 1999; Power et al., 2003). However, studies employing M_1R-selective antagonists or M_1R-deficient mice report no impairments in cued fear memory (Fornari et al., 2000; Anagnostaras et al., 2003). Given that stimulation of M_1R in the BLA enhances consolidation without being required (Robinson et al., 2011), we hypothesized that M_1R redundantly contributes to the consolidation of fear memory.

Here, we use pharmacological, genetic and biochemical approaches in mice to demonstrate that M_1R redundantly regulates the consolidation of fear memory with β_2AR and D_5R by activating PLC. Further, we examine whether the muscarine-sensitive M-current in the BLA is a downstream target of PLC activation that is important for fear memory consolidation. Finally, we examine the relationship between the role of cyclic AMP and protein kinase A (cAMP/PKA) signaling in consolidation and that for the neuromodulatory signaling coupled to PLC.

MATERIALS AND METHODS

Animals

Stock *Chrm1*$^{-/-}$ (M_1R knockout) and wild-type (WT) mice on a C57BL/6 background were from the Jackson Laboratory and bred at the University of Pennsylvania (Gerber et

al., 2001). All other mice were on a hybrid 129/Sv x C57BL/6 background and bred locally. M_1R knockout (KO) mice were generated by mating heterozygotes or homozygotes, and genotype was determined by PCR. Animals were maintained on *ad libitum* food and water and a 12 h light/dark cycle, with lights on beginning at 7:00 A.M. Mice were 3-6 months old when tested and of either sex. No significant differences were found by sex or parental genotype, so data were combined. Studies were performed during the light phase, with most experiments taking place between 9:00 A.M. and 5:00 P.M. Studies were in accordance with NIH guidelines and had the approval of the Institutional Animal Care and Use Committee at the University of Pennsylvania.

Classical fear conditioning

Animals were habituated to handling and drug administration for two (systemic injection) or four (BLA infusion) days prior to behavioral experimentation. On habituation days, animals were handled for 4 min, and either injected with vehicle (systemic experiments) or given a simulated infusion (infusion experiments). Animals were then placed in individual plastic holding buckets (12 cm diameter) with bedding and lids for 30–60 min. Prior to behavioral experimentation, animals were held in the buckets for 30–60 min. For conditioning, animals were placed in the training apparatus (ENV-010MC with ENV-414S, Med Associates) for 2 min, after which an 84 dB, 4.5 kHz tone was activated for 30 s that co-terminated with a 2 s footshock (moderate = 0.4 mA or strong = 1 mA). Animals were removed from the apparatus and injected or infused 30 s after shock, and then returned to the home cage. The apparatus was cleaned with Versa-Clean (Thermo Fisher Scientific) between subjects. Individual subjects were tested for either contextual or cued fear memory, but not both, the day after training. Contextual fear was tested for 5 min in the conditioning apparatus in the absence of the tone. Cued fear was tested in a Plexiglas cylinder (21 cm diameter, 24 cm tall) with

green wire grid floor and vertical green and white wall stripes 240° around that was cleaned with lemon-scented Ajax between subjects. After 2 min, the training tone was turned on for 3 min. Percent freezing was estimated by scoring the presence or absence of nonrespiratory movement every 5 s.

BLA infusions

Two guide cannulae mounted on a base plate (C315GS system, Plastics One) were implanted under pentobarbital anesthesia (72.5 mg/kg) using a stereotax (SAS75/EM40M, Cartesian Research). The guides were placed 1.25 mm posterior to bregma and 3.5 mm bilateral. The guide and dummy cannulae projected 3 mm below the base plate. Habituation of the animals to the investigator and the infusion procedure began a couple of days later with a 4 min handling session followed by 3 min of immobilization (gently holding the nape of the neck and body) that mimicked infusion. Handling sessions were conducted on each of the 4 d preceding training and once more 1 h before training. Immediately after training, mice were infused bilaterally using injection cannulae that extended 2.8 mm below the tip of the guide cannulae. All BLA infusions were 0.2 µl/BLA at 0.08 µl/min, and infusion cannulae were left in place for 30 s after infusion.

Drugs

Cevimeline HCl, telenzepine 2HCl, SCH 23390 HCl, ICI 118,551 HCl, XE991 2HCl, SKF 83959 HBr, procaterol HCl (all Tocris Bioscience), retigabine 2HCl (Ryan Scientific), ±3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP, Abcam), Sp-8-Br-cAMPS (Sp8, Santa Cruz Biotechnology) and myr-PKI[14-22]amide (PKI, Invitrogen) were administered intraperitoneally or infused into the BLA immediately after training. Drugs were dissolved in 0.9% saline (SKF 83959 contained 0.1 mg/ml ascorbic acid and

< 1% DMSO, pH 7.4, Sigma). Vehicle was saline with or without 0.1 mg/ml ascorbic acid and < 1% DMSO. Systemic injection volumes were 10 µl/g body weight.

IP_3 levels

Mice were anesthetized with CO_2, killed by cervical dislocation and brains were rapidly removed, frozen in 2-methylbutane on dry ice and stored at −80°C. Two frozen coronal sections (400 µm) that contained the BLA were cut by cryostat (HM505E, Microm) from each mouse, and a 0.5 mm diameter punch of BLA tissue was collected bilaterally from each slice. The four punches per mouse were pooled and homogenized on ice with three 2 s pulses (5 s interval) in 125 µl of 4% perchloric acid using a Sonic Dismembrator 100 set on level 3 (Thermo Fisher Scientific). After 15 min on ice, samples were stored overnight at −80°C. The next day samples were centrifuged at 4°C and 2000 × *g* for 15 min, and the pellet was stored at −80°C for subsequent Bradford assay to determine total protein. Supernatants were neutralized on ice with 10 M KOH (to precipitate the perchloric acid) and centrifuged at 4°C and 2000 × *g* for 15 min. Supernatant (100 µl) was then used in the [^{3}H]-IP_3 radioreceptor assay (PerkinElmer) according to instructions.

Statistics

Data were analyzed with Statistica 9.1 (StatSoft, Tulsa, OK) using one- or two-way ANOVA with alpha = 0.05. The Bartlett *Chi*-square test was employed to analyze homogeneity of variances. Post-hoc comparisons were made using the Newman-Keuls test. Data are presented as mean ± standard error. For all figures, * indicates $P < 0.05$, ^ indicates $P < 0.01$, and # indicates $P < 0.001$. Comparisons marked as significant are to the reference group except where indicated.

RESULTS

Activating M_1R in the BLA enhances fear memory consolidation

To determine whether signaling by M_1R influences the consolidation of classical auditory fear conditioning, we explored whether immediate post-training administration of cevimeline (Cev), an M_1R-selective agonist, affected freezing in response to the training tone the following day. Mice were trained with a moderate shock intensity (0.4 mA) that elicits relatively low levels of freezing in response to the training tone. Systemic injection of Cev caused a dose-dependent increase in freezing in response to the tone during testing (Fig. 2.1*A*). These data suggest that signaling by M_1R can enhance cued fear memory consolidation.

Given that the BLA is a crucial site for fear memory consolidation (Pape and Pare, 2010; Ouyang et al., 2012), we next tested whether the effects of systemically injected Cev could be replicated by infusing it into the BLA immediately after moderate conditioning. Compared to vehicle-treated controls, mice given BLA infusions of Cev exhibited dose-dependent increases in freezing to the training tone during testing (Fig. 2.1*B*). These data suggest that the consolidation enhancement observed after systemic injection of Cev is mediated by the BLA.

Cev potently activates M_1R and, to a lesser extent, M_3- and M_5-muscarinic receptors (Heinrich et al., 2009). To determine whether the enhancing effects of Cev on consolidation require M_1R, we tested the effect of Cev on mice congenitally lacking M_1R. M_1R KO mice injected with Cev (1 mg/kg) immediately after moderate fear conditioning did not exhibit a significant increase in freezing in response to the training tone during testing, while WT littermates exhibited enhancements similar to those described earlier (Fig. 2.1*C*). These data indicate that the consolidation enhancements observed in response to post-training administration of Cev are mediated specifically by M_1R.

M_1R redundantly modulates consolidation with D_5R and β_2AR

The $G_{q/11}$ protein coupled to M_1R activates PLC (Caulfield, 1993), whose activation by β_2AR and D_5R in the BLA promotes consolidation (Ouyang et al., 2012). To determine whether M_1R redundantly regulates fear memory consolidation with β_2AR and D_5R, we systemically administered the M_1R antagonist telenzepine (Tzp) alone or in combination with an antagonist of β_2AR or D_5R (Schudt et al., 1988).

When combined with the $D_{1,5}$R antagonist SCH 23390 (SCH; 30 µg/kg) after conditioning with strong (1 mA) footshock, Tzp dose-dependently decreased the amount of freezing mice exhibited in response to the training tone during testing the next day (Fig. 2.2*A*). The smallest maximally effective dose of Tzp, when combined with SCH, was 1 mg/kg. Neither Tzp nor SCH significantly affected freezing when administered alone. These data suggest that M_1R and D_5R redundantly contribute to signaling mechanisms required for fear memory consolidation.

Given that the β_2AR antagonist ICI 118,511 (ICI) also inhibits consolidation only when co-administered with SCH immediately after training (Ouyang et al., 2012), we next examined whether Tzp inhibits consolidation when combined with ICI. Mice injected with a combination of Tzp (1 mg/kg) and ICI (30 µg/kg) immediately after strong fear conditioning exhibited significantly less freezing in response to the training tone the following day compared to saline- or ICI-treated controls (Fig. 2.2*B*). Further, co-administering all three antagonists (Tzp, ICI and SCH) inhibited consolidation more effectively than Tzp+ICI treatment. Given these observations, it is plausible that M_1R contributes to consolidation with β_2AR and D_5R by converging on a common signaling mechanism in the BLA.

To determine the effect of Tzp in the BLA, we infused Tzp either alone or in combination with SCH or ICI (both at 50 ng/BLA), the latter of which affect consolidation when delivered together but not alone (Ouyang et al., 2012). Mice infused with Tzp into the BLA immediately after strong fear conditioning exhibited dose-dependent decreases in freezing in response to the training tone the next day, but only when Tzp was combined with either SCH or ICI (Fig. 2.2*C*). Infusion of Tzp alone at the lowest maximally effective dose (1 µg/BLA) from combination treatment did not affect consolidation (Fig. 2.2*C*). These data suggest that M_1R signaling important for consolidation of cued fear memory occurs in the BLA.

To confirm that M_1R mediates the impairing effects of Tzp described above, we examined whether M_1R KO mice exhibit impairments in response to post-training administration of β_2AR or D_5R antagonists. As previously reported (Anagnostaras et al., 2003), vehicle-treated M_1R KO and WT mice exhibited comparable freezing to the training tone (Fig. 2.2*D*). However, mice lacking M_1R exhibited significantly impaired consolidation in response to either ICI or SCH treatment that was not observed in WT littermates. These data further support the idea that M_1R signals redundantly with β_2AR and D_5R to mediate cued fear memory consolidation.

M_1R redundantly activates PLC with β_2AR and D_5R

Co-administration of β_2AR and D_5R antagonists immediately after fear conditioning impairs consolidation, and it also blocks PLC activation in the BLA that occurs 30 min after conditioning (Ouyang et al., 2012). Given that M_1R activates PLC (Caulfield, 1993) and also increases IP_3-dependent calcium release in the BLA (Power and Sah, 2007), we asked whether the behaviorally relevant Tzp and Cev treatments described above (Fig. 2.1 and 2.2) influence PLC activity in the BLA. PLC hydrolyzes the membrane phospholipid phosphatidylinositol-bisphosphate (PIP_2), generating the second

messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Thus we measured levels of IP_3 as a means of assessing PLC activity.

IP_3 levels in the BLA increase 30 min after systemic injection of a β_2AR or D_5R agonist (Ouyang et al., 2012). Here, we observed that systemically injecting a dose of Cev that enhances consolidation also increases IP_3 levels in the BLA (Fig. 2.3*A*). This observation is consistent with the hypothesis that M_1R contributes to consolidation by activating PLC in the BLA. To determine whether PLC activation by M_1R might be important for its role in consolidation, we examined how the M_1R antagonist Tzp affects IP_3 levels in the BLA 30 min after strong fear conditioning (Ouyang et al., 2012). Immediate post-training injection of Tzp had no effect on the increase in IP_3 levels at 30 min compared to vehicle-treated controls (Fig. 2.3*B*). However, IP_3 levels were significantly decreased when Tzp treatment was combined with either SCH or ICI, the latter of which have no effect on IP_3 levels when administered alone (Ouyang et al., 2012). These data suggest that M_1R contributes to fear memory consolidation by redundantly activating PLC with β_2AR and D_5R.

KCNQ potassium channels modulate fear memory consolidation in the BLA

Agonists of muscarinic receptors increase the excitability of neurons in the BLA by inhibiting the M-current (Womble and Moises, 1992, 1993), which is observed in several brain structures important for learning (Halliwell and Adams, 1982; Shen et al., 2005; Santini and Porter, 2010). The M-current is a voltage-dependent potassium current conducted through non-inactivating KCNQ channels that become active between resting membrane potential and action potential threshold (Brown and Adams, 1980). When active, these channels suppress depolarization by EPSPs and promote spike accommodation by enhancing the afterhyperpolarization (Brown and Yu, 2000). Importantly, M-current conductance by KCNQ channels depends on sufficient levels of

PIP_2, which is hydrolyzed by PLC (Suh and Hille, 2002; Suh et al., 2006; Telezhkin et al., 2012).

Given that activating M_1R and other neuromodulatory receptors coupled to PLC enhances fear memory consolidation (Ouyang et al., 2012), we asked whether directly inhibiting the M-current with the KCNQ channel blocker XE991 would have a similar effect. Systemically injecting mice with XE991 immediately after moderate fear conditioning caused a dose-dependent enhancement of consolidation (Fig. 2.4*A*). As with Cev, directly infusing XE991 into the BLA enhanced consolidation (Fig. 2.4*B*). These data suggest that inhibition of the M-current in the BLA increases the strength of fear memory consolidation.

Having observed that pharmacologically blocking the M-current in the BLA enhances consolidation, we next explored whether enhancing the M-current would inhibit consolidation. To do this, we examined whether retigabine (Ret), a drug that maintains the open-state of KCNQ channels (Tatulian et al., 2001; Wuttke et al., 2005), would inhibit consolidation. Administering Ret immediately after strong fear conditioning, either by systemic injection or BLA infusion, dose-dependently inhibited freezing in response to the training tone the following day (Fig. 2.4*C* and 2.4*D*).

So far our experiments have addressed the roles of PLC-coupled neuromodulators and the M-current in cued fear memory consolidation. However, we hypothesized that our findings in the BLA would apply to classically conditioned fear memory in general. To test this, we repeated two of our pharmacological experiments, but instead examined contextual fear memory one day after training. Post-training infusion of either Tzp+ICI or Ret into the BLA impaired the consolidation of contextual fear memory (Fig. 2.4*E*), demonstrating that consolidation of hippocampus-dependent fear memory is also

sensitive to the impairing effects of dual neuromodulator blockade or augmentation of the M-current in the BLA.

Regulation of consolidation by PLC and PLC-coupled neuromodulators requires normal KNCQ channel activity

To test whether the effects of pharmacological manipulation of β_2AR, D_5R and M_1R are mediated by downstream effects on the M-current, we examined whether the impairing effects of co-antagonist treatment could be reversed by co-administration of the M-current blocker XE991. XE991 alone had no effect on consolidation when administered immediately after strong fear conditioning (Fig. 2.5*A*). However, XE991 rescued consolidation from the impairing effects of Tzp+SCH and SCH+ICI. These data suggest that inhibition of the M-current is sufficient for the enhancement of consolidation mediated by β_2AR, D_5R and M_1R.

