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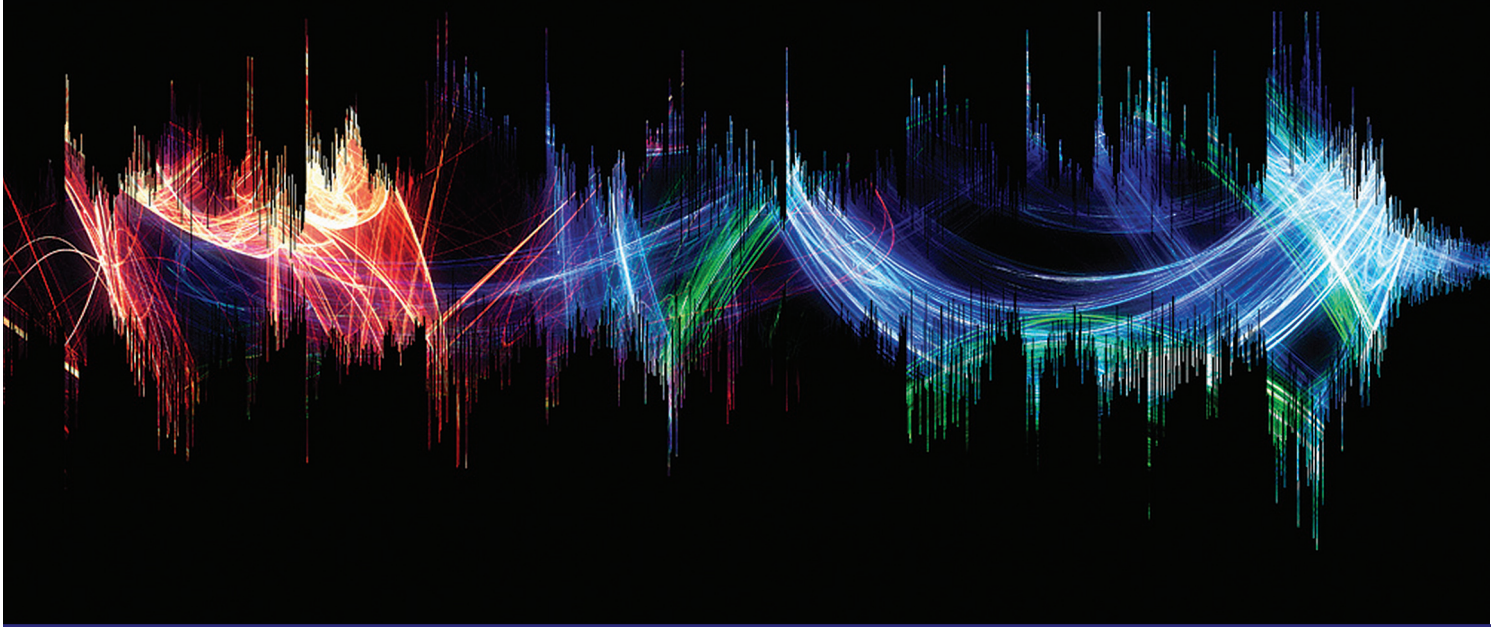

11

Brain Mechanisms for Learning and Using Safety Signals

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Introduction

Discriminating between safety and danger is elementary to the well-being of species across the animal kingdom. Distinguishing predator from prey, poison from sustenance, and friend from foe are all vital to survival. Unfortunately, exposure to trauma often precipitates mental illness, including posttraumatic stress disorder (PTSD), in which an individual's capacity to use safety signals is compromised (Jovanovic, Kazama, Bachevalier, & Davis, 2012). Thus, trauma can lead to the expression of fear or anxiety under relatively safe circumstances (Rauch et al., 2000). Changes in fear systems are hypothesized to be central to the pathophysiology of PTSD (Rauch, Shin, & Phelps, 2006); however, existing theoretical models emphasize stressor-induced changes in the neural systems underlying danger learning and recall without considering how trauma may alter the brain systems that are needed to identify, recall, and utilize safety signals.

