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Invited review

Pavlovian fear memory circuits and phenotype models of PTSD

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ABSTRACT

Pavlovian fear conditioning, also known as classical fear conditioning is an important model in the study of the neurobiology of normal and pathological fear. Progress in the neurobiology of Pavlovian fear also enhances our understanding of disorders such as posttraumatic stress disorder (PTSD) and with developing effective treatment strategies. Here we describe how Pavlovian fear conditioning is a key tool for understanding both the neurobiology of fear and the mechanisms underlying variations in fear memory strength observed across different phenotypes. First we discuss how Pavlovian fear models aspects of PTSD. Second, we describe the neural circuits of Pavlovian fear and the molecular mechanisms within these circuits that regulate fear memory. Finally, we show how fear memory strength is heritable; and describe genes which are specifically linked to both changes in Pavlovian fear behavior and to its underlying neural circuitry. These emerging data begin to define the essential genes, cells and circuits that contribute to normal and pathological fear.

This article is part of a Special Issue entitled 'Post-Traumatic Stress Disorder'.

1. Introduction

Posttraumatic Stress Disorder (PTSD) is a debilitating and often chronic condition resulting from exposure to life threatening trauma and stress. Although classified as an anxiety disorder, accumulating data suggests that PTSD is more appropriately a disease of learning and memory (Elzinga and Bremner, 2002). Normal fear memory and fear responses are an essential part of any person's or animal's survival mechanism. Using fear memory, an organism can learn to associate and remember new environmental cues with physical danger. Fear pathology such as PTSD can be characterized by pathology in fear memory where responses are amplified and fail to extinguish and thus become debilitating. These disorders can be thought of as either pathology of the acquisition of fear memory or as pathology in the expression of an otherwise normal fear memory.

With this in mind, a key challenge in identifying the etiology of PTSD is to first understand the genetic and cellular systems which regulate fear memory itself. One approach to this problem is to develop animal models that might allow us to understand these fundamental systems. One promising animal model focuses on learning of fearful memories. In this paradigm, neutral stimuli are associated with unpleasant events and this association is remembered. Fearful memories can persist indefinitely if not extinguished. Thus, the life of a fear memory has a temporal sequence of acquisition, consolidation and extinction. Importantly, fear memories also have magnitude: they can be weak or strong. The strength of a fear memory depends on the conditions which initiated it and also on the brain which encodes the memory. Thus, there are many aspects of fearful memories that may share common mechanisms with PTSD.

Understanding individual differences in the acquisition, consolidation and extinction of fearful memories will provide data on how different cellular mechanisms produce different phenotypes of fear memory. Isolation of key cellular mechanisms regulating differences in fear memory is essential to identifying the genes encoding these differences in behavior. Thus an important strategy in identifying genes regulating differences in fear memory is to first isolate the cellular responses that positively or negatively modulate the strength of fear memory consolidation and the resulting behavioral response (Ponder et al., 2007a; Camp et al., 2009). Thus, an important strategy in identifying genes regulating differences in fear memory is to first isolate the cellular basis of fear memory phenotypes (Ponder et al.,

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2007a; Camp et al., 2009). Isolating the cellular mechanism of fear learning requires a fear behavior paradigm that is both robust and amenable to the current tools of cellular neuroscience. Classical fear conditioning, also known as Pavlovian fear conditioning, has been extensively used to study the cellular basis of fear memory (LeDoux, 2000) and more recently to understand the genetic basis of different fear memory phenotypes (DuBois et al., 2006; Ponder et al., 2007a; Stoppel et al., 2006). Here we focus on the neurobiology of Pavlovian fear as a powerful vehicle for the understanding of pathological fear.

Studies using phenotypic differences in conditioned fear and genetic screening are beginning to attempt to identify genes involved in Pavlovian fear (Holmes et al., 2002; Ponder et al., 2007a; Camp et al., 2009; Ploski et al., 2010; Jovanovic and Ressler, 2010). These studies are addressing how differences in relative 'strength' of a Pavlovian fear memory may be driven by different genes. These methods include direct comparison of existing mouse strains, short term selective breeding for phenotypes, and chromosome substitution strains (Holmes et al., 2002; Ponder et al., 2007a,b; Camp et al., 2009; Ploski et al., 2010; Jovanovic and Ressler, 2010). These methods combined with a large body of data on the neural networks and cellular plasticity mechanisms of Pavlovian fear have a significant potential to begin to explain why some individuals form stronger and longer lasting Pavlovian fear memories to traumatic events.

Interpretation of genetic studies of various measures of fear learning are made possible by advances in our knowledge of the neural circuits of Pavlovian fear conditioning and mechanism of neuronal plasticity learning and memory. The last 20 years has seen the identification of the circuits to and from the amygdala as well as emerging data of circuits within the amygdala which encode Pavlovian fear memories. Moreover the key cellular mechanisms which control synaptic plasticity have been identified. Together this knowledge will allow cellular mechanisms unique to Pavlovian fear to be identified. In this review we bring together the cellular mechanism of Pavlovian conditioned fear with the emerging data on the genetics of fear phenotypes for multiple facets of Pavlovian learning and memory and make the case that this knowledge is fundamental to our understanding of fear phenotypes and fear pathology, particularly PTSD.