Next, we asked whether potentiating the M-current with Ret could block the enhancing effects of β_2AR, D_5R and M_1R agonists on consolidation. Ret alone had no effect on consolidation when administered immediately after moderate fear conditioning (Fig. 2.5*B*). However, Ret completely blocked the enhancements of consolidation elicited by agonists of M_1R, β_2AR and D_5R. These data suggest that the ability of PLC activation by M_1R, β_2AR and D_5R to promote consolidation requires inhibition of the M-current.

To determine whether the effects of PLC activation on consolidation in the BLA are indeed mediated through inhibition of the M-current, we challenged the impairing effects of direct PLC inhibition with M-current blockade by XE991. As previously reported (Ouyang et al., 2012), infusing the PLC inhibitor edelfosine into the BLA immediately after strong fear conditioning impaired consolidation (Fig. 2.5*C*). However,

co-infusion with XE991 prevented this impairment, suggesting that the lack of consolidation observed in response to PLC inhibition is due to a failure to close KCNQ channels. Conversely, enhancement of consolidation by infusing the PLC activator m-3M3FBS (3M3) was inhibited by co-administration of the KCNQ channel opener Ret (Fig. 2.5*D*). These data further support the notion that PLC promotes consolidation in large part by closing KCNQ channels and inhibiting the M-current.

Finally, we examined whether pharmacologically manipulating KCNQ channels would influence a distinct mechanism relevant to consolidation: that mediated by glutamatergic NMDA (GluN) receptor ion channels. Pre-training administration of antagonists or blockers of GluN impairs acquisition/consolidation of cued fear memory (Rodrigues et al., 2001; Bauer et al., 2002; Goosens and Maren, 2004). We first confirmed that injection of the competitive GluN antagonist ±3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP; 10 mg/kg) 1 h before strong fear conditioning decreases freezing in response to the training tone the following day (Fig. 2.5*E*). Consistent with a critical role for activation of GluN during acquisition, the effect of CPP was greatly reduced when administered immediately after conditioning. Interestingly, immediate post-training injection of XE991 partially rescued the impairing effect of pre-training CPP injection, while concurrent pre-training administration of CPP+XE991 resulted in even greater but incomplete rescue. These results suggest that KCNQ channels may also contribute to some of the earliest post-acquisition events that promote consolidation of fear memory.

Receptors that regulate PLC and KCNQ channels influence consolidation downstream of cAMP/PKA.

Results from prior studies indicate a critical role for cAMP/PKA-dependent signaling in the BLA during fear memory consolidation (Schafe and LeDoux, 2000). Given the

prominent role for neuromodulator-regulated PLC activity in consolidation, we explored the relationship between such signaling and that for cAMP/PKA. We first examined the enhancement of consolidation mediated by administration of the membrane-permeable cAMP analog Sp-8-Br-cAMPS (Sp8). Sp8 was infused into the BLA immediately after moderate fear conditioning. As expected, Sp8 enhanced cued fear memory consolidation (Fig. 2.6*A*). Interestingly, co-administration of Tzp+ICI prevented Sp8-induced consolidation enhancement, suggesting that cAMP signaling is upstream of M_1R/β_2AR activation. Given this observation, we asked whether augmenting cAMP signaling would rescue the consolidation impairment elicited by these receptor antagonists. Sp8 and Tzp+ICI were co-administered after strong fear conditioning. The consolidation impairment by Tzp+ICI was not rescued by Sp8 (Fig. 2.6*B*), further supporting the idea that an important effect of cAMP on consolidation is upstream of M_1R/β_2AR signaling.

Complementary to the above, we confirmed the requirement for cAMP/PKA signaling in consolidation by infusing the PKA inhibitor PKI immediately after strong fear conditioning (Fig. 2.6*C*). Interestingly, co-administration of either the M_1R agonist Cev, the PLC agonist 3M3, or the KCNQ channel blocker XE991 rescued the impairment of consolidation induced by PKI. Additionally, PKI did not block the enhancement of consolidation induced by Cev (Fig. 2.6*D*). Taken together, these data indicate that an important role for cAMP/PKA signaling in fear memory consolidation is upstream of neuromodulatory receptor activation that is coupled to PLC and the M-current.

DISCUSSION

Most research into the role of M_1R in fear memory consolidation suggests that stimulation of M_1R enhances but is not required for consolidation (Robinson et al., 2011).

To address this seeming inconsistency, the present study explored whether M_1R contributes to the redundant activation of PLC by β_2AR and D_5R in the BLA that is necessary for consolidation (Ouyang et al., 2012). We show here that an M_1R-selective antagonist does not inhibit fear memory consolidation in the BLA unless co-administered with an antagonist of either β_2AR or D_5R. We also demonstrate that antagonists of β_2AR and D_5R impair consolidation in M_1R KO but not WT mice. These data support the existence of redundant signaling by M_1R, β_2AR and D_5R in fear memory consolidation. Further, we show that consolidation enhancement induced by an M_1R agonist is lost in M_1R KO mice, indicating that other $G_{q/11}$-coupled muscarinic receptors, such as M_3R, are not sufficient for the role of muscarinic receptors in promoting consolidation (Caulfield, 1993).

β_2AR and D_5R stimulation elevates BLA IP_3 and contributes to the increase in IP_3 observed 30 min after conditioning (Ouyang et al., 2012). Here, we show that activation of M_1R also elevates BLA IP_3, and that M_1R antagonism, when combined with that for either β_2AR or D_5R, inhibits conditioning-induced IP_3 increases. These observations suggest an important role for activation of BLA PLC by M_1R during consolidation. Anticipated mechanisms through which PLC regulates consolidation include those mediated by IP_3 and DAG generated via hydrolysis of PIP_2. IP_3 enhances intracellular calcium release that, together with DAG, could drive a number of relevant signaling pathways, including protein kinase C (PKC) and calcium- and calmodulin-dependent protein kinase II (Miller et al., 2002; Bonini et al., 2005). Indeed, activation of muscarinic receptors induces intracellular calcium release in BLA neurons (Power and Sah, 2007). However, broad electrophysiological effects of IP_3R antagonists (Ozaki et al., 2002) preclude pharmacological characterization of behavioral roles for IP_3-dependent

mechanisms. Alternatively, conditional genetic approaches related to IP_3 signaling should be insightful (Chen et al., 2012).

In addition to the generation of IP_3 and DAG, breakdown of PIP_2 by PLC can affect the activity of a variety of ion channels (Suh and Hille, 2008; Logothetis et al., 2010). We examined KCNQ potassium channels here because they are inhibited by muscarinic agonists in the BLA (Womble and Moises, 1992, 1993), their inhibition by M_1R is well-characterized (Suh and Hille, 2002; Kosenko et al., 2012), and selective KCNQ channel modulators have been validated in subunit KO mice (Tzingounis and Nicoll, 2008). Our experiments demonstrate that pharmacologically modulating the M-current in the BLA strongly affects consolidation of fear memory.

Inhibiting the M-current increases neuronal excitability by diminishing the afterhyperpolarization and reducing spike accommodation (Aiken et al., 1995; Peters et al., 2005). Our data and recent reports that XE991 enhances learning and memory (Santini and Porter, 2010; Fontan-Lozano et al., 2011) support the hypothesis that increased neuronal excitability following acquisition promotes consolidation (Giese et al., 2001). Of special note, increased firing rates have been observed in cat BLA neurons 30-50 min after inhibitory avoidance conditioning (Pelletier et al., 2005), a time interval similar to the period over which conditioning-induced increases in BLA IP_3 occur (Ouyang et al., 2012).

The M-current limits membrane depolarization by excitatory postsynaptic potentials (EPSPs), and thus reduction in the M-current could play a role in shaping EPSPs induced by fear conditioning (George et al., 2009; Shah et al., 2011). Increased excitability induced by KCNQ blockade may also enhance activation of L-type voltage-gated calcium channels that promote consolidation in the BLA (Bauer et al., 2002;

Shinnick-Gallagher et al., 2003; McKinney and Murphy, 2006). Interestingly, enhanced excitability during the consolidation period that is sensitive to M-current manipulation also may result from internalization of $GABA_A$ receptor subunits, suggesting that enhanced excitability may have multiple mechanisms and be of general importance (Chhatwal et al., 2005; Mou et al., 2011).

The observation that modulating BLA KCNQ channel activity can completely reverse the effects on consolidation of PLC manipulation suggests that consolidation may not require effects of IP_3 and DAG on targets other than the M-current, at least around 30 min after conditioning. PKC, which can be activated by elevated calcium secondary to IP_3 and/or by DAG, may also be required for fear memory consolidation. When infused into the BLA, an inhibitor of the PKCα and PKCβ isozymes impairs consolidation of inhibitory avoidance memory (Bonini et al., 2005), and a role for BLA PKC in the maintenance of fear memory is likely (Serrano et al., 2008). Among PKC isozymes, PKCβ but not PKCγ or PKCδ appears to be critical because only PKCβ KO mice exhibit deficits in cued fear memory (Abeliovich et al., 1993; Weeber et al., 2000; Selcher et al., 2002). While specific roles and mechanisms for PKC in fear memory have not been delineated, PKC could contribute to consolidation in parallel with PIP_2 depletion by facilitating suppression of the M-current (Hoshi et al., 2003).

Notably, other ion channels that regulate excitability can be modulated by PLC activity. Decreases in PIP_2 inhibit voltage-dependent calcium channels, transient receptor potential channels, and inwardly rectifying potassium channels (Keselman et al., 2007; Suh and Hille, 2008). In addition, PKC can regulate the activity of multiple ion channels (Dai et al., 2009). Our observation that agonists of β_2AR, D_5R and M_1R lose their enhancing effects on consolidation when co-administered with the KCNQ channel opener retigabine suggests that modulation of other ion channels is insufficient for

neuromodulator-enhanced consolidation. Conversely, the observation that XE991 overcomes the impairment of consolidation by receptor antagonist treatment suggests that blockade of KCNQ channels is sufficient to promote neuromodulator-mediated consolidation. Based on our findings, we predict that future studies will demonstrate suppression of the M-current in the BLA by agonists of β_2AR and D_5R.

Our finding an interaction between the role of GluN and KCNQ channels in fear memory consolidation was unexpected. GluN channels are thought to contribute to acquisition and/or the beginning of memory consolidation by promoting some of the earliest signaling events that result from the convergence of conditioning and reinforcing sensory input to the BLA (Rodrigues et al., 2001; Bauer et al., 2002; Goosens and Maren, 2004). Our data are consistent with this idea. Interestingly, pre-training and, to some extent, even immediate post-training blockade of KCNQ channels considerably reduces the impairing effect of pre-training GluN antagonism on consolidation. As a potential intervening mechanism that could explain these findings, it is possible that the calcium influx mediated by GluN channels activates PLC, which in turn suppresses KCNQ channel activity that is relevant to consolidation. Isozymes PLCδ and PLCη are activated by calcium rather than G protein subunits (Delmas et al., 2004; Cockcroft, 2006), and specific roles for the coupling of GluN and PLC activity have been identified (Codazzi et al., 2006; Horne and Dell'Acqua, 2007). Mechanisms for the role of GluN in consolidation that are in addition to the suppression of the M-current are expected, and are supported by the observation that XE991 incompletely reverses the impairing effects of GluN receptor antagonism.

With respect to cAMP/PKA signaling in fear memory consolidation, our findings suggest that cAMP/PKA has an important role upstream of the neuromodulatory receptors that activate PLC. It may be that a prominent role for cAMP/PKA signaling in

consolidation is the presynaptic regulation of neurotransmitter release within the BLA approximately 30 min after conditioning, when IP_3 levels rise. Indeed, cAMP/PKA signaling plays a critical role in presynaptically expressed LTP (Castillo et al., 2002; Lonart et al., 2003; Bayazitov et al., 2007; Fourcaudot et al., 2008). Such a role may be at least partly responsible for the enhanced neurotransmitter release observed *in vivo* following conditioning in some paradigms (Tronel et al., 2004; Guzman-Ramos et al., 2010, 2012). Thus, we propose that cAMP/PKA regulates fear memory consolidation in part by augmenting the release of neuromodulators that activate and M_1R, β_2AR and D_5R. Our results do not exclude a postsynaptic role for cAMP/PKA signaling in consolidation, but do indicate that such signaling is not downstream of neuromodulator-driven PLC activity during this phase of consolidation.

In summary, we report a role for M_1R in the consolidation of fear memory as a redundant contributor to requisite PLC activity in the BLA (Fig. 2.7). We propose that PLC increases neuronal excitability in the BLA by suppressing KCNQ channel activity. Our experiments with retigabine offer the first report of an effect of this drug on learning and memory. Developed as anticonvulsants, retigabine and ezogabine were recently approved by the FDA as antiepileptics (Orhan et al., 2012). Our observation that retigabine can inhibit the consolidation of fear memory suggests that these drugs might have an additional application in preventing PTSD when given shortly after a traumatic experience.

FIGURE LEGENDS

Figure 2.1. Muscarinic stimulation promotes consolidation via M_1R. All treatments were given immediately after conditioning with moderate shock (0.4 mA). Animals were tested for retention in a novel context the following day. *(A)* The M_1R agonist cevimeline dose-dependently increases freezing in response to the training tone [$F(3,16) = 5.09$, $P = 0.012$ for the main effect of dose; n = 5/group]. *(B)* Directly infusing cevimeline into the basolateral amygdala (BLA) dose-dependently enhances freezing in response to the training tone [$F(3,16) = 8.49$, $P = 0.001$ for the main effect of dose; n = 5/group]. *(C)* Either vehicle or cevimeline (Cev, 1 mg/kg) was administered to M_1R knockout (KO) and wild-type (WT) littermate control mice. Only WT mice exhibit increases in freezing in response to drug treatment [$F(1,16) = 17.86$, $P = 0.0006$ for the main effect of treatment; $F(1,16) = 10.31$, $P = 0.0054$ for the main effect of genotype; and $F(1,16) = 4.83$, $P = 0.043$ for the interaction of treatment and genotype; n = 5/group]. *$P < 0.05$, ^$P < 0.01$.

Figure 2.2. M_1R signals redundantly with β_2AR and D_5R to promote fear memory consolidation. All treatments were administered immediately after conditioning with strong shock (1 mA). Retention testing occurred the following day in a novel context. *(A)* Tzp dose-dependently inhibits freezing to the training tone during testing when combined with SCH (30 μg/kg). Neither SCH nor Tzp affect consolidation on their own [$F(5,24) = 12.37$, $P < 0.0001$ for the main effect of treatment; n = 5/group]. *(B)* ICI (30 μg/kg) impairs consolidation only when combined with Tzp. In addition, significantly lower freezing is observed when all three receptor antagonists are combined [$F(3,20) = 30.82$, $P < 0.0001$ for the main effect of dose; n = 5-8/group]. *(C)* Combining Tzp treatment with either SCH or ICI (both 50 ng) impairs fear memory consolidation when infused directly into the BLA [$F(5,22) = 5.78$, $P = 0.0012$ for the main effect of dose; n = 5/group]. *(D)* Vehicle, ICI or SCH (both 30 μg/kg) was administered to M_1R KO and WT

littermate mice. ICI and SCH impair consolidation only in M_1R KO mice [$F(2,32) = 3.96$, $P = 0.0292$ for the main effect of treatment; $F(1,32) = 15.19$, $P = 0.005$ for the main effect of genotype; and $F(2,32) = 3.42$, $P = 0.045$ for the interaction of treatment and genotype; n = 6-7/group]. *$P < 0.05$, ^$P < 0.01$, #$P < 0.001$.

