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Cholinergic regulation of fear learning and extinction

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Abstract

Cholinergic activation regulates cognitive function, and particularly long term memory consolidation. Here we present an overview of the anatomical, neurochemical, and pharmacological evidence supporting the cholinergic regulation of Pavlovian contextual and cue conditioned fear learning and extinction. Basal forebrain cholinergic neurons provide inputs to neocortical regions and subcortical limbic structures such as the hippocampus and amygdala. Pharmacological manipulations of muscarinic and nicotinic receptors support the role of cholinergic processes in the amygdala, hippocampus, and prefrontal cortex in modulating the learning and extinction of contexts or cues associated with threat. Additional evidence from lesion studies and analysis of in vivo acetylcholine release using microdialysis similarly support a critical role of cholinergic neurotransmission in cortico-amygdalar or cortico-hippocampal circuits during acquisition of fear extinction. Although a few studies suggest a complex role of cholinergic neurotransmission in the cellular plasticity needed for extinction learning, more work is needed to elucidate the exact cholinergic mechanisms and physiological role of muscarinic and nicotinic receptors in these fear circuits. Such studies will be important for elucidating the role of cholinergic neurotransmission in disorders such as post-traumatic stress disorder (PTSD) that involve deficits in extinction learning as well as developing novel therapeutic approaches for such disorders.

Graphical abstract

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INTRODUCTION

Overview of cholinergic systems and cognitive functions

A variety of different types of evidence implicate cholinergic signaling—particularly via activation of muscarinic cholinergic receptors (mAChR)—in cognitive function, and more specifically long term memory consolidation (see (Gold 2003; Power et al. 2003b; Robinson et al. 2011; Tinsley 2004)). In addition, it appears that acetylcholine may interact with other modulators, including the noradrenergic, glucocorticoid, and histaminergic systems, to facilitate aversive learning in tasks dependent on regions such as the basolateral amygdala (Power et al. 2003b). This cholinergic regulation is seen with both systemic and region-specific pharmacological manipulations in various learning tasks, including Pavlovian fear learning and extinction paradigms. Although other conditioned tasks are also affected by these pharmacological manipulations, we will focus our review specifically on Pavlovian contextual and cue-conditioned fear responses and extinction. Other reviews more completely cover cholinergic modulation of additional conditioned learning responses, such as inhibitory avoidance, (Gold 2003; Gould and Leach 2014; Power et al. 2003b; Robinson et al. 2011; Tinsley 2004). In fear conditioning procedures animals are conditioned by pairing a neutral stimulus, such as a tone (the conditioned stimulus or CS) with an aversive stimulus such as a footshock (the US or unconditioned stimulus). The pairing of the CS and US enables both the context and the CS (the tone), even when the CS is presented in a novel context, to elicit a defensive response such as freezing (Fendt and Fanselow 1999). Repeated re-exposure to the context or the CS in the absence of the US results in the extinction of the response (Baldi and Bucherelli 2015). A variety of studies have demonstrated key roles for plasticity in the amygdala, hippocampus and prefrontal cortex in driving these conditioned fear and extinction responses (Baldi and Bucherelli 2015; Fendt and Fanselow 1999; Milad and Quirk 2012). Moreover, studies suggest that extinction learning involves distinct neuronal populations and signaling processes from the original learning of the contextual or cue-conditioned responses (Herry et al. 2008; Tronson et al. 2009), and extinction of contextual or cued fear responses appears to involve prefrontal-amygdalar and prefrontal-

hippocampal circuits (see (Baldi and Bucherelli 2015; Orsini and Maren 2012; Rozeske et al. 2015)).

Anatomy of cholinergic regulation of amygdala, hippocampus, and prefrontal cortex

Mammalian forebrain cholinergic neurons have been broadly grouped into four clusters, using the nomenclature of Mesulam and colleagues (Mesulam et al. 1983b): the first three subgroups (Ch1–3) consist of neurons in the medial septum (MS) and vertical and horizontal limbs of the diagonal band of Broca (DBB), and provide cholinergic innervation of the hippocampus and olfactory bulb. The fourth group (Ch4) of basal forebrain cholinergic neurons includes a loosely-clustered arrangement of cholinergic neurons located in the nucleus basalis magnocellularis (nBM) and rostrally contiguous ventral pallidum/substantia innominata (VP/SI) (Mesulam et al. 1983a; Mesulam et al. 1983b). These neurons project diffusely to all layers and areas of the neocortical mantle (Bigl et al. 1982; Struble et al. 1986), where the primary physiological effect of acetylcholine (ACh) is to modulate the response of pyramidal cells to other—particularly glutamatergic—cortical input (McCormick 1993; Metherate and Ashe 1993). This innervation of the neocortex by basal forebrain cholinergic neurons is an important mediator of cortical activation in support of cognitive function. Correspondingly, electrical stimulation of the basal forebrain increases cortical acetylcholine release and desynchronizes the cortical electroencephalogram (Kurosawa et al. 1989; Metherate et al. 1992; Rasmusson et al. 1992). It has long been appreciated that the basal forebrain plays an important role in cognitive, and particularly attentional, function. For example, a series of important primate studies from the 1970's showed that (putatively cholinergic) basal forebrain neurons respond to food-related visual stimuli only when the animal is hungry (Burton et al. 1976; Mora et al. 1976; Rolls et al. 1977; Rolls et al. 1979). These observations provided a clear demonstration that the interoceptive state of an animal modulates the ability of exteroceptive cues to drive forebrain attentional systems. However, interest in the cognitive functions of the basal forebrain cholinergic system (BFCS) accelerated greatly in the 1980s, spurred by the discovery that cholinergic cell loss is the primary neurotransmitter pathological hallmark of Alzheimer's disease (McGeer et al. 1984; Whitehouse et al. 1982). Since that time, there has been much interest in the anatomical and molecular substrates that mediate cholinergic regulation of attention, learning and memory in a variety of paradigms.

As the rostral-most component of the cortical mantle, the prefrontal cortex (PFC) derives its major cholinergic innervation from the Ch4 group of basal forebrain neurons. Some reports in rodents suggest that a smaller portion of cholinergic innervation of the medial prefrontal cortex additionally arises from the diagonal band of Broca and medial septum, as well as the pedunculopontine and laterodorsal tegmental areas of the brain stem (Eckenstein et al. 1988; Lamour et al. 1984). Studies using immunotoxic lesions of cholinergic neurons in these areas similarly suggest cholinergic projections into the prelimbic and infralimbic cortex might be segregated throughout this continuum. These studies using immunotoxic lesions demonstrated significant loss of acetylcholinesterase staining in both hippocampus and prefrontal cortex following injections into the basal forebrain or MS/vertical DBB. In contrast, targeting the nBM/horizontal DBB predominately affected cholinergic projections to the prelimbic cortex, but spared hippocampal projections (Knox and Keller 2015).

In addition to the neocortex, the BFCS is also the primary source of cholinergic innervation of subcortical limbic structures such as the hippocampus and the amygdala. In fact, cholinergic projections from the Ch4 subgroup form the densest source of neuromodulatory inputs to the amygdala—especially the basolateral complex (BLA)(Muller et al. 2011). Although the functions of these cholinergic inputs are understudied, given the role of the BFCS in attention one might predict that amygdalar acetylcholine enhances the attentional processing of emotional states and the association of these states with neutral stimuli in the environment. Cholinergic inputs from the BFCS target both calcium/calmodulin kinase (CaMK)-positive (putatively glutamatergic) pyramidal neurons and parvalbumin-positive interneurons in the BLA, although the preponderance of cholinergic innervation is to glutamatergic projection neurons of the BLA (Muller et al. 2011).

Anatomical studies suggest that M1 and M2 muscarinic receptors are selectively expressed in different components of the microcircuits in the amygdala, hippocampus, and prefrontal cortex that receive cholinergic inputs from BFCS. In the BLA, studies by McDonald and colleagues have shown pyramidal cells express relatively high levels of M1 and M2 mAChR, although M1 receptors are localized in the perikarya while both M1 and M2 receptors are found in dendritic shafts and spines of these cells (McDonald and Mascagni 2010; Muller et al. 2013; Muller et al. 2016). In the prefrontal cortex and hippocampus, studies have similarly suggested M1 (and M3) receptors are predominantly localized postsynaptically on glutamatergic pyramidal neurons (Rouse et al. 1998; Rouse et al. 1999; Volpicelli and Levey 2004). In the BLA, non-pyramidal inhibitory interneurons also express M1 and M2 mAChRs, although further studies are needed to determine if mAChR expression differs between interneuronal cell types as is seen in the hippocampus (Hajos et al. 1998). In addition, cholinergic inputs from the BFCS into the BLA only express M2 receptors, suggesting a role in autoreceptor control of acetylcholine release in this region, while GABAergic projections from the BFCS express both M1 and M2 muscarinic receptor subtypes (Muller et al. 2013; Muller et al. 2016). This has similarly been demonstrated in hippocampus and prefrontal cortex, where cholinergic inputs from BFCS and MS have presynaptic M2 receptors (Rouse et al. 1999; Volpicelli and Levey 2004). The functional role of muscarinic receptors on different cell types in the amygdala was similarly suggested using optogenetic stimulation of cholinergic fibers in the BLA, which influenced both interneurons and pyramidal cells. Although the physiological effects differed according to cell type and stimulation parameters, the net effect of optogenetic activation was enhancement of the “signal to noise ratio”, similar to cholinergic modulation of neocortical pyramidal cells (Unal et al. 2015). Muscarinic activation also increases excitability of infralimbic neurons by decreasing both M-type potassium conductance and potassium channel afterhyperpolarizations (AHPs) (Santini and Porter 2010; Santini et al. 2012). However, muscarinic activation also decreases synaptic efficacy of hippocampal inputs into prelimbic cortex via M2 receptor activation (Wang and Yuan 2009). Interestingly, a subset of BLA-projecting cholinergic neurons from the basal forebrain may also be glutamatergic as evidenced by immunohistochemical colocalization of choline acetyltransferase and the vesicular glutamate transporter-3 (Nickerson Poulin et al. 2006).

While cholinergic innervation of the cortical mantle is diffuse, reciprocal excitatory inputs from cortex back to the basal forebrain derive disproportionately from limbic- and

paralimbic-associated areas, including prefrontal and agranular insular regions (Carnes et al. 1990; Mesulam 2013; Zaborszky et al. 1997). Additional major sources of inputs to the basal forebrain derive from the nucleus accumbens (Mogenson et al. 1983; Zaborszky and Cullinan 1992) and parts of the amygdala (Grove 1988), although inputs from the central nucleus may be restricted to a caudal part of the basal forebrain cholinergic system (Gastard et al. 2002). The Ch4 neurons also receive input from brain stem monoamines, including constituents of the ascending reticular activating system and its 'rostral extension' in the ventral tegmental area (Jones and Cuello 1989; Smiley et al. 1999; Zaborszky 1989), and the hypothalamus (Zaborszky and Cullinan 1989). Thus, the BFCS is situated at the intersection of cortical, subcortical, diencephalic, and brain stem systems that form limbic and paralimbic circuits, and is therefore ideally located to modulate cognitive correlates of emotional behaviors, including Pavlovian conditioning and extinction.

