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Reward-related coherence between the basolateral amygdala and nucleus accumbens

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Reward-related coherence between the basolateral amygdala and nucleus accumbens

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Abstract

Reward-related coherence between the basolateral amygdala and nucleus accumbens
By Chia-Chun Hsu

The basolateral complex (BLC) of the amygdala plays an important role in processing emotional information. Specifically, the basolateral amygdala (BLA), a subnucleus of the BLC, integrates the majority of the sensory information and sends projections to other brain structures in the limbic system, including the nucleus accumbens (NAc). The BLA is involved in processing both negative and positive emotional information. Importantly, abnormal neural activities in the BLA were reported after acute and chronic stress, including hyper-excitability and neural oscillations at 2-4 Hz. On the other hand, the BLA→NAc projection can promote reward-seeking behavior. We hypothesized that the BLA and NAc coordinate neural activity upon the presentation of reward-associated cues. This dissertation first reports that BLA-NAc coherence in the theta band (5-8 Hz) increases in response to food-associated cues. Meanwhile, the modulatory strength of theta-gamma (50-110 Hz) phase amplitude cross-frequency coupling (PAC) in the NAc decreases, and both neuromodulations disappear upon extinction. Interestingly, access to a novel conspecific enhanced power in theta and gamma oscillations in both the BLA and NAc, but we identified no changes in theta coherence. Together, these findings suggest a role for theta-frequency synchronization between the BLA and NAc in processing select, but not all, reward-related information and suggest that theta oscillations may mediate communication between the BLA and NAc during exposure to reward-related cues. Finally, I proposed three potential future directions to extend our understanding of the functional role of BLA-NAc theta coherence in reward-related processing.

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To the memory of my grandfather, Jing-Chang Lu

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Chapter 1: Cellular Physiology of the Basolateral Complex of the Amygdala: Historical Perspectives

CONTEXT, AUTHOR'S CONTRIBUTION, AND ACKNOWLEDGEMENT OF REPRODUCTION

In the past five decades, the cellular physiology of the basolateral complex of the amygdala (BLC) has been intensively studied, particularly with regards to its implication for post-traumatic stress disorder (PTSD). The dissertation initiates by reviewing the cellular physiology of the BLC in the context of stress and fear and implications for PTSD. In the next chapter, I will discuss more recent findings regarding modulation of BLC physiology by positive emotional valance. The present chapter was conceptualized, researched, organized, and written by the dissertation author, Brendan O'Flaherty and Anzar Abbas, with editorial feedback from Dr. Donald Rainnie. This chapter is reproduced with minor edits from Rainnie DG, O'Flaherty B, Hsu C-C, Abbas A, "Cellular Physiology of the Basolateral Complex of the Amygdala and its Modulation by Stress." In: *Neurobiology of PTSD: From Brain to Mind*. Oxford University Press. 2016.

INTRODUCTION

The brain regions most frequently implicated in PTSD are the prefrontal cortex (PFC), the hippocampus, and the amygdala. The amygdala in particular is thought to underlie several important features of PTSD, including a general state of hyperarousal, impaired extinction of fear, and overgeneralization of learned fear. The purpose of this review is to update and expand on this previous work (Rainnie, 2008), with a focus on how new and more powerful scientific techniques have improved our understanding of the role of the amygdala in PTSD.

MICROCIRCUITRY AND INTRINSIC CONNECTIVITY

Microcircuitry

The amygdala is comprised of at least 13 sub-regions that can be differentiated by their cytoarchitecture, connectivity, and/or neurotransmitter content (Amaral et al., 1992; Mcdonald, 1998; Fanselow and Ledoux, 1999; LeDoux, 2000). Nevertheless, three functionally distinct clusters have been identified within the amygdala: the basolateral complex (BLC), the centromedial nuclei, and the superficial nuclei (Mcdonald, 1998).

Although dysfunction of several amygdaloid nuclei have been implicated in the etiology of PTSD, in this chapter I will focus on recent studies investigating the role of stress-induced dysfunction of the BLC in PTSD. The BLC is a "cortical-like" structure (Carlsen and Heimer, 1988) that is composed of the lateral (LA), basolateral (BLA), and accessory basal (AB) nuclei. In addition, the BLA has been further split into the magnocellular (anterior) and parvocellular (posterior) subnuclei. The BLC serves as the primary site of sensory input into the amygdala, and is critical for fear learning, extinction, consolidation, and reconsolidation (LeDoux, 2000; Merlo et al., 2014). Consequently, understanding how information is processed within this structure is critical for understanding the mechanisms contributing to the core features of PTSD, such as extinction deficits and overgeneralization of learned fear. Notably, all three nuclei of the BLC share common cell types, a canonical intrinsic neural circuitry, and are reciprocally connected, so for the purposes of this review the three subregions will be referred to collectively as the BLC (see Figure 1-1).

Sensory information enters the BLC through a variety of pathways, including glutamatergic thalamic and cortical inputs (LeDoux et al., 1990; Romanski and LeDoux, 1993) Importantly, information flow into the amygdala is topographically organized and consists of

unimodal and multimodal sensory input from all exteroceptive and interoceptive modalities (Mcdonald, 1998; Sah et al., 2003). The BLC also receives cortical input thought to represent higher order cognitive processing (Stefanacci and Amaral, 2002). Hence, the topographic organization of BLA inputs would suggest that the different subnuclei of the BLC may play different roles in fear memory formation and extinction (Pitkänen et al., 1997; Mcdonald, 1998; Sah et al., 2003; Johansen et al., 2010; Onishi and Xavier, 2010; Tye et al., 2011; Duvarci and Pare, 2014) For example, while the LA receives the densest auditory afferents, the AB receives the densest olfactory input, suggesting divergent roles in auditory and olfactory fear conditioning respectively (Jones et al., 2005). Consistent with this premise, results from fear conditioning studies suggest that the BLC allows both serial and parallel processing of sensory information to initiate unique aspects of fear behavior (Cardinal et al., 2002). Indeed, efferents of each of the BLC sub-regions innervate many non-overlapping target regions, further supporting the idea of functional specialization within the nuclei of the BLC (Pitkänen et al., 2000).

Within the amygdala, information flow occurs through a series of highly ordered connections that are conserved across species (Phelps and LeDoux, 2005). In general, information flows from more posterior regions to anterior regions within each nucleus of the BLC (Pitkänen et al., 1997). However, additional subtlety of information processing is achieved via more lateral connections. For example, the magnocellular and parvocellular subdivisions of the BLA are reciprocally connected, whereas the magnocellular and parvocellular subdivisions of the AB are not. Finally, interdivisional connections are precisely targeted and mostly non-overlapping indicating that information flow through the amygdala is split into multiple parallel and serial pathways, perhaps representing unique stimulus qualities. For a detailed treatment of intra-amygdaloid connectivity, the reader is referred to the reviews (Pitkänen et al., 1997; Duvarci and Pare, 2014). While the intrinsic circuitry of the BLC most likely reflects modality-

specific information processing, it probably also reflects the need to separate those pathways regulating aversive conditioning from those contributing to appetitive conditioning in which the BLC is also implicated (Holland and Gallagher, 2004; Murray, 2007; Stuber et al., 2011a). Topographically organized input into the BLC thus suggests that the BLC can be split into functionally distinct sub-networks. Because PTSD involves dysfunction in several aspects of fear learning, it is necessary to understand the specific structure and function of each BLC sub-network in order to treat PTSD more effectively. Notably, the functional sub-specialization suggested from preclinical studies is also observed in the human amygdala, suggesting animal studies of BLC connectivity have clinical relevance (Bzdok et al., 2013).

Neuronal Diversity in the BLC

The BLC consists of two broad classes of neurons: excitatory glutamatergic principal neurons, and inhibitory GABAergic local circuit interneurons. Excitatory principal neurons compose the primary output neurons of the BLC, and make up approximately 80-85% of the BLC's neuronal population (McDonald, 1984). Alterations in the firing patterns of these neurons most likely represents the engram of fear learning (Huff et al., 2013; Nonaka et al., 2014). Although early cytochemical studies hinted at the existence of multiple classes of BLC principal neurons (e.g. magnocellular vs parvocellular), much of the original immunohistochemical and electrophysiological studies of BLC principal neurons found evidence for only one, or at most, two subclasses (Millhouse and DeOlmos, 1983; Rainnie et al., 1993; Repa et al., 2001; Równiak et al., 2003; Sah et al., 2003). However, more recent studies together with advances in viral gene transfection and optogenetics are beginning to tell a much more complex story. For example, two distinct subpopulations of BLC principal neurons are reported to fire selectively during either fear learning or extinction, respectively (Herry et al., 2008; Barberini et al., 2012), suggesting

that specialized subnetworks of non-overlapping principal neurons may exist that encode different aspects of affective memory formation (Lalumiere, 2014). Consistent with this premise, in the Thy1-EYFP transgenic mouse line (Feng et al., 2000) only ~ 60 % of BLC principal neurons express EYFP driven by the Thy-1 promoter, suggesting that these neurons may be genetically distinct from the remaining 40% of BLC principal neurons that do not express EYFP. Significantly, optogenetic stimulation of Thy-1 EYFP expressing BLC neurons had no effect on fear memory acquisition, but enhanced extinction of fear memory (Jasnow et al., 2013), supporting the idea that a sub-network of BLC principal neurons is explicitly involved in extinction memory formation. Additionally, these BLC sub-networks may have different efferent targets, as BLC principal neurons that project to the prelimbic PFC activate during times of fear learning, while BLC principal neurons targeting the infralimbic PFC activate during extinction learning (Senn et al., 2014). Because PTSD patients have well-characterized deficits in extinction learning, it is possible that stress-induced dysfunction of this cellular population is a key component in the etiology of PTSD.

It is becoming increasingly apparent that BLC principal neurons are an anatomically, and functionally heterogeneous population, sub-networks of which most likely differentially regulate appetitive and aversive memory formation and extinction. Similar heterogeneity in the genetic, morphological, and functional diversity has been reported for CA1 principal neurons in the hippocampus (Deguchi et al., 2011; Mizuseki et al., 2011; Graves et al., 2012). Understanding the role of these different BLC neuronal subpopulations in fear memory formation and extinction is a key step towards altering the balance of BLC activity in PTSD patients. Selective enhancement of BLC principal neurons involved in extinction, for example, may help restore proper extinction learning in PTSD patients, whereas inhibition of fear memory formation neurons may help treat hyperarousal or fear generalization.

The remaining 15 -20% of BLC neurons are GABAergic interneurons, which can be roughly divided into four mutually exclusive subgroups: (1) parvalbumin immunoreactive interneurons making up ~ 40% of the total population (PV)(Sorvari et al., 1995; Smith et al., 1998; McDonald and Betette, 2001), which can be further subdivided into at least 2 subtypes based on their electrophysiological properties (Rainnie et al., 2006; Spampanato et al., 2011), (2) somatostatin interneurons making up 20% of this neuronal population (SOM) (McDonald et al., 1995; Równiak et al., 2008; Mascagni et al., 2009); (3) cholecystokinin interneurons representing 20% of BLC interneurons (CCK) (McDonald, 1985; McDonald and Mascagni, 2001), and (4) calretinin and vasoactive intestinal peptide interneurons that represent the remaining 20% (CR/VIP); (McDonald, 1994; Sorvari et al., 1996; Kemppainen and Pitkänen, 2000).

Interneurons control the output of principal neurons at all levels in the BLC, and form both feedforward and feedback inhibitory circuits. Interneurons control global BLC activity in at least to two ways: (1) by controlling the net excitatory tone of the BLC; and (2) by playing a key role in precisely organizing the firing pattern of BLC principal neurons. Classically, GABAergic interneurons were seen as modulators of overall BLC excitation. For example, early studies showed that infusion of the $GABA_A$ receptor agonist, muscimol, into the amygdala prevented fear memory acquisition by inhibiting BLC principal neurons (Helmstetter and Bellgowan, 1994; Wilensky et al., 1999). However, the view that all interneurons inhibit net BLC activity is overly simplistic. Different subtypes of interneurons have different functional roles within the BLC: for example, while most PV interneurons inhibit BLC principal neurons, CR/VIP interneurons (as well as a small population of PV interneurons) selectively inhibit other interneurons (Wolff et al., 2014). When activated, CR/VIP interneurons would thus disinhibit BLC principal neurons, increasing their activity and promoting fear learning. Though the organization of interneurons in the BLC is complex, it is clear that dysregulation of inhibitory interneurons can alter the net

excitatory tone of BLC principal neurons. This is important as increased baseline activity in BLC principal neurons could lead to hyperarousal in PTSD patients. Indeed, dysregulation of BLC inhibition has been shown to lead to increased anxiety-like behavior in several pathological conditions (Almeida-Suhett et al., 2014; Prager et al., 2014).