Figure 2.3. M_1R redundantly activates BLA phospholipase C induced by fear conditioning. *(A)* BLA samples were taken 30 min after vehicle or cevimeline (Cev, 1 mg/kg) injection. Cev elevates IP_3 in the BLA [$t(9) = 2.27$, $P = 0.0496$; n = 10/group]. *(B)* BLA samples were taken 30 min after fear conditioning using 1 mA shock. Tzp (1 mg/kg) blocks conditioning-induced IP_3 levels only when combined with SCH or ICI (both 30 μg/kg) [$F(3,36) = 4.04$, $P = 0.0142$ for main effect of treatment; n = 10/group]. *$P < 0.05$.

Figure 2.4. The M-current restrains fear memory consolidation in the BLA. All treatments were administered immediately after conditioning, with testing performed the following day. *(A,B)* Mice were conditioned with 0.4 mA shock. *(A)* The KCNQ channel blocker XE991 dose-dependently increases freezing in response to the training tone [$F(3,30) = 4.81$, $P = 0.0075$ for main effect of dose; n = 8-10/group]. *(B)* Bilateral infusion of XE991 into BLA dose-dependently enhances cued fear memory consolidation [$F(3,19) = 10.37$, $P = 0.0003$ for main effect of dose; n = 5-6/group]. *(C,D)* Animals were conditioned with 1 mA shock. *(C)* The KCNQ channel opener retigabine dose-dependently inhibits freezing in response to the training tone [$F(4,25) = 3.82$, $P = 0.0147$ for main effect of dose; n = 6/group]. *(D)* Bilateral infusion of retigabine into the BLA dose-dependently inhibits fear memory consolidation [$F(3,16) = 8.28$, $P = 0.0015$ for main effect of dose; n = 5/group]. *(E)* Infusing Tzp+ICI (1 μg + 50 ng) or retigabine (Ret, 1 μg) into the BLA immediately after conditioning impairs contextual fear memory the

following day [F(2,12) = 9.83, *P* = 0.003 for main effect of treatment; n = 5/group]. *P < 0.05; ^P < 0.01; #P < 0.001.

Figure 2.5. Manipulating the M-current blocks the effects of neuromodulatory and GluN receptor manipulations on consolidation. All treatments were administered immediately after conditioning, with cued fear testing performed the next day. *(A)* XE991 (1 mg/kg) has no effect on freezing in response to the training tone when conditioned with strong shock (1 mA). As expected, immediate post-training injection of Tzp+SCH or Tzp+ICI (both 1 mg/kg + 30 μg/kg) inhibits freezing in response to the training tone, while XE991 (1 mg/kg) blocks these inhibitory effects [F(2,24) = 11.19, *P* = 0.0004 for main effect of antagonist treatment,; F(1,24) = 27.00, *P* < 0.0001 for main effect of XE991 treatment; F(2,24) = 11.03, *P* = 0.0004 for interaction of antagonist treatment and XE991 treatment; n = 5/group]. *(B)* Retigabine (Ret, 4 mg/kg) has no effect on freezing in response to the training tone when mice are conditioned with moderate shock (0.4 mA). Agonists of M_1R (cevimeline = Cev, 1 mg/kg), β_2AR (procaterol = Proc, 50 μg/kg) and D_5R (SKF83959 = SKF, 2 mg/kg) enhance freezing in response to the training tone the next day, while Ret blocks these enhancing effects [F(3,42) = 2.89, *P* = 0.0468 for the main effect of agonist; F(1,42) = 20.54, *P* < 0.0001 for the main effect of Ret; and F(3,42) = 3.85, *P* = 0.0161 for the interaction between agonist and Ret; n = 6-7/group]. *(C,D)* All treatments were infused into the BLA immediately after conditioning. *(C)* XE991 (XE, 1 μg) has no effect on freezing in response to the training tone during testing when mice are conditioned with 1 mA shock. The PLC inhibitor edelfosine (Edel, 2 ng) significantly inhibits freezing in response to the training tone the next day, but co-administration with XE reverses that inhibition [F(3,16) = 12.33, *P* = 0.002 for main effect of treatment; n = 5/group]. *(D)* Ret (1 μg) has no effect on freezing in response to the training tone during testing when mice are conditioned with 0.4 mA shock. The PLC agonist m-3M3FBS (3M3, 0.2 ng)

significantly enhances freezing in response to the training tone the next day, but co-administration with Ret reverses that enhancement [F(3,16) = 14.23, *P* < 0.0001 for main effect of treatment; n = 5/group]. *(E)* All subjects were injected both 60 min before and immediately after (+1 min) conditioning with 1 mA shock. The GluN antagonist CPP (C, 10 mg/kg) significantly inhibits consolidation when given before but not after training. XE991 (1 mg/kg) rescues CPP-induced impairment when injected with CPP before training and when injected immediately after training [F(4,20) = 6.76, *P* = 0.0013 for main effect of treatment; n = 5/group]. **P* < 0.05; ^*P* < 0.01; #*P* < 0.001.

Figure 2.6. A critical aspect of cAMP/PKA signaling in fear memory consolidation is upstream of signaling by neuromodulators, PLC and KCNQ channels. All compounds were infused directly into the BLA immediately after fear conditioning, and cued fear testing was performed one day later. *(A,D)* Mice were conditioned with 0.4 mA shock. *(A)* The cAMP analog Sp-8-Br-cAMPS (Sp8, 0.5 µg) increases freezing in response to the training tone. The impairing effect of Sp8 is mitigated by co-infusion with either combined $M_1R+\beta_2$ antagonists (Tzp+ICI, 1 µg + 50 ng), a PLC antagonist (Edel, 2 ng), or a KCNQ channel opener (Ret, 1 µg) [F(4,20) = 6.25, *P* = 0.002 for main effect of treatment; n = 5/group]. *(B,C)* Mice were conditioned with 1 mA shock. *(B)* The impairing effect of Tzp+ICI (1 µg + 50 ng) on consolidation is not reversed by Sp8 (2 µg) [F(2,12) = 6.26, *P* = 0.0138 for main effect of treatment; n = 5/group]. *(C)* The impairing effect of the protein kinase A inhibitor PKI (0.5 µg) is blocked by co-infusion with either an M_1R agonist (Cev, 1 µg), a PLC agonist (3M3, 0.2 ng), or a KCNQ channel blocker (XE, 1 µg) [F(4,20) = 13.82, *P* < 0.0001 for main effect of treatment; n = 5/group]. *(D)* The M_1R agonist Cev (1 µg) enhances consolidation, and this effect is not significantly altered by co-infusion with PKI (0.5 µg) [F(2,12) = 7.02, *P* = 0.0096 for main effect of treatment; n = 5/group]. **P* < 0.05; ^*P* < 0.01; #*P* < 0.001.

Figure 2.7. A model for an important aspect of neuromodulatory control over fear memory consolidation. At least three neuromodulatory receptors (M_1, β_2, D_5) redundantly couple to PLC in the BLA to promote consolidation. A critical downstream effector of PLC activity is the KCNQ channel that underlies the M-current. Reduction in the M-current is hypothesized to enhance neuronal excitability and consolidation during a period beginning ~30 min after acquisition. While the exact mechanism for KCNQ channel closure following acquisition has not been determined, it is likely to be a combination of PLC-mediated signaling events that include a reduction in PIP_2 levels, an increase in intracellular calcium, and activation of PKC. ACh = acetylcholine; CaM = calmodulin; DA = dopamine; DAG = diacylglycerol; ER = endoplasmic reticulum; IP_3 = inositol 1,4,5-trisphosphate; NE = norepinephrine; PIP_2 = phosphatidylinositol 4,5-bisphosphate; PKC = protein kinase C; PLC-β = phospholipase C-β.

LIST OF FIGURES

Figure 2.1

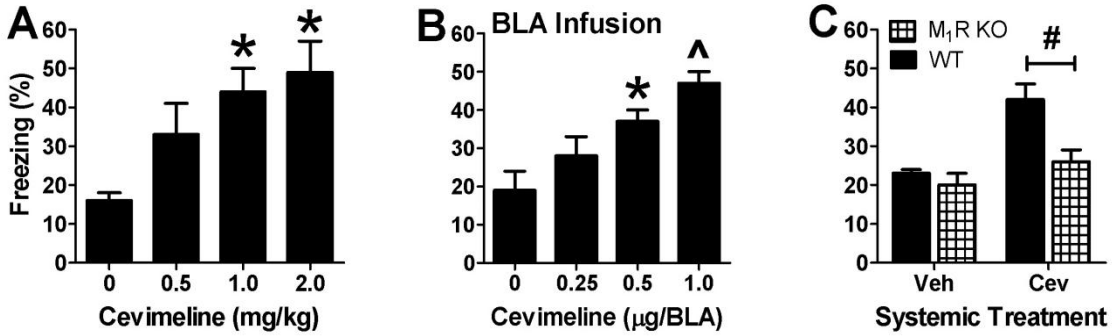
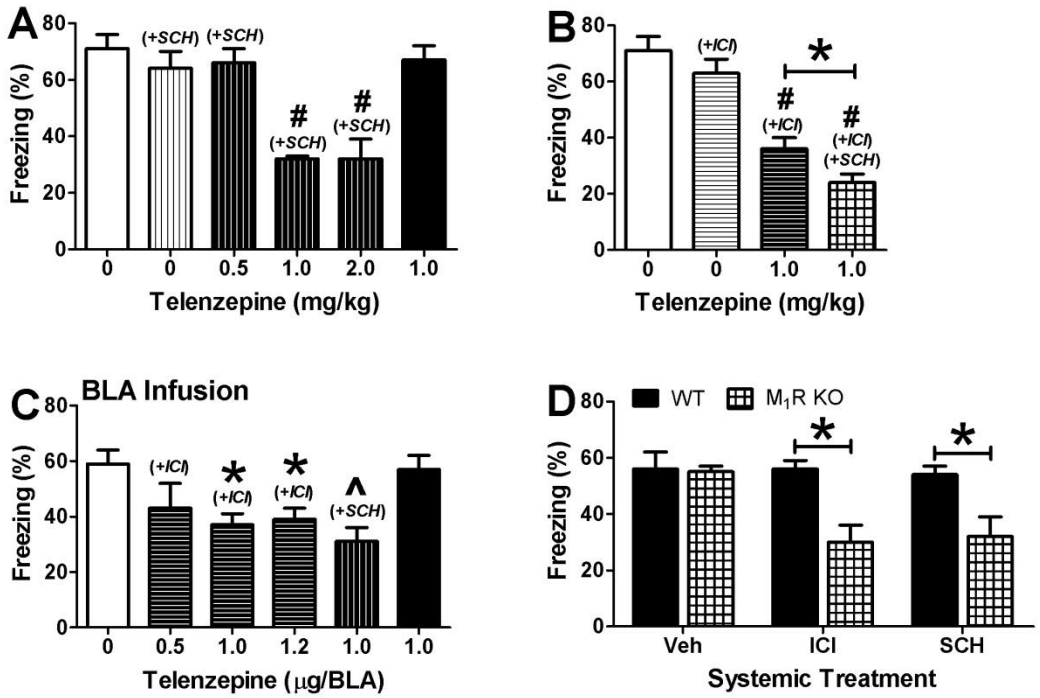


Figure 2.2



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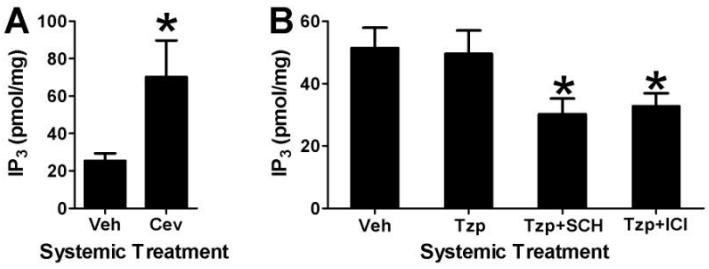

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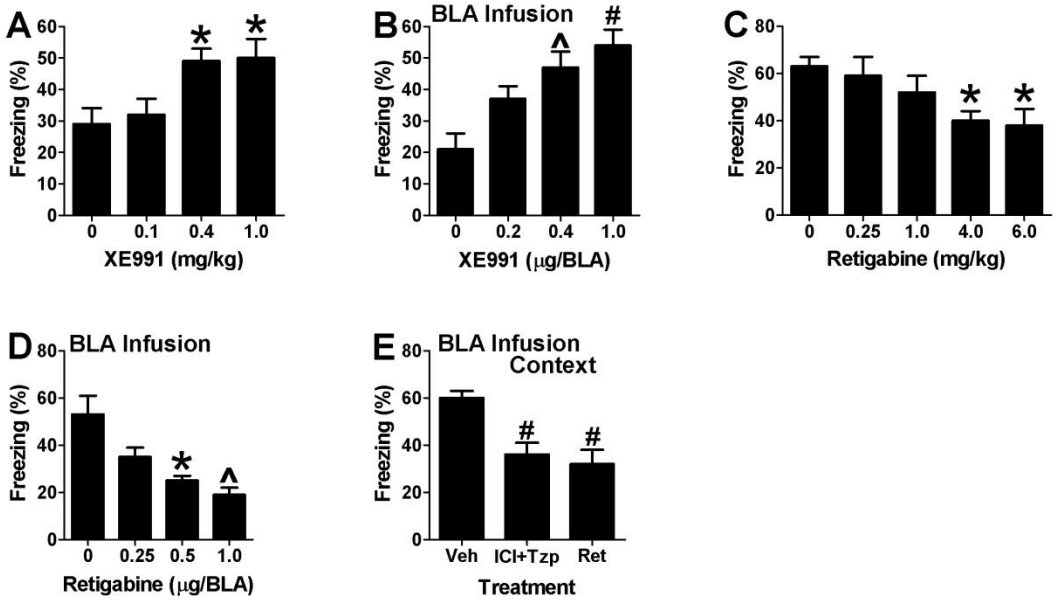