Cholinergic regulation of conditioned fear and extinction

Prefrontal cortical inputs to the amygdala play a critical role in the adaptive behavioral responses to threatening stimuli, and particularly in extinction of the learned responses to such threats (Baldi and Bucherelli 2015; Milad and Quirk 2012). As described above, the basolateral amygdala receives very dense cholinergic innervation from basal forebrain, suggesting that cholinergic neurotransmission also plays a central role in regulating amygdalar function. Further, the cholinergic innervation of prefrontal cortex and amygdala derives from a separate sub-region of the basal forebrain than the cell groups that innervate the hippocampus. This anatomical segregation may, in part, underlie pharmacological findings (reviewed below) indicating distinct cholinergic regulation of contextually conditioned fear versus cue-conditioned responses, as well as differential regulation during extinction learning (see Figure 1).

Pharmacological studies support a role for muscarinic receptors (mAChRs) in the amygdala, hippocampus, and prefrontal cortex in regulating the formation and extinction of fear memories. Pharmacological interventions with muscarinic agonists or antagonists support the general premise that cholinergic activation of mAChR contributes to memory processes during the acquisition and/or consolidation of fear learning and fear extinction. The role of nicotinic receptors (nAChR) in fear learning and extinction is less clear, with modulatory effects generally reported for nAChR agonists (e.g., nicotine) but not antagonists.

Cholinergic regulation of contextual fear responses

Although there is some inconsistency in the literature, mAChR activation in the hippocampus appears particularly critical in the acquisition of contextual fear learning (see Tables 1 and 2). Several studies using various conditioning protocols have indicated that the systemic administration of the mAChR antagonist scopolamine before training can reduce the acquisition of conditioned fear, as well as subsequent freezing responses when animals are re-exposed to the context (one day or one week later; see Table 1) (Anagnostaras et al. 1995; Anagnostaras et al. 1999; Lindner et al. 2006; Rudy 1996). Further, administration of the M1-selective mAChR antagonist dicyclomine before training or testing similarly blocked contextual fear responses, and sub-effective doses of dicyclomine also attenuated contextual fear responses when combined with sub-effective doses of the NMDA antagonist

MK801 (Figueredo et al. 2008; Fornari et al. 2000; Soares et al. 2006). Similar decreases in contextual freezing were seen after scopolamine administration within three hours after the conditioning paradigm, although one of these studies used juvenile rats (Bucherelli et al. 2006; Rudy 1996). As seen in Table 1, however, other studies failed to see effects of scopolamine given after the training session on contextual fear responses even with high doses or in juvenile rats (Anagnostaras et al. 1995; Anagnostaras et al. 1999; Young et al. 1995). Post-training administration of dicyclomine also failed to alter contextual fear responses (Soares et al. 2006). These differences in the effects of post-training scopolamine have primarily been attributed to the variable conditioning protocols, including the number and timing of the tone-shock pairings during acquisition, as well as the injection time points, dose, and potentially age or sex of the subjects (see (Tinsley 2004)). The inhibitory effects of systemic scopolamine on contextual fear conditioning were not seen with methylscopolamine, suggesting that the effects were due to central rather than peripheral block of mAChR since methylscopolamine does not cross the blood brain barrier (Anagnostaras et al. 1999).

Activation of nicotinic cholinergic receptors also modulates fear conditioned responses (see (Gould and Leach 2014; Kutlu and Gould 2015) and Table 1). Systemic administration of nicotine before both the training and the testing session enhanced contextual fear responses in a dose dependent manner (Davis et al. 2006; Gould and Wehner 1999; Wehner et al. 2004). These effects were seen both one and seven days after training, but only when nicotine was administered before both the training and testing sessions (Gould and Higgins 2003; Gould and Wehner 1999). Unlike what is seen with muscarinic antagonists, systemic pre-training administration of the nAChR antagonists mecamylamine or dihydro- β -erythroidine (DH β E) did not alter contextual fear conditioned responses on their own, but could block the actions of nicotine (Davis and Gould 2006; Feiro and Gould 2005; Gould and Higgins 2003; Gould and Wehner 1999). The lack of effects with nicotinic antagonists might suggest that effects of endogenous release of acetylcholine associated with fear conditioning are mostly mediated via mAChRs, or that nicotinic effects depend on co-incident activation of both muscarinic and nicotinic receptors. The need for co-activation of both receptors is supported by a study in which the combined administration of subthreshold doses of mecamylamine and scopolamine was able to decrease contextual and cued fear responses in young, but not old, mice (Feiro and Gould 2005). The lack of nAChR antagonist effects might also be related to cholinergic effects at different nAChR subtypes that influence fear learning at different time points during acquisition or consolidation, as suggested by microinjection studies (see (Vago and Kesner, 2007) below). Nicotine's effects appear to be mediated via β 2-containing receptors, since the ability of nicotine to enhance fear responses was blocked by the α 4 β 2 antagonist Dh β E and contextual freezing was increased by administration of the partial α 4 β 2 agonist ABT-089 given both pre-training and pre-testing (Davis and Gould 2006; Yildirim et al. 2015). In contrast, varenicline, which is a partial α 4 β 2 agonist, but a full α 7 agonist, failed to affect contextual freezing when given before testing, before training or both (Raybuck et al. 2008). Further, mice lacking the β 2 subunit of the nAChR showed reduced contextual fear responses as well as a lack of nicotine's effects on contextual fear responses (Davis and Gould 2007; Wehner et al. 2004). Mice lacking the α 7, β 3, or β 4 nAChR subunits did not show any deficits in contextual fear,

although administration of the $\alpha 7$ selective antagonist methyllycaconitine (MLA) into the ventral hippocampus blocked the influences of systemic nicotine on contextual fear responses (Kenney et al. 2012; Wehner et al. 2004) and systemic administration of MLA (without nicotine) increased contextual fear responses (Davis and Gould 2006). An agonist at homomeric $\alpha 7$ nAChR receptors (ABT-107), however, failed to increase contextual fear (Yildirim et al. 2015). Interestingly, decreased contextual fear responses were seen in mice during spontaneous or precipitated nicotine withdrawal, and this reduction during nicotine withdrawal was not seen in mice lacking the $\beta 2$ nAChR subunit (Portugal et al. 2008). Nicotine withdrawal effects are also reversed with the $\alpha 4\beta 2$ selective partial agonists ABT-089 and varenicline, as well as acetylcholinesterase inhibitors such as donepezil and galantamine (Poole et al. 2014; Raybuck et al. 2008; Wilkinson and Gould 2011; Yildirim et al. 2015). Much higher doses of donepezil or galantamine were needed to enhance conditioned fear responses in non-dependent animals, and these effects were associated with enhanced acquisition of conditioned responses and/or unconditioned freezing (Poole et al. 2014; Wilkinson and Gould 2011). Thus, although nicotine can modify contextual fear responses, only a few studies show effects of nAChR antagonists or acetylcholinesterase inhibitors (see Table 1). The more consistent effects of scopolamine on contextual fear conditioning suggest that cholinergic influences on contextual fear conditioning are mediated primarily through muscarinic receptor activation, or that different nAChR subtypes have opposing effects on conditioned fear responses.

Microinjection studies further implicate cholinergic processes in the hippocampus and amygdala in regulating the acquisition and consolidation of contextual fear learning (see Table 2). Microinjection of scopolamine into the hippocampus blocked acquisition and/or expression of contextual freezing responses if administered before or immediately after the conditioning paradigm (Chang and Liang 2012; Gale et al. 2001; Rogers and Kesner 2004; Wallenstein and Vago 2001). Similarly, immediate post-training microinjection of scopolamine into the basolateral amygdala attenuated contextual fear responses 72 hours later (Passani et al. 2001). Unfortunately, results of this study were slightly confounded by performing the post-training microinjection under ketamine anesthesia. In addition, post-training injections of the muscarinic agonist oxotremorine into the basolateral amygdala enhanced contextual responses (Cangioli et al. 2002; Vazdarjanova and McGaugh 1999). In contrast, pre-training scopolamine injections into the lateral, as opposed to the basolateral amygdala, did not attenuate contextual fear responses (Baysinger et al. 2012). In studies that observe post-training effects of scopolamine, administration of the mAChR antagonist more than 6 hours after conditioning sessions failed to affect contextual fear responses (Chang and Liang 2012; Rudy 1996).

Microinjection studies also suggest the modulatory influences of nAChR activation are dependent upon regionally-specific interactions with different receptor subtypes (see Table 2), which may explain the lack of robust or consistent effects following the systemic administration of non-selective nAChR antagonists. Pre-training microinjections of nicotine into the dorsal hippocampus enhanced contextual fear conditioning, while nicotine injections into the ventral hippocampus decreased this response, and injections into the prefrontal cortex had no effect (Kenney et al. 2012; Raybuck and Gould 2010). Injections of the non-selective antagonist mecamylamine into the dorsal hippocampus either pre- or immediately

post-training attenuated contextual fear responses, but not if mecamylamine was administered 6 hours after training. In contrast, pre-training administration of the $\alpha 7$ selective antagonist MLA in the dorsal or ventral hippocampus had no effect on contextual fear responses (Kenney et al. 2012; Vago and Kesner 2007), but post-training injections of MLA up to 6 hours after training decreased contextual freezing (Vago and Kesner, 2007). Further, blocking $\alpha 7$ nAChRs in ventral hippocampus along with systemic administration of nicotine before training enhanced contextual freezing, suggesting that blocking these receptors in ventral hippocampus permitted actions of nicotine in the dorsal hippocampus to enhance contextual fear responses (Kenney et al. 2012). In the dorsal hippocampus, injections of nicotine or nAChR antagonists administered only before testing did not alter contextual fear responses. In contrast, injections of DH β E in the ventral hippocampus, and MLA or DH β E into the prefrontal cortex, were able to decrease contextual fear responses when administered prior to the retention test (Kenney et al. 2012; Raybuck and Gould 2010). Such opposing effects of nAChRs in different brain areas may help explain the lack of effects on contextual fear conditioning with systemic administration of nicotinic receptor blockers (especially non-selective blockers).

Cholinergic regulation of cue-conditioned fear and extinction

The role of cholinergic processes in mediating the formation or expression of cued fear responses in delay conditioning protocols is less clear (see Tables 1 and 2). Although a few studies have also demonstrated that systemic administration of scopolamine before (Anagnostaras et al. 1999; Feiro and Gould 2005; Rudy 1996; Young et al. 1995) or just after (Rudy 1996) conditioning attenuated freezing responses to an auditory CS, this appears to require higher doses of scopolamine and the post-training effects of scopolamine are not uniformly observed (see (Anagnostaras et al. 1999; Tinsley 2004). Further, administration of the M1 mAChR antagonist dicyclomine, even at doses four times those needed to attenuate contextual fear responses, failed to modify freezing to the tone (Fornari et al. 2000). In general, systemic administration of nicotine, or subtype selective agonists, at doses that enhance contextual fear conditioning failed to modify cue-conditioned responses (Gould et al. 2004; Gould and Wehner 1999; Wehner et al. 2004; Yildirim et al. 2015). Although increased responses to the CS were seen in some studies with high doses of nicotine, these appeared to be non-specific effects potentially related to nicotine-induced increases in startle responses (Gould and Higgins 2003). Pre-training administration of mecamylamine similarly failed to alter conditioned responses to the CS (Feiro and Gould 2005). Administration of a combination of mAChR and nAChR antagonists (MEC and atropine) into the BLA administered before training, however, decreased cue-induced freezing during recall and enhanced extinction learning. Interestingly, administration of atropine alone had no effect on these responses, implicating nicotinic effects in this study (Jiang et al. 2016). Administration of scopolamine, nicotine, or nicotinic antagonists directly into the hippocampus also failed to attenuate cue-conditioned responses, supporting the role of the hippocampus primarily in contextual information processing (Gale et al. 2001; Kenney et al. 2012; Rogers and Kesner 2004; Vago and Kesner 2007; Wallenstein and Vago 2001). Although the neural underpinnings of trace conditioning differs from delayed cue or contextual conditioning, a few studies have implicated cholinergic (both muscarinic and

nicotinic) mechanisms in this type of protocol as well (Baysinger et al. 2012; Raybuck and Gould 2010).