Recently GABAergic interneurons have also been shown to play a number of critical organizational roles in the BLC, such as gating LTP at thalamo- and cortico-BLC synapses, and determining the time window during which inputs are able to generate action potentials in principal neurons (Ehrlich et al., 2009). Notably, individual PV interneurons innervate multiple principal neurons (Muller et al., 2005; Rainnie et al., 2006), and physiologically distinct PV interneurons are electrically coupled by gap junctions to form discrete functional syncytia (Muller et al., 2005). PV interneurons are thus well-positioned to coordinate the firing of large numbers of BLC principal neurons through rhythmic inhibition. Moreover, PV interneurons have also been shown to control spike timing precision of BLC principal neurons, thus contributing to the formation of synchronized oscillations of the BLC, which are thought to play an important role in fear learning (Ryan et al., 2012). Interestingly, abnormally strong synchronized neural activity in the amygdala has been found in humans with PTSD; BLC oscillations may play a role in the etiology of PTSD, possibly due to changes in inhibitory circuitry (Zhong et al., 2014). The implications of BLC oscillations on the wider fear network will be discussed below.

In the hippocampus, a subpopulation of PV interneurons preferentially innervate amygdala-projecting CA1 principal neurons and receive preferential input from CA1 principal neurons that project to the mPFC (Lee and Byeon, 2014), suggesting that functional sub-networks of principal neurons and PV interneurons may segregate different streams of information processing. Consequently, stress-induced dysregulation of BLC inhibition would be predicted to have a deleterious effect on BLC function.

Although the nuclei of the BLC receive topographically organized sensory input as described above, principal neurons and interneurons are organized in a more-or-less common intrinsic circuitry throughout the BLC. For example, principal cells project predominantly to other principal cells, but also heavily to interneurons. Most interneurons in turn are predominantly driven by input from BLC principal cells, and form feedback and feedforward connections with both principal neurons and other inhibitory neurons. Despite the fact that BLC principal neurons project heavily to other principal neurons, paradoxically BLC principal neurons have limited excitatory drive (Smith and Paré, 1994). This finding may be due to the fact that BLC principal neurons project preferentially to proximal interneurons and distal principal neurons, activating feed-forward inhibitory mechanisms that prevent local runaway excitation while allowing associative interactions (Pape and Pare, 2010). Importantly, the common circuitry found throughout the BLC is in no way at odds with the idea of specialized BLC sub-networks, as identical neural circuits can be adapted for different purposes by altering the incoming information.

Physiology of Pavlovian Fear Conditioning

The BLC has traditionally been thought of as the region where associative fear learning, extinction learning, consolidation, and expression take place (Davis, 1992; Davis et al., 1994; Fanselow and Ledoux, 1999). Growing evidence suggests that PTSD is associated with generalization of fear learning, as well as deficits in fear extinction. Hence, understanding the mechanisms by which fear learning and extinction occur may offer novel avenues for therapeutic intervention in PTSD. The most commonly used approach to examining this hypothesis has been to study Pavlovian fear conditioning in rodents (Hitchcock and Davis, 1987; Rogan et al., 1997; Guarraci et al., 1999; Rothbaum and Davis, 2003). Here, a noxious unconditioned stimulus (US),

usually a shock, is repeatedly paired with a normally innocuous conditioned stimulus (CS), e.g. a tone or a light, until the CS takes on the affective salience of the US. The most widely accepted cellular substrates for this associative learning are long-term potentiation (LTP) and long-term depression (LTD) (Eichenbaum, 1996; Matynia et al., 2002). Specifically, the "cellular hypothesis" of fear learning postulates that fear learning occurs through associative LTP at BLC principal neurons (Blair et al., 2001; Goosens and Maren, 2002).

The "cellular hypothesis" specifically postulates that when subcortical afferent synapses carrying information about the CS fire concurrently with thalamic afferent synapses carrying information about the US, the subcortical CS synapses are strengthened such that the CS can now recruit downstream target structures previously only recruited by the US (Sigurdsson et al., 2007). Although both thalamic and cortical synapses are capable of undergoing LTP (Ehrlich et al., 2009), recent studies have shown that the mechanism of induction of LTP at thalamic synapses are primarily NMDA-dependent and postsynaptic, whereas cortical synapses undergo an NMDA-independent presynaptic LTP (Fonseca, 2013). Because associative LTP is the mechanism by which the BLC pairs a US with a CS, dysregulation of associative LTP in the BLC may mediate the overgeneralization of fear learning seen in PTSD. For example, if associative LTP occurred at improper times, an inappropriate CS (for example, a car backfiring) may become paired with a US (for example, a memory of combat) and provoke an inappropriate fear reaction. Hence, a better understanding of how associative LTP occurs in amygdala circuits may facilitate more targeted pharmacotherapy for stimulus generalization. For a more detailed treatment of the role of amygdala LTP in the physiology of fear learning, I direct the reader to the following review (Pape and Pare, 2010).

While LTP is often thought to underlie aspects of fear learning, LTD and/or depotentiation has been considered as a substrate for fear extinction (Dalton et al., 2008, 2012).

Indeed, recent evidence suggests that induction of LTP at thalamic synapses to LA neurons, representing memory consolidation, can be deconsolidated or reversed, potentially weakening the fear memory trace (Kim et al., 2010; Hong et al., 2011). Importantly, extinction does not appear to be an erasure of an existing fear memory. Instead, extinction is thought to involve learned inhibition of fear pathways. However, it is likely that both LTP and LTD play a key role in fear memory formation and extinction (Nabavi et al., 2014). Notably, manipulations that selectively inhibit LTD are reported to reduce behavioral flexibility (Kim et al., 2011a; Mills et al., 2014). As extinction learning is a form of behavioral flexibility, the apparent deficit in extinction learning in PTSD may be expected to correlate with reduced LTD in the BLC. Consistent with this premise, fear extinction has been shown to reduce the efficacy of afferent input (LTD) from the medial prefrontal cortex (mPFC) to the BLC (Cho et al., 2013).

Significantly, the response of BLC principal neurons to synaptic input has been shown to be frequency-dependent. As noted above, high frequency (> 50 Hz) stimulation of afferent pathways routinely elicits a dopamine D1 receptor- and N-Methyl-D-aspartic acid (NMDA) receptor-dependent form of LTP (Li et al., 2011); however, at stimulation frequencies of ~ 10 Hz LTP is occluded by an Metabotropic glutamate receptor (mGluR) $_{2/3}$-dependent form of long-term depression (LTD) (Li and Rainnie, 2014). At stimulation frequencies < 10 Hz, LTD transitions into an NMDA receptor-dependent form of LTD in BLC principal neurons. Significantly, repeated stress causes a leftward shift in the LTD-LTP frequency-response stimulation curve in BLC neurons (see Figure 1-2; Li et al., unpublished observations), consistent with a loss of $mGluR_{2/3}$-dependent LTD. Such a leftward shift in the stimulus response curve would facilitate LTP induction in relatively weak inputs to the BLC and may contribute to stimulus generalization that is a key feature of PTSD. Consistent with this premise, an inbred mouse strain that show extinction deficits also exhibit fear generalization to ambiguous contexts

and cues (Camp et al., 2012). Of note, chronic corticosterone exposure, as would be predicted to happen during prolonged or traumatic stress, reduces extinction learning and $mGluR_{2/3}$ receptor expression (Gourley et al., 2009). Hence, prolonged stress exposure may effectively remove a key component of bidirectional synaptic plasticity in the BLC.

Less is known about the cellular mechanisms of fear memory consolidation, the process through which fear memories are converted from short-term to long-term memory. Like consolidation processes observed in the hippocampus, fear memory consolidation requires *de novo* mRNA and protein synthesis in the amygdala (Duvarci et al., 2008). There is little evidence to suggest that the consolidation process is dysregulated in PTSD. However, a similar process called reconsolidation, which occurs in parallel with extinction learning with the presentation of a CS, may play a role in PTSD extinction deficits. Reconsolidation and extinction appear to be mutually exclusive cellular processes in the BLC, therefore abnormal reconsolidation of a fear memory could interfere with extinction learning (Merlo et al., 2014). Pharmacological inhibition of reconsolidation could theoretically improve extinction learning, however attempts in humans have so far been unsuccessful (Wood et al., 2015).

Finally, LTP and LTD are subject to neuromodulatory influences that can influence the amplitude and induction threshold of both LTP and LTD. It appears that virtually all forms of LTP and LTD are subject to neuromodulatory influences (Krasne et al., 2011). For example, dopamine plays an important role in gating LTP, as application of dopamine in the BLA suppresses feedforward inhibition, disinhibiting BLA principal neurons and allowing LTP to occur (Bissière et al., 2003). Dopamine has also been shown to gate LTD in the striatum (Calabresi et al., 1992). Similarly, norepinephrine also gates LTP by reducing inhibition in the BLC (Tully et al., 2007). Furthermore, these neuromodulatory systems are vital for fear conditioning, as depletion of norepinephrine and dopamine in the BLC impairs the ability to

acquire learned fear (Selden et al., 1991). Because of the profound effect of the neuromodulatory systems on LTP within the BLC, dysregulation of these systems could dysregulate plasticity in the BLC, potentially leading to deficits in extinction. For a more detailed treatment of neuromodulation in the BLC, see below.

The BLC and Pathological Stress

Although fear learning is an adaptive process, prolonged or traumatic stress can cause maladaptive changes in the BLC network, leading to the development of PTSD. Hence, hyperactivation of the amygdala has been reported during symptom provocation in several imaging studies of PTSD (Linnman et al., 2011; Sripada et al., 2013; Stevens et al., 2014), and amygdala hyperactivation is thought to contribute to the symptoms of hyper-arousal and stimulus generalization that are characteristics of the disorder (Huang et al., 2014). Similar observations have been observed in studies in rodents where BLC principal neurons are reported to show increased firing rates in response to depolarizing current injection following chronic stress (Rosenkranz et al., 2010). Three general mechanisms could account for BLC hyper-excitability: (1) increased net excitatory drive within the BLC, (2) a reduction in net inhibitory drive, or (3) an increase in the intrinsic excitability of BLC principal neurons. A growing body of evidence suggests that all three mechanisms may contribute to BLC hyper-excitability, indicating a series of complex interactions in response to pathological stress.

Firstly, studies have shown that pathological stress results in an increase in excitatory drive to BLC neurons. Fear conditioning is reported to increase the magnitude of excitatory synaptic potentials at cortico-amygdala synapses (Sui et al., 2014), as well as lower the stimulation threshold required for LTP induction in BLC principal cells (Li et al., unpublished

observations). It is possible that in PTSD, a traumatic stressor results in an inappropriate magnitude of LTP in the BLC, or an abnormally low threshold for LTP induction. Indeed, the amygdala has been shown to be particularly susceptible to repeated electrical stimulation (kindling), which by inducing inappropriate LTP in the amygdala, can induce seizure activity in rodents faster than stimulation of any other brain region (Racine et al., 1972; Schubert et al., 2005). Notably, kindled rats show symptoms similar to PTSD, including extinction deficits and enhanced reinstatement of previously extinguished fear (Kellett and Kokkinidis, 2004).

Because the amygdala is so susceptible to pathological LTP, and because such LTP results in PTSD-like symptoms in rodents, it is likely that synaptic plasticity becomes dysregulated in PTSD. Numerous studies have described an increase in the frequency (Hubert et al., 2013; Padival et al., 2013b; Hetzel and Rosenkranz, 2014) or amplitude (Masneuf et al., 2014; Sui et al., 2014) of excitatory synaptic events recorded in BLC principal neurons following stress manipulations. However, studies that found amplitude changes generally did not find changes in the frequency of excitatory events, and vice versa. It is thus possible that excitatory drive is affected differently in different principal neuron populations, or affected differently by different stressors. Repeated stress has been shown to increase the dendritic spine:dendrite ratio of AMPA receptors of BLC principal neurons of adult rats (Hubert et al., 2013), and increase the number of NMDA receptors in dendritic spines of adolescent rats, also indicating that synaptic plasticity becomes dysregulated in PTSD, leading to an increased excitatory synaptic drive (Gan et al., 2014).

Another potential mechanism for increasing excitatory drive onto BLC principal neurons is through morphological plasticity. Numerous studies have reported an increase in dendritic arborization in BLC principal neurons in response to stress (Vyas et al., 2002, 2006; Mitra et al., 2005; Hill et al., 2011, 2012; Adamec et al., 2012; Boyle, 2013; Padival et al., 2013b). Increased

dendritic arborization would increase the receptive field of BLC neurons and allow them to form new synapses with axon terminals, thereby increasing excitatory drive. Notably, such morphological changes are associated with extinction deficits (Maroun et al., 2013a), and appear to be irreversible (Vyas et al., 2004). However, stress-induced remodeling of the dendritic arborization of BLC neurons remains controversial. Some studies report no change in dendritic arborization (Hubert et al., 2014), dendritic retraction (Maroun et al., 2013b; Grillo et al., 2015), and/or principal neuron atrophy (Grillo et al., 2015) in response to chronic stress. A possible answer to this conundrum may lie in the potential heterogeneity of BLC principal neurons. Herry and coworkers reported distinct populations of BA neurons encode fear conditioning and extinction (Herry et al., 2008). It is possible that, principal neurons within the BA may also differentially respond to chronic stress.