A safety signal is a cue that can, when presented in compound or in juxtaposition to fear evoking stimuli, reduce the behavioral or physiological expression of fear. On the other hand, stimuli that are contemporaneous to a noxious event become learned danger signals and later presentations of these stimuli will elicit responses that prepare the subject for impending threat. Although there are innate safety cues that vary by species, in this chapter we concentrate on safety cues that are learned. We summarize the behavioral paradigms that have been used to investigate the neural basis of safety signals, review the existing data on the neural basis of safety signals, and suggest a hypothetical model to drive future research. The existing work suggests a mosaic, an incomplete picture, of the neural circuitry involved in learning and using safety signals.

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In the laboratory, danger and safety signals are established using Pavlovian learning techniques. To establish danger, a neutral stimulus (the conditioned stimulus or CS) is paired with a mild electric shock (the unconditioned stimulus or US). When the CS is later presented, it elicits fear, typically observed as behavioral freezing in laboratory rats. Unlike danger learning, which is evident after a single CS–US pairing, safety learning occurs more gradually. Cues presented without consequence (i.e., no aversive US) under conditions when there is a nonzero probability that an aversive stimulus is imminent may become safety signals. Safety learning may constitute two processes, the first entails the discriminative learning that allows the subject to distinguish between the danger and safe cues. Safe cues may then become conditioned fear inhibitors.

Based in part on the experimental and theoretical work of Pavlov (1927), Konorski (1948) and Rescorla (1969) outlined empirical requirements for establishing a conditioned inhibitor. Presenting the excitatory cue and inhibitor in compound, a so-called summation test, is the most direct method to test conditioned inhibition. In this sense, conditioned inhibition of fear occurs when a safety cue (often termed CS−, or B) indicating the absence of danger can reduce fear in the presence of a danger cue (often termed CS+, or A). In fact, the safety cue, B, must possess such a strong association contrasting the danger cue, A, that there is diminished fear learning when B is paired with the aversive US compared to a neutral and novel cue (Hammond, 1968). That is, once a cue is a conditioned inhibitor, pairing the inhibitor with an unconditioned excitor results in impaired excitation learning compared to a neutral cue. People (Jovanovic et al., 2005), monkeys (Winslow, Noble, & Davis, 2008), and rats (Myers & Davis, 2004) are all able to discriminate between danger and safety in laboratory settings.

Current Models

The primary condition necessary for a stimulus to become a safety signal is that it occurs together with an excitatory fear stimulus, but is then followed by the absence of shock when shock is otherwise expected (Table 11.1). Thus, safety signals lead to fear reduction because they predict that the risk of harm is minimal and can be established in a variety of approaches. The conditioned inhibition and feature-negative discrimination procedures entail presenting danger cue A alone on reinforced trials or with a second cue B in compound, or preceding, respectively, on safe trials. While these procedures result in fear inhibitors, concerns regarding external inhibition (Myers & Davis, 2004) and Rescorla's criteria for conditioned inhibition are not consistently met (Falls & Davis, 1997).

Differential conditioning procedures entail pairing the discrete cue A with an aversive US, while cue B is presented during the same conditioning session but is never paired with the US. A variation of differential inhibition paradigm is

Table 11.1 Laboratory Procedures for Safety Learning. Schematics Outline Cue Presentations, Where Red Squares Are Danger Cues (A), Green Squares Are Safety Cues (B), Blue Squares Are Cues A and B Presented in Compound, and Gray Squares Are Transfer Cues (X). Presentations of the Aversive US Are Signified by Lightning Bolts (Often Electric Shock or Air Puff in Laboratory Settings). Citations Indicate Relevant Publications that Utilize a Particular Method.