In contrast, activation of mAChR, particularly in the prefrontal cortex and amygdala, appears to play an important role in the encoding of extinction learning, including extinction of responses to a conditioned cue (see Table 3). In animals conditioned to an auditory CS, administration of the mAChR antagonist scopolamine immediately before or after extinction training attenuated extinction recall; these effects were seen with both systemic administration and local administration into the infralimbic prefrontal cortex (Santini et al. 2012). Unfortunately, the testing of extinction in the same context as training in this study cannot completely rule out cholinergic influences on contextual fear processes during extinction recall (Santini et al., 2012). In an intriguing study by Fanselow and colleagues, low systemic doses of scopolamine (0.1 mg/kg) prior to extinction training appeared to shift the nature of the extinction memory. Although animals treated with low doses of scopolamine showed a slower rate of long-term extinction memory formation (e.g., the need for more extinction training sessions), there was an attenuation of fear renewal even if subjects were tested in a novel context. These effects were not observed with post-training injections of scopolamine (Zelikowsky et al. 2013), suggesting that pre-training administration of mAChR antagonists may be related to modulating contextual information during these paradigms (see discussion below). Further, administration of mAChR agonists can also improve extinction learning or recall. The muscarinic agonist cevimeline enhanced extinction learning when the drug was given either before or immediately after daily extinction training sessions (Santini et al. 2012), although these studies were done with animals undergoing extinction in the training context so effects on contextual information cannot be dismissed. Administration of the mAChR agonist oxotremorine unilaterally into the basolateral amygdala, however, also improved extinction of contextual fear (Boccia et al. 2009). Although data with classical conditioning is limited, post-training systemic and intracerebral injections of oxotremorine into the basolateral amygdala similarly accelerated the extinction of amphetamine conditioned place preference (Schroeder and Packard 2004), perhaps suggesting a generalized effect of muscarinic activation in extinction processes.

The effects of nicotine on extinction, however, appear to be dependent upon the extinction protocol, when nicotine is administered, and if extinction is tested in the same or different contexts (see Table 3), and reports assessing effects of nAChR antagonists in extinction trials are lacking. Nicotine given before the extinction trials impaired extinction to the context and appeared to delay extinction over multiple trials. This study suggested that nicotine might have enhanced the recall of the original fear memory during these trials (Kutlu and Gould 2014). Chronic nicotine administration given two weeks prior to the conditioning protocol also impaired between-trial extinction to the CS, but not extinction to the context (Tian et al. 2008). Nicotine administered before extinction training trials enhanced between-trial extinction of responses to the cue over five days and reduced renewal of the original fear memory (Elias et al. 2010). Interestingly, administration of the nicotine metabolite cotinine, considered a positive modulator of $\alpha 7$ containing nAChRs, also enhanced fear extinction in mice (Barreto et al. 2015; Zeitlin et al. 2012).

As summarized in Figure 1, overall these studies suggest that muscarinic receptor activation in the prefrontal cortex and basolateral amygdala enhance memory consolidation during extinction training. In contrast, it appears the effects of nicotinic receptor activation activity might either delay or enhance extinction depending on the timing of agonist administration, or perhaps the subtype selectivity of the agonist.

Neurochemical studies of cholinergic regulation of fear learning and extinction

As summarized in Figure 1, pharmacological evidence supports the notion that cholinergic, and particularly muscarinic, activation enhances the consolidation of memory formation (Power et al. 2003a). Others have also suggested that the release of acetylcholine is important in activating neural systems during learning and may mediate individual differences seen in several learning paradigms, including aversive conditioning (Gold 2003). Post-training activation of mAChR, or other treatments that increase ACh release in the basolateral amygdala, such as glucose or H3 histamine agonists, enhance contextual fear responses (Cangioli et al. 2002; Passani et al. 2001; Santini et al. 2012) as well as accelerating or improving extinction of conditioned responses (Boccia et al. 2009; Santini et al. 2012; Schroeder and Packard 2004). Similarly, muscarinic antagonists can delay or attenuate extinction learning (Maruki et al. 2003; Santini et al. 2012; Zelikowsky et al. 2013), suggesting muscarinic cholinergic activation is critical in this process. Activation of nAChRs with nicotine or cotinine can also enhance extinction learning (Barreto et al. 2015; Elias et al. 2010; Zeitlin et al. 2012), so it is possible acetylcholine release could enhance both muscarinic and nicotinic neurotransmission in regions like the prefrontal cortex, hippocampus, and amygdala that are critical for extinction processes.

In support of this notion, several microdialysis studies have demonstrated an association between acetylcholine release in the hippocampus, prefrontal cortex, and amygdala and associative learning responses, although only a few studies have specifically examined acetylcholine efflux during Pavlovian fear conditioning or extinction. Increases in acetylcholine efflux are seen during fear conditioning procedures, and in hippocampus unpaired shocks and tones led to higher efflux than tones paired with shocks (Calandreau et al. 2006; Nail-Boucherie et al. 2000). Exposure to a conditioned cue or context, or objects in a novel object task, also increases acetylcholine in the hippocampus and/or prefrontal cortex (Acquas et al. 1996; Nail-Boucherie et al. 2000; Stanley et al. 2012), and there is increased acetylcholine released in the prefrontal cortex during extinction of an operant task (Izaki et al. 2001). In addition, activation of histaminergic H3 receptors increases acetylcholine release in the basolateral amygdala, and improves the expression of fear memories (Cangioli et al. 2002). These studies are consistent with the notion that cholinergic systems originating in the basal forebrain are activated and enhance acetylcholine efflux—presumably resulting in increased endogenous tone on cholinergic receptors—in cortical, hippocampal, and amygdalar projection areas during exposure to conditioned cues or contexts, and also during extinction training. Recent studies by Jiang and coworkers (2016) using pharmacological and optogenetic techniques to activate or inhibit cholinergic inputs into the BLA during training support this notion. Co-administration of nAChR and mAChR antagonists into the BLA reduced freezing to the CS as well as enhancing extinction (Jiang et al. 2016). Optogenetic activation failed to change cue-induced freezing during the retention trial, but

attenuated extinction training, while optogenetic inhibition decreased freezing during training and cue presentation twenty-four hours later (Jiang et al. 2016).

Studies diminishing acetylcholine efflux using immunotoxic lesions of cholinergic neurons support the role of cholinergic inputs to hippocampus and/or cortical-amygdalar regions in modulating specific aspects of fear extinction processes, but not the acquisition or consolidation of contextual or cued fear learning. Although lesions of the basal forebrain cholinergic system using 192-IgG saporin failed to alter cue-conditioned responses, these lesions spared cholinergic projections to the amygdala (Conner et al. 2003). In addition, immunotoxic lesions of the BFCS either pre-conditioning or post-conditioning did not significantly diminish either acquisition or expression of contextual freezing, although pre-testing lesions did significantly reduce ultrasonic vocalizations upon return to the context (Frick et al. 2004). Immunotoxic lesions of the medial septum that reduced cholinergic inputs to hippocampus failed to shift acquisition of contextual fear responses, or modify cFos activation of CA1 neurons associated with that response. In contrast, these medial septal lesions specifically attenuated extinction of contextual fear responses, and the induction of ERK positive neurons in the dorsal hippocampus associated with extinction processes (Tronson et al. 2009). Similarly, lesions of either the basal forebrain or the medial septum/ventral DBB regions that reduced cholinergic inputs to the prefrontal cortex and hippocampus led to generalization of contextual fear memories that were resistant to extinction, as well as an attenuated acquisition of cued fear extinction. Interestingly, these changes in extinction of contextual fear memories or acquisition of cued fear extinction were not seen with specific lesions of the nBM and horizontal limb of the DBB (Knox and Keller 2015). Importantly, lesions of these regions did not modulate acquisition of conditioned responses. Although these studies by Knox and Keller (2015) did not analyze decrements in cholinergic inputs to the amygdala, they contribute to the notion that cortico-hippocampal circuits play a role in acquisition, expression, and generalization of contextual fear memories, and that contextual information is important during extinction learning (Rozeske et al. 2015).

Several studies have examined changes in various markers of cholinergic function associated with fear learning or extinction paradigms, and these studies have suggested that individual differences these indices of cholinergic function may be correlated with behavioral responses during learning tasks, including conditioned fear responses. The expression of choline acetyltransferase (ChAT) mRNA is enhanced by cue conditioning, although these changes were seen in the caudal nBM regions that specifically projected to the auditory cortex (Oh et al. 1992). Further, although no studies have examined receptor changes after classical conditioning procedures, immunoreactive mAChR staining was increased in the central amygdala and decreased in the corticomedial amygdala after training in an active avoidance paradigm, and these changes persisted for at least 25 days (Rooszendaal et al. 1997). Such changes in mAChR staining were suggestive of functional activation of mAChRs in the amygdala in response to a conditioning protocol. Further, correlative relationships have been observed between various indices of cholinergic function and behavioral endpoints in several aversive learning tasks (Gold 2003; McIntyre et al. 2002; van der Zee et al. 1997). In an operant task, acetylcholine release in the prefrontal cortex was negatively correlated with lever presses during extinction, suggesting heightened

acetylcholine release was associated with enhanced extinction learning (Izaki et al. 2001). This finding is also consistent with other studies demonstrating that cognitive and motoric correlates of acetylcholine release in the cortex can be dissociated (Himmelheber et al. 2000). In addition, muscarinic receptor immunoreactivity in the central amygdala of naïve rats showed large individual variations, and mAChR immunoreactivity was positively correlated with immobility in a conditioned task (van der Zee and Luiten 1999; van der Zee et al. 1997). Interestingly, these mAChR positive cells are GABAergic neurons that also express nAChRs and are densely packed in the lateral portions of the central nucleus (van der Zee et al. 1997). While few human studies have directly investigated cholinergic systems associated with fear extinction, a recent neuroimaging study suggests that the BFCS enhances amygdala connectivity with both the PFC and the hippocampus during the processing of biologically salient stimuli in humans, and the magnitude of this effect was predicted by functional variation within the choline transporter gene (Gorka et al. 2015).

Physiology of cholinergic regulation of fear learning and extinction

Physiological studies have begun to elucidate region-specific responses that will be needed to integrate pharmacological and neurochemical findings into understanding how cholinergic modulation of neuronal circuits regulates fear learning and extinction. The demonstration that fear conditioning and fear extinction processes involve segregated cell types and signaling cascades within the neuronal networks make this a challenging undertaking that will clearly require innovative approaches (Herry et al. 2008; Tronson et al. 2009). In auditory fear learning, cholinergic projections activate layer 1 neurons in auditory cortex during contingent presentation of footshock with tones, and this activation disinhibits pyramidal neurons via parvalbumin-positive inhibitory interneurons of layers 2/3 (Letzkus et al. 2015; Letzkus et al. 2011). Cholinergic disinhibition of pyramidal neurons was blocked by a combination of mecamylamine (MEC) and MLA, implicating nAChRs in these effects. Microinjection of nAChR antagonists in auditory cortex also blocked freezing to conditioned auditory cues, although freezing to both the CS+ and the CS- was reduced (Letzkus et al. 2015; Letzkus et al. 2011). The authors speculate that cholinergic regulation of pyramidal neuron outputs via disinhibition is seen in other areas with microcircuits similar to those in cortex involving parvalbumin interneurons, such as basolateral amygdala (Letzkus et al. 2015).