In addition to dendritic outgrowth, several studies have reported an increase in the number of dendritic spines in response to chronic stress (Vyas et al., 2003, 2006; Mitra et al., 2005; Qin et al., 2011; Heinrichs et al., 2013; Maroun et al., 2013b; Padival et al., 2013a, 2013b) or a single prolonged stressor (Cui et al., 2008) or prolonged corticosteroid exposure (Gourley et al., 2013). As dendritic spines are the main source of excitatory synaptic input onto BLC principal neurons, an increased number of spines could potentially increase frequency of excitatory synaptic events observed following chronic stress (Radley et al., 2007; Hubert et al., 2013; Padival et al., 2013b). However, many of the increases in spine density are reported on proximal dendrites that have a much higher variability in spine density than distal dendrites, and not in distal dendrites. Moreover, to date there is no clear evidence that these spines are fully functional. Hence, it remains unclear if increases in spine numbers are in any way responsible for increased principal neuron excitatory drive. Nevertheless, it remains a potential mechanism through which BLC excitatory drive is increased after pathological stress, and may play a role in

the development of PTSD.

In addition to influencing the strength and/or number of synaptic inputs onto BLC principal neurons, stress can also alter intrinsic properties of principal neurons. For instance, repeated stress has been shown to influence the excitability of LA and BA neurons in adolescent rats through modulation of post-spike afterhyperpolarizing potentials (AHPs), although the effect was only seen in LA neurons of adult rats (Hetzel and Rosenkranz, 2014). Notably, the mechanism of this excitability change depends on age: in adolescent rats, the change was mediated by a decrease in the medium AHP (mAHP), while in adult rats, the change in BA neuron excitability was mediated by a decrease in the slow AHP (sAHP). Interestingly, adolescents that were resistant to the development of PTSD-like symptoms showed a stronger sAHP in BA principal neurons and stronger mAHP in LA principal neurons, reducing neuronal excitability and possibly conferring a stress-resistance phenotype. It may thus be possible to modulate the intrinsic excitability of BLC neurons through the calcium-activated potassium channels responsible for the AHP in rodents (Faber and Sah, 2002). In addition, chronic stress may also regulate neuronal excitability by regulating key intracellular signaling cascades in BLC neurons. As noted above, chronic stress can reduce the threshold for LTP induction in BLC principal neurons (Figure 1-2, Li et al., unpublished observations).

LTP induction in BLC neurons is dependent on activation of the dopamine D1 receptor - adenylate cyclase (AC) – cyclic adenosine monophosphate (cAMP) signaling cascade (Li et al., 2011). In neurons, termination of this signaling cascade is brought about by the activity of the Type 4 family of phosphodiesterase enzymes (PDE4s) (Duman et al., 1999; Pérez-Torres et al., 2000; Li et al., 2009). We have generated evidence that intracellular blockade of PDE4s with the selective inhibitor, rolipram 100 nM, mimics the effect of chronic stress on lowering the threshold for LTP induction in BLC neurons in a D1 receptor-dependent manner (Figure 1-3, Li

et al, unpublished observations). Together, these data suggest that lowering the activity of PDE4s in BLC neurons may contribute to the hyper-activation of the BLC observed in PTSD and potentially also to stimulus generalization.

Reduction in BLC GABAergic inhibition could also underlie the hyperexcitability of the BLC seen in PTSD. Increased GABAergic activity has been negatively correlated with an anxiety-like phenotype (Roozendaal et al., 2009). Most BLC principal neurons express a robust and tonic GABA current, affecting their excitability (Marowsky et al., 2012), and it is possible to induce an anxiety-like phenotype by decreasing GABAergic tone (Shekhar et al., 1999; Rainnie et al., 2004). Moreover, rats exposed to the nerve agent, soman, display fear-like behavior likely due to interneuron death in the BLC (Prager et al., 2014) . Decreased GABAergic tone in the BLC has also been observed in a rodent model of traumatic brain injury, leading to an anxiety-like phenotype (Almeida-Suhett et al., 2014). Significantly, repeated administration of CRF, an anxiogenic neuropeptide, can also reduce phasic inhibition in the BLC, leading to an anxiety-like phenotype (Rainnie et al., 2004), and many studies have reported that chronic stress decreases tonic GABA inhibition in the BLC, thus disinhibiting BLC principal neurons and contributing to the anxiety-like phenotype (Rodríguez Manzanares et al., 2005; Reznikov et al., 2009; Liu et al., 2014). GABAergic interneurons are also subject to morphological changes as a result of chronic stress. For example, in mice subjected to repeat restraint stress, BLC interneurons showed reduced dendritic arborization, dendritic spine density, and synaptic input, all of which would function to reduce the excitatory drive onto inhibitory interneurons (Gilabert-Juan et al., 2011). This loss of interneuron excitatory drive could disorganize the activity of the BLC, as well as lead to amygdala hyperexcitability, in turn leading to increased fear learning and decreased extinction learning, as seen in PTSD. GABAergic inhibition is also involved in suppressing LTP in BLC principal neurons (Ehrlich et al., 2009), reduction of which could lead to increased

excitability.

EXTRINIC CONNECTIVITY OF BLC

Besides serving as the main processing center for sensory input in the amygdala, the BLC also projects to multiple brain regions that are implicated in the etiology of PTSD, including the hippocampus, medial prefrontal cortex, nucleus accumbens, and bed nucleus of stria terminalis (Pikkarainen et al., 1999; Aggleton, 2000). As a growing body of evidence suggests that aberrant BLC activity and plasticity is involved in the etiology of PTSD, the question arises: how do changes in BLC activity affect downstream target structures? In this section, I will review recent findings on BLC regulation of downstream targets.

Hippocampus

The BLC projects to the entorhinal cortex (EC), CA1, and CA3 regions of the hippocampal formation and has been shown to play a critical role in hippocampal plasticity and emotional memory (Akirav and Richter-Levin, 2002; Nakao et al., 2004; Kim et al., 2005). For example, lesions or pharmacological inactivation of BLC can impair hippocampal LTP and emotional memory consolidation (Kim et al., 2001; Vouimba et al., 2004; Farmer and Thompson, 2012). Moreover, intra-BLC infusion of a β-adrenergic receptor agonist enhanced arousal, emotional memory, and the expression of *Arc*, a plasticity-related molecular marker, in the dorsal hippocampus (Barsegyan et al., 2014). Consistent with this observation, Bass and colleagues demonstrated that brief bursts of electrical stimulation at 50 Hz applied to the BLC enhanced hippocampal-dependent episodic memory (Bass et al., 2012). Furthermore, inactivation of the BLC suppresses proliferation and activation of newborn neurons that are

associated with fear memory formation in the dentate gyrus (DG), suggesting that the BLC directly modulates hippocampal neurogenesis (Kirby et al., 2011). Together, these data indicate that the function and physiology of the hippocampus can be directly regulated by the BLC, suggesting the dysfunctional BLC may lead to short-term memory loss (Bremner et al., 1993), fear memory extinction deficits (Rothbaum and Davis, 2003; Quirk et al., 2006; Parsons and Ressler, 2013), as well as the aberrant changes in the hippocampus in PTSD patients (Shin et al., 2006).

Notably, not all stimulation paradigms in BLC facilitate hippocampal LTP. Other studies have shown that different BLC stimulation parameters can either impair, facilitate, or have no effect on DG or CA1 LTP (Akirav and Richter-Levin, 1999, 2006; Frey et al., 2001; Tsoory et al., 2007), suggesting that the timing, intensity, and frequency of BLC stimulation are critical factors in regulating DG and CA1 LTP. Hence, the memory enhancing effect of electrical stimulation shown by Bass and colleagues (discussed above) could be a region-specific response to BLC stimulation. Moreover, Vouimba and Richter-Levin recently reported that a non-linear relationship exists between the frequencies used for BLC stimulation and its effect on later LTP induction in the CA1 (Vouimba and Richter-Levin, 2013). Hence, even within a single hippocampal region, the response to BLC stimulation appears to be frequency dependent. The differential effects of BLC stimulation parameters on hippocampal plasticity could be considered as reflective of altered states of BLC activation and/or distinct signals that encode different aspects of emotional information, as different stressors have been shown to enhance, decrease, or have no effect on BLC activity (Shors, 1999; Vouimba et al., 2004). Consistent with this premise, different stress manipulations have different effects on LTP induction in CA1 and DG (Diamond and Rose, 1994; Izaki and Arita, 1996; Shors, 1999; Wang et al., 2000; Sacchetti et al., 2002; Vouimba et al., 2004). Furthermore, the effects of BLC stimulation is not static, but

dynamic, such that an initial period of BLC stimulation can prevent a subsequent stressor, or a second period of BLC activation, from suppressing LTP in the ventral hippocampus to medial prefrontal cortex (mPFC) pathway (Richter-Levin and Maroun, 2010). Together these data suggest that there might be specific stimulation frequencies and windows of time in which the BLC can modulate emotional memory formation and extinction, which offers important insights into the potential mechanisms of an ongoing clinical trial using deep-brain stimulation of the BLC to treat treatment-refractory PTSD (Koek et al., 2014).

One caveat to the above experiments is that electrical stimulation is non-specific and cannot discriminate between direct local activation/inactivation of different cell types or fibers of passage. However, with the recent development of cell type-specific optogenetics, I are now able to functionally dissect specific neural circuits throughout the CNS (Yizhar et al., 2011). Recently, Felix-Ortiz and colleagues demonstrated that optogentic inhibition of inputs from BLC projection neurons to the ventral hippocampus (vHPC) decreased anxiety-like behavior in the elevated plus maze (EPM) and open field test (OFT), whereas optogenetic activation of BLC inputs to vHPC increased anxiety-like behavior, as well as decreased feeding during a novelty-suppressed feeding (NSF) test (Felix-Ortiz et al., 2013). In a similar manner, specific optogenetic silencing of the BLC-EC pathway during fear acquisition impairs fear expression (Sparta et al., 2014). Together, these findings further suggest that hyper-activation of the BLC in PTSD could have profound effects on hippocampal circuits involved in fear and anxiety, leading to hyperarousal and fear extinction deficits characteristic of PTSD.

Medial prefrontal cortex

The medial prefrontal cortex (mPFC) is reciprocally connected with the BLC. Hence, BLC projection neurons innervate layer 2 and 5 projection neurons of the mPFC which in turn project to cortical, autonomic, and subcortical regions including the BLC (Bacon et al., 1996; Gabbott et al., 2012; Little and Carter, 2013). Intriguingly, the BLC input also selectively innervates a sub-population of parvalbumin-containing interneurons in the mPFC (Gabbott et al., 2006). Hence, depending on the pattern of activation, BLC output neurons can recruit either feed-forward activation and/or inhibition in the mPFC (Bacon et al., 1996; Cho et al., 2013; Dilgen et al., 2013). Significantly, compared with cortical-projecting neurons, BLC inputs preferentially activate mPFC principal neurons that project back to the BLC (Little and Carter, 2013), suggesting that reciprocal cross-talk between the BLC and the mPFC is an essential component in regulating affective state. However, the innervation of the mPFC to the BLC is not uniform, while the basal nucleus receives a dense innervation from the mPFC, the innervation to the lateral nucleus is much lighter (Aggleton, 2000; Hübner et al., 2014). Like the BLC projections to the mPFC, layer 2 and 5 mPFC projection neurons innervate both principal neurons and PV interneurons in the BLC (Gabbott et al., 2005; Little and Carter, 2013), suggesting that mPFC inputs to the BLC can regulate BLC activity in the same way that BLC activity can regulate activity in the mPFC (Hübner et al., 2014).

Recent studies have shown that the reciprocal connectivity between the BLC and mPFC is important for extinction of fear memory, a deficit of which is a hallmark of PTSD (Quirk et al., 2000; Milad and Quirk, 2002; Milad et al., 2004). Notably, Cho and colleagues have shown that fear extinction resulted in reduced excitatory drive from mPFC projection neurons onto BLC principal neurons, without affecting the strength of the inhibitory drive. Thus, following extinction learning, the balance of excitatory/inhibitory drive in the BLC will shift in favor of

inhibition (Cho et al., 2013). However, the mPFC is not a homologous unit and is comprised of the prelimbic cortex (PL) and infralimbic cortex (IL), among other regions. Although the PL-BLC and IL-BLC pathways have similar projection patterns, as well as EPSC/IPSC ratios (Cho et al., 2013), they are thought to play different roles in fear memory formation and extinction. Hence, electrical stimulation of the IL facilitates fear extinction, whereas stimulation of the PL enhances fear memory formation (Vidal-Gonzalez et al., 2006; Sierra-Mercado et al., 2011). Consistent with this observation, reduction of brain-derived neurotrophic factor (BDNF) in the PL impairs fear expression but not extinction (Choi et al., 2012a). In contrast, infusion of BDNF into the IL prior to fear conditioning has no effect on either fear acquisition or expression, but post-conditioning BDNF infusion decreases fear expression, even in absence of extinction training (Peters et al., 2010).

Importantly, Senn and colleagues have shown that PL- or IL-projecting BLC neurons, are intermingled within the BLC, and also have distinct roles in fear expression and extinction (Senn et al., 2014). Thus, fear conditioning caused c-fos expression primarily in PL-projecting BLC neurons, whereas fear extinction resulted in c-fos expression mainly in IL-projecting neurons. Corresponding with changes in c-fos expression, around one-third of recorded IL-projecting BLC neurons selectively fired to an extinguished CS, whereas one-fourth of PL-projecting neurons exclusively fired to a shock-linked CS. Moreover, during fear extinction training the proportion of active BLC-PL projecting neurons gradually decreased, whereas the proportion of active BLC-IL neurons gradually increased. Finally, optogenetic inactivation of BLC-IL projection neurons during extinction training resulted in decreased extinction memory. These data are consistent with the observations of Jasnow and colleagues who showed that optogenetic activation of a subpopulation of BLC neurons enhanced extinction learning (Jasnow et al., 2013).