2. Modeling PTSD – the importance of Pavlovian fear conditioning

PTSD is a debilitating and often chronic condition resulting from exposure to life threatening trauma and stress. While often associated with traumatic wartime experiences, it affects both military persons and civilians (Ursano et al., 2009). Understanding how the brain is involved in PTSD is critical to more effective treatment. A prominent animal model used to study different aspects of PTSD is Pavlovian fear conditioning.

Pavlovian fear conditioning is a behavioral method by which a rodent or other organism learns to fear a particular stimulus. An emotionally neutral stimulus such as a light or a tone (conditioned stimulus; CS) is presented to the rodent at the same time as a small electric shock (unconditioned stimulus; US). After one or more presentations of the CS and US together, the animal will respond in a manner appropriate for the US to the CS alone. Pavlovian fear conditioning can be said to have a degree of etiological validity for the study of PTSD because the initial formation of memories that later develop to become PTSD involve the same process of Pavlovian fear. That is, the sites, sound and smell of traumatic events become potent memories from which classic PTSD symptoms stem. Pavlovian fear can refer to both fear of the CS or of the context or place that the fear was acquired. Thus a clinically pathological fear of places or stimuli such as a sounds or smells have their roots a basic mechanisms of Pavlovian fear (Johnson and LeDoux, 2004; Johnson et al., 2009). Thus, studies of the neural networks, cellular mechanism and genetics of Pavlovian fear in animal models are particularly important for the understanding of the fundamental mechanism of PTSD.

The study of neurobiology underlying Pavlovian fear conditioning provides a unique opportunity to understand the neural circuits in the mammalian brain responsible for an essential survival behavior. Extensive data supports the hypothesis that humans and other organisms undergo learning during Pavlovian conditioning and that this learning is driven by plasticity in neural synapses (Miserendino et al., 1990; McKernan and Shinnick-Gallagher, 1997; LeDoux, 2000; Walker and Davis, 2002; Maren and Quirk, 2004; Sah and Westbrook, 2008). Fear responses can be both unconditioned (innate rather than learned) as well as conditioned (in response to a learned association). Therefore, not only does the study of conditioned fear allow for the identification of intrinsic unconditioned responses within the mammalian brain but it also allows for the identification of circuits, synapses and mechanisms that undergo learning and memory (LeDoux, 2000; Johnson and LeDoux, 2004; Johnson et al., 2008; Johnson et al., 2009).

PTSD is also a disorder of fear extinction. Fear extinction is a process of new learning. During this learning a subject is exposed to the CS in the absence of the US. As a result the CS gradually stops eliciting the previously learned conditioned response (CR). Several lines of evidence indicate that extinction is new learning (Myers and Davis, 2007). In classic studies by Davis et al., they found extinction learning is dependent upon NMDA receptors in the amygdala suggesting that synaptic plasticity also occurs as in the initial fear learning (Sotres-Bayon et al., 2006; Myers and Davis, 2007; Sotres-Bayon et al., 2007). However, not all learning occurring during extinction takes place in the amygdala. A growing body of evidence indicates that plasticity also occurs in the medial prefrontal cortex (mPFC). Studies using lesions of the mPFC (Morgan et al., 1993; Myers and Davis, 2007), as well as direct electrophysiological recordings (Milad and Quirk, 2002), indicate a key role for the mPFC in extinction.

The Pavlovian fear paradigm also allows for the study of the cellular and genetic basis of conditioned fear across the fear learning cycle. This is important to the study of basic mechanisms of fear development and loss which occur under both normal and pathological conditions, including fear integration with the learning environment. Each of these stages has important applicability to PTSD. First, pre-exposure to both contextual and cued stimuli, also known as habituation, reduces the development of fear through the process of latent inhibition (Lubow and Moore, 1959). The influence of pre-exposure on fear learning has direct clinical applicability to stress inoculation and pre-exposure therapy also known as Pre-traumatic Vaccination Intervention (PTV) (Essar et al., 2010). These pre-exposure and inoculation methods may be important preventative strategies for inhibiting the development of PTSD (Essar et al., 2010). However, the development of latent inhibition is dependent upon the number and duration of pre-exposures. Thus any use for inoculation requires careful experimentation. Second, the conditioning paradigm, including the number and contingency of CS and US associations influence the level of fear memory strength (Rescorla, 1968; Bauer et al., 2001). Thus, the relative intensity of the US and the CS as well as the number of and timing of the US and CS pairing combine to form the conditioned 'memory strength'. Memory strength is important for PTSD because PTSD may occur in individuals who form particularly strong and thus more difficult to extinguish fear memories. Recent work from Norrholm et al. (2011) showed that PTSD patients