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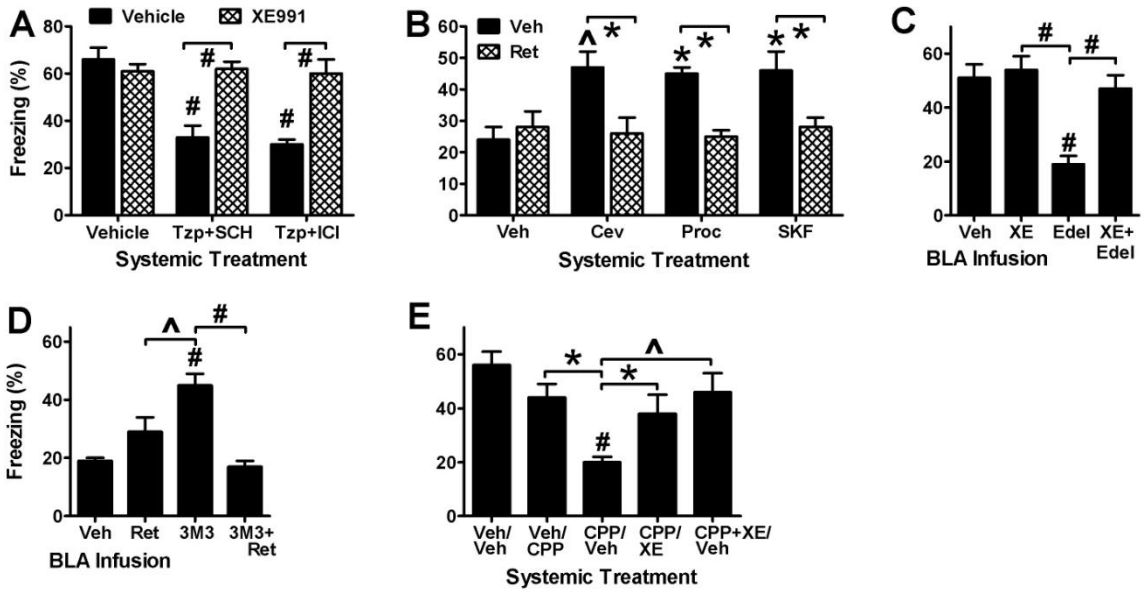
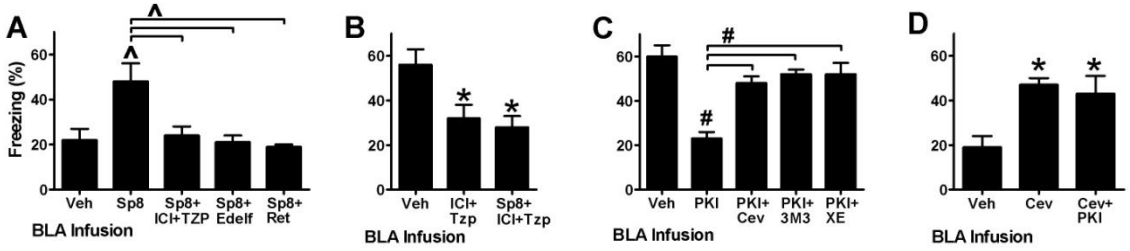


Figure 2.6



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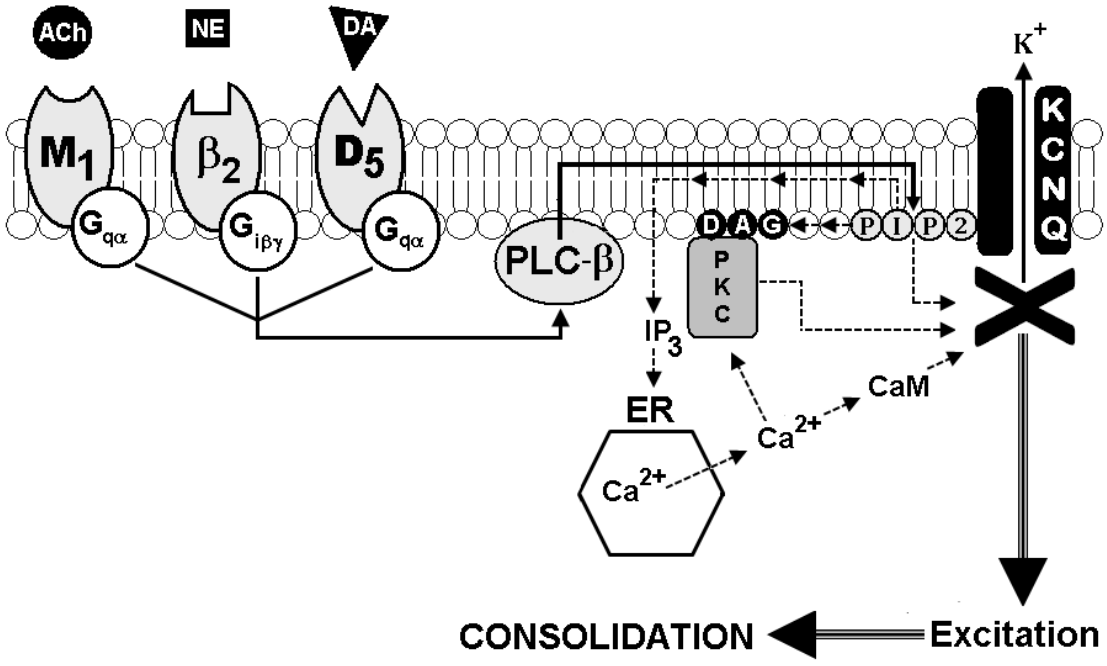
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KCNQ
IP3
ER
Ca2+
Ca2+
CaM
CONSOLIDATION
Excitation

CHAPTER 4: GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

"For small creatures such as we the vastness is bearable only through love."

- Carl Sagan, "Contact" (1985)

General Conclusions

This dissertation set out to explore the neuromodulatory signaling pathways that are essential to the consolidation of fear memory in the basolateral amygdala (BLA). In doing so, this work revealed a novel role for phospholipase C (PLC) as a crucial intracellular signaling molecule in consolidation and as a target of neuromodulatory receptors that facilitate fear memory consolidation. In addition, a novel role for the M_1 muscarinic receptor (M_1R) in fear memory consolidation was discovered, wherein M_1R, β_2-adrenergic (β_2AR) and D_5-dopaminergic (D_5R) receptors redundantly activate PLC activity necessary for consolidation. The discovery of an essential role for M_1R in fear memory consolidation as part of a redundant neuromodulatory framework gave way to the observation that inhibition of the muscarinic-controlled M-current contributes to the effects of PLC and β_2AR/D_5R on fear memory consolidation. Together, these observations provide an expanded and novel model for how the release of neuromodulators induced by a frightening experience drives the powerful consolidation of fear memory in the BLA. In this model, neuromodulators released during fear increase the excitability of neurons in the BLA by activating PLC and inhibiting KCNQ/M-current (KCNQ/M) channels, which likely increases neuronal firing and activity-dependent consolidation processes within those neurons. In other words, fear exacerbates consolidation by putting the BLA in an excitable state that would otherwise not persist into the consolidation window after an experience.

β_2AR, D_5R and M_1R control fear memory by redundantly activating PLC.

M_1R had previously been shown to be both unnecessary for the consolidation of cued fear memory but also facilitative of it when activated exogenously (Vazdarjanova and McGaugh, 1999; Anagnostoras et al., 2003). Here, the contradictory nature of M_1R's contribution to fear memory consolidation in the BLA is resolved by its redundant relationship with β_2AR and D_5R as activators of PLC. Naturally, this begs the question of whether redundant signaling between β_2AR/D_5R/M_1R also includes other neuromodulatory receptors that activate PLC, such as the α_1-adrenergic receptor, 5-HT_{2A} serotonergic receptor, and group I metabotropic glutamate receptors (mGluR). Among these, 5-HT_{2A} may be the best candidate for inclusion in the redundancy model, as α_1-adrenergic receptors inhibit consolidation in the BLA (Lazzaro et al., 2010), and mGluRs are believed to facilitate acquisition, but not consolidation (Rodrigues et al., 2002). Serotonin levels increase in the BLA after a stressful experience (Kawahara et al., 1993; Yokoyama et al., 2005), and 5-HT_{2A} is expressed on pyramidal neurons there (McDonald and Mascagni, 2007). Interestingly, as reported for β_2AR, D_5R and M_1R (Chapter 2; Chapter 3) post-training administration of a 5-HT_{2A} agonist enhances fear memory, while 5-HT_{2A}-deficient mice do not exhibit any impairments (Weisstaub et al., 2006; Zhang et al., 2013), and 5-HT_{2A} receptors are known to increase neuronal excitability by diminishing potassium conductance in the BLA (Yamamoto et al., 2007; Villalobos et al., 2011).

Previous to the work described here, a potential contribution of PLC to memory consolidation, fear memory or otherwise, had seldom been explored (Buckley and Caldwell, 2004; Weeber and Caldwell, 2004; Baker et al., 2008). This is surprising given that $G_{q/11}$- coupled receptors such as 5-HT_{2A} and M_1R have been observed to play some role in fear memory consolidation (Vazdarjanova and McGaugh, 1999; Zhang et al.,

2013). While the behavioral effects of PLC manipulations described here clearly implicate PLC as an important contributor to consolidation, the dynamics of IP_3 levels increasing so distinctly 30 minutes after fear conditioning and then quickly dissipating is peculiar. Moreover, if NMDA receptors (NMDA-R) also activates PLC, as some studies have shown (Horne et al., 2007) and as described here in the relationship between NMDA-R antagonism and KCNQ/M channel blockade (Chapter 3), one might expect to see a rapid elevation in IP_3 as well. One way to assess the importance of PLC at other time-points would be to administer a PLC antagonist after 30-40 minutes post-training, when IP_3 elevations appear to be absent. It is currently unclear whether subsequent IP_3 spikes indicative of PLC activity important for consolidation occur after the last post-training time point that was measured (60 min), which delayed PLC antagonism would still prevent. Therefore, experiments administering delayed PLC antagonist treatment go hand in hand with additional experiments assessing IP_3 levels beyond 60 minutes after conditioning.

Redundant activation of PLC by β_2AR, D_5R and M_1R controls fear memory consolidation by interacting with KCNQ/M channels.

The work described here not only identifies PLC activity as a requisite for fear memory consolidation in the BLA and as a point of convergence for neuromodulatory facilitation of consolidation, but it also potentially explains one manner in which PLC affects consolidation. Here, evidence suggests that PLC controls consolidation through its inhibitory effect on KCNQ/M channels. Inhibition of I_{KM} increases the excitability of pyramidal neurons by decreasing afterhyperpolarization (AHP; Womble and Moises, 1993; Gu et al., 2005) and promoting afterdepolarization (ADP; Yue and Yaari, 2004; Vervaeke et al., 2006; Chen and Yaari, 2008), which together increase firing by inhibiting spike frequency accommodation. However, a number of ion channels other than

KCNQ/M contribute to AHP and ADP, and several of these are known to be regulated by PIP_2 hydrolysis, β_2AR, D_5R and M_1R.

The effects of PLC and of the neuromodulatory receptors described here are known to influence the activity of a number of ion channels other than KCNQ/M. In recent years, the effects of PIP_2 on the activity of a variety of ion channels has generated much interest (Suh and Hille, 2008). While M_1R is the hallmark modulator of KCNQ/M channels and I_{KM}, it also is known to regulate the small-conductance calcium activated potassium current (Power and Sah, 2008) and a voltage-independent potassium leak current (Womble and Moises, 1992). Dopamine and adrenergic agonists have both been observed to increase the excitability of pyramidal neurons in the BLA (Kroner et al., 2005; Yamamoto et al., 2007; Abraham et al., 2008), however, it is unclear whether their effects are mediated strictly by I_{KM}. Future electrophysiological experiments in the BLA with agonists of β_2AR/D_5R/M_1R should shed light on whether those neuromodulatory receptors indeed exert their effects on consolidation through inhibition of I_{KM} and/or other ion channels. However, the observation described here that blockade of I_{KM} masks the effects of β_2AR/D_5R/M_1R and PLC agonists suggests that any effects of those agonists on other ion channels is not sufficient to alter consolidation.

The idea that multiple neuromodulatory receptors converge on PLC to promote, among other potential effects, increased excitability of BLA neurons obviously requires that all neuromodulatory receptors involved are expressed on the same neurons and within close proximity to one another and to KCNQ channels. While β_2AR, D_5R and M_1R have all been observed on excitatory pyramidal neurons in the BLA (Muly et al., 2009; Farb et al., 2010; Muller et al., 2013), co-expression of all three receptors on the same neurons within the same space is difficult to assess. Interestingly, in BLA neurons agonists of β-adrenergic and muscarinic receptors potentiate one another's effect on

neuronal excitability (Power and Sah, 2008), suggesting that they co-localize on neurons in the BLA. The particular model described here could be studied electrophysiologically by determining whether agonists of β_2 AR/D_5R/M_1R exert similar effects in the same BLA neuron.

As an alternative mechanism to receptor clustering at the synaptic locus of plasticity in the BLA, the combined effects of β_2AR/D_5R/M_1R on KCNQ/M channel inhibition could be mediated by more widespread effects of the receptors throughout a neuron. Inhibition of KCNQ/M channels has been observed to promote global neuronal depolarization and back-propagating action potentials that enhance LTP independent of NMDA-R opening by presynaptic stimulation (Tsubokawa et al., 1997; Petrovic et al., 2012). In this case, neuromodulatory receptors could exert their effect on excitability and consolidation through more diffuse expression patterns across a neuron, rather than requiring receptor clustering at dendritic spines. This explanation is supported by the observation that XE991 partially rescues consolidation from impairments induced by NMDA-R blockade (Chapter 3), and also that KCNQ/M channels are primarily expressed near the neuronal soma (Cooper et al., 2001; Chung et al., 2006; Hu et al., 2007), where β_2AR, D_5R and M_1R have all been observed in BLA neurons (Muly et al., 2009; Farb et al., 2010; Muller et al., 2013). Furthermore, in the BLA, calcium release from intracellular stores induced by a muscarinic agonist occurs only in the neuronal soma and proximal dendrites (Power and Sah, 2007), and not in distal dendrites.

As the issue of localizing the neuromodulatory receptors and KCNQ/M channels important for controlling consolidation, it is unclear precisely how KCNQ/M channels promote consolidation within the complex circuitry of the BLA. KCNQ/M channels have been observed to exert effects on plasticity both presynaptically and postsynaptically (Vervaeke et al., 2006; Petrovic et al., 2012). Moreover, pharmacological modulators of

KCNQ/M channels can regulate the release of neurotransmitters from presynaptic neurons (Martire et al., 2004; Jensen et al., 2011). Therefore, it is feasible that KCNQ/M channels mediate their effects on consolidation in the BLA by enhancing the release of neurotransmitters and neuromodulators from presynaptic terminals, which would subsequently promote intracellular consolidation pathways in postsynaptic neurons. While increased presynaptic release could contribute to consolidation, several previous studies suggest that muscarinic receptors and KCNQ/M channels directly modulate the excitability of postsynaptic pyramidal neurons there (Washburn and Moises, 1992; Womble and Moises, 1992; Power and Sah, 2008). Although, given that M_1R has been observed on presynaptic terminals, the effects of neuromodulators on KCNQ/M channels may be widespread within the BLA.

The PLC-KCNQ/M pathway regulates consolidation downstream of cAMP/PKA.

One of the most surprising observations described here is that of the relationship between the PLC-KCNQ/M and cAMP-PKA signaling pathways. The cAMP-PKA pathway has traditionally been the primary second messenger pathway associated with driving the protein synthesis and gene expression believed to be required for consolidation (Hernandez and Abel, 2008). While certain forms of LTP depend on cAMP/PKA in postsynaptic neurons and others do not (Duffy and Nguyen, 2003), no definitive evidence supports a requisite postsynaptic role for cAMP/PKA in consolidation. Indeed, LTP is more reliably blocked when cAMP-PKA is inhibited in both presynaptic and postsynaptic neurons (Blitzer et al., 1995), and presynaptic cAMP-PKA is known to be important for the release of neurotransmitters (Fourcadot et al., 2008). Assuming a postsynaptic role for both KCNQ/M and cAMP/PKA, consolidation impairments induced by pharmacological I_{KM} enhancement should have been overcome by postsynaptic elevations of cAMP activity that promote the activation of important intracellular

consolidation pathways, which they were not. In the context of these findings, our observation that modulators of PLC and of KCNQ/M channels could mask the effects of cAMP/PKA modulators further supports the importance of presynaptic cAMP/PKA to consolidation in the BLA. This of course presumes that KCNQ/M channels exert their effects on consolidation directly through postsynaptic BLA neurons, which, as mentioned previously, could be better supported by further electrophysiological studies.

Inhibition of I_{KM} promotes learning and memory.