Method	Description		Citations
Conditioned inhibition	A + /AB − where A and B are presented in compound		Josselyn, Falls, Gewirtz, Pistell, and Davis (2005) and Sangha, Chadick, and Janak (2013)
Feature negative discrimination	A + /BA − where B precedes A on nonreinforced trials		Falls and Davis (1995), Falls and Davis (1997), Heldt, Coover, and Falls (2002), Waddell, Heldt, and Falls (2003), Heldt and Falls (2006) and Campeau et al. (1997)
Differential inhibition	A + /B −		Schiller, Levy, Niv, LeDoux, and Phelps (2008), Genud-Gabai, Klavir, and Paz (2013), Sangha et al. (2013), Likhtik, Stujenske, Topiwala, Harris, and Gordon (2014)
Differential inhibition with transfer cue	AX + /BX − where X is presented in compound or in serial with A and B. X is a transfer cue which carries the expectation of danger on nonreinforced trials		Myers and Davis (2004), Jovanovic et al. (2005, 2009, 2010), Winslow et al. (2008), Toufexis, Myers, Bowser, and Davis (2007), Gutman et al. (2010), Foilb and Christianson (2016), Chen, Foilb, and Christianson (2016), Foilb, Flyer-Adams, Maier, and Christianson (2016), Sarlitto, Foilb, and Christianson (in prep)
Explicitly unpaired	B is temporally distant from reinforcement		Rogan, Leon, Perez, and Kandel (2005), Pollak et al. (2008), Amano et al. (2010), Ostroff, Cain, Bedont, Monfils, and Ledoux (2010)
Backwards conditioning	B signals the end of the aversive reinforcement		Christianson et al. (2008, 2011)
			

conditional discrimination, often termed AX + /BX −. Originally used by Wagner, Logan, Haberlandt, and Price (1968) to study the associative strength acquired by X, it was later described by Myers and Davis (2004) as an ideal method to produce conditioned inhibition. Myers and Davis found that simultaneous presentations of X with each cue resulted in minimal external inhibition, which is present in the traditional conditioned inhibition procedure. Differential conditioning paradigms result in fear discrimination and conditioned inhibition in healthy human participants (Jovanovic et al., 2005). Our own lab uses a serial variation of the AX + /BX − paradigm in which

cue X immediately precedes both the danger cue A or safety cue B during conditioning (Foilb & Christianson, 2016; Foilb et al., 2016). In explicitly unpaired conditioning, discrete cue A is omitted and B is instead presented at a temporally distal position to an unsignaled US (Miller, Hallam, Hong, & Dufore, 1991). Similarly, in backwards conditioning, a CS is presented immediately after the aversive US. In this procedure, the CS comes to signal the onset of a US-free period, giving it inhibitory associative strength. In both explicitly unpaired and backwards conditioning paradigms, the conditioning context somewhat serves as the danger signal.

Once learned, assessing the discriminative stimulus or fear inhibitor is achieved in one of several types of recall tests. A fear discrimination test simply entails presenting the subject with the danger and safe cues at separate times and observing fear evoked to each; safety signals will not evoke fear. In rats, fear is often assessed as freezing, a defensive behavior observed as complete immobility (Fanselow & Bolles, 1979; Fanselow, 1980, 1984), conditioned suppression of feeding (Estes & Skinner, 1941; Hammond & Maser, 1970), or fear-potentiated startle (Brown, Kalish, & Farber, 1951). To test that a cue has gained inhibitory properties, Pavlov (1927) introduced the summation test. In summation tests, the putative conditioned fear inhibitor and a conditioned danger CS are presented in compound. If the cue is indeed a conditioned inhibitor, there will be significantly reduced fear to the AB compound cue than to A alone. Hammond (1968) introduced the "retardation-of-acquisition test" in which the putative conditioned inhibitor is paired with an aversive US, essentially reversing the stimulus outcome expectancy present during initial conditioning. If a cue has become a conditioned fear inhibitor, new danger learning will occur more slowly due to the preexisting inhibitory relationship between the safety cue and the US.

Mechanisms

Fear Circuitry

Because inhibition of a fear response, such as freezing, is central to the operational definition of a safety signal, understanding the brain mechanisms of danger learning and the expression of fear are prerequisite to understanding safety. CS–US pairings are present in the majority of safety learning protocols (Table 11.1), so fear conditioning is inherent in safety learning experiments. Here, we provide a very concise overview of the brain mechanisms of fear expression.