develop increased memory strength and fear load after fear conditioning. Third, testing of fear learning associated with the environment, also known as contextual conditioning and of cue learning (Lubow and Moore, 1959; Fanselow and LeDoux, 1999; Jovanovic and Ressler, 2010) provides important information about hippocampal versus amygdala based aspects of learning. Both nuclei are implicated in clinical studies of PTSD. A fourth aspect of the fear learning cycle is extinction (Morgan and LeDoux, 1999; Myers and Davis, 2007; Maren and Quirk, 2004). Extinction is a process of new learning and occurs any time that the CS is presented in the absence of the US. Thus after a fear memory is formed the continual presentation of a CS alone will weaken the fear memory. This process appears to be impaired in PTSD (Ursano et al., 2009). Fifth, reconsolidation of fear memory occurs after a Pavlovian fear memory is recalled. At recall the memory becomes labile and must be re-consolidated again using the same cellular processes as the original consolidation (see below) (Nader et al., 2000; Monfils et al., 2009) Pharmacological augmentation of reconsolidation of conditioned fear memories is proposed to promote the formation of revised non pathological memories during therapy in PTSD patients (See Dębiec et al., 2011, for discussion). It is during reconsolidation that new learning is incorporated into existing memory, modifying the memory over time. Treatments for pathological fear including repeated exposures, script-driven imagery and guided visualization attempt to take advantage of reconsolidation to reduce the emotional impact of fearful and traumatic memories. In summary, Pavlovian fear conditioning methods provides vital information on the ongoing transformation of fear memory over time.

Other forms of fear learning and anxiety testing are also proving useful in studying the neurobiology of fear. An important and well studied model is fear potentiated startle (Davis, 1992; Myers and Davis, 2007; Jovanovic and Ressler, 2010). Fear potentiated startle, like Pavlovian fear, is amygdala dependant and has been used in human studies in which PTSD patients show non-normal responding including heightened autonomic responses (Jovanovic and Ressler, 2010). Both Pavlovian fear conditioning and fear potentiated startle can be performed on both animals and humans (Delgado et al., 2006). In contrast, tests for anxiety are not common to both animals and humans. Human anxiety is most commonly measured using questionnaires. Tools for measuring anxiety in rodents, including open field and elevated plus maze are also used to study some aspects of fear and PTSD-associated anxiety behaviors (Sullivan et al., 2004). However, because Pavlovian fear is the simplest form of fear learning (LeDoux, 2000; Johnson and LeDoux, 2004), it is an essential paradigm for subsequent studies of the neurobiology and genetics of fear. Thus, while Pavlovian fear is not the only fear learning paradigm applicable to pathological fear and PTSD, it provides the most robust and well understood of the fear learning and anxiety paradigms.

3. Measures of fear memory strength in humans and animals

The study of fear neurobiology has focused on the behavioral and physiological responses elicited by threatening stimuli (LeDoux, 1994, 2000; Johnson and LeDoux, 2004). In addition, subjective states sometimes described as 'feeling' can also be defined as fear. Here we discuss the circuits that are important for learning about new threats and for producing responses to those threats. These responses include activation of the hypothalamic-pituitary-adrenal (HPA) axis, reduced tolerance to pain, activation of the autonomic nervous system, and defensive behaviors (freezing and then fight or flight) (LeDoux, 2000). The fear learning circuits that are studied most heavily produce changes in freezing behavior (LeDoux, 2007). Freezing behavior is used to understand the neural circuits, cellular mechanisms and genetics of fear. Recent data using animal models of Pavlovian fear illustrate the potential for changes in the circuits and synapses of the amygdala that may control the strength of fear memory (Reijmers et al., 2007; Bergstrom et al., 2011) Moreover different phenotypes may be driven by memory specific genes (Holmes et al., 2002; Ponder et al., 2007a; Camp et al., 2009; Jovanovic and Ressler, 2010).

One characteristic of PTSD is its interaction with underlying fear memory strength. What defines the strength of a memory? In PTSD, memory of an event can be defined by two parts: the quality of the memory and the stability of the memory. Quality of a memory can be construed as the combination of both the emotional and narrative vividness of an incident – the encoded emotional quality, as well as a defined set of facts of the event. Stability determines the duration of the intact, organized memory over time. One could say that PTSD might involve the "over-vividness" of the emotional quality of a traumatic memory: the incident may be dramatically provoked without conscious effort, yet lacks a narrative partner for the sake of cohesion. Additionally, the stability of the intact memory is jeopardized by various physiological effects of the disorder. One measure of stability is the disorganization of the memory over time, which can be measured as a scalable variable. In one study by Halligan, Michael, Clark, & Ehlers (Halligan et al., 2003), memory disorganization is measured along two different scales. In one, the Trauma Memory Questionnaire, disorganization is scaled by the deficits in recall observed when participants are asked to describe their traumatic memories. These deficits are measured by the incompleteness and disorganization of the memory.

A separate measure of disorganization is taken by asking a subject to recall the memory (Foa et al., 1995). This narrative is divided into chunks (i.e., a clause containing a single thought or action), with each chunk scored according to its amount of repetitions and the participant's reported uncertainty about specific facts of the memory. These "negative" qualities are subtracted from chunks that indicate an understanding of what was happening during the narrative. Combined, these three characteristics form a score, and thus a scalable variable, that may be used to measure memory disorganization. In this sense, it is possible to study the memory strength of a particular traumatic memory and monitor its change with therapy. These studies reveal that PTSD can be characterized by strong memories, where the number of trauma related memory chunks decreases with therapeutic success.