The observation that inhibiting I_{KM} promotes the consolidation of fear memory contributes to a surprisingly small volume of literature reporting effects of pharmacological KCNQ/M channel modulators on memory formation. A handful of studies have revealed an enhancing effect of pre-training KCNQ/M channel blockers (i.e. XE991 or linopiridine) on hippocampus-dependent memory (Fontana et al., 1994; Fontan-Lozano et al., 2011) and of the extinction fear conditioning in the infralimbic cortex (Santini and Porter, 2010). On the other hand, no studies have explored the effects of KCNQ/M channel openers, such as retigabine, on animal models of learning and memory. Therefore, the findings described here present several new avenues of research.

Given that consolidation consists of several general principles, and that a number of these carry over to the reconsolidation of memory, modulators of KCNQ/M channels may be discovered to have effects on learning and memory beyond just the consolidation of fear memory. While different in several ways, the consolidation and reconsolidation of fear memory share several requisite molecular underpinnings (Alberini, 2005), and PLC or I_{KM} may have similar effects on reconsolidation as they have on consolidation. Additionally, given the observations that PLC and KCNQ/M channels can influence synaptic plasticity *in vitro* (Horne et al., 2007; Petrovic et al., 2012), it is

possible that PLC and I_{KM} regulate memory consolidation in general, and affect memory formation independent of the site of plasticity.

Potential therapeutic implications for KCNQ/M channel openers.

The study of fear memory most closely translates clinically to understanding how the recurrent and intrusive memories characteristic of post-traumatic stress disorder (PTSD) develop and persist. The observation here that systemic administration of retigabine reliably inhibits the consolidation of ordinarily powerful fear memory provides a potential early response treatment for individuals who have suffered severe trauma. Interestingly, retigabine (also known as ezogabine) is currently approved by the FDA as an anti-convulsant for the prevention of *mal petit* seizures (Orhan, 2012). In this therapeutic setting, retigabine is administered daily, which has recently been reported by the FDA to have some negative side effects when taken for extended periods of time. This would not appear to present a problem if used as a preventative early response treatment.

Some support for the use of anti-convulsants to treat PTSD has grown in the clinical community due to some effectiveness of a variety of compounds in clinical studies. However, many of these studies were performed with very low numbers of subjects (Berlin, 2007). Additionally, further research exploring the effects of KCNQ/M modulators on fear memory reconsolidation could provide some support for the use of retigabine in reconsolidation-based re-exposure therapies (Bentz et al., 2010). Conversely, some research suggests that XE991 can enhance the efficacy of fear memory extinction training, which further supports the idea of using KCNQ/M channel modulators as a clinical treatment for PTSD (Santini and Porter, 2010).

REFERENCES

Abeliovich A, Paylor R, Chen C, Kim JJ, Wehner JM, Tonegawa S (1993) PKC gamma mutant mice exhibit mild deficits in spatial and contextual learning. Cell 75:1263-1271.

Abercrombie ED, Jacobs BL (1987) Single-unit response of noradrenergic neurons in the locus coeruleus of freely moving cats. I. Acutely presented stressful and nonstressful stimuli. J Neurosci 7:2837-2843.

Abraham PA, Xing G, Zhang L, Yu EZ, Post R, Gamble EH, Li H (2008) beta1- and beta2-adrenoceptor induced synaptic facilitation in rat basolateral amygdala. Brain Res 1209:65-73.

Abramson CI, Bitterman ME (1986) Latent inhibition in honeybees. Anim Learn Behav 14:184-189.

Acquas E, Wilson C, Fibiger HC (1996) Conditioned and unconditioned stimuli increase frontal cortical and hippocampal acetylcholine release: effects of novelty, habituation, and fear. J Neurosci 16:3089-3096.

Aggleton JP, Neave N, Nagle S, Hunt PR (1995) A comparison of the effects of anterior thalamic, mamillary body and fornix lesions on reinforced spatial alteration. Behav Brain Res 68:91-101.

Aiba A, Chen C, Herrup K, Rosenmund C, Stevens CF, Tonegawa S (1994) Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. Cell 79:365-375.

Aiken SP, Lampe BJ, Murphy PA, Brown BS (1995) Reduction of spike frequency adaptation and blockade of M-current in rat CA1 pyramidal neurones by linopirdine (DuP 996), a neurotransmitter release enhancer. Br J Pharmacol 115:1163-1168.

Alberini CM (2005) Mechanisms of memory stabilization: are consolidation and reconsolidation similar or distinct processes? Trends Neurosci 28:51-56.

Anagnostaras SG, Murphy GG, Hamilton SE, Mitchell SL, Rahnama NP, Nathanson NM, Silva AJ (2003) Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. Nat Neurosci 6:51-58.

Anstrom KK, Woodward DJ (2005) Restraint increases dopaminergic burst firing in awake rats. Neuropsychopharmacology 30:1832-1840.

Asan E (1997) Ultrastructural features of tyrosine-hydroxylase-immunoreactive afferents and their targets in the rat amygdala. Cell Tissue Res 288:449-469.

Asan E (1998) The catecholaminergic innervation of the rat amygdala. Adv Anat Embryol Cell Biol 142:1-118.

Aston-Jones G, Rajkowski J, Kubiak P, Alexinsky T (1994) Locus coeruleus neurons in monkey are selectively activated by attended cues in a vigilance task. J Neurosci 14:4467-4480.

Aston-Jones G, Rajkowski J, Ivanova S, Usher M, Cohen J (1998) Neuromodulation and cognitive performance: recent studies of noradrenergic locus ceruleus neurons in behaving monkeys. Adv Pharmacol 42:755-759.

Bading H. (2000) Transcription-dependent neuronal plasticity the nuclear calcium hypothesis. Eur J Biochem 267:5280-5283.

Bae YS, Lee TG, Park JC, Hur JH, Kim Y, Heo K, Kwak JY, Suh PG, Ryu SH (2003) Identification of a compound that directly stimulates phospholipase C activity. Mol Pharmacol 63:1043-1050.

Baker KD, Edwards TM, Rickard NS (2008) Inhibition of mGluR1 and IP3Rs impairs long-term memory formation in young chicks. Neurobiol Learn Mem 90:296-274.

Barros DM, Pereira P, Medina JH, Izquierdo I (2002) Modulation of working memory and of long- but not short-term memory by cholinergic mechanisms in the basolateral amygdala. Behav Pharmacol 13:163-167.

Bauer EP, Schafe GE, LeDoux JE (2002) NMDA receptors and L-type voltage-gated calcium channels contribute to long-term potentiation and different components of fear memory formation in the lateral amygdala. J Neurosci 22:5239-5249.

Bayazitov IT, Richardson RJ, Fricke RG, Zakharenko SS (2007) Slow presynaptic and fast postsynaptic components of compound long-term potentiation. J Neurosci 27:11510-11521.

Berlin HA (2007) Antiepileptic drugs for the treatment of post-traumatic stress disorder. Curr Psychiatry Rep 9:291-300.

Bentz D, Michael T, de Quervain DJ, Wilhelm FH (2010) Enhancing exposure therapy for anxiety disorders with glucocorticoids: from basic mechanisms of emotional learning to clinical applications. J Anxiety Disord 24:223-230.

Bernabeu R, Bevilaqua L, Ardenghi P, Bromberg E, Schmitz P, Bianchin M, Izquierdo I, Medina JH (1997) Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. Proc Natl Acad Sci U S A 94:7041-7046.

Blanchard DC, Blanchard RJ (1969) Passive and active reactions to fear-eliciting stimuli. J Comp Physiol Psychol 68:129-135.

Blanchard DC, Blanchard RJ (1972) Innate and conditioned reactions to threat in rats with amygdaloid lesions. J Comp Physiol Psychol 81:281-290.

Bliss TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 232:33-356.

Blitzer RD, Wong T, Nouranifar R, Iyengar R, Landau EM (1995) Postsynaptic cAMP pathway gates early LTP in hippocampal CA1 region. Neuron 15:1403-1414.

Bonini JS, Cammarota M, Kerr DS, Bevilaqua LR, Izquierdo I (2005) Inhibition of PKC in basolateral amygdala and posterior parietal cortex impairs consolidation of inhibitory avoidance memory. Pharmacol Biochem Behav 80:63-67.

Brambilla R, Gnesutta N, Minichiello L, White G, Roylance AJ, Herron CE, Ramsey M, Wolfer DP, Cestari V, Rossi-Arnaud C, Grant SG, Chapman PF, Lipp HP, Sturani E, Klein R. A role for the Ras signalling pathway in synaptic transmission and long-term memory. Nature 390:281-286.

Bromberg-Martin ES, Matsumoto M, Hikosaka O (2010) Dopamine in motivational control: rewarding, aversive, and alerting. Neuron 68:815-834.

Brown BS, Yu SP (2000) Modulation and genetic identification of the M channel. Prog Biophys Mol Biol 73:135-166.

Brown DA, Adams PR (1980) Muscarinic suppression of a novel voltage-sensitive K+ current in a vertebrate neurone. Nature 283:673-676.

Buckley CT, Caldwell KK (2004) Fear conditioning is associated with altered integration of PLC and ERK signaling in the hippocampus. Pharmacol Biochem Behav 79:633-640.

Buckley NJ, Bonner TI, Brann MR (1988) Localization of a family of muscarinic receptor mRNAs in rat brain. J Neurosci 8:4646-4652.

Burke A, Heuer F, Reisberg D (1992) Remembering emotional events. Mem Cognit 20:277-290.

Bush DE, Caparosa EM, Gekker A, LeDoux J (2010) Beta-adrenergic receptors in the lateral nucleus of the amygdala contribute to the acquisition but not the consolidation of auditory fear conditioning. Front Behav Neurosci 4:154.

Cahill L, Prins B, Weber M, McGaugh JL (1994) Beta-adrenergic activation and memory for emotional events. Nature 371:702-704.

Carew TJ, Hawkins RD, Kandel ER (1983) Differential classical conditioning of a defensive withdrawal reflex in Aplysia californica. Science 219:397-400.

Carlsen J, Zaborszky L, Heimer L (1985) Cholinergic projections from the basal forebrain to the basolateral amygdaloid complex: A combined retrograde fluorescent and immunohistochemical study. J Comp Neurol 234:155-167.

Castillo PE, Schoch S, Schmitz F, Sudhof TC, Malenka RC (2002) RIM1alpha is required for presynaptic long-term potentiation. Nature 415:327-330.

Caulfield MP (1993) Muscarinic receptors--characterization, coupling and function. Pharmacol Ther 58:319-379.

Chen N, Sugihara H, Sharma J, Perea G, Petravicz J, Le C, Sur M (2012) Nucleus basalis-enabled stimulus-specific plasticity in the visual cortex is mediated by astrocytes. Proc Natl Acad Sci U S A 109:E2832-2841.

Chen S, Yaari Y (2008) Spike Ca2+ influx upmodulates the spike afterdepolarization and bursting via intracellular inhibition of KV7/M channels. J Physiol 586:1351-1363.

Chhatwal JP, Myers KM, Ressler KJ, Davis M (2005) Regulation of gephyrin and GABAA receptor binding within the amygdala after fear acquisition and extinction. J Neurosci 25:502-506.

Chiodo LA, Antelman SM, Caggiula AR, Lineberry CG (1980) Sensory stimuli alter the discharge rate of dopamine (DA) neurons: evidence for two functional types of DA cells in the substantia nigra. Brain Res 189:544-549.

Chipkin RE, Iorio LC, Coffin VL, McQuade RD, Berger JG, Barnett A (1988) Pharmacological profile of SCH39166: a dopamine D1 selective benzonaphthazepine with potential antipsychotic activity. J Pharmacol Exp Ther 247:1093-1102.

Chruscinski AJ, Rohrer DK, Schauble E, Desai KH, Bernstein D, Kobilka BK (1999) Targeted disruption of the beta2 adrenergic receptor gene. J Biol Chem 274:16694-16700.

Chung HJ, Jan YN, Jan LY (2006) Polarized axonal surface expression of neuronal KCNQ channels is mediated by multiple signals in the KCNQ2 and KCNQ3 C-terminal domains. Proc Natl Acad Sci U S A 103:8870-8875.

Ciliax BJ, Nash N, Heilman C, Sunahara R, Hartney A, Tiberi M, Rye DB, Caron MG, Niznik HB, Levey AI (2000) Dopamine D(5) receptor immunolocalization in rat and monkey brain. Synapse 37:125-145.

Cockcroft S (2006) The latest phospholipase C, PLCeta, is implicated in neuronal function. Trends Biochem Sci 31:4-7.

Codazzi F, Di Cesare A, Chiulli N, Albanese A, Meyer T, Zacchetti D, Grohovaz F (2006) Synergistic control of protein kinase Cgamma activity by ionotropic and

metabotropic glutamate receptor inputs in hippocampal neurons. J Neurosci 26:3404-3411.

Cooper EC, Harrington E, Jan YN, Jan LY (2001) M channel KCNQ2 subunits are localized to key sites for control of neuronal network oscillations and synchronization in mouse brain. J Neurosci 21:9529-9540.

Dai H, Kaneko K, Kato H, Fujii S, Jing Y, Xu A, Sakurai E, Kato M, Okamura N, Kuramasu A, Yanai K (2007) Selective cognitive dysfunction in mice lacking histamine H1 and H2 receptors. Neurosci Res 57:306-313.

Daoudal G, Debanne D (2003) Long-term plasticity of intrinsic excitability: learning rules and mechanisms. J Neurosci 10:456-465.

Dascal N (2001) Ion-channel regulation by G proteins. Trends Endocrinol Metab 12:391-398.

Debiec J, LeDoux JE (2004) Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. Neuroscience 129:267-272.

Delmas P, Crest M, Brown DA (2004) Functional organization of PLC signaling microdomains in neurons. Trends Neurosci 27:41-47.

Dolmetsch RE, Pajvani U, Fife K, Spotts JM, Greenberg ME (2001) Signaling to the nucleus by an L-type calcium channel-calmodulin complex through the MAP kinase pathway. Science 294:333-339.

Dooley DJ, Bittiger H, Reymann NC (1986) CGP 20712 A: a useful tool for quantitating beta 1- and beta 2- adrenoceptors. Eur J Pharmacol 130:137-139.

Duvarci S, Nader K, LeDoux JE (2008) De novo mRNA synthesis is required for both consolidation and reconsolidation of fear memories in the amygdala. Learn Mem 15:747-755.

Duffy SN, Nguyen PV (2003) Postsynaptic application of a peptide inhibitor of cAMP-dependent protein kinase blocks expression of long-lasting synaptic potentiation in hippocampal neurons. J Neurosci 23:1142-1150.

Ehlert, FJ, Roeske WH, Yamamura HI (1995) Molecular biology, pharmacology, and brain distribution of subtypes of the muscarinic receptor." Psychopharmacology: the fourth generation of progress. Raven Press, New York: pp 111-124.

Ehrlich I, Humeau Y, Grenier F, Ciocchi S, Herry C, Luthi A (2009) Amygdala inhibitory circuits and the control of fear memory. Neuron 62:757-771.

El-Ghundi M, Fletcher PJ, Drago J, Sibley DR, O'Dowd BF, George SR (1999) Spatial learning deficit in dopamine D(1) receptor knockout mice. Eur J Pharmacol 383:95-106.

Fadok JP, Dickerson TM, Palmiter RD (2009) Dopamine is necessary for cue-dependent fear conditioning. J Neurosci 29:11089-11097.

Farb CR, Chang W, LeDoux JE (2010) Ultrastructural characterization of noradrenergic axons and Beta-adrenergic receptors in the lateral nucleus of the amygdala. Front Behav Neurosci 4:162.

Ferraguti F, Shigamoto R (2006) Metabotropic glutamate receptors. Cell Tissue Res 326:483-504.

Ferry B, McGaugh JL (1999) Clenbuterol administration into the basolateral amygdala post-training enhances retention in an inhibitory avoidance task. Neurobiol Learn Mem 72:8-12.