Danger learning occurs primarily within the amygdala where neuroplasticity binds the CS and US in association as a result of their temporal contiguity. A subsystem of the amygdala termed the basolateral amygdala (BLA), consisting of the lateral, basolateral, and basomedial nuclei, receives sensory inputs, including nociceptive information, from diverse brain areas, including the thalamus, neocortex, olfactory cortex, and hippocampus (Kim & Jung, 2006;

LeDoux, 1996; LeDoux, Cicchetti, Xagoraris, & Romanski, 1990; Mascagni, McDonald, & Coleman, 1993; McDonald, 1998; Romanski & LeDoux, 1993; Stefanacci et al., 1992; Turner & Zimmer, 1984). Some neurons in the lateral amygdala are also directly responsive to pain (Romanski, Clugnet, Bordi, & LeDoux, 1993). This makes the BLA a likely site of convergence for information about the CS and US (LeDoux, 2000). Indeed, when the CS and US are coincident, synaptic plasticity occurs in the BLA such that subsequent presentations of the CS alone, evoke stronger BLA activation (Quirk, Repa, & LeDoux, 1995; Rogan, Stäubli, & LeDoux, 1997) than an unconditioned CS. Many manipulations that prevent BLA excitability or plasticity—from inhibitory drugs, to lesions, to optogenetic silencing—all interfere with the learning and later expression of conditioned fear (Cousens & Otto, 1998; Lalumiere, 2014; Maren, Aharonov, & Fanselow, 1996). Excitation within the BLA begins a cascade of circuit activation via projections to the central amygdala (CeA) and bed nucleus of the stria terminalis (BNST), which in turn project to the hypothalamus and brainstem areas which are the proximate mediators of specific fear responses (LeDoux, Iwata, Cicchetti, & Reis, 1988; Maren, 2001; Swanson & Petrovich, 1998) including freezing, autonomic arousal, hormone release, analgesia, and startle (Davis, 1992; Kapp, Whalen, Supple, & Pascoe, 1992; LeDoux et al., 1988; Van de Kar, Piechowski, Rittenhouse, & Gray, 1991). More specifically, the projection from BLA to CeA to mediates fear responses to cues of short duration; whereas prolonged fear responses are mediated by BLA to BNST projections (Davis, Walker, Miles, & Grillon, 2010). A fundamental assumption taken when considering the mechanisms of safety signals is that they inhibit fear responses by modulation of this fear circuitry.

Safety Signals in Basolateral Amygdala

The BLA is the site of neuroplasticity for fear learning, and it is necessary for expression of conditioned fear. Therefore, safety signals might also utilize the BLA for both learning and recall, where a safety signal would be expected to reduce BLA activity. Many studies have in fact found evidence that safety signals impact responding in the BLA.

Using monkeys, Genud-Gabai et al. (2013) made single unit neuronal recordings during a differential inhibition procedure. They found that discrimination involved amygdala neurons firing in the presence of both A and B cues. Sangha et al. (2013) found similar results when looking at safety encoding in the amygdala of rats. A combination of conditioned inhibition procedure and differential inhibition methods were used during sessions of *in vivo* single unit recording, where A trials were paired with shock and the AB compound without shock, as well as some trials where B was presented alone in the absence of shock. Over a third of neurons sampled altered firing selectively to either fear or safety stimuli. About a quarter of these neurons selectively altered firing during safe signals—the AB compound or B alone—particularly during later conditioning

sessions. Ostroff et al. (2010) compared spine morphology of lateral amygdala in rats after explicitly unpaired conditioning and found decreased synapse size and weakened amygdala synapses with safety learning, while fear learning produces the opposite (Ostroff, Cain, Jindal, Dar, & Ledoux, 2012). Kazama, Heuer, Davis, and Bachevalier (2012) performed neonatal amygdala lesions in rhesus monkeys that were then trained as adults on the differential inhibition paradigm adapted for monkeys (Winslow et al., 2008). Neonatal amygdala lesions delayed the acquisition of learned fear, but did not impair discrimination between safety and danger, or summation. Taken together, correlational evidence indicates both neuronal encoding and synaptic plasticity within the BLA, yet safety learning was intact after amygdala lesions. It is likely that safety signal learning and recall require a subset of fear-inhibiting amygdala neurons but this hypothesis has not yet been tested mechanistically.