In hippocampus, exposure to a novel environment causes remapping of place cells and a shift in theta (4–12 Hz) oscillations toward encoding (Douchamps et al. 2013). Fear conditioning also induces a synchrony of theta oscillations between hippocampus (CA1), lateral amygdala, and prefrontal cortex; this synchrony is disrupted by repeated exposure to the conditioned context and partially rebounds during extinction recall (Lesting et al. 2011). Extinction of contextually conditioned freezing to a predator odor induces a re-mapping by some hippocampal place cells, while some place cells remain stable, suggesting that these shifts may help in the modification of pre-existing contextual memories as well as the formation of new ones during extinction (Wang et al. 2015). Further, the remapping of hippocampal place cells in a novel environment, as well as the shifts in theta oscillations toward encoding (pyramidal-layer theta peak), are both disrupted with the muscarinic antagonist scopolamine, suggesting a key role for cholinergic inputs in modulating these

responses and providing contextual information during fear learning and extinction (Douchamps et al. 2013).

In the prefrontal cortex, activation of mAChRs increases excitability of infralimbic neurons via decreased M-type potassium currents and potassium channel afterhyperpolarizations (AHPs), and these changes are linked to modulating extinction recall (Santini and Porter 2010; Santini et al. 2012). Activation of mAChRs in prelimbic cortex by carbachol causes both acute and long term depression (LTD) of synaptic efficacy from hippocampal, but not cortical, inputs. Interestingly the effects of carbachol required activation of the hippocampal inputs for induction of LTD, and appeared to be mediated via M2 muscarinic receptors (both presynaptic and postsynaptic) in this region (Wang and Yuan 2009).

In the BLA, optogenetic stimulation of basal forebrain cholinergic inputs shows differential activity-dependent effects on pyramidal neurons, suggesting that cholinergic regulation of this region enhances the signal to noise ratio, activity, and plasticity (Jiang et al. 2016; Unal et al. 2015). At low firing rates in pyramidal cells, optogenetic activation of the basal forebrain decreased activity of these neurons, while during periods of higher baseline activity basal forebrain activation induced an early nAChR activation of GABAergic interneurons followed by direct M1 mAChR activation of an inward rectifying potassium channel (Unal et al. 2015). Interestingly, these effects on pyramidal neurons were not observed with carbachol application in slices, suggesting that such divergent effects of cholinergic stimulation may be dependent upon endogenous cholinergic release acting on the circuit as a whole, as opposed to specific stimulation of a single mAChR or nAChR receptor type. This is similar to findings in prelimbic cortex using carbachol (Wang and Yuan 2009). In support of the notion, mice with M1 or M2 mAChR deficiencies, or mice lacking nAChR subtypes generally failed to demonstrate alterations in cue conditioning (Anagnostaras et al. 2003; Bainbridge et al. 2008), although one report showed a decrease in cue conditioning in M1 knockout mice (Miyakawa et al. 2001). Further, Young and Thomas (2014) demonstrated that muscarinic effects on consolidation of cued fear memories act in concert with signaling induced by β -adrenergic and dopaminergic receptors (Young and Thomas 2014), again suggesting cholinergic regulation may need to modulate circuit dynamics during fear extinction processes. In further support of this, studies from another group using *in vivo* and *ex vivo* optogenetic stimulation demonstrated that after a brief (400 msec) pause, that stimulation of cholinergic inputs induced a sustained increase in putative BLA pyramidal cell firing via enhanced glutamatergic neurotransmission, and also decreased the threshold for long-term potentiation (LTP) (Jiang et al. 2016). These effects of cholinergic stimulation on BLA neurons were blocked by co-administration of MEC and atropine, although many of these effects seem to be predominantly via nAChR activation (Jiang et al. 2016). This is consistent with prior work of this group showing acute nicotine administration facilitated glutamatergic post-synaptic currents in the BLA as well as facilitating synaptic responses during activation of cortical inputs (Jiang and Role 2008).

In summary, evidence suggests that cholinergic processes are involved in extinction learning, and activation of mAChRs and nAChRs in the cortico-hippocampal or cortico-amygdalar circuits may regulate activity of these regions during acquisition of contextual or cued fear extinction, respectively (see Figure 1). The indication that fear conditioning induces a

coupling of theta oscillations between the CA1 hippocampus, infralimbic prefrontal cortex, and lateral amygdala, which is uncoupled during fear extinction paradigms and only partially rebounds during extinction recall, supports the possibility that cholinergic mechanisms in these separate regions regulate synchrony in this network (Lesting et al. 2013; Lesting et al. 2011). Validation of this concept will require additional studies using pharmacological manipulations or optogenetic stimulation of the cholinergic system during analysis of theta oscillations, but the work in hippocampus suggests that cholinergic regulation of theta oscillations is likely (Douchamps et al. 2013).

Conclusions

Both the acquisition of fear conditioning and the extinction of conditioned fear responses require plasticity of associations between sensory stimuli and behavioral processes. It is well-established that cholinergic-receptive brain regions such as the prefrontal cortex, hippocampus and amygdala play crucial roles in fear learning and extinction although the specific contribution of cholinergic mechanisms to these phenomena remains poorly understood. A growing literature implicates a fundamental role for cholinergic regulation in the consolidation of learning responses, including fear learning and especially in acquisition of fear extinction (see Figure 1). The basal forebrain projects to the regions known to be involved in fear extinction learning and recall, and several emerging lines of evidence suggest that cholinergic modulation of the cortico-hippocampal-amygdalar circuit may regulate specific aspects of fear learning and extinction. Additional studies will be needed to determine the exact mechanisms and relative involvement of acetylcholine release, as well as activation of mAChR and nAChR activation on distinct signaling pathways and different cell types, in fear learning and extinction processes. Furthermore, it will be interesting to examine whether individual differences in pre- or postsynaptic indices of cholinergic neurotransmission may mediate variations in animal models of fear extinction and, perhaps, may have translational potential for disorders of fear and anxiety involving memory processes, such as post-traumatic stress disorder.

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LITERATURE CITED

- Acquas E, Wilson C, Fibiger HC. Conditioned and unconditioned stimuli increase frontal cortical and hippocampal acetylcholine release: effects of novelty, habituation, and fear. *J Neurosci*. 1996; 16(9): 3089–3096. [PubMed: 8622138]
- Anagnostaras SG, Maren S, Fanselow MS. Scopolamine selectively disrupts the acquisition of contextual fear conditioning in rats. *Neurobiol Learn Mem*. 1995; 64(3):191–194. [PubMed: 8564372]
- Anagnostaras SG, Maren S, Sage JR, Goodrich S, Fanselow MS. Scopolamine and Pavlovian fear conditioning in rats: dose-effect analysis. *Neuropsychopharmacology*. 1999; 21(6):731–744. [PubMed: 10633479]

- Anagnostaras SG, Murphy GG, Hamilton SE, Mitchell SL, Rahnama NP, Nathanson NM, Silva AJ. Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. *Nat Neurosci*. 2003; 6(1):51–58. [PubMed: 12483218]
- Bainbridge NK, Koselke LR, Jeon J, Bailey KR, Wess J, Crawley JN, Wrenn CC. Learning and memory impairments in a congenic C57BL/6 strain of mice that lacks the M2 muscarinic acetylcholine receptor subtype. *Behav Brain Res*. 2008; 190(1):50–58. [PubMed: 18346798]
- Baldi E, Bucherelli C. Brain sites involved in fear memory reconsolidation and extinction of rodents. *Neurosci Biobehav Rev*. 2015; 53:160–190. [PubMed: 25887284]
- Barreto GE, Yarkov A, Avila-Rodriguez M, Aliev G, Echeverria V. Nicotine-Derived Compounds as Therapeutic Tools Against Post-Traumatic Stress Disorder. *Curr Pharm Des*. 2015; 21(25):3589–3595. [PubMed: 26166610]
- Baysinger AN, Kent BA, Brown TH. Muscarinic receptors in amygdala control trace fear conditioning. *PLoS One*. 2012; 7(9):e45720. [PubMed: 23029199]
- Bigl V, Woolf NJ, Butcher LL. Cholinergic projections from the basal forebrain to frontal, parietal, temporal, occipital, and cingulate cortices: a combined fluorescent tracer and acetylcholinesterase analysis. *Brain Res Bull*. 1982; 8(6):727–749. [PubMed: 6182962]
- Boccia MM, Blake MG, Baratti CM, McGaugh JL. Involvement of the basolateral amygdala in muscarinic cholinergic modulation of extinction memory consolidation. *Neurobiol Learn Mem*. 2009; 91(1):93–97. [PubMed: 18706510]
- Bucherelli C, Baldi E, Mariottini C, Passani MB, Blandina P. Aversive memory reactivation engages in the amygdala only some neurotransmitters involved in consolidation. *Learn Mem*. 2006; 13(4):426–430. [PubMed: 16882859]
- Burton MJ, Rolls ET, Mora F. Effects of hunger on the responses of neurons in the lateral hypothalamus to the sight and taste of food. *Exp Neurol*. 1976; 51(3):668–677. [PubMed: 819286]
- Caberlotto L, Fuxe K, Rimland JM, Sedvall G, Hurd YL. Regional distribution of neuropeptide Y Y2 receptor messenger RNA in the human post mortem brain. *Neuroscience*. 1998; 86(1):167–178. [PubMed: 9692752]
- Calandreau L, Trifilieff P, Mons N, Costes L, Marien M, Marighetto A, Micheau J, Jaffard R, Desmedt A. Extracellular hippocampal acetylcholine level controls amygdala function and promotes adaptive conditioned emotional response. *J Neurosci*. 2006; 26(52):13556–13566. [PubMed: 17192439]
- Cangioli I, Baldi E, Mannaioni PF, Bucherelli C, Blandina P, Passani MB. Activation of histaminergic H3 receptors in the rat basolateral amygdala improves expression of fear memory and enhances acetylcholine release. *Eur J Neurosci*. 2002; 16(3):521–528. [PubMed: 12193196]
- Carnes KM, Fuller TA, Price JL. Sources of presumptive glutamatergic/aspartatergic afferents to the magnocellular basal forebrain in the rat. *J Comp Neurol*. 1990; 302(4):824–852. [PubMed: 1982006]
- Chang SD, Liang KC. Roles of hippocampal GABA(A) and muscarinic receptors in consolidation of context memory and context-shock association in contextual fear conditioning: a double dissociation study. *Neurobiol Learn Mem*. 2012; 98(1):17–24. [PubMed: 22543193]
- Conner JM, Culberson A, Packowski C, Chiba AA, Tuszynski MH. Lesions of the Basal forebrain cholinergic system impair task acquisition and abolish cortical plasticity associated with motor skill learning. *Neuron*. 2003; 38(5):819–829. [PubMed: 12797965]
- Davis JA, Gould TJ. The effects of DHBE and MLA on nicotine-induced enhancement of contextual fear conditioning in C57BL/6 mice. *Psychopharmacology (Berl)*. 2006; 184(3–4):345–352. [PubMed: 15988571]
- Davis JA, Gould TJ. beta2 subunit-containing nicotinic receptors mediate the enhancing effect of nicotine on trace cued fear conditioning in C57BL/6 mice. *Psychopharmacology (Berl)*. 2007; 190(3):343–352. [PubMed: 17136517]
- Davis JA, Porter J, Gould TJ. Nicotine enhances both foreground and background contextual fear conditioning. *Neurosci Lett*. 2006; 394(3):202–205. [PubMed: 16260086]
- Douchamps V, Jeewajee A, Blundell P, Burgess N, Lever C. Evidence for encoding versus retrieval scheduling in the hippocampus by theta phase and acetylcholine. *J Neurosci*. 2013; 33(20):8689–8704. [PubMed: 23678113]