Together, the studies described above have revealed the complexity of the circuits between the IL/PL and BLC, and suggest that parallel pathways into and out of the BLC dictate the behavioral response to affective stimuli. Although the circuits are composed of neurons with seemingly similar electrophysiological phenotypes, each circuit has its own distinctive role in fear expression and extinction, depending on their site of origin and projection target. Understanding this circuitry will allow future studies to focus on the circuit- and cellular mechanisms of the transition between fear memory formation and extinction. For instance: what governs the shift in activation from PL-projecting BLC neurons to IL-projecting neurons during fear extinction? These studies would provide us with a more comprehensive understanding about local and long-range communication between BLC circuits and the mPFC and how dysfunction of this circuitry may contribute to the etiology of PTSD.

Topographical aBLC/pBLC projection

As PL and IL neurons of the mPFC play different roles in fear learning and extinction respectively, a growing body of evidence suggests that selective activation in the rostro-caudal axis of the BLC may differentially influence affective behavior. For instance, the anterior BLC (aBLC) sends strong projections to the anterodorsal BNST, but only minimal projections to the oval nucleus of the BNST, and activation of which appears to be anxiolytic (Kim et al., 2013). Conversely, we have data showing that the posterior BLC (pBLC) sends strong projections to the oval nucleus of the BNST (see Figure 1-4), and activation of this pathway is thought to be anxiogenic. Similarly, the aBLC is reported to preferentially innervate the core of the nucleus accumbens (NAc), whereas the pBLC preferentially targets the shell of the NAc (Shinonaga et al., 1994). Notably, the core and shell of the NAc respond differentially to both appetitive and aversive cues (Bassareo and Di Chiara, 1999; Di Chiara, 2002), further supporting the premise

that the aBLA and pBLC may modulate opposing aspects of affective behavior. As substance abuse is often comorbid with PTSD, the balance between two BLC-NAc projections might be dysregulated in PTSD patients.

The aBLC also receives dense inputs from ventral hippocampus and agranular insula, and has strong reciprocal connectivity with the mPFC (Shinonaga et al., 1994; Wright et al., 1996), unlike the pBLC. Hence, the anterior and posterior BLC appear to engage different cortical and sub-cortical circuits to modulate behavior (see Figure 1-5). Consistent with this observation, Thy1-expressing neurons, a subgroup of projection neurons that facilitate fear extinction are predominantly found in the aBLC (Jasnow et al., 2013). Future studies may now focus on how the different topographical projections from the BLC may be dysregulated in animal models of PTSD.

Functional relevance of topographically organized/output of the BLC

As noted above, while the target for many of the reciprocal BLC and mPFC efferents are the dendritic spines of principal neurons, both neuronal populations also appear to selectively innervate PV-containing interneurons. We have shown that in the BLC, PV interneurons are a critical component of the intrinsic circuitry that function to establish spike-timing precision in ensembles of BLC principal neurons, and that these interneurons can entrain principal neuron firing particularly in the delta (1.5-4 Hz) and theta (4-10 Hz) frequency bands (Ryan et al., 2012). However, beta (10-30 Hz) and gamma (30-80 Hz) oscillations have also been observed in the BLC following activation of GluR5-containing kainate receptors (Paré et al., 2002; Randall et al., 2011). One consistent underlying principle of all neural oscillations is that they can effectively bias incoming information to be either amplified (if synaptic input arrives during a

receptive phase), or ignored (if an input of the same strength is received during a phase of suppressed responsivity) (Uhlhaas and Singer, 2012). The coherent oscillation of a neuronal population is now recognized as a common feature of all cortical-like brain structures and disruption of critical dynamic neural systems has been observed in nearly every major category of mental illness, including anxiety disorder and PTSD (Woodward et al., 2000; Ehlers et al., 2006; Veltmeyer et al., 2006; Gordon et al., 2010; Buzsáki and Watson, 2012). Significantly, the frequencies of oscillation band peaks are conserved among human and rodents (Buzsáki and Watson, 2012), which provides the construct validity for using rats as model organisms to study neural oscillopathy in PTSD.

Delta (1.5-4 Hz) / Theta (4-10Hz) band oscillations

Consistent with *in vitro* observations, synchronized theta activity (4-5 Hz) has been observed in *in vivo* local field potential (LFP) recordings from the amygdala and hippocampus during the retrieval of fear memory (Seidenbecher et al., 2003; Pape et al., 2005; Narayanan et al., 2007). Furthermore, coherent theta band oscillations occur between the medial prefrontal cortex, the amygdala, and the hippocampus during fear memory recall, and this coordinated activity diminishes over the course of extinction (Sangha et al., 2009; Likhtik et al., 2014), suggesting that synchronized neural activity in these three regions contributes to the expression of fear memory. Similar coherent oscillations between the mPFC and BLC have been observed in the high delta range (2-5 Hz) during fear acquisition and recall in rats (Madsen & Rainnie, unpublished observations), further suggesting that the neural mechanisms of fear memory formation and recall are conserved across species.

Significantly, the directionality of coherent activity plays a critical role in the response to conditioned stimuli. Hence, during fear acquisition and recall, a zero-lag coherent theta oscillation is observed between the IL, CA1, and lateral nucleus (LA) of the BLC. However, upon fear extinction, the theta oscillation of CA1 and LA was seen to be driven by the activity of the IL (Lesting et al., 2013). Hence, theta coherence may be a canonical mechanism to entrain distant neuronal cell populations, but the behavioral response to entrainment is primarily determined directionality. Recently, Courtin and colleagues reported that optgenetic inhibition of PV interneurons in the mPFC induced freezing behavior in naïve mice, fear reinstatement in fear-extinguished mice, as well as conditioned place aversion. Brief optogenetic inhibition also caused a transient increase in high-theta oscillations (8-12 Hz) in the dorsal mPFC (Courtin et al., 2014a). Given the differential role of activation of the PL and IL during fear acquisition and extinction, respectively, it is possible that disinhibition of PL principal neurons acts to mimic the entrainment of BLC oscillations during fear acquisition. Similarly, Popa and colleagues have reported that the strength of BLA to mPFC theta coherence during bouts of REM sleep following fear conditioning is positively correlated with successful consolidation of the fear memory (Popa et al., 2010). It is possible that consolidation of extinction requires a similar process and as sleep disturbance are reported by 70-91% of PTSD patients (Maher et al., 2006), this may prevent adequate consolidation of fear extinction in PTSD.

Gamma (30-80 Hz) band oscillations

As seen elsewhere in the brain (Wang and Buzsáki, 1996; Edden et al., 2009), gamma band oscillations are frequently observed in the BLC and are thought to play a key role in fear memory formation. For a comprehensive review of the role of gamma oscillations in the amygdala see Headley and Paré (2013) (Headley and Paré, 2013). In brief, the authors present

strong evidence that negatively valanced emotional stimuli can elicit increased gamma power oscillations in the amygdala and cortex, which was most prominently observed upon retrieval of conditioned fear. During fear conditioning, the power of gamma band oscillations in the BLC increase (Madsen & Rainnie, unpublished observations) (Courtin et al., 2014c). Significantly, the relative power of the BLC gamma band oscillation to extinction training is predictive of the level of spontaneous fear recovery (Courtin et al., 2014c). As deficits in extinction learning are thought to play a key role in the etiology of PSTD, understanding how gamma oscillations may persist in the face of extinction training may open up new avenues for clinical intervention in PTSD. Finally, Stujenske and colleagues have reported an increased power of BLC fast-gamma (70-120 Hz) band oscillations when mice receive a safety cue (Stujenske et al., 2014a), suggesting that different frequency bands of gamma oscillations may recruit different components of the fear/extinction circuits.

Cross-frequency coupling

What has received less attention, until recently, is the power relationship between the different frequency bands in the BLC. As is often the case, oscillations at different frequencies are present simultaneously in the BLC and the phase of a slower oscillation often modulates the amplitude of a faster oscillation, a phenomenon known as cross-frequency coupling (CFC) (Canolty and Knight, 2010a; Aru et al., 2014). Hence, CFC establishes a hierarchical organization of neural oscillations, similar to the syntactical relationships in all languages between letters, words, and sentences (Buzsáki and Watson, 2012). Notably, Stujenske and colleagues have shown that during presentation of a fear-conditioned CS, a strong theta-fast gamma coupling occurs that decreases the power of the fast-gamma (70-120 Hz) band oscillation. On the other hand, when presented with a safety signal CS, the power of fast-gamma

band increased as the coupling between theta and fast gamma decreased (Stujenske et al., 2014a). Significantly, the increased power of 70-120 Hz gamma band was also found after fear extinction or in the safe zones of an open field. The elevated gamma power was associated with an increased entrainment of mPFC theta to BLC gamma band. Hence, the amplitude of fast gamma oscillations in the BLC can be dynamically regulated by the degree of coupling with the lower theta frequency oscillation.

Consistent with the observations described in the prior paragraph, we have identified two distinct pairs of frequency bands that show CFC in the mPFC during late stage of fear-conditioning, while CFCs disappear during fear extinction in rats (Madsen & Rainnie, unpublished data). Here, a delta oscillation shows CFC with a low (30-45 Hz) gamma oscillation, and a theta oscillation shows CFC with a mid (45-60 Hz) gamma, which might be the mediators of neuronal plasticity to build the CS-US association. Moreover, the modulated mid-gamma band in the mPFC was found coherent with same frequency band in the BLC. These findings are consistent with the hypothesis proposed by Stujenske and colleagues that during fear, a strong delta/theta band emerges to synchronize the low-mid gamma oscillations between BLC and mPFC. While in a safe context, the theta band from the mPFC drives the high gamma band oscillation in the BLC to increase, and thus inhibit the fear response. Hence, once again directionality of CFC appears to be the critical factor in switching from fear learning to extinction. Understanding what drives the directionality will be key to understanding the proposed extinction deficits in PTSD.

Together, these findings suggest that the BLC uses frequency codes, in the form of coherent oscillations at delta and gamma frequencies as well as cross-frequency coupling, to communicate with remote brain structures during anxiety-like and fear-like behaviors. Neural oscillation patterns during emotion-elicited behaviors are highly conserved between humans and

rodents; hence, understanding the true relationship between directionality and frequency codes will be a necessary first step in understanding the extinction deficits of PTSD. Moreover, as more advanced non-invasive recording techniques are developed for human studies, investigations into the mechanism of neural oscillations in the BLC and mPFC during fear learning and extinction in rodents will be invaluable, since the findings could then be examined in humans with good construct validity.

NEUROMODULATION IN THE BLC

In addition to influencing other brain regions through extrinsic connections, the BLC receives neuromodulatory input from many different areas of the brain. These neuromodulators can have multiple effects on each of the different BLC cell types due to the heterogeneous expression of their cognate receptors. As a result, each neuromodulator has the ability to differentially affect gross BLC activity due to specific activation and inhibition of different cell types. While this section individually discusses the activity of different neuromodulators, it should be remembered that their activity is oftentimes concurrent and synergistic. Hence, it is important to appreciate the complexity, and state-dependent nature, of the response of BLC principal neurons due to such an intricate regulatory mechanism. All of the following neuromodulators represent potential mechanisms through which pathological stress could act on the BLC to produce the core symptoms of PTSD, hence understanding these processes is vital for the development of effective PTSD treatments.

Serotonin

The major source of serotonin (5-HT) input to the amygdala primarily comes from specific subdivisions of the dorsal raphe nucleus (Lowry et al., 2005; Hale et al., 2012; Weissbourd et al., 2014). Hence, anterograde and retrograde tracing studies have revealed clearly labeled 5-HT neurons in the dorsal and caudal DRN (Kiyasova et al., 2011; Hale et al., 2012). Significantly, experimental manipulations of these connections lead to altered stress-related behavior (Commons et al., 2003; Abrams et al., 2005; Asan et al., 2013), and 5-HT levels in the BLC increase during psychological and physiological stressors (Kawahara et al., 1993; Funada and Hara, 2001). In control animals, 5-HT release in the BLC generally decreases the excitability of principal neurons through three main mechanisms: (1) hyperpolarization of a subpopulation of BLC projection neurons through activation of 5-HT_{1A} receptors (Rainnie, 1999), (2) depolarization of GABAergic PARV interneurons through activation of 5-HT_{2A} receptors, resulting in increased inhibitory drive onto principal neurons (Jiang et al., 2009), and (3) presynaptic inhibition of glutamate release through activation of 5-$HT_{1A/B}$ receptors (Cheng et al., 1998) (Guo & Rainnie, unpublished observations). However, decreased BLC excitation in response to 5-HT can be blocked by prior application of a $GABA_A$ receptor antagonist, suggesting that the inhibitory actions driven by activation of PARV interneurons is the predominant response to 5-HT release under non-stressful conditions (Rainnie, 1999; Xiao et al., 2011). Nevertheless, selective activation of 5-HT_{1A} receptors in the BLC has also been shown to reduce anxiety-like behavior (Christianson et al., 2010; Ferreira and Nobre, 2014). Consequently, 5-HT release in the BLC has been viewed as contributing to an inhibitory feedback loop that may act to terminate an ongoing stress response as part of a coping mechanism (Rainnie, 1999; Andolina et al., 2013; Paul et al., 2014). Consistent, with this hypothesis, selective depletion of 5-HT in the BLC facilitates fear-potentiated startle (Tran et al., 2013), and local infusion of the

selective serotonin receptor uptake inhibitor (SSRI), citalopram, is reported to decrease fear conditioning (Ravinder et al., 2011; Pockros-Burgess et al., 2014).