Fontan-Lozano A, Suarez-Pereira I, Delgado-Garcia JM, Carrion AM (2011) The M-current inhibitor XE991 decreases the stimulation threshold for long-term synaptic plasticity in healthy mice and in models of cognitive disease. Hippocampus 21:22-32.

Fontana DJ, Inouye GT, Johnson RM (1994) Linopirdine (DuP 996) improves performance in several tests of learning and memory by modulation of cholinergic neurotransmission. Pharmacol Biochem Behav 49:1075-1082.

Fornari RV, Moreira KM, Oliveira MG (2000) Effects of the selective M1 muscarinic receptor antagonist dicyclomine on emotional memory. Learn Mem 7:287-292.

Fourcaudot E, Gambino F, Humeau Y, Casassus G, Shaban H, Poulain B, Luthi A (2008) cAMP/PKA signaling and RIM1alpha mediate presynaptic LTP in the lateral amygdala. Proc Natl Acad Sci U S A 105:15130-15135.

Frankland KBJ, Paxinos G (1997) The mouse brain in stereotaxic coordinates. San Diego: Academic Press.

Frankland PW, Josselyn SA, Anagnostaras SG, Kogan JH, Takahashi E, Silva AJ (2004) Consolidation of CS and US representations in associative fear conditioning. Hippocampus 14: 557-569.

Frey U, Schroeder H, Matthies H (1990) Dopaminergic antagonists prevent long-term maintenance of posttetanic LTP in the CA1 region of rat hippocampal slices. Brain Res 522:69-75.

Frey U, Huang YY, Kandel ER (1993) Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. Science 260:1661-1664.

Friedman E, Jin LQ, Cai GP, Hollon TR, Drago J, Sibley DR, Wang HY (1997) D1-like dopaminergic activation of phosphoinositide hydrolysis is independent of D1A dopamine receptors: evidence from D1A knockout mice. Mol Pharmacol 51:6-11.

Gallagher M, Kapp BS, Musty RE, Driscoll PA (1977) Memory formation: evidence for a specific neurochemical system in the amygdala. Science 198:423-425.

Galvez R, Mesches MH, McGaugh JL (1996) Norepinephrine release in the amygdala in response to footshock stimulation. Neurobiol Learn Mem 66:253-257.

Gelinas JN, Nguyen PV (2005) Beta-adrenergic receptor activation facilitates induction of a protein synthesis-dependent late phase of long-term potentiation. J Neurosci 25:3294-3303.

George MS, Abbott LF, Siegelbaum SA (2009) HCN hyperpolarization-activated cation channels inhibit EPSPs by interactions with M-type K(+) channels. Nat Neurosci 12:577-584.

Gerber DJ, Sotnikova TD, Gainetdinov RR, Huang SY, Caron MG, Tonegawa S (2001) Hyperactivity, elevated dopaminergic transmission, and response to amphetamine in

M1 muscarinic acetylcholine receptor-deficient mice. Proc Natl Acad Sci U S A 98:15312-15317.

Giese KP, Peters M, Vernon J (2001) Modulation of excitability as a learning and memory mechanism: a molecular genetic perspective. Physiol Behav 73:803-810.

Girgis M (1980) Acetylcholinesterase enzyme localization in the amygdala: a comparative histochemical and ultrastructural study. Acta Anat (Basel) 106:192-202.

Goddard GV (1964) Amygdaloid stimulation and learning in the rat. J Comp Physiol Psychol 58:23-30.

Goh JW, Pennefather PS (1989) A pertussis toxin-sensitive G protein in hippocampal long-term potentiation. Science 244:980-983.

Gold PE, Zornetzer SF, McGaugh JL (1974) Electrical stimulation of the brain: effects on memory storage. Adv Psychobiol 2:193-224.

Goosens KA, Holt W, Maren S (2000) A role for amygdaloid PKA and PKC in the acquisition of long-term conditional fear memories in rats. Behav Brain Res 114:145-152.

Goosens KA, Maren S (2001) Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. Learn Mem 8:148-155.

Goosens KA, Maren S (2004) NMDA receptors are essential for the acquisition, but not expression, of conditional fear and associative spike firing in the lateral amygdala. Eur J Neurosci 20:537-548.

Gravius A, Barberi C, Schafer D, Schmidt WJ, Danysz W (2006) The role of group I metabotropic glutamate receptors in acquisition and expression of contextual and auditory fear conditioning in rats - a comparison. Neuropharmacology 51:1146-1155.

Greba Q, Kokkinidis L (2000) Peripheral and intraamygdalar administration of the dopamine D1 receptor antagonist SCH 23390 blocks fear-potentiated startle but not shock reactivity or the shock sensitization of acoustic startle. Behav Neurosci 114:262-272.

Grillon C, Cordova J, Morgan JA, Charney DS, Davis S (2004) Effects of the beta-blocker propranolol on cued and contextual fear conditioning in humans. Psychopharmacology (Berl) 175:342-352.

Gu N, Vervaeke K, Hu H, Storm JF (2005) Kv7/KCNQ/M and HCN/h, but not KCa2/SK channels, contribute to the somatic medium after-hyperpolarization and excitability control in CA1 hippocampal pyramidal cells. J Physiol 566:689-715.

Guarraci FA, Frohardt RJ, Kapp BS (1999) Amygdaloid D1 dopamine receptor involvement in Pavlovian fear conditioning. Brain Res 827:28-40.

Guzman-Ramos K, Osorio-Gomez D, Moreno-Castilla P, Bermudez-Rattoni F (2010) Off-line concomitant release of dopamine and glutamate involvement in taste memory consolidation. J Neurochem 114:226-236.

Guzman-Ramos K, Osorio-Gomez D, Moreno-Castilla P, Bermudez-Rattoni F (2012) Post-acquisition release of glutamate and norepinephrine in the amygdala is involved in taste-aversion memory consolidation. Learn Mem 19:231-238.

Halliwell JV, Adams PR (1982) Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. Brain Res 250:71-92.

Han JH, Kushner SA, Yiu AP, Cole CJ, Matynia A, Brown RA, Neve RL, Guzowski JF, Silva AJ, Josselyn SA (2007) Neuronal competition and selection during memory formation. Science 316:457-460.

Hebb, DO (1949) The Organization of Behavior.

Heinrich JN, Butera JA, Carrick T, Kramer A, Kowal D, Lock T, Marquis KL, Pausch MH, Popiolek M, Sun SC, Tseng E, Uveges AJ, Mayer SC (2009) Pharmacological comparison of muscarinic ligands: historical versus more recent muscarinic M1-preferring receptor agonists. Eur J Pharmacol 605:53-56.

Hellendall RP, Godfrey DA, Ross CD, Armstrong DM, Price JL (1986) The distribution of choline acetyltransferase in the rat amygdaloid complex and adjacent cortical areas, as determined by quantitative micro-assay and immunohistochemistry. J Comp Neurol 249:486-498.

Hernandez PJ, Abel T (2008) The role of protein synthesis in memory consolidation: progress amid decades of debate. Neurobiol Learn Mem 89:293-311.

Herrero JL, Roberts MJ, Delicato LS, Gieselmann MA, Dayan P, Thiele A (2008) Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. Nature 454:1110-1114.

Heuer F, Resiberg D (1990) Vivid memories of emotional events: the accuracy of remembered minutiae. Mem Cognit 18:496-506.

Hicks PE, Cavero I, Manoury P, Lefevre-Borg F, Langer SZ (1987) Comparative analysis of beta-1 adrenoceptor agonist and antagonist potency and selectivity of cicloprolol, xamoterol and pindolol. J Pharmacol Exp Ther 242:1025-1034.

Holland PC, Bouton ME (1999) Hippocampus and context in classical conditioning. Curr Opin Neurobiol 9:195-202.

Holmes A, Hollon TR, Gleason TC, Liu Z, Dreiling J, Sibley DR, Crawley JN (2001) Behavioral characterization of dopamine D5 receptor null mutant mice. Behav Neurosci 115:1129-1144.

Homberg JR (2012) Serotonergic modulation of conditioned fear. Scientifica 2012:1-16.

Horne EA, Dell'Acqua ML (2007) Phospholipase C is required for changes in postsynaptic structure and function associated with NMDA receptor-dependent long-term depression. J Neurosci 27:3523-3534.

Hoshi N, Zhang JS, Omaki M, Takeuchi T, Yokoyama S, Wanaverbecq N, Langeberg LK, Yoneda Y, Scott JD, Brown DA, Higashida H (2003) AKAP150 signaling complex promotes suppression of the M-current by muscarinic agonists. Nat Neurosci 6:564-571.

Hu H, Vervaeke K, Storm JF (2007) M-channels (Kv7/KCNQ channels) that regulate synaptic integration, excitability, and spike pattern of CA1 pyramidal cells are located in the perisomatic region. J Neurosci 27:1853-1867.

Hu H, Real E, Takamiya K, Kang MG, Ledoux J, Huganir RL, Malinow R (2007) Emotion enhances learning via norepinephrine regulation of AMPA-receptor trafficking. Cell 131:160-173.

Huang YY, Kandel ER (1995) D1/D5 receptor agonists induce a protein synthesis-dependent late potentiation in the CA1 region of the hippocampus. Proc Natl Acad Sci U S A 92:2446-2450.

Huang YY, Kandel ER (1996) Modulation of both the early and the late phase of mossy fiber LTP by the activation of beta-adrenergic receptors. Neuron 16:611-617.

Huang YY, Martin KC, Kandel ER (2000) Both protein kinase A and mitogen-activated protein kinase are required in the amygdala for the macromolecular synthesis-dependent late phase of long-term potentiation. J Neurosci 20:6317-6325.

Inglis FM, Moghaddam B (1999) Dopaminergic innervation of the amygdala is highly responsive to stress. J Neurochem 72:1088-1094.

Iorio LC, Barnett A, Leitz FH, Houser VP, Korduba CA (1983) SCH 23390, a potential benzazepine antipsychotic with unique interactions on dopaminergic systems. J Pharmacol Exp Ther 226:462-468.

Izquierdo I, Dias RD (1983) The influence of adrenergic receptor antagonists on the amnestic and antiamnestic actions of adrenaline and tyramine. Psychopharmacology (Berl) 80:181-183.

Izquierdo I, da Cunha C, Rosat R, Jerusalinsky D, Ferreira MB, Medina JH (1992) Neurotransmitter receptors involved in post-training memory processing by the amygdala, medial septum, and hippocampus of the rat. Behav Neural Biol 58:16-26.

Jensen MM, Lange SC, Thomsen MS, Hansen HH, Mikkelsen JD (2011) The pharmacological effect of positive KCNQ (Kv7) modulators on dopamine release from striatal slices. Basic Clin Pharmacol Toxicol 109:339-342.

Jerusalinsky D, Ferreira MB, Walz R, Da Silva RC, Bianchin M, Ruschel AC, Zanatta MS, Medina JH, Izqueirdo I (1992) Amnesia by post-training infusion of glutamate receptor antagonists into the amygdala, hippocampus, and entorhinal cortex. Behav Neural Biol 58:76-80.

Ji JZ, Zhang XH, Li BM (2003) Deficient spatial memory induced by blockade of beta-adrenoceptors in the hippocampal CA1 region. Behav Neurosci 117:1378-1384.

Jin LQ, Goswami S, Cai G, Zhen X, Friedman E (2003) SKF83959 selectively regulates phosphatidylinositol-linked D1 dopamine receptors in rat brain. J Neurochem 85:378-386.

Joels M, Baram TZ (2009) The neuro-symphony of stress. Nat Rev Neurosci 10:459-466.

Johansen JP, Cain CK, Ostroff LE, LeDoux JE (2011) Molecular mechanisms of fear learning and memory. Cell 147:509-524.

Josselyn SA, Shi C, Carlezon WA Jr, Neve RL, Nertler EJ, Davis M (2001) Long-term memory is facilitated by cAMP response element-binding protein overexpression in the amygdala. J Neurosci 21:2404-2412.

Kalin NH, Shelton SE, Davidson RJ, Kelley AE (2001) The primate amygdala mediates acute fear but not the behavioral and physiological components of anxious temperament. J Neurosci 21:2067-2074.

Kalisch R, Holt B, Petrovic P, De Martino B, Kloppel S, Buchel C, Dolan RJ (2009) The NMDA agonist D-cycloserine facilitates fear memory consolidation in humans. Cereb Cortex 19:197-196.

Kawahara H, Yoshida M, Yokoo H, Nishi M, Tanaka M (1993) Psychological stress increases serotonin release in the rat amygdala and prefrontal cortex assessed by in vivo microdialysis. Neurosci Lett 162:81-84.

Keiper M, Stope MB, Szatkowski D, Bohm A, Tysack K, Vom Dorp F, Saur O, Oude Weernink PA, Evellin S, Jakobs KH, Schmidt M (2004) Epac- and Ca2+ -controlled activation of Ras and extracellular signal-regulated kinases by Gs-coupled receptors. J Biol Chem 279:46497-46508.

Kemppainen S, Pitkanen A (2000) Distribution of parvalbumin, calretinin, and calbindin-D(28k) immunoreactivity in the rat amygdaloid complex and colocalization with gamma-aminobutyric acid. J Comp Neurol 426:441-467.

Keselman I, Fribourg M, Felsenfeld DP, Logothetis DE (2007) Mechanism of PLC-mediated Kir3 current inhibition. Channels (Austin) 1:113-123.

Kordower JH, Bartus RT, Marciano FF, Gash DM (1989) Telencephalic Cholinergic System of the New World Monkey (Cebus-Apella) - Morphological and Cytoarchitectonic Assessment and Analysis of the Projection to the Amygdala. Journal of Comparative Neurology 279:528-545.

Kosenko A, Kang S, Smith IM, Greene DL, Langeberg LK, Scott JD, Hoshi N (2012) Coordinated signal integration at the M-type potassium channel upon muscarinic stimulation. EMBO J 31:3147-3156.

Kroner S, Rosenkranz JA, Grace AA, Barrioneuvo G (2005) Dopamine modulates excitability of basolateral amygdala neurons in vitro. J Neurophysiol 93:1598-1610.

LaLumiere RT, Buen TV, McGaugh JL (2003) Post-training intra-basolateral amygdala infusions of norepinephrine enhance consolidation of memory for contextual fear conditioning. J Neurosci 23:6754-6758.

LaLumiere RT, Nguyen LT, McGaugh JL (2005) Post-training intrabasolateral amygdala infusions of dopamine modulate consolidation of inhibitory avoidance memory: involvement of noradrenergic and cholinergic systems. Eur J Neurosci 20: 2804-2810.

Lamont EW, Kokkinidis L (1998) Infusion of the dopamine D1 receptor antagonist SCH 23390 into the amygdala blocks fear expression in a potentiated startle paradigm. Brain Res 795:128-136.

Lazzaro SC, Hou M, Cunha C, LeDoux JE, Cain CK (2010) Antagonism of lateral amygdala alpha1-adrenergic receptors facilitates fear conditioning and long-term potentiatIon. Learn Mem 17:489-493.

LeDoux JE, Iwata J, Cicchetti P, Reis DJ (1988) Different projections of the central amygdaloid nucleus mediate autonomic behavioral correlates of conditioned fear. J Neurosci 8:2517-2529.

LeDoux JE, Cicchetti P, Xagoraris A, Romanski LM (1990) The lateral amydaloid nucleus: sensory interface of the amygdala in fear conditioning. J Neurosci 10:1062-1069.

LeDoux JE (2000) Emotion circuits in the brain. Ann Rev Neurosci 23:155-184.