- Eckenstein FP, Baughman RW, Quinn J. An anatomical study of cholinergic innervation in rat cerebral cortex. *Neuroscience*. 1988; 25(2):457–474. [PubMed: 2456488]
- Elias GA, Gulick D, Wilkinson DS, Gould TJ. Nicotine and extinction of fear conditioning. *Neuroscience*. 2010; 165(4):1063–1073. [PubMed: 19931599]
- Feiro O, Gould TJ. The interactive effects of nicotinic and muscarinic cholinergic receptor inhibition on fear conditioning in young and aged C57BL/6 mice. *Pharmacol Biochem Behav*. 2005; 80(2):251–262. [PubMed: 15680178]
- Fendt M, Fanselow MS. The neuroanatomical and neurochemical basis of conditioned fear. *Neurosci Biobehav Rev*. 1999; 23(5):743–760. [PubMed: 10392663]
- Figueredo LZ, Moreira KM, Ferreira TL, Fornari RV, Oliveira MG. Interaction between glutamatergic-NMDA and cholinergic-muscarinic systems in classical fear conditioning. *Brain Res Bull*. 2008; 77(2–3):71–76. [PubMed: 18582541]
- Fornari RV, Moreira KM, Oliveira MG. Effects of the selective M1 muscarinic receptor antagonist dicyclomine on emotional memory. *Learn Mem*. 2000; 7(5):287–292. [PubMed: 11040260]
- Frick KM, Kim JJ, Baxter MG. Effects of complete immunotoxin lesions of the cholinergic basal forebrain on fear conditioning and spatial learning. *Hippocampus*. 2004; 14(2):244–254. [PubMed: 15098729]
- Gale GD, Anagnostaras SG, Fanselow MS. Cholinergic modulation of pavlovian fear conditioning: effects of intrahippocampal scopolamine infusion. *Hippocampus*. 2001; 11(4):371–376. [PubMed: 11530841]
- Gastard M, Jensen SL, Martin JR 3rd, Williams EA, Zahm DS. The caudal sublentiform region/anterior amygdaloid area is the only part of the rat forebrain and mesopontine tegmentum occupied by magnocellular cholinergic neurons that receives outputs from the central division of extended amygdala. *Brain Res*. 2002; 957(2):207–222. [PubMed: 12445963]
- Gold PE. Acetylcholine modulation of neural systems involved in learning and memory. *Neurobiol Learn Mem*. 2003; 80(3):194–210. [PubMed: 14521863]
- Gorka AX, Knodt AR, Hariri AR. Basal forebrain moderates the magnitude of task-dependent amygdala functional connectivity. *Social cognitive and affective neuroscience*. 2015; 10(4):501–507. [PubMed: 24847112]
- Gould TJ, Feiro O, Moore D. Nicotine enhances trace cued fear conditioning but not delay cued fear conditioning in C57BL/6 mice. *Behav Brain Res*. 2004; 155(1):167–173. [PubMed: 15325790]
- Gould TJ, Higgins JS. Nicotine enhances contextual fear conditioning in C57BL/6J mice at 1 and 7 days post-training. *Neurobiol Learn Mem*. 2003; 80(2):147–157. [PubMed: 12932430]
- Gould TJ, Leach PT. Cellular, molecular, and genetic substrates underlying the impact of nicotine on learning. *Neurobiol Learn Mem*. 2014; 107:108–132. [PubMed: 23973448]
- Gould TJ, Wehner JM. Nicotine enhancement of contextual fear conditioning. *Behav Brain Res*. 1999; 102(1–2):31–39. [PubMed: 10403013]
- Grove EA. Neural associations of the substantia innominata in the rat: afferent connections. *J Comp Neurol*. 1988; 277(3):315–346. [PubMed: 2461972]
- Hajos N, Papp EC, Acsády L, Levey AI, Freund TF. Distinct interneuron types express m2 muscarinic receptor immunoreactivity on their dendrites or axon terminals in the hippocampus. *Neuroscience*. 1998; 82(2):355–376. [PubMed: 9466448]
- Herry C, Ciochi S, Senn V, Demmou L, Muller C, Luthi A. Switching on and off fear by distinct neuronal circuits. *Nature*. 2008; 454(7204):600–606. [PubMed: 18615015]
- Himmelheber AM, Sarter M, Bruno JP. Increases in cortical acetylcholine release during sustained attention performance in rats. *Brain Res Cogn Brain Res*. 2000; 9(3):313–325. [PubMed: 10808142]
- Izaki Y, Hori K, Nomura M. Elevation of prefrontal acetylcholine is related to the extinction of learned behavior in rats. *Neurosci Lett*. 2001; 306(1–2):33–36. [PubMed: 11403951]
- Jiang L, Kundu S, Lederman JD, Lopez-Hernandez GY, Ballinger EC, Wang S, Talmage DA, Role LW. Cholinergic Signaling Controls Conditioned Fear Behaviors and Enhances Plasticity of Cortical-Amygdala Circuits. *Neuron*. 2016; 90(5):1057–1070. [PubMed: 27161525]

- Jiang L, Role LW. Facilitation of cortico-amygdala synapses by nicotine: activity-dependent modulation of glutamatergic transmission. *J Neurophysiol.* 2008; 99(4):1988–1999. [PubMed: 18272879]
- Jones BE, Cuello AC. Afferents to the basal forebrain cholinergic cell area from pontomesencephalic–catecholamine, serotonin, and acetylcholine–neurons. *Neuroscience.* 1989; 31(1):37–61. [PubMed: 2475819]
- Kenney JW, Raybuck JD, Gould TJ. Nicotinic receptors in the dorsal and ventral hippocampus differentially modulate contextual fear conditioning. *Hippocampus.* 2012; 22(8):1681–1690. [PubMed: 22271264]
- Knox D, Keller SM. Cholinergic neuronal lesions in the medial septum and vertical limb of the Diagonal Bands of Broca induce contextual fear memory generalization and impair acquisition of fear extinction. *Hippocampus.* 2015
- Kurosawa M, Sato A, Sato Y. Stimulation of the nucleus basalis of Meynert increases acetylcholine release in the cerebral cortex in rats. *Neurosci Lett.* 1989; 98(1):45–50. [PubMed: 2565563]
- Kutlu MG, Gould TJ. Acute nicotine delays extinction of contextual fear in mice. *Behav Brain Res.* 2014; 263:133–137. [PubMed: 24487010]
- Kutlu MG, Gould TJ. Nicotine modulation of fear memories and anxiety: Implications for learning and anxiety disorders. *Biochem Pharmacol.* 2015; 97(4):498–511. [PubMed: 26231942]
- Lamour Y, Dutar P, Jobert A. Cortical projections of the nucleus of the diagonal band of Broca and of the substantia innominata in the rat: an anatomical study using the anterograde transport of a conjugate of wheat germ agglutinin and horseradish peroxidase. *Neuroscience.* 1984; 12(2):395–408. [PubMed: 6205320]
- Lesting J, Daldrup T, Narayanan V, Himpe C, Seidenbecher T, Pape HC. Directional theta coherence in prefrontal cortical to amygdalo-hippocampal pathways signals fear extinction. *PLoS One.* 2013; 8(10):e77707. [PubMed: 24204927]
- Lesting J, Geiger M, Narayanan RT, Pape HC, Seidenbecher T. Impaired extinction of fear and maintained amygdala-hippocampal theta synchrony in a mouse model of temporal lobe epilepsy. *Epilepsia.* 2011; 52(2):337–346. [PubMed: 21054349]
- Letzkus JJ, Wolff SB, Luthi A. Disinhibition, a Circuit Mechanism for Associative Learning and Memory. *Neuron.* 2015; 88(2):264–276. [PubMed: 26494276]
- Letzkus JJ, Wolff SB, Meyer EM, Tovote P, Courtin J, Herry C, Luthi A. A disinhibitory microcircuit for associative fear learning in the auditory cortex. *Nature.* 2011; 480(7377):331–335. [PubMed: 22158104]
- Lindner MD, Hogan JB, Hodges DB Jr, Orie AF, Chen P, Corsa JA, Leet JE, Gillman KW, Rose GM, Jones KM, Gribkoff VK. Donepezil primarily attenuates scopolamine-induced deficits in psychomotor function, with moderate effects on simple conditioning and attention, and small effects on working memory and spatial mapping. *Psychopharmacology (Berl).* 2006; 188(4):629–640. [PubMed: 17004085]
- Maruki K, Izaki Y, Akema T, Nomura M. Effects of acetylcholine antagonist injection into the prefrontal cortex on the progress of lever-press extinction in rats. *Neurosci Lett.* 2003; 351(2):95–98. [PubMed: 14583390]
- McCormick DA. Actions of acetylcholine in the cerebral cortex and thalamus and implications for function. *Prog Brain Res.* 1993; 98:303–308. [PubMed: 8248519]
- McDonald AJ, Mascagni F. Neuronal localization of m1 muscarinic receptor immunoreactivity in the rat basolateral amygdala. *Brain Struct Funct.* 2010; 215(1):37–48. [PubMed: 20503057]
- McGeer PL, McGeer EG, Suzuki J, Dolman CE, Nagai T. Aging, Alzheimer's disease, and the cholinergic system of the basal forebrain. *Neurology.* 1984; 34(6):741–745. [PubMed: 6539435]
- McIntyre CK, Pal SN, Marriott LK, Gold PE. Competition between memory systems: acetylcholine release in the hippocampus correlates negatively with good performance on an amygdala-dependent task. *J Neurosci.* 2002; 22(3):1171–1176. [PubMed: 11826146]
- Mesulam MM. Cholinergic circuitry of the human nucleus basalis and its fate in Alzheimer's disease. *J Comp Neurol.* 2013; 521(18):4124–4144. [PubMed: 23852922]
- Mesulam MM, Mufson EJ, Levey AI, Wainer BH. Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus

- basalis (substantia innominata), and hypothalamus in the rhesus monkey. *J Comp Neurol*. 1983a; 214(2):170–197. [PubMed: 6841683]
- Mesulam MM, Mufson EJ, Wainer BH, Levey AI. Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1–Ch6). *Neuroscience*. 1983b; 10(4):1185–1201. [PubMed: 6320048]
- Metherate R, Ashe JH. Nucleus basalis stimulation facilitates thalamocortical synaptic transmission in the rat auditory cortex. *Synapse*. 1993; 14(2):132–143. [PubMed: 8392756]
- Metherate R, Cox CL, Ashe JH. Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. *J Neurosci*. 1992; 12(12):4701–4711. [PubMed: 1361197]
- Milad MR, Quirk GJ. Fear extinction as a model for translational neuroscience: ten years of progress. *Annu Rev Psychol*. 2012; 63:129–151. [PubMed: 22129456]
- Miyakawa T, Yamada M, Duttaroy A, Wess J. Hyperactivity and intact hippocampus-dependent learning in mice lacking the M1 muscarinic acetylcholine receptor. *J Neurosci*. 2001; 21(14):5239–5250. [PubMed: 11438599]
- Mogenson GJ, Swanson LW, Wu M. Neural projections from nucleus accumbens to globus pallidus, substantia innominata, and lateral preoptic-lateral hypothalamic area: an anatomical and electrophysiological investigation in the rat. *J Neurosci*. 1983; 3(1):189–202. [PubMed: 6822855]
- Mora F, Rolls ET, Burton MJ. Modulation during learning of the responses of neurons in the lateral hypothalamus to the sight of food. *Exp Neurol*. 1976; 53(2):508–519. [PubMed: 824153]
- Muller JF, Mascagni F, McDonald AJ. Cholinergic innervation of pyramidal cells and parvalbumin-immunoreactive interneurons in the rat basolateral amygdala. *J Comp Neurol*. 2011; 519(4):790–805. [PubMed: 21246555]
- Muller JF, Mascagni F, Zaric V, McDonald AJ. Muscarinic cholinergic receptor M1 in the rat basolateral amygdala: ultrastructural localization and synaptic relationships to cholinergic axons. *J Comp Neurol*. 2013; 521(8):1743–1759. [PubMed: 23559406]
- Muller JF, Mascagni F, Zaric V, Mott DD, McDonald AJ. Localization of the M2 muscarinic cholinergic receptor in dendrites, cholinergic terminals and non-cholinergic terminals in the rat basolateral amygdala: An ultrastructural analysis. *J Comp Neurol*. 2016
- Nail-Boucherie K, Dourmap N, Jaffard R, Costentin J. Contextual fear conditioning is associated with an increase of acetylcholine release in the hippocampus of rat. *Brain Res Cogn Brain Res*. 2000; 9(2):193–197. [PubMed: 10729702]
- Nickerson Poulin A, Guerri A, El Mestikawy S, Semba K. Vesicular glutamate transporter 3 immunoreactivity is present in cholinergic basal forebrain neurons projecting to the basolateral amygdala in rat. *J Comp Neurol*. 2006; 498(5):690–711. [PubMed: 16917846]
- Oh JD, Woolf NJ, Roghani A, Edwards RH, Butcher LL. Cholinergic neurons in the rat central nervous system demonstrated by in situ hybridization of choline acetyltransferase mRNA. *Neuroscience*. 1992; 47(4):807–822. [PubMed: 1579211]
- Orsini CA, Maren S. Neural and cellular mechanisms of fear and extinction memory formation. *Neurosci Biobehav Rev*. 2012; 36(7):1773–1802. [PubMed: 22230704]
- Passani MB, Cangioli I, Baldi E, Bucherelli C, Mannaioni PF, Blandina P. Histamine H3 receptor-mediated impairment of contextual fear conditioning and in-vivo inhibition of cholinergic transmission in the rat basolateral amygdala. *Eur J Neurosci*. 2001; 14(9):1522–1532. [PubMed: 11722614]
- Poole RL, Connor DA, Gould TJ. Donepezil reverses nicotine withdrawal-induced deficits in contextual fear conditioning in C57BL/6J mice. *Behav Neurosci*. 2014; 128(5):588–593. [PubMed: 24911319]
- Portugal GS, Kenney JW, Gould TJ. Beta2 subunit containing acetylcholine receptors mediate nicotine withdrawal deficits in the acquisition of contextual fear conditioning. *Neurobiol Learn Mem*. 2008; 89(2):106–113. [PubMed: 17584502]
- Power AE, McIntyre CK, Litmanovich A, McGaugh JL. Cholinergic modulation of memory in the basolateral amygdala involves activation of both m1 and m2 receptors. *Behav Pharmacol*. 2003a; 14(3):207–213. [PubMed: 12799522]

- Power AE, Vazdarjanova A, McGaugh JL. Muscarinic cholinergic influences in memory consolidation. *Neurobiol Learn Mem.* 2003b; 80(3):178–193. [PubMed: 14521862]
- Rasmusson DD, Clow K, Szerb JC. Frequency-dependent increase in cortical acetylcholine release evoked by stimulation of the nucleus basalis magnocellularis in the rat. *Brain Res.* 1992; 594(1): 150–154. [PubMed: 1467935]
- Raybuck JD, Gould TJ. The role of nicotinic acetylcholine receptors in the medial prefrontal cortex and hippocampus in trace fear conditioning. *Neurobiol Learn Mem.* 2010; 94(3):353–363. [PubMed: 20727979]
- Raybuck JD, Portugal GS, Lerman C, Gould TJ. Varenicline ameliorates nicotine withdrawal-induced learning deficits in C57BL/6 mice. *Behav Neurosci.* 2008; 122(5):1166–1171. [PubMed: 18823172]
- Robinson L, Platt B, Riedel G. Involvement of the cholinergic system in conditioning and perceptual memory. *Behav Brain Res.* 2011; 221(2):443–465. [PubMed: 21315109]
- Rogers JL, Kesner RP. Cholinergic modulation of the hippocampus during encoding and retrieval of tone/shock-induced fear conditioning. *Learn Mem.* 2004; 11(1):102–107. [PubMed: 14747523]
- Rolls ET, Roper-Hall A, Sanghera MK. Activity of neurones in the substantia innominata and lateral hypothalamus during the initiation of feeding in the monkey [proceedings]. *J Physiol.* 1977; 272(1):24P.
- Rolls ET, Sanghera MK, Roper-Hall A. The latency of activation of neurones in the lateral hypothalamus and substantia innominata during feeding in the monkey. *Brain Res.* 1979; 164:121–135. [PubMed: 106927]
- Roosendaal B, van der Zee EA, Hensbroek RA, Maat H, Luiten PG, Koolhaas JM, Bohus B. Muscarinic acetylcholine receptor immunoreactivity in the amygdala–II. Fear-induced plasticity. *Neuroscience.* 1997; 76(1):75–83. [PubMed: 8971760]
- Rouse ST, Gilmor ML, Levey AI. Differential presynaptic and postsynaptic expression of m1-m4 muscarinic acetylcholine receptors at the perforant pathway/granule cell synapse. *Neuroscience.* 1998; 86(1):221–232. [PubMed: 9692756]
- Rouse ST, Marino MJ, Potter LT, Conn PJ, Levey AI. Muscarinic receptor subtypes involved in hippocampal circuits. *Life Sci.* 1999; 64(6–7):501–509. [PubMed: 10069516]
- Rozeske RR, Valerio S, Chaudun F, Herry C. Prefrontal neuronal circuits of contextual fear conditioning. *Genes Brain Behav.* 2015; 14(1):22–36. [PubMed: 25287656]
- Rudy JW. Scopolamine administered before and after training impairs both contextual and auditory-cue fear conditioning. *Neurobiol Learn Mem.* 1996; 65(1):73–81. [PubMed: 8673409]
- Santini E, Porter JT. M-type potassium channels modulate the intrinsic excitability of infralimbic neurons and regulate fear expression and extinction. *J Neurosci.* 2010; 30(37):12379–12386. [PubMed: 20844133]
- Santini E, Sepulveda-Orengo M, Porter JT. Muscarinic receptors modulate the intrinsic excitability of infralimbic neurons and consolidation of fear extinction. *Neuropsychopharmacology.* 2012; 37(9): 2047–2056. [PubMed: 22510723]
- Schroeder JP, Packard MG. Facilitation of memory for extinction of drug-induced conditioned reward: role of amygdala and acetylcholine. *Learn Mem.* 2004; 11(5):641–647. [PubMed: 15466320]
- Smiley JF, Subramanian M, Mesulam MM. Monoaminergic-cholinergic interactions in the primate basal forebrain. *Neuroscience.* 1999; 93(3):817–829. [PubMed: 10473248]
- Soares JC, Fornari RV, Oliveira MG. Role of muscarinic M1 receptors in inhibitory avoidance and contextual fear conditioning. *Neurobiol Learn Mem.* 2006; 86(2):188–196. [PubMed: 16647280]
- Stanley EM, Wilson MA, Fadel JR. Hippocampal neurotransmitter efflux during one-trial novel object recognition in rats. *Neurosci Lett.* 2012; 511(1):38–42. [PubMed: 22306091]
- Struble RG, Lehmann J, Mitchell SJ, McKinney M, Price DL, Coyle JT, DeLong MR. Basal forebrain neurons provide major cholinergic innervation of primate neocortex. *Neurosci Lett.* 1986; 66(2): 215–220. [PubMed: 2425286]
- Tian S, Gao J, Han L, Fu J, Li C, Li Z. Prior chronic nicotine impairs cued fear extinction but enhances contextual fear conditioning in rats. *Neuroscience.* 2008; 153(4):935–943. [PubMed: 18440720]

- Tinsley MR, Q JJ, Fanselow MS. The role of muscarinic and nicotinic cholinergic neurotransmission in aversive conditioning: comparing pavlovian fear conditioning and inhibitory avoidance. *Learn Mem.* 2004; 11(1):35–42. [PubMed: 14747515]
- Tronson NC, Schrick C, Guzman YF, Huh KH, Srivastava DP, Penzes P, Guedea AL, Gao C, Radulovic J. Segregated populations of hippocampal principal CA1 neurons mediating conditioning and extinction of contextual fear. *J Neurosci.* 2009; 29(11):3387–3394. [PubMed: 19295145]
- Unal CT, Pare D, Zaborszky L. Impact of basal forebrain cholinergic inputs on basolateral amygdala neurons. *J Neurosci.* 2015; 35(2):853–863. [PubMed: 25589777]
- Vago DR, Kesner RP. Cholinergic modulation of Pavlovian fear conditioning in rats: differential effects of intrahippocampal infusion of mecamylamine and methyllycaconitine. *Neurobiol Learn Mem.* 2007; 87(3):441–449. [PubMed: 17178240]
- van der Zee EA, Luiten PG. Muscarinic acetylcholine receptors in the hippocampus, neocortex and amygdala: a review of immunocytochemical localization in relation to learning and memory. *Prog Neurobiol.* 1999; 58(5):409–471. [PubMed: 10380240]
- van der Zee EA, Roozendaal B, Bohus B, Koolhaas JM, Luiten PG. Muscarinic acetylcholine receptor immunoreactivity in the amygdala–I. Cellular distribution correlated with fear-induced behavior. *Neuroscience.* 1997; 76(1):63–73. [PubMed: 8971759]
- Vazdarjanova A, McGaugh JL. Basolateral amygdala is involved in modulating consolidation of memory for classical fear conditioning. *J Neurosci.* 1999; 19(15):6615–6622. [PubMed: 10414989]
- Volpicelli LA, Levey AI. Muscarinic acetylcholine receptor subtypes in cerebral cortex and hippocampus. *Prog Brain Res.* 2004; 145:59–66. [PubMed: 14650906]
- Wallenstein GV, Vago DR. Intrahippocampal scopolamine impairs both acquisition and consolidation of contextual fear conditioning. *Neurobiol Learn Mem.* 2001; 75(3):245–252. [PubMed: 11300731]
- Wang L, Yuan LL. Activation of M2 muscarinic receptors leads to sustained suppression of hippocampal transmission in the medial prefrontal cortex. *J Physiol.* 2009; 587(Pt 21):5139–5147. [PubMed: 19752120]
- Wang ME, Yuan RK, Keinath AT, Ramos Alvarez MM, Muzzio IA. Extinction of Learned Fear Induces Hippocampal Place Cell Remapping. *J Neurosci.* 2015; 35(24):9122–9136. [PubMed: 26085635]
- Wehner JM, Keller JJ, Keller AB, Picciotto MR, Paylor R, Booker TK, Beaudet A, Heinemann SF, Balogh SA. Role of neuronal nicotinic receptors in the effects of nicotine and ethanol on contextual fear conditioning. *Neuroscience.* 2004; 129(1):11–24. [PubMed: 15489024]
- Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR. Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science.* 1982; 215(4537):1237–1239. [PubMed: 7058341]
- Wilkinson DS, Gould TJ. The effects of galantamine on nicotine withdrawal-induced deficits in contextual fear conditioning in C57BL/6 mice. *Behav Brain Res.* 2011; 223(1):53–57. [PubMed: 21514327]
- Yildirim E, Connor DA, Gould TJ. ABT-089, but not ABT-107, ameliorates nicotine withdrawal-induced cognitive deficits in C57BL/6J mice. *Behav Pharmacol.* 2015; 26(3):241–248. [PubMed: 25426579]
- Young MB, Thomas SA. M1-muscarinic receptors promote fear memory consolidation via phospholipase C and the M-current. *J Neurosci.* 2014; 34(5):1570–1578. [PubMed: 24478341]
- Young SL, Bohenek DL, Fanselow MS. Scopolamine impairs acquisition and facilitates consolidation of fear conditioning: differential effects for tone vs context conditioning. *Neurobiol Learn Mem.* 1995; 63(2):174–180. [PubMed: 7663891]
- Zaborszky L. Afferent connections of the forebrain cholinergic projection neurons, with special reference to monoaminergic and peptidergic fibers. *EXS.* 1989; 57:12–32. [PubMed: 2533086]
- Zaborszky L, Cullinan WE. Hypothalamic axons terminate on forebrain cholinergic neurons: an ultrastructural double-labeling study using PHA-L tracing and ChAT immunocytochemistry. *Brain Res.* 1989; 479(1):177–184. [PubMed: 2924147]