4. The neural circuits of Pavlovian fear

The neural circuits underlying classical fear conditioning are very well characterized. The neurobiology is best understood for an auditory stimulus although both visual (Shi and Davis, 2001) and olfactory (Rosenkranz and Grace, 2002) stimuli have also been studied. For auditory fear conditioning, auditory CS and nociceptive US converge in the amygdala, specifically in its lateral nucleus (Lateral Amygdala, LA) (Romanski et al., 1993; LeDoux, 2003). Fear conditioning is dependent on nociceptive inputs from the spinothalamic tract that terminate in the amygdala. These inputs may enter the amygdala via the thalamus or via the cortex (Romanski et al., 1993; Li et al., 1996; Shi and Davis, 2001). Although nociceptive inputs also terminate in the central amygdala directly from the spinal cord and the parabrachial area (Bernard and Besson, 1990; Bernard et al., 1990), evidence suggests that the convergent input in the LA is most likely the initial site of US–CS convergence and learning (Romanski et al., 1993; LeDoux, 2003; Lanuza et al., 2008) (see also Herry et al., 2008). More recent data has begun to reveal the circuits and networks interconnected with the amygdala that are responsible for fear conditioning associated behaviors,

including contextual fear learning and fear extinction. The connectivity between the amygdala and meso temporal structures including the hippocampus is very important for the contextual elements of fear learning. Lesions of the hippocampus disrupt the parallel contextual learning component during CS plus US convergence (Anagnostaras et al., 2001; Herry et al., 2008). However, here we focus on the details of the micro circuitry and neuronal responses underlying US-CS convergence in amygdala because it is better understood.

Classical fear conditioning depends on input from a previously neutral stimulus. Auditory CS is the most studied and understood sensory CS in mammalian classical fear conditioning. In the case of auditory signals considerable evidence demonstrates convergent acoustic input to the LA. The acoustic signal conveying the tone CS enters the LA via the auditory thalamus and the auditory association cortex (Romanski et al., 1993; LeDoux, 2003). Both of these pathways enter the LA, where they converge with US signals (Fig. 1). The two auditory routes are generally thought to provide different aspects of the CS to the LA, with the thalamic input providing a rudimentary version of the CS and the cortical input providing more detail. One interpretation of the basis for these two anatomically distinct pathways proposed by LeDoux et al. is that the direct thalamic route to the amygdala can provide a fast, and possibly life saving, sensory signal to warn the amygdala of potential danger (LeDoux, 2000). While the input via the cortex arrives some 30 ms later, it can provide more sensory detail

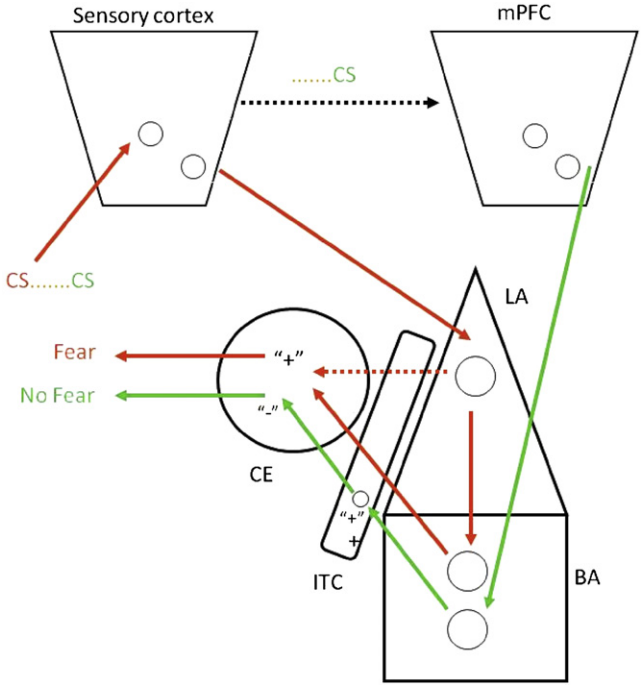

Fig. 1. Key nuclei involved in the recall of Pavlovian fear and its extinction. A sensory signal from a previously conditioned stimulus (CS) arrives in the LA via the sensory cortex (and also directly via sensory thalamus – not shown). In the LA potentiated synapses activate LA neurons propagating the signal to the CE to initiate fear behavior. The LA also sends local axons to the BA. BA neurons, like LA neurons, respond to the CS. Upon multiple presentations of the CS in the absence of the US, the CS no longer activates fear. When fear extinction occurs, infralimbic (IL) neurons in the medial prefrontal cortex (mPFC) increase their firing in response to the CS. The IL response shunts fear output in the BA nucleus. Activating a separate population of BA neurons the extinction response next activates GABAergic intercalated neurons (ITC) which inhibit the CE and thus shut off fear behavior. This model indicates fear memory remains in the LA and BA and is suppressed during extinction (adapted from Johnson and LeDoux, 2010).

(Armony et al., 1995; Li et al., 1996; Quirk et al., 1997; Armony et al., 1998; LeDoux, 2000).