Safety Signals and Fear Expression Circuits

The principle outputs of the BLA that initiate and maintain fear responses are the CeA and the BNST. The CeA receives sensory and visceral information from the BLA, and projects to the hypothalamus and brainstem areas responsible for the fear response (LeDoux et al., 1988; Maren, 2001; Swanson & Petrovich, 1998). Falls and Davis (1995) made lesions to the CeA after extensive training using the feature-negative discrimination procedure. Since lesions of the CeA block the expression of fear-potentiated startle to A, additional A and shock training was conducted until fear returned, but no additional training to the B cue was performed. Animals with amygdala lesions were able to inhibit fear-potentiated startle to the AB compound, indicating that CeA is not critical for the expression of conditioned of fear (Falls & Davis, 1995).

Campeau et al. (1997) quantified neuronal activation following feature-negative conditioning. Presentation of the AB and B cues led to activation in the dorsal caudate nucleus of the striatum and BNST. Providing a backwards CS also leads to differential activation of the BNST, without effect on the CeA (Christianson et al., 2010). BNST efferents are very similar to those of the CeA and so this region is involved in the sustained expression of fear (for review, see Walker & Davis, 2008). Safety signals likely alter the expression of fear through a circuit involving the BNST and a better understanding of how safety signals affect the different nuclei and neuronal subgroups within this heterogenous region should be a rich area for future research.

Safety Signals and Sensory Systems

As safety signals must be encoded into the central nervous system through sensory systems, it is easy to assume that interfering with the subject's sensory capacity to hear, see, smell, or touch the safety signal would impede safety learning and recall. Falls and colleagues employed a feature-negative paradigm

with a combination of visual and auditory cues as the CSs to test the role of sensory systems. Somewhat surprisingly, neither lesions to the auditory thalamus (Heldt & Falls, 1998) or perirhinal cortex (Falls & Davis, 1997) affected AB summation tests. However, Waddell et al. (2003) later found that lesions of the superior colliculus, a brainstem center for visual processing, prevented inhibition in a summation test. What is fascinating about this result is that the inhibitory stimulus was an auditory cue and not a visual cue! A complementary study found that lesions to the inferior colliculus prevented the expression of summation to an auditory cue (Heldt & Falls, 2003) suggesting damage to fibers of passage could account for the interference caused by superior colliculus lesions. While interruption of sensory relay is a parsimonious account of these studies, it is not yet possible to rule out a role for elementary sensory structures in safety signal learning or recall (Heldt & Falls, 2003).

Although the results of the studies of Falls and colleagues suggest the thalamus may not be a *necessary* component for safety signal recall, a set of experiments by Rogan et al. (2005) suggests that thalamic inputs to the BLA are differentially altered by either danger or safety cues. Whereas danger cues potentiated, safety signals dampened auditory evoked potentials in the BLA suggesting depotentiation at auditory-BLA synapses. That corresponding changes in excitatory synapses are also observed in the BLA after either danger or safety learning (Ostroff et al., 2012) suggests that learned safety signals may not excite the BLA to the same degree as a danger signal.

Our lab hypothesized that insular cortex may play a role in conditioned inhibition because of its role in sensory integration (Benison, Rector, & Barth, 2007; Rodgers, Benison, Klein, & Barth, 2008) and bidirectional amygdala connectivity (Shi & Cassell, 1998a; Shi & Cassell, 1998b). Using a backwards CS paradigm in which the safety signal prevented the development of learned helplessness, we found that lesions and inactivation of posterior insular cortex completely eliminated the stress protective effects of the safety signal (Christianson et al., 2008, 2011). To investigate the generality of this role of insula in safety learning, we recently reported that blockade of N-methyl-D-aspartate (NMDA) receptors in the posterior insula prevented acquisition of inhibition of fear by the safety signal on later AB summation tests after serial differential inhibition conditioning (Foilb et al., 2016). Interestingly, when rats were trained drug free, later inhibition of insular cortex paradoxically reduced fear expression yet did not influence conditioned inhibition of fear in a AB summation test. Thus, the insular cortex seems to be important to learning about safety signals, but not for their recall.