- Zaborszky L, Cullinan WE. Projections from the nucleus accumbens to cholinergic neurons of the ventral pallidum: a correlated light and electron microscopic double-immunolabeling study in rat. *Brain Res.* 1992; 570(1–2):92–101. [PubMed: 1617433]
- Zaborszky L, Gaykema RP, Swanson DJ, Cullinan WE. Cortical input to the basal forebrain. *Neuroscience.* 1997; 79(4):1051–1078. [PubMed: 9219967]
- Zeitlin R, Patel S, Solomon R, Tran J, Weeber EJ, Echeverria V. Cotinine enhances the extinction of contextual fear memory and reduces anxiety after fear conditioning. *Behav Brain Res.* 2012; 228(2):284–293. [PubMed: 22137886]
- Zelikowsky M, Hast TA, Bennett RZ, Merjanian M, Nocera NA, Ponnusamy R, Fanselow MS. Cholinergic blockade frees fear extinction from its contextual dependency. *Biol Psychiatry.* 2013; 73(4):345–352. [PubMed: 22981655]

Significance Statement

This study reviews the impact of the cholinergic system that helps regulate brain circuits involved in the learning and extinguishing of fear memories. This report reviews the anatomic, pharmacologic, and neurochemical evidence that suggests a key role of cholinergic regulation, and particularly activation of muscarinic cholinergic receptors, in modulating cortico-amygdalar and cortico-hippocampal circuits that contribute to the acquisition of fear extinction.

Figure 1.
Panel A: Diagram illustrates the primary cholinergic inputs from the medial septum (MS) into the hippocampus, and the basal forebrain cholinergic system (BFCS) into the prefrontal cortex (PFC) and amygdala. Panel C: During Pavlovian fear conditioning a conditioned stimulus (CS), such as a tone, is paired with an unconditioned stimulus such as a footshock. Animals show conditioned freezing upon return to the conditioning context (Context A), or cue-conditioned freezing upon presentation of the tone in a novel environment (Context B). Repeated exposure to the tone elicits extinction learning, which involves cortico-amygdalar circuits, and results in reduced freezing behaviors. Panel B: Pharmacological interventions with muscarinic (mAChR) or nicotinic (nAChR) agonists and antagonists suggest that muscarinic activation enhances contextual fear conditioning primarily through interactions in the hippocampus, while muscarinic activation through the BFCS inputs into PFC and

amygdala enhance fear extinction processes. Nicotinic agonists generally enhance contextual fear conditioning and extinction, but the effects of nAChR antagonists appear to be dependent upon receptor subtype in specific sub-regions of these brain areas. See Tables 1, 2, and 3 or summaries of these effects and references.

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TABLE 1

Effects of Systemic Administration of Muscarinic and Nicotinic Receptor Agonists or Antagonists on Fear Learning

MUSCARINIC							Reference
Receptor	Agonist or Antagonist	Drug	Subjects	Timing	Drug Effects at Training	Effect on Contextual Fear Conditioning	
Muscarinic	Antagonist	SCOP	Long Evans rats, juvenile male/female	Pre-training	Decreased	Decreased	(Rudy 1996)
Muscarinic	Antagonist	SCOP	Long Evans rats, male	Pre-training		No effect	(Young et al. 1995)
	Antagonist	SCOP	Long Evans rats, female	Pre-training		Decreased	(Anagnostaras et al. 1995)
Muscarinic	Antagonist	SCOP	Long Evans rats, female adult, juvenile	Pre-training	Decreased	Decreased	(Anagnostaras et al. 1999)
Muscarinic	Antagonist	SCOP	C57BL/6J mice, young and old	Pre-training		Decreased	(Feiro and Gould 2005)
Muscarinic	Antagonist	SCOP	Sprague Dawley, male	Pre-training		Decreased	(Lindner et al. 2006)
Muscarinic	Antagonist**	Methyl SCOP	Long Evans rats, female adult, juvenile	Pre-training		No effect	(Anagnostaras et al. 1999)
Muscarinic	Antagonist**	Methyl-SCOP	Long Evans rats, male	Pre-training	Decreased	Increased	(Young et al. 1995)
Muscarinic	M1 antagonist	DICYCLO	Wistar rats, male	Pre-training	Decreased	Decreased	(Figueredo et al. 2008; Fornari et al. 2000; Soares et al. 2006)
Muscarinic	M1 antagonist	DICYCLO	Wistar rats, male	Pre-training		Decreased	(Fornari et al. 2000)
Muscarinic	M1 antagonist	DICYCLO	Wistar rats, male	Pre-training	No Effect	Decreased	(Figueredo et al. 2008)
Muscarinic	M1 antagonist	DICYCLO	Wistar rats, male	Pre-testing (context)		Decreased	(Soares et al. 2006)
Muscarinic	Antagonist	SCOP	Long Evans rats, male	Post-training		No effect	(Young et al. 1995)
Muscarinic	Antagonist	SCOP	Long Evans rats, female adult, juvenile	Post-training		No effect	(Anagnostaras et al. 1995;

Receptor	Agonist or Antagonist	Drug	Subjects	Timing	Drug Effects at Training	Effect on Contextual Fear Conditioning	Effect on Cued Fear Conditioning	Reference
								Anagnostaras et al. 1999)
Muscarinic	Antagonist	SCOP	Long Evans rats juvenile, male/female	Post-training, <3 h		Decreased	Decreased	(Rudy 1996)
Muscarinic	M1 antagonist	DICYCLO	Wistar rats, male	Post-training		No effect	Not tested	(Soares et al. 2006)
NICOTINIC								
Nicotinic	Agonist	NIC	C57BL/6J mice, male	Pre-training and pre-testing		Increased	No Effect	(Davis and Gould 2006; Davis et al. 2006; Gould and Higgins 2003; Gould and Wehner 1999; Wehner et al. 2004)
Nicotinic	Agonist	NIC	C57BL/6J mice, male	Pre-training and pre-testing		Increased	Increased	(Gould and Higgins 2003)
Nicotinic	Agonist	NIC	C57BL/6J mice, male and female	Pre-training and pre-testing		Not Tested	No Effect	(Gould et al. 2004)
Nicotinic	Agonist	NIC	Mice with knockouts for $\alpha 7$, $\beta 3$, or $\beta 4$ nACHR	Pre-training and pre-testing		Increased	No Effect	(Wehner et al. 2004)
Nicotinic	Agonist	NIC	Mice with knockout for $\beta 2$ nACHR	Pre-training and pre-testing		No Effect (decreased without NIC)	No Effect	(Wehner et al. 2004)
Nicotinic	Agonist	NIC	C57BL/6J mice, male	Pre-training		No Effect	No Effect (some doses increased)	(Gould and Higgins 2003; Gould and Wehner 1999)
Nicotinic	Agonist	NIC	C57BL/6J mice, male	Pre-testing		No Effect	No Effect	(Gould and Higgins 2003; Gould and Wehner 1999)
Nicotinic	Positive modulator, $\alpha 7$	Cotinine	C57BL/6J mice, male	Pre-training		No Effect		(Zeitlin et al. 2012)
Nicotinic	Partial agonist, $\alpha 4\beta 2$	ABT-089	C57BL/6J mice, male	Pre-training and pre-testing	No Effect	Increased	No Effect	(Yildirim et al. 2015)
Nicotinic	Partial agonist $\alpha 4\beta 2$, full agonist $\alpha 7$	Varenicline	C57BL/6J mice, male	Pre-training and pre-testing	No Effect	No Effect	No Effect	(Raybuck et al. 2008)

Receptor	Agonist or Antagonist	Drug	Subjects	Timing	Drug Effects at Training	Effect on Contextual Fear Conditioning	Effect on Cued Fear Conditioning	Reference
Nicotinic	Agonist, homomeric $\alpha 7$	ABT-107	C57BL/6J mice, male	Pre-training and pre-testing	No Effect	No Effect	No Effect	(Yildirim et al. 2015)
Nicotinic	Antagonist	MEC	C57BL/6J mice, young and old	Pre-training		No Effect	No Effect	(Feiro and Gould 2005).
Nicotinic	Antagonist	MEC	C57BL/6 mice, male	Pre-training and pre-testing		No effect alone, blocked NIC effects	No effect	(Gould and Wehner 1999)
Nicotinic	Antagonist	DH β E	C57BL/6 mice, male	Pre-training and pre-testing		No effect alone, blocked NIC effects	No effect	(Davis and Gould 2006)
Nicotinic	Antagonist, $\alpha 7$ selective	MLA	C57BL/6 mice, male	Pre-training and pre-testing		Increased, did not block NIC effects	No effect	(Davis and Gould 2006)
Nicotinic	Agonist	NIC	C57BL/6J mice, male	Post-training		No Effect	No Effect	(Gould and Higgins 2003; Gould and Wehner 1999)
Nicotinic	Agonist	MEC	C57BL/6J mice, male	Post-training		No Effect	No Effect	(Gould and Higgins 2003)
<i>Acetylcholinesterase Inhibitors</i>								
Muscarinic & Nicotinic	ACHE Inhibitor	Donepezil	C57BL/6J mice	Pre-training and Pre-testing	Increased	Increased	No Effect	(Poole et al. 2014)
Muscarinic & Nicotinic	ACHE Inhibitor	Galantamine	C57BL/6J mice	Pre-training and Pre-testing		No Effect	No Effect	(Wilkinson and Gould 2011)

Drug Abbreviations: ABT-089 = 2-methyl-3-(2-(S)-pyrrolidinylmethoxy)pyridine, pozzanicline, partial agonist at $\alpha 4\beta 2^*$ nAChRs, high selectivity for $\alpha 6\beta 2^*$ and $\alpha 4\alpha 5\beta 2$ nAChR; ABT-107 = 5-(6-[(3R)-1-azabicyclo[2,2,2]oct-3-yl]oxy]pyridazin-3-yl)-1H-indole, agonist at homomeric $\alpha 7$ nAChRs; ACHE=Acetylcholinesterase; DICYLO= dicyclomine; DH β E= dihydro- β -erythroidine; MEC= mecamlamine; MLA=methyllycaconitine; NIC=Nicotine; PHYO=Physostigmine; SCOP=scopolamine; methyl-SCOP methylscopolamine. **methylscopolamine does not cross blood brain barrier (BBB). Blank slots (under acquisition) are data not shown/reported or not applicable (injections are post-training). Pre-training and pre-testing indicates injections at both time points.