Either the thalamo-LA or cortico-LA pathway is sufficient for conditioning to simple auditory stimuli. However the cortical pathway appears to be required for learning about more complex stimuli (LeDoux, 2000, 2007). The two sensory pathways both arrive in the LA, and both synapse onto LA principal neurons where they contact different types of dendritic spines (Humeau et al., 2005). While there is spatial convergence of these sensory inputs, there is also temporal divergence with the two inputs being separated in time. One possible mechanism that may allow for the two temporally segregated sensory inputs to converge in time as well as in space is a recurrent network in the LA. This network may allow for thalamo-LA signals to feedback to the superior parts of the LA during conditioning where they will meet incoming cortical signals (Johnson et al., 2008). It is not known if this recurrent feedback is directed to the same neurons which received the cortical input or to adjacent neurons. Thus, at the same time that signals originating from the thalamus to the LA are feeding back within the LA, coincident cortical signals are also reaching the LA. This allows for temporal convergence of thalamic and cortical originated signals.

The initial signal from the thalamus, which in turn feeds back in the LA, also potentially provides the initial signal output from the LA. This output signal initiates the behavioral manifestations of fear. Activation of the bodily responses is controlled via the central nucleus of the amygdala, which receives direct projections from the LA (Fig. 1). The central nucleus (CE) in turn projects to the various brainstem areas that control the different responses (LeDoux, 2000, 2007, Pare et al., 2004). The brain's fear system thus appears to require a series of sequentially connected nuclei, with the apex for sensory convergence and motor activation located in the LA.

Because the LA is an apex in processing sensory input and producing fear output it is a strong candidate for the storage of at least some associative memories linking the US and CS in conditioned fear. A likely mechanism for memory storage is synaptic plasticity in neurons. Moreover, in the study of conditioned fear, plasticity in neurons correlates with fear learning (McKernan and Shinnick-Gallagher, 1997; Rosenkranz and Grace, 2002; Doyere et al., 2007). The LA is capable of considerable plasticity during fear conditioning. For example, the firing rates of cells in the LA increase before, during, and following conditioning trials (Quirk et al., 1995; Quirk et al., 1997; Repa et al., 2001). These changes precede the expression of fear responses, suggesting that plastic changes in LA neurons drive fear behavior.

Neurons of the LA which undergo plasticity as a result of fear conditioning show heterogeneity in learning behavior. One population of LA neurons show transient increases in firing rates during learning. These rapid changes are extinguished when the CS is presented without the US. A second population of neurons requires more training trials of CS–US pairings before firing rates increase, but following learning these plastic changes endure through extinction trials (Repa et al., 2001). The transiently plastic neurons are located superiorly in the dorsal LA whereas long-term plastic cells are in a more inferior part of the dorsal LA (the LA has a dorsal nucleus (LAd), and cells were distributed dorsally and ventrally within the LAd). The finding of heterogeneity of cell learning behavior and anatomical distribution suggests a complex network within the LA, such that dorsally located cells may act as "trigger cells" for longer-term learning in ventral cells (Repa et al., 2001). These cell populations are anatomically distributed across the recurrent feedback network of the LAd (Johnson et al., 2008 and Johnson et al., 2009). The micro anatomy and network behavior of the LA may contribute to differences in learning properties amongst LA neurons. In addition to LA neurons, neurons of the basal nucleus of the amygdala (BA) which is also an output target of LA, also show

plasticity after fear learning (Herry et al., 2008). These BA neurons increase their firing rate after fear conditioning and decrease their firing rate after the extinction of fear learning.

Details of the micro network underlying conditioned and unconditioned fear need to be known for the mechanisms and etiology of normal and pathological fear, including PTSD, to be fully understood and efficaciously treated. Given current knowledge of the micro networks of fear, changes or differences would be predicted in persons with PTSD. Several influential studies have shown differences in fear learning in PTSD patients. Changes included increased amygdala activation and reduced prefrontal activation during conditioned fear and extinction acquisition (see Bremner et al., 2005; Milad et al., 2009). These data support a theory of PTSD that includes increased activation of the amygdala leading to increased fear and reduced activation of the prefrontal cortex also leading to increased fear through impaired extinction. This theory is supported by the known micro circuitry of fear described above. Further knowledge of the micro circuitry and its changes in PTSD will continue to refine this theory.

The amygdala and prefrontal cortex and their reciprocal connections are key components of the fear network underlying both fear acquisition and fear extinction. Phenotypical differences in these networks likely contribute to phenotypical differences in conditioned fear behavior. Moreover, pathology in these networks may contribute to fear pathology including PTSD. Emerging evidence indicates that differences in neuronal structure in these networks are associated with differences in fear behaviors. A key study by Holmes et al. (Wellman et al., 2007) used a 5-TT Transporter (5-HTT) knock out mouse that showed a phenotype deficit in fear extinction recall. Associated with this behavioral change were structural changes in the mPFC and amygdala. Neurons showed increased dendrite length and increased spine density in these structures respectively. The electrophysiological profile of these structural changes is not yet known; however, based on known fear circuitry it may be predicted that amygdala neurons will show increased while mPFC neurons will show reduced excitation in response to fear and extinction learning. In addition a reduced fear extinction phenotype was also produced by the same authors (Izquierdo et al., 2006) using behavioral stress. In those studies a brief uncontrollable stress led to mPFC dendrite retraction and an associated reduction in fear extinction. Taken together this emerging evidence associates changes in fear behavior, especially fear extinction, with structural and functional changes in mouse models and PTSD patients.