Fear Modulatory Circuits: Prefrontal Cortex, Hippocampus, and Striatum

The prefrontal cortex (PFC), dorsal and ventral hippocampus, and regions of striatum are critical for executive function, episodic memory, and reward

seeking behaviors, respectively (for reviews, see Hart, Leung, & Balleine, 2014; Kesner & Churchwell, 2011). The ventral medial PFC (vmPFC) plays a critical role in the extinction of fear (for review, see Milad, Rosenbaum, & Simon, 2014), which has often thought to be closely related to the inhibition of fear by a safety signal. Further, bilateral lesions of vmPFC in dogs disrupt conditioned inhibition of appetitive conditioning (Konorski, 1967), however vmPFC inactivation appeared to have no effect after either differential inhibition (Gewirtz, Falls, & Davis, 1997) or backwards CS procedures (Christianson et al., 2008).

Sangha, Robinson, Greba, Davies, and Howland (2014) dissected the contributions of prefrontal cortex subregions—prelimibic (PL) and infralimbic (IL). Inactivation of PL led to a reduction of freezing to the danger cue A, but did not alter freezing to B or AB cues compared to vehicle animals. Consistently, Likhtik et al. (2014) observed a strong correlation between PL and BLA synchrony and behavioral discrimination during a differential inhibition task in mice. Inactivation of the IL before recall testing resulted in reduced freezing to A, which abolished discrimination between the A and AB cues. These results are especially interesting in light of compelling support for fear promoting and fear inhibiting roles of the PL (Sotres-Bayon & Quirk, 2010) and IL (Sierra-Mercado, Padilla-Coreano, & Quirk, 2011), respectively. Our lab looked at the role of ventrolateral orbital frontal cortex (vlOFC) in fear discrimination using a serial AX + /BX − differential inhibition procedure (Sarlitto, Foilb, & Christianson, in prep). vlOFC has been implicated in value-based decision making (Sul, Kim, Huh, Lee, & Jung, 2010), as well as in switching between cognitive tasks (Wilson, Takahashi, Schoenbaum, & Niv, 2014). Based on these functions, we hypothesized that vlOFC would be recruited during fear discrimination recall to facilitate changes in behavioral freezing. Temporary inactivation of the vlOFC with muscimol before a discrimination recall test impaired discrimination resulting in greater fear to the safety cue B. While future work is required, there is evidence that the vmPFC and vlOFC contribute to different aspects of recall of both danger and safety signals, perhaps as a consequence of a more general function in response selection.

Hippocampal regions have also been implicated in fear discrimination behavior. Pretraining lesions to the hippocampus did not impact discrimination performance in a feature-negative paradigm potentiated startle task but posttraining lesions impaired safety recall, such that there was no reduction of fear when B was presented with A. Interestingly, with additional training, animals could successfully be retrained to make this discrimination (Heldt et al., 2002). Our lab followed up on these results with a focus on ventral hippocampus. We used a serial differential inhibition procedure and freezing as a behavioral measure of fear to assess the role of ventral hippocampus in safe/danger discrimination learning (Chen et al., 2016). Temporary inhibition of the ventral hippocampus via muscimol injections prior to conditioning prevented danger learning, as subsequent presentations of A and B cues evoked little fear. Rats were later

retrained and the ventral hippocampus was inactivated prior to a discrimination test, but no was apparent. Although contradictory to the results of Heldt et al. (2002), these results support the existing literature implicating a role for ventral hippocampus in fear acquisition (see Anagnostaras, Gale, & Fanselow, 2001 for review), as well as to the discrimination of fear contexts (Orsini, Kim, Knapska, & Maren, 2011). While the results of this study indicate that ventral hippocampus may be part of the fear circuit, it doesn't appear to directly encode excitatory and inhibitory associations of discrete CSs.