In contrast, animals that have undergone prolonged uncontrollable stress show an increased excitability of BLC principal neurons in response to 5-HT (Christianson et al., 2010). Moreover, Maier and colleagues have shown that inescapable, but not escapable, stress leads to an increase of 5-HT neuronal activation in the DRN, resulting in an elevation of 5-HT levels in target regions such as the BLC (Maier and Watkins, 2005; Christianson et al., 2010). However, stress-induced dynamic changes in the expression of a number of serotonergic receptors in the BLC are also thought to contribute to the alteration in the response to 5-HT input. For example, inescapable stress has been reported to cause a down-regulation in the activation of GABAergic BLC interneurons due to a decrease in 5-HT_{2A} receptor expression, thus contributing to the increased excitability of BLC principal neurons (Jiang et al., 2009). It has also been suggested that a down-regulation of 5-HT_{1A} receptors may be a mechanism behind stress-induced anxiety-like behavior (Akimova et al., 2009). However, there are also 5-HT receptors that normally act to increase BLC excitability, such as 5-HT_{2C} receptors, activation of which increases anxiety-like behavior (Pockros-Burgess et al., 2014; Zangrossi and Graeff, 2014). Hence, the behavioral consequences of activation of 5-HT_{2C} receptors may be unmasked by the down-regulation of inhibitory modulators of BLC activity in response to uncontrollable stress. Consistent with this premise, local infusion of the selective 5-HT_{2C} receptor antagonist, SB 242,084, into the BLC prevented the enhancement of anxiety-like behavior in response to uncontrollable stress (Christianson et al., 2010). A similar stress-induced dysregulation of 5-HT receptor expression/function may be the basis behind altered BLC activity in PTSD.

Dopamine

Like serotonin, dopamine (DA) is released into the BLC in response to stressful stimuli. Most dopamine input into the BLC arrives via afferents arising from the ventral tegmental area (VTA) and substantia nigra (SN) (Inglis and Moghaddam, 1999; Muller et al., 2009; Takahashi et al., 2010). Recent evidence suggests that dopamine D1 receptor activation in the BLC is necessary and sufficient for the acquisition and expression of Pavlovian fear conditioning (Fadok et al., 2009). However, the cellular mechanisms mediating dopaminergic regulation of fear conditioning are only now beginning to be understood.

As noted above, it is understood that fear learning and consolidation occurs, at least in part, through LTP induction in the BLC (Rogan et al., 1997; Goosens and Maren, 2002; Bissière et al., 2003). Given that D1 receptors in the BLC are co-distributed with NMDA receptors on the dendritic spines of principal neurons, and that spines are the primary site of afferent innervation of principal neurons, locally released DA is in a unique position to modulate NMDA currents, and thus have a strong influence on LTP induction in sensory pathways (Pickel et al., 2006; Muly et al., 2010). Consistent with this hypothesis, we have shown that local DA release onto BLC principal neurons is a necessary pre-requisite for LTP induction (Li et al., 2011). However, DA release in the BLC can have multiple effects at both pre- and postsynaptic loci, as well as on different cell types. Nonetheless, the net effect of BLC dopamine release is to (1) facilitate transmission of salient sensory input, (2) enhance synaptic plasticity, and (3) synchronize the firing activity of ensembles of principal neurons.

Firstly, dopamine has been reported to modulate sensory input into the BLC by both direct and indirect mechanisms in a pathway-specific manner. Hence, early studies reported that dopamine acts to reduce the efficacy of synaptic input from the mPFC to the BLC, while at the same time enhancing sensory cortical input (Rosenkranz and Grace, 2001; Grace and

Rosenkranz, 2002). Subsequent studies revealed that DA acts to facilitate sensory input by reducing the expression of feed-forward inhibitory drive onto BLC principal neurons (Bissière et al., 2003; Marowsky et al., 2005). More recently, we have shown that activation of presynaptic D1 receptors also functions to increase glutamate release from cortical afferents onto BLC principal neurons, an effect that can be overcome by activation of presynaptic mGluR2/3 receptors (Li and Rainnie, 2014). Thus, DA appears to act presynaptically to selectively enhance the efficacy of salient sensory input.

At the postsynaptic side, Kroner and colleagues reported that dopamine acts to enhance the net excitability of BLC principal neurons by decreasing a delayed outward potassium current and by increasing the membrane input resistance, through activation of D1 and D2 receptors respectively (Kröner et al., 2005). Significantly, the postsynaptic response to dopamine is similar in rats and non-human primates (Muly et al., 2010), suggesting that this response is conserved across species. In addition, evidence suggests that dopamine can also increase the rate of action potential firing in BLC principal neurons by attenuating the medium afterhyperpolarizing potential (mAHP) that follows each action potential (Ryan & Rainnie, unpublished observations). More recently, we have shown that postsynaptic D1 receptor activation is necessary for LTP induction in BLC principal neurons, and that this effect is mediated via activation of the adenylate cyclase signaling cascade (Li et al., 2011). Significantly, we have also shown that spike-timing precision in BLC principal neurons is tightly regulated by the ability of this same adenylate cyclase signaling cascade to amplify an intrinsic sub-threshold membrane potential oscillation (SMPO) (Ryan et al., 2012).

To summarize, local dopamine release enhances salient sensory input, facilitates LTP, and enhances spike-timing precision and firing rate in BLC principal neurons. At the same time, dopamine has also been shown to increase burst firing in a subpopulation of BLC parvalbumin

(PV) interneurons through D1 receptor activation (Kröner et al., 2005; Muly et al., 2010). While PV interneuron activation might seem counter-intuitive given the overall excitatory influence of DA in the BLC, at the circuit level, PV interneurons play a major role in entraining spike firing in ensembles of BLC principal neurons (Ryan et al., 2012). Dopamine fibers heavily innervate PV interneurons in the BLC (Pinard et al., 2008), and increased burst firing of this cell population would effectively increase the frequency of coordinated output to downstream target structures. Finally, activation of BLC PV interneurons has been reported to inhibit SOM interneurons and thereby disinhibit sensory input arriving at distal dendrites of BLC principal neurons, further contributing the enhancement of signal processing in the BLC (Wolff et al., 2014). Taken together, these data strongly suggest that the overall effect of DA on amygdala activity is to place the BLC circuit into an optimal state to influence fear learning. Given that the general role of DA in the BLC is to augment and synchronize principal neuron activity, it is not hard to assert that continuous enhancement of DA levels in the amygdala could be a factor behind the overall increased excitability of BLC output during stress disorders such as PTSD.

Norepinephrine

Afferents from the locus coeruleus (LC) are the main source of norepinephrine (NE) input into the BLC (Chen and Sara, 2007), and NE release in the BLC has been shown to be critical for the consolidation of memory, particularly emotional memory (McGaugh et al., 2002; Mcintyre et al., 2003; McGaugh, 2004; van Stegeren, 2008; Joëls et al., 2011). Consistent with this observation, projections from the LC target both BLC principal neurons and interneurons (Zhang et al., 2013), and NE levels increase in the amygdala during stress. However, despite the growing body of evidence supporting a role for NE in memory consolidation, the cellular

mechanisms underlying this response are complex, involving multiple receptor subtypes and concurrent release of other neurotransmitters.

For example, NE release in the BLC has been reported to result in an overall inhibition of BLC firing activity (Buffalari and Grace, 2007; Chen and Sara, 2007), which theoretically would not favor memory consolidation. However, subpopulations of BLC neurons were shown to either decrease or increase their firing rates, suggesting that NE may differently effect principal neurons and interneurons depending on their NE receptor expression patterns (Buffalari and Grace, 2007). Consistent with this observation, $\alpha 1_A$ receptor activation increases GABAergic inhibition of principal neurons through a direct postsynaptic membrane depolarization and increased firing activity of BLC interneurons (Braga et al., 2004; Miyajima et al., 2010). However, the increased firing activity is only seen in a subset of BLC interneurons (Kaneko et al., 2008). If these interneurons were say the PV interneurons, this could effectively enhance synchronized firing activity in ensembles of BLC principal neurons (see above). In contrast, β receptors are thought to be preferentially expressed in principal neurons and are reported to cause an increase in neuronal firing activity in response to local NE release (Buffalari and Grace, 2007; Hurlemann et al., 2010). However, another study found that BLC principal neurons had no direct response to NE (Kaneko et al., 2008). The disparity between these two observations most likely results from the use of NE versus the selective β-adrenergic receptor agonist, isoprotenerol, to examine the response to β receptor activation. In our hands, isoprotenerol routinely evokes a depolarizing response in BLC principal neurons, and markedly facilitates the SMPO that determines spike-timing precision (Ryan & Rainnie, unpublished observations). Hence, activation of β receptors may mimic many of the same processes activated by D1 receptor activation and act to facilitate synchronized firing activity in ensembles of BLC principal neurons.

Notably, in animals that have undergone chronic stress, NE causes an overall increase in BLC activity (Buffalari and Grace, 2009), and recordings from BLC principal neurons of stressed animals showed no significant NE-induced GABAergic activity compared to controls (Braga et al., 2004). A proposed mechanism for the alteration in the NE response is a stress-induced decrease in $\alpha 1_A$ receptor activity either though desensitization, downregulation, or internalization of the receptor, or a change in its intracellular signaling pathways (Braga et al., 2004). Another possibility could include an increase in the response to β receptor activation. Consistent with the latter hypothesis, recent evidence suggests that the response of BLC circuits to NE release may be dependent on the timing of its release in relation to corticosteroid release, (for review see Joëls et al., 2011). Hence, concurrent release of corticosteroids and NE act synergistically to enhance emotional memory formation and facilitate LTP induction in the BLC (Sarabdjitsingh et al., 2012). Significantly, the stress-induced facilitation of LTP was attenuated by prior application of corticosteroid- or β-receptor antagonists. Moreover, if corticosteroid release occurs asynchronously it can prevent or suppress the effects of β receptor activation, thus allowing for a bidirectional modulation of the NE response in the BLC. Release of NE is also associated with increased attention and vigilance (Goddard et al., 2010). As some of the cardinal signs of PTSD are hypercortisol secretion, hypervigilance, and stimulus generalization, it is possible that synergistic release of cortisol and NE in the BLC could contribute to hypervigilance and stimulus generalization.

Acetylcholine

A defining feature of the BLC is that it receives dense cholinergic input from the basal forebrain (Woolf, 1991). The vast majority of cholinergic inputs (~89%) target principal neurons, whereas ~7 % target PV interneurons (Muller et al., 2011). Significantly, cholinergic

modulation of BLC activity has been shown to play a critical role in memory consolidation (Gold, 2003; McGaugh, 2004). While ACh release in the BLC can activate nicotinic and muscarinic receptors, it is the activation of BLC muscarinic receptors that is believed to play the predominant role in modulating memory consolidation (Power et al., 2003). Indeed, local infusion of antagonists at either the muscarinic M1 or M2 receptor blocks the memory enhancing effect of ACh in the BLC. However, recent anatomical studies have suggested that M1 and M2 receptors are differentially expressed in distinct cell populations of the BLC. Hence, M1 receptors are expressed by all BLC principal neurons, where they are found in dendrites and spines, but also in the dendrites of a small population of interneurons (McDonald and Mascagni, 2010; Muller et al., 2013). M1 receptors were also observed in axon terminals forming asymmetric, presumed glutamatergic, synapses with BLC principal neurons suggesting both pre- and postsynaptic loci for ACh modulation of BLC activation. Intriguingly, M2 receptor expression is highest in the rostral BLC and is most commonly seen in somatostatin (SOM) and neuropeptide Y (NPY) containing interneurons, but not PV containing neurons (McDonald and Mascagni, 2011). Consistent with these observations, M1 but not M2 receptor agonists induce a form of LTP in BLC principal neurons (Park et al., 2004), which would be consistent with the memory enhancing effects of M1 activation seen in behavioral studies. Elsewhere in the brain, chronic stress has been shown to facilitate cholinergic-modulated LTP (Pavlovsky et al., 2012). If a similar process occurs in the BLC in response to chronic stress, it could result in enhanced fear memory consolidation and attenuated extinction that is thought to contribute to the pathophysiology of PTSD.

Cholinergic input to the BLC can also influence activity via activation of nicotinic acetylcholine receptors (nAChRs). Activation of nAChRs has been shown to cause depolarization and increased firing activity in BLC interneurons through activation of α_7 and or

$\alpha_3\beta_4$ subunit containing nAChRs (Zhu et al., 2005; Klein and Yakel, 2006; Pidoplichko et al., 2013). These receptors have also been shown to be present on glutamatergic terminals forming synapses with BLC interneurons, indicating another possible mechanism for nAChRs to induce interneuron firing. The high sensitivity of α_7-containing nAChRs receptors to ACh suggests that there may be continuous basal activation of α_7-containing interneurons to control and regulate principal neuron activity (Pidoplichko et al., 2013). However, nAChRs are also expressed by BLC principal neurons and behave similarly to depolarize the membrane potential and increase firing activity (Pidoplichko et al., 2013). Additionally, nAChRs are found on axon terminals of afferent projections to the BLC, and activation of these receptors increases EPSC frequency in BLC principal neurons (Barazangi and Role, 2001; Jiang and Role, 2008). Hence, nAChR activation may have a complementary action to that of muscarinic receptor activation in the BLC. Finally, nAChRs are reported to significantly increase dopamine release in the BLC (Palotai et al., 2013). If ACh release is enhanced following chronic stress, a concurrent increase in local dopamine release may be expected. Significantly, LTP induced in BLC principal neurons by M1 or D1 receptor activation are both dependent on activation of the adenylate cyclase-cAMP signaling cascade (Park et al., 2004; Li et al., 2011). Hence, concurrent activation of these two receptor systems may contribute to the etiology of PTSD following exposure to prolonged or traumatic stress.