5. Molecular mechanisms of fear memory and its network allocation

One factor that contributes to the persistence of a fear memory is the strength of associative learning. The molecular and physiological mechanisms that mediate fear memory strength are not understood. Recently, the strength of a conditioned memory was shown to depend on particular NMDA receptor subtypes (Zhang et al., 2008). When various subunits of the NMDA receptor were pharmacologically blocked prior to one or five CS/US presentations, blockage of the freezing response was found only after NR2B blockade. This finding was supported in a mouse with transgenetic over-expression of NR2B C-terminal in the BLA (Zhang et al., 2008). In a recent experiment, 1 but not 10 CS/US pairing was susceptible to pharmacological blockade of auditory fear reconsolidation (Wang et al., 2009). Together, these data suggest that different molecular mechanisms may mediate fear learning that depend on the strength of conditioning.

Fear memory is formed and stored in the LA and depends upon the phosphorylation of the p42/44 mitogen activated protein kinase (pMAPK). Fear learning potentiates glutamatergic synaptic currents at thalamic and cortical afferents to the LA. Increased glutamatergic current may result in an increase in $Ca2+$ influx. The phosphorylation of MAPK is triggered by synaptic activated $Ca2+$ influx and leads to the induction of new protein synthesis. Fear memory storage most likely depends on receptor and structural changes on postsynaptic principle neurons as a result of new protein synthesis. Phosphorylation of MAPK is an essential component in both consolidation of new fear memory and reconsolidation of previously stored and retrieved memory (Schafe et al., 2000; Schafe et al., 2008; Kelly et al., 2003). pMAPK is a vital component in fear memory storage and its activation is therefore an indicator of the neural network underlying the storage of fear memory. The down-stream target of pMAPK is the cyclic-AMP response element binding protein (CREB). CREB mediates activity dependent gene transcription upon which physical memory storage depends. A considerable number of studies of associative learning in both invertebrate and mammalian models show the essential role of CREB in the induction of long-term memory (Han et al., 2007). CREB is a necessary component of the consolidation signaling cascade and a reliable indicator for Pavlovian fear conditioning (Josselyn et al., 2001).

Identification of these essential molecular mechanisms provides useful tools for examining neuron allocation properties of the micro network that encodes Pavlovian fear memories in the LA. An important question to resolve is the proportion, stability and allocation of the cellular network within the LA that encodes the consolidation of fear (Johnson and LeDoux, 2004; Johnson et al., 2008, 2009; Bergstrom et al., 2011). Recent studies using the molecular knowledge described above are beginning to answer these questions. First, several key studies conclude the proportion of LA neurons needed to encode a Pavlovian fear memory is between 20 and 30% of glutamatergic principal neurons (Rumpel et al., 2005; Han et al., 2007; Bergstrom et al., 2011). Second, Han et al. also found that neurons with elevated CREB are preferentially recruited into the micro network (Han et al., 2007). That study identified neuronal competition as a key principle for neuron allocation. More recently the properties of neuronal allocation in LA, for Pavlovian fear, in a naturalistic, non alerted, neuronal network were identified. Across different animals a stable pattern of pMAPK expressing neurons is allocated to encode Pavlovian fear memories (see Fig. 2 and also Bergstrom et al., 2011). Thus, these molecular and cellular tools help to identify key properties of both how and where Pavlovian fear memories are stored and allocated during memory consolidation (Johnson and LeDoux, 2004, 2010; Johnson et al., 2009; Bergstrom et al., 2011).

These molecular and network studies provide a strong cellular framework from which to understand changes in memory strength. Pavlovian fear memory to a cued auditory stimulus both correlates with and depends on cellular levels of pMAPK (Schafe et al., 2000; Bergstrom et al., 2011). These studies demonstrate that direct correlation of molecular signals in the LA can account for differences in memory strength. A key question is whether the same or similar cellular mechanisms may underlie genetic variation in Pavlovian fear memory strength and PTSD.

6. Genetics of Pavlovian fear phenotypes

Following involvement in a traumatic incident most people do not develop PTSD. The fraction of individuals who develop PTSD as a result of a trauma is estimated to be in the range of 15–30% (Ursano et al., 2009). Importantly, the predisposition to develop PTSD is heritable, meaning that it is partially influenced by the genotype of the individual (Stein et al., 2002). Individuals also differ greatly in their capacity to develop fearful associations, including