TABLE 2

Effects of Intracerebral Administration of Muscarinic and Nicotinic Receptor Agonists or Antagonists on Fear Learning

Receptor	Agonist or Antagonist	Drug	Brain Area	Subjects	Timing	Effect on acquisition	Effect on Contextual Fear Conditioning	Effect on Cued Fear Conditioning	Reference
MUSCARINIC									
Muscarinic	Agonist	OXO	BLA *	Wistar rats, male	Post training		Increased		(Cangioli et al. 2002)
Muscarinic	Agonist	OXO	BLA *	Sprague Dawley rats, male	Post training		Increased	Not tested	(Vazdarjanova and McGaugh 1999)
Muscarinic	Antagonist	SCOP	BLA	Wistar rats, male	Post-training		Decreased	Not tested	(Bucherelli et al. 2006)
Muscarinic	Antagonist	SCOP	BLA *	Wistar rats, male	Post training		Decreased		(Passani et al. 2001)
Muscarinic	Antagonist	Atropine	BLA	ChAT-CRE mice	Pre-training		Not tested	No effect	(Jiang et al. 2016)
Muscarinic+Nicotinic	Antagonist	Atropine + MEC	BLA	ChAT-CRE mice	Pre-training		Not tested	Decreased	(Jiang et al. 2016)
Muscarinic	Antagonist	SCOP	Lateral amygdala	Sprague Dawley rats, male	Pre-training		No effect	No effect	(Baysinger et al. 2012)
Muscarinic	Antagonist	SCOP	CA3 HIPPO	Long Evans rats	Pre-training	Decreased	Decreased	No effect	(Rogers and Kesner 2004)
Muscarinic	Antagonist	SCOP	Dorsal HIPPO	Sprague Dawley rats, female	Pre-training		Decreased	No effect	(Wallenstein and Vago 2001)
Muscarinic	Antagonist	SCOP	Dorsal HIPPO	Long Evans rats	Pre-training	Decreased	Decreased	No effect	(Gale et al. 2001)
Muscarinic	Antagonist	SCOP	Dorsal HIPPO	Sprague Dawley rats, female	Post-training		Decreased	No effect	(Wallenstein and Vago 2001)
Muscarinic	Antagonist	SCOP	Dorsal HIPPO	Wistar rats, male	Post-training		Decreased	Not tested	(Chang and Liang 2012)
Muscarinic	Antagonist	SCOP	Dorsal HIPPO	Wistar rats, male	Post-training (6–12 hrs)		No effect	Not tested	(Chang and Liang 2012)
NICOTINIC									
Nicotinic	Agonist	NIC	Dorsal HIPPO	C57BL/6J mice, male	Pre-training, or pre-training and pre-testing		Increased	Not tested	(Raybuck and Gould 2010)

Receptor	Agonist or Antagonist	Drug	Brain Area	Subjects	Timing	Effect on acquisition	Effect on Contextual Fear Conditioning	Effect on Cued Fear Conditioning	Reference
									(Kenney et al. 2012)
Nicotinic	Agonist	NIC	Dorsal HIPPO	C57BL/6J mice, male	Pre-testing		No effect	Not tested	(Raybuck and Gould 2010) (Kenney et al. 2012)
Nicotinic	Antagonist	MEC	Dorsal HIPPO	Long Evans rats, male	Pre-training		Decreased	No effect	(Vago and Kesner 2007)
Nicotinic	Antagonist	DH β E	Dorsal HIPPO	C57BL/6J mice	Pre-training, pre-testing, or pre-training and pre-testing		No effect	Not tested	(Raybuck and Gould 2010)
Nicotinic	Antagonist	MEC	Dorsal HIPPO	Long Evans rats, male	Pre-training		Decreased	No effect	(Vago and Kesner 2007)
Nicotinic	Antagonist	MEC	Dorsal HIPPO	Long Evans rats, male	Post-training		Decreased	No effect	(Vago and Kesner 2007)
Nicotinic	Antagonist	MEC	Dorsal HIPPO	Long Evans rats, male	Post-training, 6 hr		No effect	No effect	(Vago and Kesner 2007)
Nicotinic	Antagonist, α 7 selective	MLA	Dorsal HIPPO	Long Evans rats, male	Pre-training		No effect	No effect	(Vago and Kesner 2007)
Nicotinic	Antagonist, α 7 selective	MLA	Dorsal HIPPO	Long Evans rats, male	Post-training		Decreased	No effect	(Vago and Kesner 2007)
Nicotinic	Antagonist, α 7 selective	MLA	Dorsal HIPPO	Long Evans rats, male	Post-training, 6 hr		Decreased	No effect	(Vago and Kesner 2007)
Nicotinic	Agonist	NIC	Ventral HIPPO	C57BL/6J mice, male	Pre-training, pre-training and pre-testing	Decreased	Decreased	No effect	(Raybuck and Gould 2010) (Kenney et al. 2012)
Nicotinic	Agonist	NIC	Ventral HIPPO	C57BL/6J mice, male	Pre-testing		Decreased		
Nicotinic	Antagonist	DH β E	Ventral HIPPO	C57BL/6J mice, male	Pre-training	No Effect	No effect	Not tested	(Raybuck and Gould 2010) (Kenney et al. 2012)
Nicotinic	Antagonist	DH β E	Ventral HIPPO	C57BL/6J mice	Pre-training		Increased	Not tested	(Kenney et al. 2012)
Nicotinic	Antagonist	DH β E	Ventral HIPPO	C57BL/6J mice, male	Pre-testing		Decreased	Not tested	(Kenney et al. 2012)
Nicotinic	Antagonist, α 7 selective	MLA	Ventral HIPPO	C57BL/6J mice, male	Pre-training or pre-testing		No Effect		(Kenney et al. 2012)

Receptor	Agonist or Antagonist	Drug	Brain Area	Subjects	Timing	Effect on acquisition	Effect on Contextual Fear Conditioning	Effect on Cued Fear Conditioning	Reference
Nicotinic	Agonist	NIC	PFC	C57BL/6J mice, male	Pre-training or pre-training and pre-testing		No Effect	No Effect	(Raybuck and Gould 2010)
Nicotinic	Antagonist	DHβE	PFC	C57BL/6J mice, male	Pre-training or pre-training and pre-testing		No Effect	Not tested	(Raybuck and Gould 2010)
Nicotinic	Antagonist	DHβE	PFC	C57BL/6J mice	Pre-testing		Decreased	Not tested	(Raybuck and Gould 2010)
Nicotinic	Antagonist, α7 selective	MLA	PFC	C57BL/6J mice, male	Pre-training or pre-training and pre-testing		No Effect	Not tested	(Raybuck and Gould 2010)
Nicotinic	Antagonist, α7 selective	MLA	PFC	C57BL/6J mice, male	Pre-testing		Decreased	Not tested	(Raybuck and Gould 2010)
Acetylcholinesterase Inhibitors									
Muscarinic & Nicotinic	ACHE inhibitor	PHYSO	CA3 HIPPO	Long Evans rats	Pre-training	No significant effect	Decreased (slightly)	No Effect	(Rogers and Kesner 2004)

Post-training drug injections are within 1 hour after training unless indicated. Pre-training and pre-testing refers to injections at both time points.

Drug Abbreviations: ACHE=Acetylcholinesterase; ChAT=Choline Acetyltransferase; DHβE= dihydro-β-erythroidine; MEC= mecamylamine; MLA=methyllycaconitine; NIC=Nicotine; OXO= Oxotremorine; PHYSO=Physostigmine; SCOP=scopolamine

Brain Region Abbreviations: BLA=Basolateral Amygdala; BLA* indicates BLA injections under ketamine anesthesia; HIPPO=Hippocampus; PFC=Prefrontal Cortex

TABLE 3
Summary of Muscarinic and Nicotinic Receptor Agonists and Antagonists on Fear Extinction

Receptor	Agonist or Antagonist	Drug	Route or Brain Site	Subjects	Timing	Effect on Extinction	Reference
<i>MUSCARINIC</i>							
Muscarinic	Agonist	Cevimeline	Systemic	Sprague Dawley rats, male (<40 days old)	Pre-extinction training	Enhanced extinction	(Santini et al. 2012)
Muscarinic	Antagonist	SCOP	Systemic, low dose	Long Evans, male	Pre-extinction training	Slower rate of extinction, less renewal and no context dependence	(Zelikowsky et al. 2013)
Muscarinic	Antagonist	SCOP	Systemic, high dose	Long Evans, male	Pre-extinction training	Impaired extinction	(Zelikowsky et al. 2013)
Muscarinic	Antagonist	SCOP	Systemic	Sprague Dawley rats, male (<40 days old)	Pre-extinction training	Impaired extinction	(Santini et al. 2012)
Muscarinic	Antagonist	SCOP	PFC (IL)	Sprague Dawley rats, male (<40 days old)	Pre-extinction training	Impaired extinction	(Santini et al. 2012)
Muscarinic	Agonist	Cevimeline	Systemic	Sprague Dawley rats, male (<40 days old)	Post-extinction training	Enhanced extinction	(Santini et al. 2012)
Muscarinic	Agonist	Oxotremorine	BLA(unilateral)	Sprague Dawley rats, male	Post-extinction training	Enhanced extinction (context)	(Boccia et al. 2009)
Muscarinic	Antagonist	SCOP	systemic	Sprague Dawley rats, male (<40 days old)	Post-extinction training	Impaired extinction	(Santini et al. 2012)
Muscarinic	Antagonist	SCOP	Systemic	Long Evans, male	Post-extinction training	No Effect	(Zelikowsky et al. 2013)
Muscarinic	Antagonist	SCOP	PFC (IL)	Sprague Dawley rats, male (<40 days old)	Post-extinction training	Impaired Extinction	(Santini et al. 2012)
<i>NICOTINIC</i>							
Nicotinic	Agonist	Nicotine	Systemic	C57BL/6J mice, male	Pre- extinction training	Enhanced extinction (CS)	(Elias et al. 2010)
Nicotinic	Agonist	Nicotine	Systemic	C57BL/6J mice, male	Both Pre-training and Pre-extinction	Impaired (delayed) extinction (CS)	(Elias et al. 2010)
Nicotinic	Agonist	Nicotine	Systemic	C57BL/6J mice, male	Pre- extinction training	Impaired extinction to context, not cue	(Kuulu and Gould 2014)
Nicotinic	Positive modulator, $\alpha 7$	Cotinine	Systemic	C57BL/6J mice, male	Post-extinction training	Enhanced extinction (context)	(Zeitlin et al. 2012)

SCOP=scopolamine, BLA = Basolateral Amygdala, PFC(IL) = Intralimbic Prefrontal Cortex

Table is formatted in the following order: mACHR agonists injected pre-extinction training, mACHR antagonists injected pre-extinction training, mACHR agonists injected post-extinction training, mACHR antagonists injected post-extinction training, and nicotinic agonists injected systemically before extinction training. Unless noted, changes refer to extinction of cue (CS)-conditioned freezing.