CONCLUSION

The BLC becomes hyperexcitable after pathological stress, due to changes in principal neuron morphology, plasticity, and physiology, as well as defects in the inhibitory network. Of particular interest are changes that occur to a specialized sub-network in the BLC thought to

underlie extinction learning. The hyperexcitability and defective extinction machinery within the BLC are thought to underlie the hyperarousal, stimulus generalization, and extinction deficits observed in patients with PTSD. The dysregulated microcircuitry in the BLC will in turn affect the wider fear network, including the hippocampus, PFC, and the BNST. Dysregulating the topographically organized BLC output to the wider fear network induces the imbalance of the fear-on and the extinction circuit. In particular, oscillations between the BLC and the PFC critical for fear learning and extinction change after pathological stress, which is thought to contribute to overgeneralization and extinction deficits. Furthermore, neuromodulatory input into the BLC has the ability to significantly influence its neuronal activity, bringing about remarkable changes to how the amygdala processes information.

The complex interplay between the different neuromodulatory systems results in the overall excitability and activity of the BLC. Disruptions in these systems can transform how BLC principal neurons behave and modulate their output in a way that can lead to the symptoms and behavior seen in PTSD. Hence, alterations in BLC circuitry and function may lie at the core of the amygdala's role in the etiology of PTSD. A better understanding of BLC circuitry alteration during fear learning and extinction is critical for working towards an effective treatment for this disorder.

In next chapter, I will discuss additional functions of the BLC, specifically, reward-related behavior and social behavior. I report in chapter 2 that coherent activity between the BLA, a sub-nucleus of the BLC, and NAc plays an important role in appetitive CS-US conditioning. Moreover, strength of theta-gamma phase-amplitude CFC in the NAc was lower during CS presentation in the NAc. Importantly, neither neural activities was observed during social interaction.

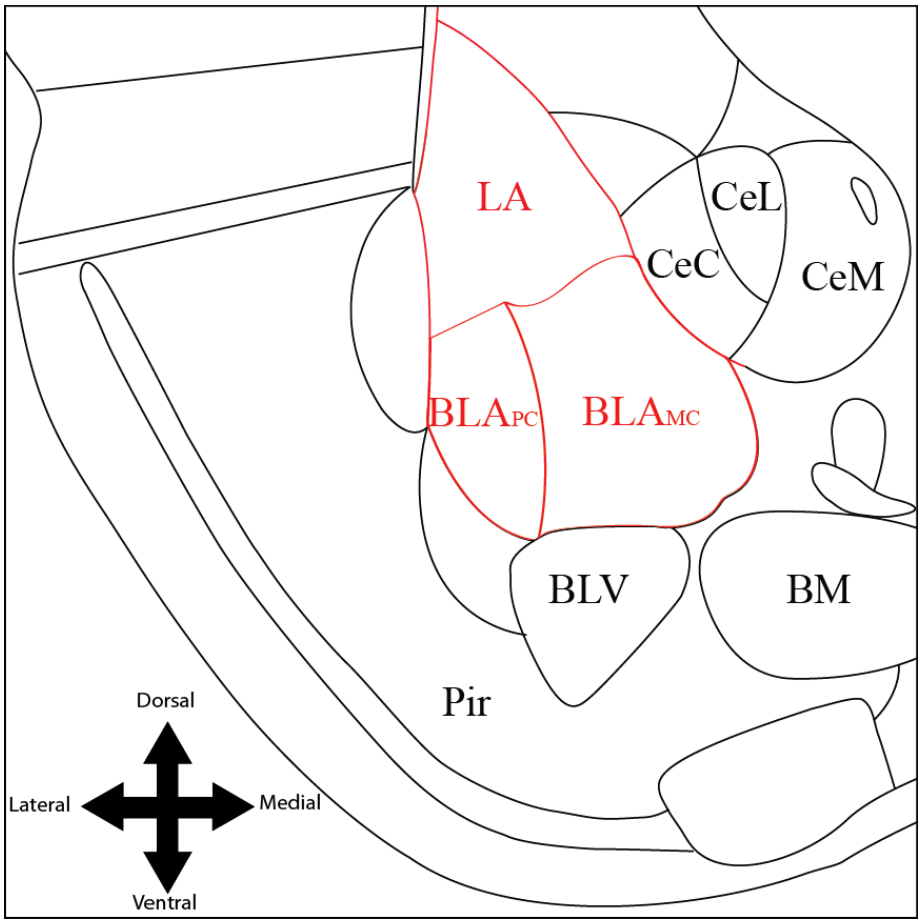

Figure 1-1. Anatomical structure of the basolateral complex. Subdivisions of the basolateral complex are shown in red. Abbreviations: LA, lateral amygdala; BLA_{PC}, basolateral amygdala, parvocellular division; BLA_{MC}, basolateral amygdala, magnocellular division; BLV, basolateral amygdala, ventral nucleus; BM, basomedial amygdaloid nucleus; CeC, central amygdala, capsular division; CeL, central amygdala, lateral division; CeM, central amygdala, medial division; Pir, piriform cortex. BLA_{MC} and BLA_{PC} divisions correspond to the anterior and posterior divisions of the BLA respectively. Adapted from Paxinos and Watson, 2013.

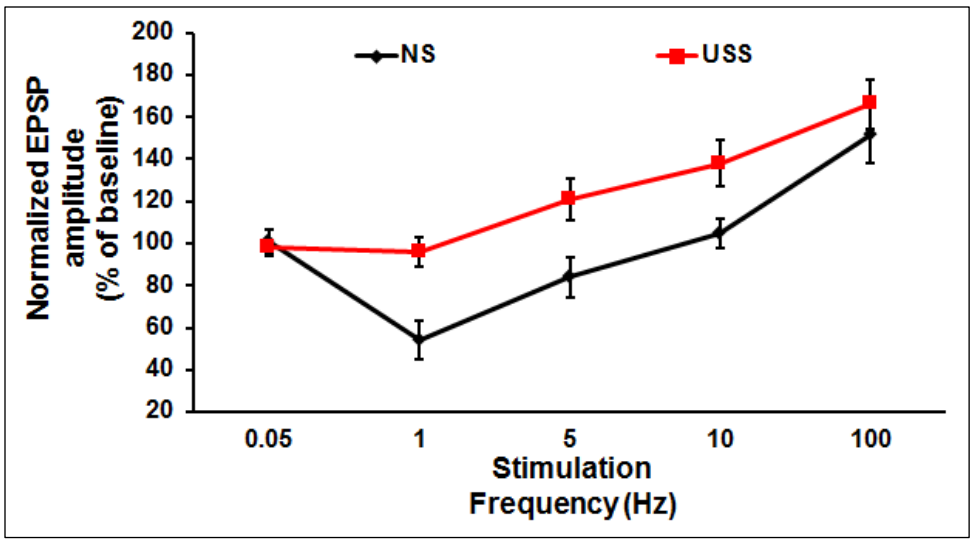

Figure 1-2. Synaptic plasticity in the BLC is regulated by prior stress exposure. Repeated stress causes a leftward shift in the frequency-response curve for the induction of LTD or LTP. In control rats (black line), frequencies below 10 Hz induce LTD, while frequencies above 10 Hz induce LTP. In rats exposed to repeated unpredictable shock stress (red line), frequencies below 5 Hz induce LTD, while frequencies above 5 Hz induce LTP. Note that 0.05 Hz (the stimulation frequency used to monitor strength of synaptic transmission throughout the experiments) had no effect on synaptic transmission in both Non-stressed (NS) and Unconditioned-shock-stressed (USS) animals.

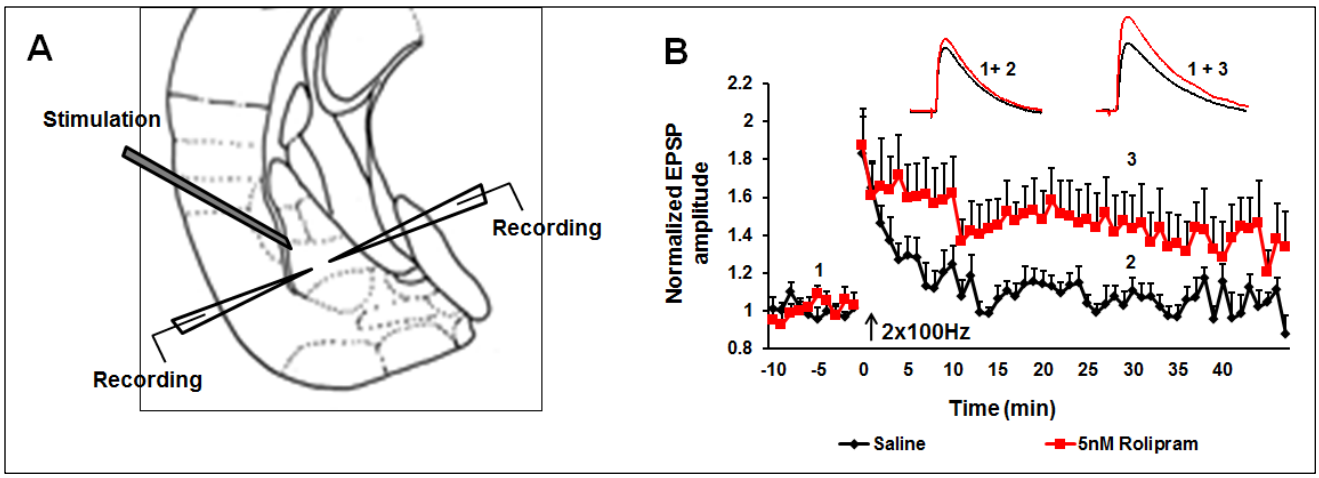

Figure 1-3. Intracellular inhibition of PDE4 causes a cell-specific induction of LTP in response to 2 times high-frequency stimulation (2xHFS). Intracellular application of the specific PDE4 inhibitor, rolipram (5 nM), caused a significant, cell-specific, reduction in the threshold for LTP induction in response to 2xHFS at 100 Hz. (A) A schematic showing the placement of recording electrodes for LTP induction in BLA. (B) Simultaneous whole-cell patch clamp recordings in BLA principal neurons were done in the absence (black) or presence (red) of intracellular 5 nM rolipram. 2xHFS induced LTP in cells injected with intracellular 5 nM rolipram, but not in control cells.

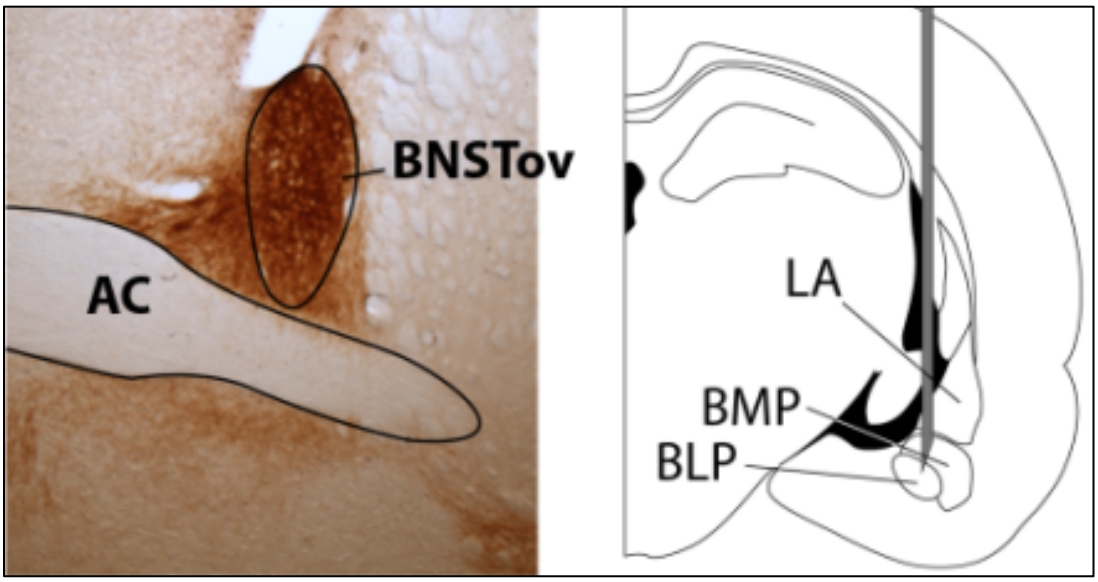

Figure 1-4. The connectivity between the posterior basolateral amygdala (BLP) and the bed nucleus of the stria terminalis (BNST). Anterograde tracer injection (biotinylated dextran-amine 10,000 MWt) into the BLP (right) resulted in dense terminal staining in the oval subnucleus of the BNST (BNSTov) (left). Abbreviations: AC, the anterior commissure; BNSTov: the oval subnucleus of the BNST; LA, the lateral amygdala; BMP, the basomedial amygdala, posterior; BLP, the basolateral amygdala, posterior.