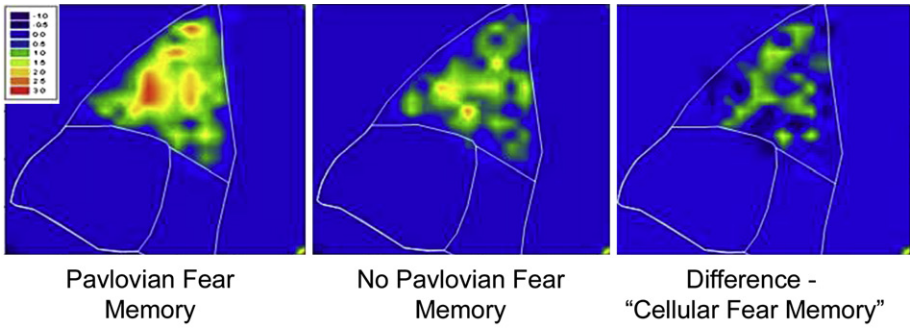

Fig. 2. Allocation of Pavlovian fear memory within micro networks of the amygdala. Micro maps of density of neurons immunoreactive for pMAPK in the dorsal sub nucleus of the rat lateral amygdala. Maps show neuron density as color change from black (low) to red (high). Neuron density is greatest in animals forming a Pavlovian fear memory compared to control animals exposed to same unpaired stimuli. These data show that fear memory is discretely allocated in micro regions of the lateral amygdala and that fear memory encoding includes neuron number. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Pavlovian fear. In humans, studies of identical and non-identical twins show that differences in the strength of Pavlovian fearful associations are somewhat heritable (Hettema et al., 2003, 2008). The degree of heritability in PTSD symptoms is consistently in the range of 30–40% in twin studies (True et al., 1993; Stein et al., 2002; Hettema et al., 2003). Similar results are observed from mass casualty studies where natural disaster produces a discrete traumatic event for a substantial population (Goenjian et al., 2008; Bailey et al., 2010). Moreover, a recent report in a population of earthquake survivors, intrusive memory symptoms (category B in the DSM-IV) had the highest degree of heritability at 75% (Bailey et al., 2010). Whether this can be replicated in other populations remains to be determined. There is also modest evidence from studies in humans that laboratory measures of Pavlovian fear are positively correlated with anxiety disorders (Lissek et al., 2005) and positively correlated with PTSD symptomology (Orr et al., 2000; Peri et al., 2000). However self-reported fearfulness shows a modest but negative correlation with fear conditioning (Hettema et al., 2008), which is perhaps counter-intuitive and cautions against using fear conditioning as an endophenotype for all self-reported fearfulness, and emphasizes the need for careful evaluation of similar claims. Thus, there is possibility of genetic overlap between laboratory tests of fear learning and anxiety disorders in humans.

Heritability of individual variability in associative fear traits has been established in mice and rats through the use of inbred strains and selected lines. Inbred strains and selected lines differ in a variety of parameters related to Pavlovian Fear conditioning (Caldarone et al., 1997; Wehner et al., 1997; Balogh and Wehner, 2003; Ponder et al., 2007b; Cohen et al., 2008; Camp et al., 2009; Brigman et al., 2009; Holmes et al., 2002), as well as fear potentiated startle (McCaughran et al., 2000; Ponder et al., 2007a; Lopez-Aumatell et al., 2008). The advantage of the analysis of inbred strains is that the differences in fear related traits between strains provide direct evidence of heritability. In addition, selection studies demonstrate that significant differences in Pavlovian fear conditioning can be obtained using selective breeding, which further establishes their genetic underpinnings (Radcliffe et al., 2000; Ponder et al., 2007a; 2008). These animal studies, together with human data strongly support a genetic contribution to individual variability in Pavlovian fear.

The search for the underlying genes takes two directions. First, the identification of strain differences in Pavlovian Fear learning opens the doors to forward genetic approaches that seek to dissect the specific genes that give rise to these strain differences. This can be accomplished by using techniques such as quantitative trait locus (QTL) mapping (Caldarone et al., 1997; Wehner et al., 1997; Balogh and Wehner, 2003; DuBois et al., 2006; Lopez-Aumatell et al., 2008), selective breeding (Radcliffe et al., 2000; Ponder et al., 2007a; 2008) and chemical mutagenesis (Reijmers et al., 2006). The second direction uses a reverse genetic approach in which specific mutations are induced and their phenotypic consequences are observed. These approaches have identified multiple classes of molecules critical for fear memory including neurotrophic factors like brain-derived neurotrophic factor (BDNF), cell structural components such as neural cell adhesion molecule (NCAM), neurotransmitter systems, and intracellular signaling molecules of the CAMKIV, PKA and MAPK signaling pathways (See Fig. 3) (Stork and Pape, 2002; Stoppel et al., 2006; Amstadter et al., 2009). In general, increased expression of genes supportive of neuronal outgrowth, dendritic complexity, synaptic strength and LTP and/or decreased expression of negative regulators of neuron excitation and growth encourage strong memory formation.

Whether or not PTSD is an extreme manifestation of a conditioned response is testable in the laboratory using classical conditioning techniques similar to those used in laboratory animals. Clinical studies in PTSD patients reliably indicate they are more "conditionable" than non-PTSD individuals, take longer to