Like Heldt et al. (2002) and Pollak et al. (2008) found evidence for a role of hippocampus in safety learning. Comparing animals that underwent an explicitly unpaired procedure to those that experienced fear conditioning, they found that animals in the safety learning condition had increased hippocampal newborn cell survival, with no changes in neurogenesis, compared to fear conditioned animals. Ablation of hippocampal neurogenesis by X-irradiation delayed safety learning. With this study, it is worth considering whether the explicitly unpaired paradigm recruited the hippocampus because safety is learned as a consequence of the temporal distance between the aversive US and the safety cue which could be hippocampally mediated whereas differential conditioning or feature-negative procedures, that appear to be hippocampal-independent, the temporal distance between cues that predict danger (transfer cues) and safety cues is small, often overlapping.

Josselyn et al. (2005) hypothesized that the nucleus accumbens (NAcc) may be necessary for the fear modulating effects of a safety signal. The NAcc plays a role in modulating the motivational responding in appetitive conditioning (for review, see Castro, Cole, & Berridge, 2015) and is situated to perform a similar task in fear and safety learning, as it receives information from many neural structures involved in conditioned fear (McDonald, 1991). With this information, they tested the role of the NAcc in inhibition of conditioned fear by using the conditioned inhibition procedure, pairing A with shock in phase one, and an A and B compound with no shock in phase 2. Using three independent manipulations of NAcc activity: lesion, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor blockade, or amphetamine injection, none altered the fear-potentiated startle response, or conditioned inhibition of startle during AB summation trials.

Also focused on striatum, Rogan et al. (2005) recorded tone-evoked synaptic responses in the caudate putamen of mice in an explicitly unpaired paradigm. In contrast to auditory evoked responses to the safety signal in the amygdala, which decrease after conditioning, in the caudate putamen, tone-responses were enhanced with safety conditioning and weakened with fear conditioning. This was interpreted as plasticity associated with reward, but the necessity of caudate putamen, or any other striatal region outside of the NAcc requires further mechanistic inquiry.

Neurotransmitter Systems

Serotonin plays a role in conditioned inhibition of appetitive learning (Lister, Pearce, Butcher, Collard, & Foster, 1996) and conditioned analgesia (Watkins et al., 1998). In each case, destruction of serotonergic neurons impaired the effect of a conditioned inhibitor. Regarding fear discrimination, Berg, Schoenbaum, and McDannald (2014) recently reported impairment in differential learning to a partially reinforced safety signal after lesions to the serotonergic dorsal raphe nucleus (DRN). This work suggests a role of the DRN, and likely serotonin, in using prediction errors to update associations between CS and US in discrimination learning. There is substantial literature implicating serotonin (5-HT) in the modulation of fear, with the general consensus that 5-HT release, and action at 5-HT2C receptors in the amygdala enhances the expression of fear (Baratta et al., 2016; Campbell & Merchant, 2003; Christianson et al., 2010; Greenwood, Strong, Brooks, & Fleshner, 2008; Martin, Ballard, & Higgins, 2002). Accordingly, 5-HT2C antagonist administration serial differential inhibition procedure improved fear discrimination and facilitated conditioned inhibition in summation tests (Foilb & Christianson, 2016). Given the vast number of neurotransmitter systems implemented in the modulation of fear and anxiety, future investigations of drugs that could reduce fear, may make for useful therapeutics to augment safety learning.

Interestingly, sex differences have also been found in safety learning, indicating a potential role for sex-related hormones in facilitating fear discrimination. Females are faster than males to acquire safe/danger discrimination, yet show a lack of retardation of fear acquisition to conditioned inhibitor (Day, Reed, & Stevenson, 2016). The mechanisms for this sex difference are unknown but may involve different actions of estrogens in males and females (Toufexis et al., 2007). Further investigation on how females learn about safety differently from males is a pressing need as it may help explain why more women than men are diagnosed with PTSD (Jovanovic & Norrholm, 2011; Lebron-Milad & Milad, 2012; McLean, Asnaani, Litz, & Hofmann, 2011).