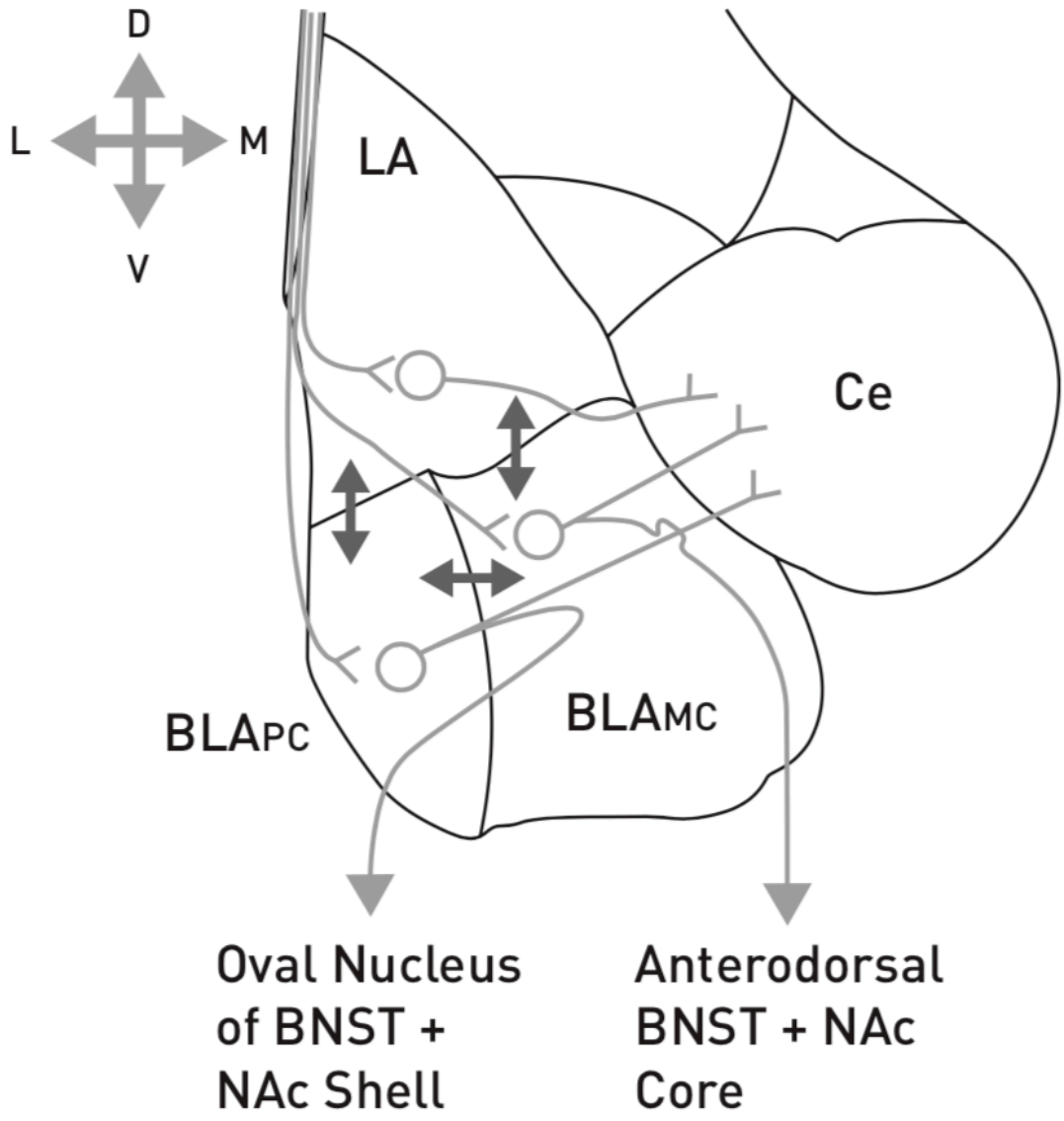

Figure 1-5. Diagram illustrating functionally distinct subnetworks in the BLC. Thalamo-cortical afferents project to all parts of the BLC, including the LA, BLA_{PC}, and BLA_{MC}. These BLC subdivisions are reciprocally connected, represented by purple arrows. The LA, BLC_{PC}, and BLA_{MC} in turn send efferents to the CeA. Additionally, the BLA_{PC} sends projections to the oval nucleus of the BNST and NAc shell, promoting anxiety-like behavior. The BLA_{MC} sends projections to the anterodorsal BNST and NAc core, decreasing anxiety-like behavior. Abbreviations: BLC, the basolateral complex of the amygdala; LA, lateral amygdala, BLA_{PC},

basolateral amygdala, parvo- cellular division; BLA_{MC}, basolateral amygdala, magnocellular division; CeA, central amygdala, BNST, bed nucleus of the stria terminalis; NAc, nucleus accumbens.

Chapter 2: Amygdalo-striatal coherence with reward-related cues

CONTEXT AND AUTHOR'S CONTRIBUTION

The following chapter describes a form of synchronized activity between the basolateral amygdala (BLA) and the nucleus accumbens (NAc) in response to reward-related cues. We also examined other neural activities, including power spectral density and phase amplitude cross-frequency coupling (PAC), in the BLA and NAc when explicit stimuli that were associated with food or social conspecifics were presented. The dissertation author contributed to the paper by designing and conducting the experiments, analyzing the data and writing the manuscript under the guidance of Dr. Donald Rainnie and Dr. Shannon Gourley. Dr. Teresa Madsen contributed to designing experiments and analyzing data and Elizabeth O'Gorman performed some behavioral and histological analysis.

ABSTRACT

Recognizing reward-related stimuli is crucial for survival. Neuronal projections from the BLA to the NAc play an important role in processing reward-related cues, however, whether the NAc synchronizes with the BLA is unknown. Here we recorded local field potentials simultaneously from the BLA and NAc during appetitive conditioning and social preference tests, in which explicit stimuli were associated with food or social conspecifics, respectively. BLA-NAc coherence in the theta band increases in response to food-associated cues. Meanwhile, the modulatory strength of theta-high gamma PAC in the NAc decreases, and both neuromodulations disappear upon extinction. In contrast both theta and gamma power increase in response to a social conspecific or a stimulus associated with the conspecific, but coherence does not change. To potentially disrupt behavior and associated neural activity, a subgroup of rats was exposed prenatally to valproic acid (VPA). VPA-exposed rats demonstrated impulsive-like

behavior, but it did not affect BLA-NAc coherence. These findings reveal BLA-NAc coherence in response to select, and not all, reward-related stimuli (i.e., food-predictive, but not social, cues).

SIGNIFICANCE STATEMENT

The ability to identify food-associated cues is crucial for survival. The neural projection from the basolateral amygdala to nucleus accumbens plays an important role in cue-elicited reward seeking. Here, we identified a distinct neural pattern, namely coherent neural activity between the basolateral amygdala and nucleus accumbens, that predicted upcoming food. Coherence was not affected by pharmacologically-induced impulsive-like behavior or social cues, suggesting that food-related cues coordinate a specific neurocircuit unrelated to – or that escapes – factors that trigger impulsive action.

INTRODUCTION

The ability to recognize stimuli with positive emotional valence is crucial to survival and reproduction for animals and humans. Individuals utilize previous experiences and memories of cues associated with positive value to identify nutrients and form social networks. These cues also affect attention and motivation, as well as decision-making strategies. Deficits in recognizing reward-associated cues can impair the ability of individuals to interact with others and the environment. Abnormalities in positive memories are reported in many psychiatric disorders, including depression, PTSD and schizophrenia.

Over the past decades, researchers have made great progress in dissecting the neural circuits underlying reward processing using experimental procedures such as intra-cranial self-

stimulation, appetitive conditioning, and social preference tests (Popescu et al., 2009; Choi et al., 2012b; Izquierdo et al., 2013; Namburi et al., 2015). The basolateral amygdala (BLA) is an important brain structure for valence coding of external stimuli. The BLA processes sensory information from thalamic and sensory inputs and sends projections to many structures in the limbic system, including the nucleus accumbens (NAc) (Mcdonald, 1991; Brog et al., 1993). Multiple studies have demonstrated that NAc-projecting neurons from the BLA are involved in processing reward information. For instance, reward-predictive cues stimulate neurons in both regions (Schultz et al., 1997; McGinty et al., 2013; Terada et al., 2013). Also, BLA→NAc projections are necessary for cue-induced reward-seeking behavior (Ambroggi et al., 2008; Stuber et al., 2011b), and functionally disconnecting BLA→NAc interactions impedes reward seeking (Di Ciano and Everitt, 2004; Ambroggi et al., 2008). Further, reward-associated cues induce spiking activity in BLA→NAc neurons (Namburi et al., 2015).

Precisely coordinated neuronal activity is crucial for communication between brain structures. Neural oscillations are rhythmic fluctuations resulting from the summation of transmembrane currents and reflect excitability of groups of neurons (Buzsáki et al., 2012). Neural oscillations are thought to play an important role in memory and emotion. For instance, theta oscillations coordinate firing patterns of place cells in the hippocampus during spatial navigation tasks (Itskov et al., 2008; Mizuseki et al., 2009). Conditioned freezing is associated with synchronized activity at 4Hz between the BLA and medial prefrontal cortex (Karalis et al., 2016). Meanwhile, reward-related cues enhance gamma band coherence between the BLA and striatum (Popescu et al., 2009).

Here, we tested the hypothesis that the BLA and NAc coordinate neural activity upon the presentation of reward-associated cues. We simultaneously recorded local field potentials (LFPs), which reflect the summation of synaptic activities, from the BLA and NAc. Rats were

exposed to two potentially rewarding situations – in one case, a stimulus was associated with food, and in another, rats had access to a novel conspecific. To potentially disrupt reward processing and identify neural correlates, some rats were treated with the histone deacetylase inhibitor Valproic acid (VPA) *in utero*, which can induce social interaction deficits and impulsive phenotypes (Vorhees, 1987; Tyzio et al., 2014). We report enhanced coherence between the BLA and NAc in the theta band (5-8 Hz) during reward-related cue presentation. Meanwhile, access to a novel conspecific enhanced power in theta and gamma (50-110 Hz) oscillations in both the BLA and NAc, but we identified no changes in theta coherence. VPA induced impulsive-like behavior but otherwise had few discernible consequences. These results suggest a role for theta-frequency synchronization between the BLA and NAc in processing select, but not all, reward-related information and suggest that theta oscillations may mediate communication between the BLA and NAc during exposure to reward-related cues.

MATERIALS AND METHODS

Animals and drug treatments

The 24 rats used in this report were male offspring of pregnant Sprague-Dawley rats (Charles River Labs). Dams arrived at our facility at 5-6 days of gestation and were maintained on a standard, 12-hour light-dark cycle. On embryonic day (E) 11-13, each dam received a daily 800-900 µL gavage of either VPA (500 mg/kg; Sigma-Aldrich) dissolved in bacteriostatic 0.9% sodium chloride or bacteriostatic 0.9% sodium chloride alone (as per Barrett et al., 2017). After weaning at postnatal (P) day 21, the offspring were group-housed with 2-4 same-sex littermates and then singly-housed after surgery to prevent damage to the implant. Only one rat from any given litter was used. All rats had access to food and water *ad libitum* except during the food

restriction period described in the next section. All procedures were conducted in accordance with Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Emory University Institutional Animal Care and Use Committee (IACUC).

Experimental design

12 control and 12 VPA-exposed rats were used. Electrode implantation occurred between P70-77 (Figure 1). Two VPA-exposed rats lost the implant before experiments ended and thus were excluded from the cohort. Rats were handled daily for 3 days after surgery. After 7 days of recovery, rats were habituated to the testing chamber for 20-30 minutes, with the recording cable connected to the headstage. On post-surgical day 9, rats underwent a social preference test, described below. Food restriction started on post-surgical day 10 and was maintained until the end of our appetitive conditioning assay. Each rat was maintained at 90% of its baseline body weight. Testing began on post-surgical day 13. Rats were euthanized between post-surgical days 22-29. To prevent the potential stress of food restriction from affecting the social preference test, the social preference test preceded the appetitive conditioning experiments. Nevertheless, we will discuss the appetitive conditioning experiments first.

Surgery

Rats were anesthetized under isoflurane (1-2%) with continuous oxygen flow (1.5-2 L/minute). A single dose of meloxicam (2 mg/kg, *i.p.*, Metacam, Boehringer Ingelheim Vetmedica, Inc.) was administered for pre-surgical analgesia. Microwire electrodes (50 μm SS or Tungsten, Microprobes or Tucker-Davis Technologies) were implanted at the following angle-adjusted coordinates relative to bregma (AP/ML/DV/angle): nucleus accumbens (NAc) core:

+1.4mm/±3.52mm/-8.32 mm/10° toward the midline, and ipsilateral basolateral amygdala (BLA): -3.6 mm/±3.83 mm/-9.45 mm/6° toward the side. All coordinates were measured from the surface of bregma. Electrodes were anchored to the skull with 5 stainless steel screws and dental acrylic. Rats were allowed one week for post-surgical recovery. Metacam was administered daily for 1-2 days after surgery, depending on each animal's condition.