Lee HJ, Berger SY, Stiedl O, Speiss J, Kim JJ (2001) Post-training injections of catecholaminergic drugs do not modulate fear conditioning in rats and mice. Neurosci Lett 303:123-126.

Leonard SK, Anderson CM, Lachowicz JE, Schulz DW, Kilts CD, Mailman RB (2003) Amygdaloid D1 receptors are not linked to stimulation of adenylate cyclase. Synapse 50:320-333.

Levey AI, Kitt CA, Simonds WF, Price DL, Brann MR (1991) Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. J Neurosci 11:3218-3226.

Li XF, Stutzmann GE, LeDoux JE (1996) Convergent but temporally separated inputs to lateral amygdala neurons from the auditory thalamus and auditory cortex use different postsynaptic receptors: in vivo intracellular and extracellular recordings in fear conditioning pathways. Learn Mem 3:229-242.

Liang KC, McGaugh JL, Yao HY (1990) Involvement of amygdala pathways in the influence of post-training intra-amygdala norepinephrine and peripheral epinephrine on memory storage. Brain Res 508:225-233.

Logothetis DE, Petrou VI, Adney SK, Mahajan R (2010) Channelopathies linked to plasma membrane phosphoinositides. Pflugers Arch 460:321-341.

Lonart G, Schoch S, Kaeser PS, Larkin CJ, Sudhof TC, Linden DJ (2003) Phosphorylation of RIM1alpha by PKA triggers presynaptic long-term potentiation at cerebellar parallel fiber synapses. Cell 115:49-60.

Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM, Roder JC (1997) Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. J Neurosci 17:5196-5205.

Macphail EM (1968) Avoidance responding in pigeons. J Exp Anal Behav 11:692-632.

Mansour A, Meador-Woodruff JH, Zhou QY, Civelli O, Akil H, Watson SJ (1991) A comparison of D1 receptor binding and mRNA in rat brain using receptor autoradiographic and in situ hybridization techniques. Neuroscience 45:359-371.

Mantz J, Thierry AM, Glowinski J (1989) Effect of noxious tail pinch on the discharge rate of mesocortical and mesolimbic dopamine neurons: selective activation of the mesocortical system. Brain Res 476:377-381.

Maren S. Auditory fear conditioning increases CS-elicited spike firing in lateral amygdala neurons even after extensive overtraining. Eur J Neurosci 12:4047-4054.

Maren S, Ferrario CR, Corcoran KA, Desmond TJ, Frey KA (2003) Protein synthesis in the amygdala, but not the auditory thalamus, is required for consolidation of pavlovian fear conditioning in rats. Eur J Neurosci 18:3080-3088.

Martire M, Castaldo P, D'Amico M, Preziosi P, Annunziato L, Taglialatela M (2004) M channels containing KCNQ2 subunits modulate norepinephrine, aspartate, and GABA release from hippocampal nerve terminals. J Neurosci 24:592-597.

Marzo A, Bai J, Otani S (2009) Neuroplasticity regulation by noradrenaline in mammalian brain. Curr Neuropharmacol 7:286-295.

McDonald AJ (1982) Neurons of the lateral and basolateral amygdaloid nuclei: a Golgi study in the rat. J Comp Neurol 212:293-312.

McDonald AJ (1998) Cortical pathways to the amygdala. Prog Neurobiol 55:257-332.

McDonald AJ, Mascagni F (2002) Immunohistochemical characterization of somatostatin containing interneurons in the rat basolateral amygdala. Brain Res 943:237-244.

McDonald AJ, Mascagni F (2007) Neuronal localization of 5-HT type 2A receptor immunoreactivity in the rat basolateral amygdala. Neuroscience 146:306-320.

McDonald AJ, Mascagni F (2010) Neuronal localization of m1 muscarinic receptor immunoreactivity in the rat basolateral amygdala. Brain Struct Funct 215:37-48.

McDonald SD, Calhoun PS (2010) The diagnostic accuracy of the PTSD checklist: a critical review. Clin Psychol Rev 30:976-987.

McGaugh JL (1966) Time-dependent processes in memory storage. Science 153:1351-1358.

McGaugh JL (2000) Memory--a century of consolidation. Science 287:248-251.

McGaugh JL, Roozendaal B (2002) Role of adrenal stress hormones in forming lasting memories in the brain. Curr Opin Neurobiol 12:205-210.

McGhee LL, Maani CV, Garza TH, Desocio PA, Gaylord KM, Black IH (2009) The effect of propranolol on posttraumatic stress disorder in burned service members. J Burn Care Res 30:92-97.

McIntyre CK, Hatfield T, McGaugh JL (2002) Amygdala norepinephrine levels after training predict inhibitory avoidance retention performance in rats. Eur J Neurosci 16:1223-1226.

McKinney BC, Murphy GG (2006) The L-Type voltage-gated calcium channel Cav1.3 mediates consolidation, but not extinction, of contextually conditioned fear in mice. Learn Mem 13:584-589.

McOmish CE, Burrows EL, Howard M, Hannan AJ (2008a) PLC-beta1 knockout mice as a model of disrupted cortical development and plasticity: behavioral endophenotypes and dysregulation of RGS4 gene expression. Hippocampus 18:824-834.

McOmish CE, Burrows E, Howard M, Scarr E, Kim D, Shin HS, Dean B, van den Buuse M, Hannan AJ (2008b) Phospholipase C-beta1 knockout mice exhibit endophenotypes modeling schizophrenia which are rescued by environmental enrichment and clozapine administration. Mol Psychiatry 13:661-672.

Miller S, Yasuda M, Coats JK, Jones Y, Martone ME, Mayford M (2002) Disruption of dendritic translation of CaMKIIalpha impairs stabilization of synaptic plasticity and memory consolidation. Neuron 36:507-519.

Miserendino MJ, Sananes CB, Melia KR, Davis M (1990) Blocking of acquisition but not expression of conditioned fear- potentiated startle by NMDA antagonists in the amygdala. Nature 345:716-718.

Morris JS, DeGelder B, Weiskrantz L, Dolan RJ (2001) Differential extrageniculostriate and amygdala responses to presentation of emotional faces in a cortically blind field. Brain 124:1241-1252.

Mou L, Heldt SA, Ressler KJ (2011) Rapid brain-derived neurotrophic factor-dependent sequestration of amygdala and hippocampal GABA(A) receptors via different tyrosine receptor kinase B-mediated phosphorylation pathways. Neuroscience 176:72-85.

Muller JF, Mascagni F, McDonald AJ (2006) Pyramidal cells of the rat basolateral amygdala: synaptology and innervation by parvalbumin-immunoreactive interneurons. J Comp Physiol 494:635-650.

Muller JF, Mascagni F, McDonald AJ (2009) Dopaminergic innervation of pyramidal cells in the rat basolateral amygdala. Brain Struct Funct 213:275-288.

Muller JF, Mascagni F, Zaric V, McDonald AJ (2013) Muscarinic cholinergic receptor M1 in the rat basolateral amygdala: ultrastructural localization and synaptic relationships to cholinergic axons. J Comp Neurol 521:1743-1759.

Muly EC, Senyuz M, Khan ZU, Guo JD, Hazra R, Rainnie DG (2009) Distribution of D1 and D5 dopamine receptors in the primate and rat basolateral amygdala. Brain Struct Funct 213:375-393.

Murchison CF, Zhang XY, Zhang WP, Ouyang M, Lee A, Thomas SA (2004) A distinct role for norepinephrine in memory retrieval. Cell 117:131-143.

Nader K, LeDoux JE (1999) Inhibition of the mesoamygdala dopaminergic pathway impairs the retrieval of conditioned fear associations. Behav Neurosci 113:891-901.

Nader K, Majidishad P, Amorapanth P, LeDoux JE (2001) Damage to the lateral and central, but not other, amygdaloid nuclei prevents the acquisition of auditory fear conditioning. Learn Mem 8:156-163.

Nielsen KS, Macphail EM, Riedel G (1997) Class I mGlu receptor antagonist 1-aminoindan-1,5-dicarboxylic acid blocks contextual but not cue conditioning in rats. Eur J Pharmacol 326:105-108.

Nieoullon A (2002) Dopamine and the regulation of cognition and attention. Prog Neurobiol 67:53-83.

Nugent NR, Christopher NC, Crow JP, Browne L, Ostrowski S, Delahanty DL (2010) The efficacy of early propranolol administration at reducing PTSD symptoms in pediatric injury patients: a pilot study. J Trauma Stress 23:282-287.

O'Carroll RE, Drysdale E, Cahill L, Shajahan P, Ebmeier KP (1999) Memory for emotional material: a comparison of central versus peripheral beta blockade. J Psychopharmacol 13:32-39.

O'Donnell SR, Wanstall JC (1980) Evidence that ICI 118, 551 is a potent, highly Beta 2-selective adrenoceptor antagonist and can be used to characterize Beta-adrenoceptor populations in tissues. Life Sci 27:671-677.

Ohman A, Mineka S (2001) Fears, phobias, and preparedness: toward an evolved module of fear and fear learning. Psychol Rev 108:483-522.

Ohman A, Flykt A, Esteves F (2001) Emotion drives attention: detecting snakes in the grass. J Exp Psychol Gen 130:466-478.

Orhan G, Wuttke TV, Nies AT, Schwab M, Lerche H (2012) Retigabine/Ezogabine, a KCNQ/K(V)7 channel opener: pharmacological and clinical data. Expert Opin Pharmacother 13:1807-1816.

Ostroff LE, Cain CK, Bedont J, Monfils MH, LeDoux JE (2010) Fear and safety learning differentially affect synapse size and dendritic translation in the lateral amygdala. Proc Natl Acad Sci U S A 520:295-314.

Ou LC, Gean PW (2007) Transcriptional regulation of brain-derived neurotrophic factor in the amygdala during consolidation of fear memory. Mol Pharmacol 72:350-358.

Ouyang M, Hellman K, Abel T, Thomas SA (2004) Adrenergic signaling plays a critical role in the maintenance of waking and in the regulation of REM sleep. J Neurophysiol 92:2071-2082.

Ouyang M, Zhang L, Zhu JJ, Schwede F, Thomas SA (2008) Epac signaling is required for hippocampus-dependent memory retrieval. Proc Natl Acad Sci USA 105:11993-11997.

Ouyang M, Young MB, Lestini MM, Schutsky K, Thomas SA (2012) Redundant catecholamine signaling consolidates fear memory via phospholipase C. J Neurosci 32:1932-1941.

Overeem KA, Ota KT, Monsey MS, Ploski JE, Schafe GE (2010) A role for nitric oxide-driven retrograde signaling in the consolidation of fear memory. Front Behav Neurosci 4:2.

Ozaki H, Hori M, Kim YS, Kwon SC, Ahn DS, Nakazawa H, Kobayashi M, Karaki H (2002) Inhibitory mechanism of xestospongin-C on contraction and ion channels in the intestinal smooth muscle. Br J Pharmacol 137:1207-1212.

Pape HC, Pare D (2010) Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiol Rev 90:419-463.

Pare D, Collins DR (2000) Neuronal correlates of fear in the lateral amygdala: multiple extracellular recordings in conscious cats. J Neurosci 20:2701-2710.

Parsons RG, Ressler KJ (2013) Implications of memory modulation for post-traumatic stress and fear disorders. Nat Neurosci 16:146-153.

Pelletier JG, Pare D (2004) Role of amygdala oscillations in the consolidation of emotional memories. Biol Psychiatry 55:559-562.

Pelletier JG, Likhtik E, Filali M, Pare D (2005) Lasting increases in basolateral amygdala activity after emotional arousal: implications for facilitated consolidation of emotional memories. Learn Mem 12:96-102.

Peters HC, Hu H, Pongs O, Storm JF, Isbrandt D (2005) Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior. Nat Neurosci 8:51-60.

Petrovich GD, Risold PY, Swanson LW (1996) Organization of projections from the basomedial nucleus of the amygdala: a PHAL study in the rat. J Comp Neurol 374:387-420.

Petrovich MM, Nowacki J, Olivo V, Tsaneva-Atanasova K, Randall AD, Mellor JR (2012) Inhibition of post-synaptic Kv7/KCNQ/M channels facilitates long-term potentiation in the hippocampus. PLoS One 7(2):e30402.

Pezze MA, Feldon J (2004) Mesolimbic dopaminergic pathways in fear conditioning. Prog Neurobiol 74:301-320.

Pinard CR, Muller JF, Mascagni F, McDonald AJ (2008) Dopaminergic innervation of interneurons in the rat basolateral amygdala. Neuroscience 157:850-863.

Pitkänen A *(*2000*)* Connectivity of the rat amygdaloid complex. *in* The amygdala. A functional analysis*, ed* Aggleton JP *(*Oxford UP*,* Oxford*),* pp 31–116*.*

Ploski JE, Park KW, Ping J, Monsey MS, Schafe GE (2010) Identification of plasticity-associated genes regulated by pavlovian gear conditioning in the lateral amygdala. J Neurochem 112:636-650.

Poulin B, Butcher A, McWilliams P, Bourgognon JM, Pawlak R, Kong KC, Bottrill A, Mistry S, Wess J, Rosethorne EM, Charlton SJ, Tobin AB (2010) The M3-muscarinic receptor regulates learning and memory in a receptor phosphorylation/arrestin-dependent manner. Proc Natl Acad Sci USA 107:9440-9445.

Power AE, McIntyre CK, Litmanovich A, McGaugh JL (2003) Cholinergic modulation of memory in the basolateral amygdala involves activation of both m1 and m2 receptors. Behav Pharmacol 14:207-213.

Power JM, Sah P (2007) Distribution of IP3-mediated calcium responses and their role in nuclear signalling in rat basolateral amygdala neurons. J Physiol 580:835-857.

Power JM, Sah P (2008) Competition between calcium-activated K+ channels determines cholinergic action on firing properties of basolateral amygdala projection neurons. J Neurosci 28:3209-3220.

Powis G, Seewald MJ, Gratas C, Melder D, Riebow J, Modest EJ (1992) Selective inhibition of phosphatidylinositol phospholipase C by cytotoxic ether lipid analogues. Cancer Res 52:2835-2840.

Qu LL, Guo NN, Li BM (2008) Beta1- and beta2-adrenoceptors in basolateral nucleus of amygdala and their roles in consolidation of fear memory in rats. Hippocampus 18:1131-1139.

Quirarte GL, Roozendaal B, McGaugh JL (1997) Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdala. Proc Natl Acad Sci U S A 94:14048-14053.

Quirk GJ, Repa C, LeDoux JE (1995) Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. Neuron 15:1029-1039.

Ramos M, Goni-Allo B, Aguirre N (2005) Administration of SCH 23390 into the medial prefrontal cortex blocks the expression of MDMA-induced behavioral sensitization in rats: an effect mediated by 5-HT2C receptor stimulation and not by D1 receptor blockade. Neuropsychopharmacology 30:2180-2191.

Rainnie DG, Asprodini EK, Schinnick-Gallagher P (1993) Intracellular recordings from morphologically identified neurons of the basolateral amygdala. J Neurophysiol 69:1350-1362.

Rainnie DG, Mania I, Mascagni F, McDonald AJ (2006) Physiological and morphological characterization of parvalbumin-containing interneurons of the rat basolateral amygdala. 498:142-161.

Rao ZR, Yamano M, Wanaka A, Tatehata T, Shiosaka S, Tohyama M (1987) Distribution of cholinergic neurons and fibers in the hypothalamus of the rat using choline acetyltransferase as a marker. Neuroscience 20:923-934.

Repa JC, Muller J, Apergis J, Desrochers TM, Zhou Y, LeDoux JE (2001) Two different lateral amygdala cell populations contribute to the initiation and storage of memory. Nat Neurosci 4:724-731.