Studies of Safety Signals in Humans

As noted, the conditional discrimination protocol allows for comparative studies of discrimination and safety learning between rodents and humans (Jovanovic et al., 2005). In civilian and combat PTSD patients, healthy controls and low-symptom PTSD participants successfully discriminated between safe and danger trials, but high-symptom PTSD subjects did not, displaying comparable levels of fear to AB and AX. When asked to indicate their expectation of the danger US (an air blast), all groups showed successful by the end of conditioning (Jovanovic et al., 2009, 2010). This means that high-symptom PTSD patients were able to learn safety signals, but were unable to inhibit their physiological fear response. Although fear extinction has received considerable attention in functional magnetic resonance imaging (fMRI) studies, safety signals are less common in human neuroimaging.

Using a fear discrimination paradigm and fMRI, Schiller et al. (2008) observed differential vmPFC activation to safety cues, which is consistent with evidence of structural differences in the vmPFC are consistently reported in PTSD populations (Corbo, Clément, Armony, Pruessner, & Brunet, 2005; Etkin & Wager, 2007; Hughes & Shin, 2011). Conversely, Schiller et al. found greater amygdala, striatum, caudate putamen, thalamus, midbrain, dorsal anterior cingulate cortex, superior frontal gyrus, and insular cortex activation during presentations the danger cue. Preliminary work by Gutman et al. (2010) also correlated insular cortex volume with fear inhibition by safety signals in females with PTSD in a differential inhibition paradigm. Individuals with a higher startle response to the safety signal (i.e., poor inhibitors) had smaller insula volumes than those that attenuated their fear response in the presence of the safety signal. Future studies must evaluate insular cortex activity using fMRI and connectivity methods to better elucidate the circuits that are required to learn about safety and recall of these cues to inhibit fear responses.

The Current Model of Safety Learning and Future Directions

The nuances found in review of the mechanistic studies regarding the contributions to different brain regions to safety learning suggest that the assessment of danger and safety and the control of fear engages a network of structures, and the pattern of activity within that network determines the behavioral output of fear or inhibition (Fig. 11.1). The amygdala is likely to be a common pathway

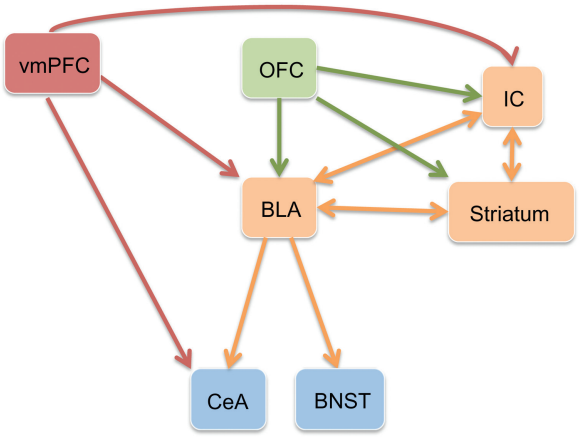

Figure 11.1 A hypothetical circuit for the processing of safety information. Danger processing regions (*red*), safety regions (*green*) regions that display altered patterns of responding due to the reception of safety information (*orange*), and regions that ultimately lead to behavioral outputs (*blue*) indicate how safety processing may occur.

by which sensory and associative information is bound and relayed to the CeA and BNST, which provide drive to the proximal mediators of fear behavior in the brainstem. The amygdala receives modulatory input from myriad cortical structures including the vmPFC (IL and PL), insular cortex, and OFC which are interconnected with each other and the striatum.

The next phase of research must seek to define the safety network by taking advantage of many new technologies along with neuroimaging, computational modeling, and network analysis. Together, this will increase our understanding of this fundamental cognitive process in health and disease. It will also facilitate translation of basic discoveries to therapeutic treatments for individuals who struggle to properly identify and utilize safety signals, such as those with high-symptom PTSD.

Highlights

- Discrimination between safety and danger is fundamental to survival, yet impaired in individuals with PTSD.
- Basic and clinical research into safety learning is still in its infancy.
- A network involving the vmPFC (fear processing), vlOFC (behavioral control), striatum, insula, BLA (integration of information), and CeA and BNST (output regions) likely encodes the meaning of safe and dangerous cues and orchestrates behaviors.
- Future work is needed to expand the safety network and determine the contributions of each node to behavior.

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