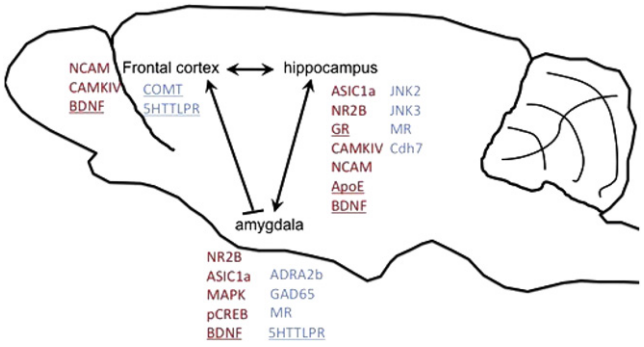

Fig. 3. Genes regulating the Pavlovian fear network. Selected genes implicated in modulating the strength and persistence of emotional memories. These lists encompass a number of different genetic models including conditional transgenic animals, viral gene targeting and human polymorphism analyses. Gene names are positioned with the brain regions within which they have been studied, or where they are proposed to have the greatest influence on fear memory. Red denotes genes in which increased gene expression enhances fear memory, blue denotes genes for which decreased expression enhances fear memory. Genes underlined have also been studied with respect to PTSD symptomology in human linkage studies. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

extinguish fear once established, and have a greater emotional valence for conditioned stimuli (Peri et al., 2000; Orr et al., 2000; Blechert et al., 2005; Norrholm et al., 2011; see above for discussion on 'fear load'). Phenotypic and genetic studies of Pavlovian fear and PTSD show a parallel association between the known circuitry of Pavlovian fear and genotype including hypo function of prefrontal cortex and enhanced amydala-centric fear conditioning. Understanding the molecular components of emotional memory acquisition and maintenance may therefore be directly applicable to understanding PTSD.

Polymorphisms in the human genome allow genes identified in animal models to be studied for memory effect in humans as well. For example, serotonin transporter (5-HTT) deficient mice have impaired extinction memory and behavioral sensitivity to stress (Wellman et al., 2007). A presumptive loss-of function short ('s') allele in the homologous human serotonin transporter 5-HTTLPR is linked to altered patterns of prefrontal-amygdala connectivity, facilitated conditioned learning and sensitivity to anxiogenic stress (Schardt et al., 2009). The prevalence of the 's' allele in PTSD patients has been examined in a number of study populations (reviewed in Cornelis et al., 2010; Amstadter et al., 2009). Although far from conclusive, these studies generally support a contribution of the short allele, in conjunction with environmental factors, to the etiology of PTSD. Another loss-of function variant in the BDNF gene has a similar learning and memory and emotional regulation phenotype to the short allele 5-HTTLPR (Frielingsdorf et al., 2010).

Carriers of $BDNF_{MET}$ are impaired in their ability to extinguish conditioned responses and show reduction in prefrontal cortical activity in functional imaging studies (Frielingsdorf et al., 2010). To date, $BDNF_{MET}$ is not shown to be overrepresented in PTSD populations (Cornelis et al., 2010). However, $BDNF_{MET}$ has implications for PTSD treatment, as desensitization strategies relying on principles of extinction of conditioned responses, may not be effective in $BDNF_{MET}$ carriers or similar phenotypes.

7. The genetics of fear circuitry

Understanding genetic contributions to fear memory and risk for PTSD in humans may be greatly aided by prospective studies of known polymorphisms in populations at high risk for trauma. A recent study showed a correlation between a single nucleotide polymorphism (SNP; rs41423247, often refered to as BclI) in the glucocorticoid receptor in a pre-surgical population and increased risk for later PTSD symptoms (Hauer et al., 2011). Additionally, as imaging techniques, particularly Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) continue to advance, it may become possible in to correlate genetic contributions directly to brain activity and later PTSD symptomology. While these types of analyses have not yet been extended to familial studies of pathological fear or PTSD, they may in the future become powerful tools for separating genetic "risk factors" for PTSD and other stress-related disorders from epigenetic consequences of trauma exposure and from PTSD symptomology.

Learning can be measured using the same principles of Pavlovian conditioning in a range or organisms from invertebrates to humans. The highly conserved nature of fear learning circuitry facilitates genetic comparisons of memory phenotypes both among individuals and across species. These individual genetic differences in memory strength and persistence may be at the root of pathological fear. Pavlovian conditioning is therefore a uniquely powerful tool for determining the basis of how normal and pathological fear memories are formed, maintained and modified over time at both a cellular and systems level within a defined neural network.

8. Conclusions

PTSD research is at a critical stage. Some evidence from animal models and humans suggest a consensus; however, considerable gaps in any overall theory of the neurobiology of PTSD remain (Ursano et al., 2009, Jovanovic and Ressler, 2010). Several important conclusions can be made about the role of Pavlovian fear and its underlying cellular mechanisms and genetics. First, different measures related to Pavlovian fear are heritable. Second, neurobiological knowledge of Pavlovian fear needs to continue to determine the mechanisms of fear memory encoding, including memory strength and memory allocation by amygdala, hippocampal and prefrontal networks. Third, the isolation of genes to date associated with both high Pavlovian fear and PTSD, suggest broad changes in neuron activity across transmitters systems and structural and signaling proteins. Pavlovian fear phenotype studies indicate some differences in gene expression in amygdala and prefrontal cortex (See above and Koenen et al., 2009); however genetic studies of fear memory strength are limited by a lack of precise details of the neural networks that determine strong fear memory phenotypes (Johnson and LeDoux, 2004, 2010; Jovanovic and Ressler, 2010; Bergstrom et al., 2011). Thus, future studies that can combine both analysis of the cellular and genetic basis of the micro networks which control the strength of fear memory will provide further important insights into normal and pathological fear memory.

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