Robinson L, Platt B, Riedel G (2011) Involvement of the cholinergic system in conditioning and perceptual memory. Behav Brain Res 221:443-465.

Rockman HA, Koch WJ, Lefkowitz RJ (2002) Seven-transmembrane-spanning receptors and heart function. Nature 415:206-212.

Rodrigues SM, Schafe GE, LeDoux JE (2001) Intra-amygdala blockade of the NR2B subunit of the NMDA receptor disrupts the acquisition but not the expression of fear conditioning. J Neurosci 21:6889-6896.

Rodrigues SM, Bauer EP, Farb CR, Schafe GE, LeDoux JE (2002) The group I metabotropic glutamate receptor mGluR5 is required for fear memory formation and long-term potentiation in the lateral amygdala. J Neurosci 22:5219-5229.

Rogan MT, LeDoux JE (1995) LTP is accompanied by commensurate enhancement of auditory-evoked responses in a fear conditioning circuit. Neuron 15:127-136.

Rohrer DK, Desai KH, Jasper JR, Stevens ME, Regula DP, Jr., Barsh GS, Bernstein D, Kobilka BK (1996) Targeted disruption of the mouse beta1-adrenergic receptor gene: developmental and cardiovascular effects. Proc Natl Acad Sci USA 93:7375-7380.

Romanski LM, LeDoux JE (1993) Information cascade from primary auditory cortex to the amygdala: corticocortical and corticoamygdaloid projections of temporal cortex in the rat. Cereb Cortex 3:515-532.

Roozendaal B, Okuda S, Van der Zee EA, McGaugh JL (2006) Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. Proc Natl Acad Sci U S A 103:6741-6746.

Roozendaal B, Castello NA, Vedana G, Barsegyan A, McGaugh JL (2008) Noradrenergic activation of the basolateral amygdala modulates consolidation of object recognition memory. Neurobiol Learn Mem 90:576-579.

Roozendaal B, McEwen BS, Chattarji S (2009) Stress, memory and the amygdala. Nat Rev Neurosci 10:423-433.

Royer S, Martina M, Pare D (1999) An inhibitory interface gates impulse traffic between input and output stations of the amygdala. J Neurosci 19:10575-10583.

Rumpel S, LeDoux J, Zador A, Malinow R (2005) Postsynaptic receptor trafficking underlying a form of associative learning. Science: 308:83-88.

Rudy JW, Matus-Amat P (2009) DHPG activation of group 1 mGluRs in BLA enhances fear conditioning. Learn Mem 16:421-425.

Sadalge A, Coughlin L, Fu H, Wang B, Valladares O, Valentino R, Blendy JA (2003) alpha 1d Adrenoceptor signaling is required for stimulus induced locomotor activity. Mol Psychiatry 8:664-672.

Sahu A, Tyeryar KR, Vongtau HO, Sibley DR, Undieh AS (2009) D5 dopamine receptors are required for dopaminergic activation of phospholipase C. Mol Pharmacol 75:447-453.

Santini E, Porter JT (2010) M-type potassium channels modulate the intrinsic excitability of infralimbic neurons and regulate fear expression and extinction. J Neurosci 30:12379-12386.

Sara SJ (2009) The locus coeruleus and noradrenergic modulation of cognition. Nat Rev Neurosci 10:211-223.

Schafe GE, Nadel NV, Sullivan GM, Harris A, LeDoux JE (1999) Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. Learn Mem 6:97-110.

Schafe GE, LeDoux JE (2000) Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. J Neurosci 20:RC96.

Schroeder BW, Schinnick-Gallagher P (2005) Fear learning induces persistent facilitation of amygdala synaptic transmission. Eur J Neurosci 22:1775-1783.

Schudt C, Auriga C, Birdsall NJ, Boer R (1988) Does the prolonged occupancy of M1 receptors by telenzepine protect them against the action of vagally released acetylcholine? Pharmacology 37 Suppl 1:32-39.

Schutsky K, Ouyang M, Castelino CB, Zhang L, Thomas SA (2011) Stress and glucocorticoids impair memory retrieval via {beta}2-adrenergic, Gi/o-coupled suppression of cAMP signaling. J Neurosci 31:14172-14181.

Selcher JC, Weeber EJ, Varga AW, Sweatt JD, Swank M (2002) Protein kinase signal transduction cascades in mammalian associative conditioning. Neuroscientist 8:122-131.

Serrano P, Friedman EL, Kenney J, Taubenfeld SM, Zimmerman JM, Hanna J, Alberini C, Kelley AE, Maren S, Rudy JW, Yin JC, Sacktor TC, Fenton AA (2008) PKMzeta maintains spatial, instrumental, and classically conditioned long-term memories. PLoS Biol 6:2698-2706.

Shah MM, Migliore M, Brown DA (2011) Differential effects of Kv7 (M-) channels on synaptic integration in distinct subcellular compartments of rat hippocampal pyramidal neurons. J Physiol 589:6029-6038.

Shen W, Hamilton SE, Nathanson NM, Surmeier DJ (2005) Cholinergic suppression of KCNQ channel currents enhances excitability of striatal medium spiny neurons. J Neurosci 25:7449-7458.

Shinnick-Gallagher P, McKernan MG, Xie J, Zinebi F (2003) L-type voltage-gated calcium channels are involved in the in vivo and in vitro expression of fear conditioning. Ann N Y Acad Sci 985:135-149.

Sibley DR, Monsma FJ, Jr. (1992) Molecular biology of dopamine receptors. Trends Pharmacol Sci 13:61-69.

Sigurdsson T, Doyere V, Cain CK, LeDoux JE (2007) Long-term potentiation in the amygdala: a cellular mechanism of fear learning and memory. Neuropharmacology 52:215-227.

Simon MI, Strathmann MP, Gautam N (1991) Diveresity of G proteins in signal transduction. Science 252:802-808.

Slotnick BM (1973) Fear behavior and passive avoidance deficits in mice with amygdala lesions. Physiol Behav 11:717-720.

Soeter M, Kindt M (2011) Noradrenergic enhancement of associative fear memory in humans. Neurobiol Learn Mem 96:263-271.

Southwick SM, Davis M, Horner B, Cahill L, Morgan CA 3rd, Gold PE, Bremner JD, Charney DC (2002) Relationship of enhanced norepinephrine activity during memory consolidation to enhanced long-term memory in humans. Am J Psychiatry 150:14201422.

Steckler T, Risbrough V (2012) Pharmacological treatment of PTSD - established and new approaches. Neuropharmacology 62:617-627.

Stein MB, Kerridge C, Dimsdale JE, Hoyt DB (2007) Pharmacotherapy to prevent PTSD: Results from a randomized controlled proof-of-concept trial in physically injured patients. J Trauma Stress 20:923-932.

Stork O, Welzl H (1999) Memory formation and the regulation of gene expression. 55:575-592.

Stratton KR, Cole AJ, Pritchett J, Eccles CU, Worley PF, Baraban JM (1989) Intrahippocampal injection of pertussis toxin blocks adenosine suppression of synaptic responses. Brain Res 494:359-364.

Suh BC, Hille B (2002) Recovery from muscarinic modulation of M current channels requires phosphatidylinositol 4,5-bisphosphate synthesis. Neuron 35:507-520.

Suh BC, Hille B (2008) PIP2 is a necessary cofactor for ion channel function: how and why? Annu Rev Biophys 37:175-195.

Suh BC, Inoue T, Meyer T, Hille B (2006) Rapid chemically induced changes of PtdIns(4,5)P2 gate KCNQ ion channels. Science 314:1454-1457.

Takahashi H, Takano H, Kodaka F, Arakawa R, Yamada M, Otsuka T, Hirano Y, Kikyo H, Okubo Y, Kato M, Obata T, Ito H, Suhara T (2010) Contribution of dopamine D1 and D2 receptors to amygdala activity in human. J Neurosci 30:3043-3047.

Tatulian L, Delmas P, Abogadie FC, Brown DA (2001) Activation of expressed KCNQ potassium currents and native neuronal M-type potassium currents by the anti-convulsant drug retigabine. J Neurosci 21:5535-5545.

Tecott LH, Logue SF, Wehner JM, Kauer JA (1998) Perturbed dentate gyrus function in serotonin 5-HT2C receptor mutant mice. Proc Natl Acad Sci USA 95:15026-15031.

Telezhkin V, Reilly JM, Thomas AM, Tinker A, Brown DA (2012) Structural requirements of membrane phospholipids for M-type potassium channel activation and binding. J Biol Chem 287:10001-10012.

Thomas SA, Palmiter RD (1997) Disruption of the dopamine beta-hydroxylase gene in mice suggests roles for norepinephrine in motor function, learning, and memory. Behav Neurosci 111:579-589.

Thomas SA, Matsumoto AM, Palmiter RD (1995) Noradrenaline is essential for mouse fetal development. Nature 374:643-646.

Tronel S, Feenstra MG, Sara SJ (2004) Noradrenergic action in prefrontal cortex in the late stage of memory consolidation. Learn Mem 11:453-458.

Tsubokawa H, Ross WN (1997) Muscarinic modulation of spike backpropagation in the apical dendrites of hippocampal CA1 pyramidal neurons. J Neurosci 17:5782-5791.

Tully K, Bolshakov VY (2010) Emotional enhancement of memory: how norepinephrine enables synaptic plasticity. Mol Brain 3:15.

Turner BH, Herkenham M (1991) Thalamoamygdaloid projections in the rat: a test of the amygdala's role in sensory processing. J Comp Neurol 313:295-325.

Tzingounis AV, Nicoll RA (2008) Contribution of KCNQ2 and KCNQ3 to the medium and slow afterhyperpolarization currents. Proc Natl Acad Sci U S A 105:19974-19979.

Ulrich-Lai YM, Herman JP (2009) Neural regulation of endocrine and autonomic stress responses. Nat Rev Neurosci 10:497-409.

Undie AS, Weinstock J, Sarau HM, Friedman E (1994) Evidence for a distinct D1-like dopamine receptor that couples to activation of phosphoinositide metabolism in brain. J Neurochem 62:2045-2048.

Vazdarjanova A, McGaugh JL (1999) Basolateral amygdala is involved in modulating consolidation of memory for classical fear conditioning. J Neurosci 19:6615-6622.

Vervaeke K, Gu N, Agdestein C, Hu H, Storm JF (2006) Kv7/KCNQ/M-channels in rat glutamatergic hippocampal axons and their role in regulation of excitability and transmitter release. J Physiol 576:235-256.

Villalobos C, Foehring RC, Lee JC, Andrade R (2011) Essential role for phosphatidylinositol 4,5-bisphosphate in the expression, regulation, and gating of the slow afterhyperpolarization current in the cerebral cortex. J Neurosci 31:18303-18312.

Vuilleumier P, Armony JL, Clarke K, Husain M, Driver J, Dolan RJ (2002) Neural response to emotional faces with and without awareness: event-related fMRI in a parietal patient with visual extinction and spatial neglect. Neuropsychologia 40:2156-2166.

Waelbroeck M, Taton G, Delhaye M, Chatelain P, Camus JC, Pochet R, Leclerc JL, De Smet JM, Robberecht P, Christophe J (1983) The human heart beta-adrenergic receptors. II. Coupling of beta 2-adrenergic receptors with the adenylate cyclase system. Mol Pharmacol 24:174-182.

Washburn MS, Moises HC (1992) Muscarinic responses of rat basolateral amygdaloid neurons recorded in vitro. J Physiol 449:121-154.

Weeber EJ, Atkins CM, Selcher JC, Varga AW, Mirnikjoo B, Paylor R, Leitges M, Sweatt JD (2000) A role for the beta isoform of protein kinase C in fear conditioning. J Neurosci 20:5906-5914.

Weeber EJ, Caldwell KK (2004) Delay fear conditioning modifies phospholipase C-beta 1a signaling in the hippocampus and frontal cortex. Pharmacol Biochem Behav 78:155-164.

Wei F, Qiu CS, Liauw J, Robinson DA, Ho N, Chatila T, Zhuo M (2002) Calcium calmodulin-dependent protein kinase IV is required for fear memory. Nat Neurosci 5:573-579.

Weisstaub NV, Zhou M, Lira A, Lambe E, Gonzalez-Maeso J, Hornung JP, Sibille E, Underwood M, Itohara S, Dauer WT, Ansorge MS, Morelli E, Mann JJ, Toth M, Aghajanian G, Sealfon SC, Hen R, Gingrich JA (2006) Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. Science 313:536-540.

West AE, Chen WG, Dalva MB, Dolmetsch RE, Kornhauser JM, Shaywitz AJ, Takasu MA, Tao X, Greenberg ME (2001) Calcium regulation of neuronal gene expression. Proc Natl Acad Sci U S A 98:11024-11031.

Wilensky AE, Schafe GE, LeDoux JE (2000) The amygdala modulates memory consolidation of fear-motivated inhibitory avoidance learning but not classical fear conditioning. J Neurosci 20:7059-7066.

Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5:483-494.

Womble MD, Moises HC (1992) Muscarinic inhibition of M-current and a potassium leak conductance in neurones of the rat basolateral amygdala. J Physiol 457:93-114.

Womble MD, Moises HC (1993) Muscarinic modulation of conductances underlying the afterhyperpolarization in neurons of the rat basolateral amygdala. Brain Res 621:87-96.

Wuttke TV, Seebohm G, Bail S, Maljevic S, Lerche H (2005) The new anticonvulsant retigabine favors voltage-dependent opening of the Kv7.2 (KCNQ2) channel by binding to its activation gate. Mol Pharmacol 67:1009-1017.

Xu M, Moratalla R, Gold LH, Hiroi N, Koob GF, Graybiel AM, Tonegawa S (1994) Dopamine D1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. Cell 79:729-742.

Yajeya J, de la Fuente Juan A, Bajo VM, Riolobos AS, Heredia M, Criado JM (1999) Muscarinic activation of a non-selective cationic conductance in pyramidal neurons in rat basolateral amygdala. Neuroscience 88:159-167.

Yamamoto R, Ueta Y, Kato N (2007) Dopamine induces a slow afterdepolarization in lateral amygdala neurons. J Neurophysiol 98:984-992.

Yokoyama M, Suzuki E, Sato T, Maruta S, Watanabe S, Miyaoka H (2005) Amygdalic levels of dopamine and serotonin rise upon exposure to conditioned fear stress without elevation of glutamate. Neurosci Lett 379:37-41.

Yu C, Yaari Y (2004) KCNQ/M channels control spike afterdepolarization and burst generation in hippocampal neurons. J Neurosci 24:4614-4624.

Zhang G, Asgeirsdottir HN, Cohen SJ, Munchow AH, Barrera MP, Stackman RW Jr (2013) Stimulation of serotonin 2A receptors facilitates consolidation and extinction of fear memory in C57BL/6J mice. Neuropharmacology 64:403-413.

Zhang W, Schneider DM, Belova MA, Morrison SE, Paton JJ, Salzman CD (2013) Functional circuits and anatomical distribution of response properties in the primate amygdala. J Neurosci 33:722-733.

Zhou Y, Won J, Karlsson MG, Zhou M, Rogerson T, Balaji J, Neve R, Poirazi P, Silva AJ (2009) CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. Nat Neurosci 12:1438-1443.

Zweifel LS, Fadok JP, Argilli E, Garelick MG, Jones GL, Dickerson TM, Allen JM, Mizumori SJ, Bonci A, Palmiter RD (2011) Activation of dopamine neurons is critical for aversive conditioning and prevention of generalized anxiety. Nat Neurosci 14:620-626.