Histology

Rats were anaesthetized under 1.5% of isoflurane with oxygen, and electrical lesioning at the each electrode tip was performed (10 μA for 20 seconds, Midgard Precision Current Source, Stoelting). Rats were then euthanized via pentobarbital (100 mg/kg, SomnaSol, Henry Schein Animal Health). After showing no response to tail pinch, rats were perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (pH 7.4 in PBS, Sigma-Aldrich). The brain was removed and stored in 4% paraformaldehyde overnight. The brain was then transferred to 30% sucrose solution in PBS until the brain sank, indicating that it had reached an isosmotic state. The brain was then sectioned into 50 μm sections using a freezing sliding microtome (Microm) and mounted on gelatin coated microscope slides (Fisher Scientific). Sections were stained by cresyl violet (Sigma-Aldrich) and imaged on a light microscope (Nikon). Electrode placements were verified according to the *Rat Brain in Stereotaxic Coordinates* (Paxinos and Watson, 2013).

Behavioral testing

Social preference test

The social preference test was conducted in an acrylic chamber (19 × 30 × 12 inch), with two small wire cages placed at opposite ends of the chamber. The headstage cable was

sufficiently long and flexible as to allow the implanted rats to freely explore the entire chamber and interact with both cages. The test was divided into 3x15-minute sessions, between which the wire cages were changed to prevent odor contamination. The first phase was **baseline**, in which both wire cages were empty, and rats were allowed to explore for 15 minutes. The second phase was **interaction**, in which the empty wire cages were replaced with a new wire cage containing a novel male adolescent rat (5 weeks old) and another empty wire cage. The side containing the adolescent rat was counterbalanced across subjects. The third phase was **retention**, in which case, the cages used in the interaction phase were again replaced with two clean empty wire cages.

Appetitive conditioning and extinction

Testing consisted of two phases: a 7-day conditioning phase and a 2-day extinction phase. It was performed in a modular test chamber (60 x 34 x 26 cm, with aluminum and polycarbonate walls, Lafayette Instruments) controlled by ABET II software (Lafayette Instruments).

Each rat underwent 60 trials/day. Each trial started with a 6-second tone at 6 kHz. A 20mg pellet (chocolate flavor, catalog ID: 1811223, TestDiet) was delivered from a food dispenser to a food magazine, coincident with the end of the tone. The food magazine was equipped with an acrylic door and an infrared beam sensor to detect magazine entry. The inter-trial interval (ITI) was randomly selected between 20-40 seconds. The 6 seconds from tone onset to 6 seconds after pellet delivery was defined as the active period of each trial. During this active period, rats were allowed to enter the magazine with no punishment. If rats entered the magazine during the inactive period between trials, the timer toward the next trial was paused, and a 3-second penalty was added. The timer resumed after the food magazine door closed, indicating that the animal had left.

During the extinction phase, the rats underwent a 60-trial training session identical to the conditioning sessions, except that food pellets were withheld.

Data acquisition

Local field potential (LFP) recordings were collected via a recording headstage with 20x gain and a MAP data acquisition system (Plexon), including a preamp with 50x gain. LFP signals of each brain region were locally referenced. Raw signals were filtered into 0.7-300 Hz to isolate LFPs. Food magazine entries and tone time stamps were recorded via ABET II software. Videos were recorded via Cineplex system (Plexon) with time stamps synchronized to the LFP recordings.

Data analysis and statistics

Social preference test. Time spent interacting with either target cage was collected using TopScan Software (CleverSys). Only direct interactions with the cage were counted. Non-direct interaction events were manually removed by a trained human reviewer. Direct interactions are defined as interactions when rats were interacting with wire cages with the nose pointing toward the cage, whereas indirect interactions are defined as interactions when rats stayed in close proximity to the wire cage, but without the nose pointing toward the cage.

Appetitive conditioning. Time stamps of tone onset and food magazine entries were exported from ABET II software. Average counts of magazine entry during tones, latency of magazine entry relative to tone onset, and magazine entries during inactive periods were computed using Excel (Microsoft). Minimum latency between food magazine entries was set at 250 milliseconds to prevent counting duplicating entries. Maximum latency of food magazine entry during tone was set at 6 seconds, which was the length of the tone. We also calculated a

behavioral enhancement ratio (BER) to evaluate how the magazine entry pattern changed from before to during tones. The BER was calculated as magazine entry counts during each tone divided by the sum of magazine entry counts 6 seconds before and magazine entry counts during each tone (Eq. 1).

$$\text{BER} = \frac{\text{Entry counts during tone}}{\text{Entry counts before tone} + \text{Entry counts during tone}}$$

In vivo electrophysiology

LFP recordings were first processed through FPalign software (Plexon) to correct filter-induced phase shifts, converted into NEX file via NeuroExplorer (Nex Technologies), and imported into Matlab (MathWorks) for analysis. LFP data were analyzed using custom Matlab scripts and FieldTrip, an open-source MATLAB toolbox (Oostenveld et al., 2011). One electrode per brain region was selected for LFP analysis based on histologically verified placements. Artifacts were detected and removed using custom written scripts based on artifact rejection functions in FieldTrip. Multi-taper-method wavelet convolution from FieldTrip was used for spectral analysis. Phase-amplitude cross-frequency coupling was assessed using the Kullback-Leibler modulation index (KL-MI) as defined in Tort et al. (Tort et al., 2010) and analyzed by a custom written MATLAB function by Dr. Teresa Madsen. To assess tone-triggered effects in appetitive conditioning experiments, oscillatory power and spectral coherence from 0.5-1 second and KL-MI from 0.2-5.8 seconds before each tone was used to normalize signals collected during the tones.

Statistical analysis

Data were analyzed in Prism (GraphPad) with an alpha level of 0.05. Two-way within-subject repeated measures (RM) ANOVA was applied to neural and behavioral results. Post-hoc comparisons were performed via planned t-tests with Bonferroni corrections. All values are expressed as mean ± standard error of the mean. Two VPA-exposed rats were excluded from analysis due to implant loss or damage that led to euthanasia in the middle of the study. One control rat was excluded from neural analysis due to poor data quality. Another control rat was excluded from neural analysis in social preference test due to loss of video and time stamp.

RESULTS

LFPs in the BLA and NAc were successfully recorded from 21 male adult Sprague-Dawley rats. Ten of these rats were exposed to the histone deacetylase inhibitor VPA *in utero*, used as a method to potentially disrupt impulse control and social interaction behavior and identify neural correlates. Figure 2-1 depicts a timeline and schematic of the appetitive conditioning and social preference tests. One electrode with correct placement per brain region was chosen for analysis. Figure 2-2 depicts the electrode placements.

Theta activities in the BLA and NAc during appetitive Pavlovian conditioning and extinction

Rats were conditioned for 7 days using a Pavlovian conditioning procedure in which a tone signaled pellet delivery. We measured head entries into a food magazine during the tone, entry latency from tone onset, and a “behavioral enhancement ratio” (BER). BER refers to the

ratio of head entries during the tone / all head entries. BER thus quantifies the effect of the tone on magazine entries. Two-way RM ANOVAs revealed main effects of session on all 3 measures during the initial conditioning phase (Fig. 2-3 a-c; entry counts: $F(6,120) = 11.20$, $p<0.0001$; entry latency: $F(6,120) = 6.681$, $p<0.0001$; BER: $F(6,120) = 16.96$, $p<0.0001$). Post-hoc analyses revealed increased magazine entry counts and BER, as well as decreased magazine entry latency, on day 7 compared to day 1 (with Bonferroni correction, $p<0.05$), evidence that rats acquired the tone-food association. No VPA treatment effects were detected in these or most subsequent analyses and will be noted only when statistical significance was identified.

Under extinction conditions (days 8-9), the food dispenser was still activated coincident with the end of the tones, but no food pellets were delivered. Two-way RM ANOVA of responding across all 9 sessions revealed main effects of session on entry counts during the tone, entry latency relative to the tone, and BER (Fig. 2-3 a-c; entry counts: $F(8,160) = 29.70$, $p<0.0001$; entry latency: $F(8,160) = 59.96$, $p<0.0001$; BER: $F(8,160) = 73.61$, $p<0.0001$). Post-hoc analyses revealed that days 1 and 7 both differed from extinction day 2 (with Bonferroni correction, $p<0.05$). Thus, rats learned that the tone was no longer associated with food pellets, indicated by a drop in magazine entries during extinction.

We next examined responding *within* sessions. Magazine entries, entry latency, and BER were compared in bins of 10 trials (60 total trials). We analyzed response patterns from conditioning day 1 and extinction day 1. Two-way RM ANOVA revealed main effects of time on all 3 measurements on conditioning day 1 (Fig. 2-3 d-f; entry counts: $F(5,100) = 9.680$, $p<0.0001$; entry latency: $F(5,100) = 13.942$, $p<0.0001$; BER: $F(5,100) = 10.26$, $p<0.0001$), as well as extinction day 1 (Fig. 2-3 g-i: entry counts: $F(5,100) = 17.79$, $p<0.0001$; entry latency: $F(5,100) = 19.26$, $p<0.0001$; BER: $F(5,100) = 35.17$, $p<0.0001$). Post-hoc multiple comparisons revealed that all 3 measurements collected during bin 1 on both conditioning day 1 and

extinction day 1 differed from all other trial bins (with Bonferroni correction, $p<0.05$). In other words, rats rapidly modified their behaviors according to conditions experienced within the first 10 trials. A non-specific interaction between time and treatment for magazine entry counts on conditioning day 1 was also detected ($F(5,100) = 2.354$, $p<0.05$), but otherwise, no group effects were identified.

To investigate potential interactions between the BLA and NAc during appetitive conditioning and extinction, spectral coherence was computed. $Coherence_{BLA\text{-}NAc}$ was normalized to the average value generated 0.5-1.5 seconds prior to the tone (baseline). $Coherence_{BLA\text{-}NAc}$ in the theta band (5-8 Hz) was enhanced during the tone presentation on conditioning day 7, when rats readily approached the food magazine, and not extinction day 2, when rats no longer approached (Fig. 2-4 a-d; $F(1,19) = 13.29$, $P = 0.0017$). Further analysis by two-way RM ANOVA across all sessions revealed a main effect of session (Fig. 2-4 e; $F(8,152) = 3.998$, $p=0.0003$), with theta $coherence_{BLA\text{-}NAc}$ on all conditioning days 1-7 significantly higher than on extinction day 2 (with Bonferroni correction, $p<0.05$). Meanwhile, theta $coherence_{BLA\text{-}NAc}$ on extinction day 1 did not differ from extinction day 2 (with Bonferroni correction, $p>0.05$). Baseline $coherence_{BLA\text{-}NAc}$ in the theta band remained unchanged across sessions (Fig. 2-4 e; $F(8,152) = 0.4954$, $p = 0.8581$), indicating that coherence changes were associated with the CS.

Next, we examined the strength of theta oscillations in the BLA and NAc, which changed significantly across sessions (Fig. 2-4 f-g; main effects for BLA: $F(8,152) = 2.796$, $p=0.0065$; NAc: $F(8,152) = 2.512$, $p=0.0136$). Moreover, an interaction between session and treatment was identified in the BLA ($F(8,152) = 2.682$; $p = 0.0087$). Post-hoc multiple comparison revealed that the strength of theta oscillations in the BLA on conditioning days 6 and 7 of VPA-treated rats was significantly higher than in control rats (with Bonferroni correction, $p<0.05$). Higher theta power in the BLA of VPA rats in the late sessions of conditioning phase suggests that,

despite no tone-related behavioral differences between groups, VPA-exposed rats might utilize different strategies to learn or maintain the association between conditioned and unconditioned stimuli (CS and US, respectively).

Previous studies indicate that phase-amplitude cross-frequency coupling (PAC) is associated with reward processing (Cohen et al., 2009a; Terada et al., 2013; Donnelly et al., 2014). Therefore, we computed the strength of PAC in the NAc during CS. Group-averaged comodulograms highlight a loss in the strength of theta (5-8 Hz) to high gamma (70-110 Hz) PAC during CS-US conditioning (*e.g.*, on day 7 here), whereas the decrease was not observed during extinction (*e.g.*, day 2 here) (Fig. 2-5 a). Statistical comparisons revealed decreased NAc theta-gamma PAC during the CS presentations throughout all conditioning sessions, returning to baseline during extinction training (Fig. 2-5 b; effect of time $F(8,152) = 5.153$; $p < 0.0001$). Post-hoc multiple comparisons indicated that the strength of theta-high gamma PAC in the NAc during all CS-US conditioning sessions was lower than during extinction day 1 (with Bonferroni correction, $p<0.05$), except conditioning day 5.

VPA-exposed rats demonstrate impulsive-like behavior

in utero VPA is thought to cause impulsive behavior (Schneider and Przewłocki, 2005; Hill et al., 2015). To investigate impulsive behavior in the present study, we compared magazine entries during the "inactive" period, defined as the interval starting 6 seconds after the tone ended and lasting until the beginning of the next tone. Two-way RM ANOVA revealed main effects of session and treatment on magazine entries during inactive periods (Fig. 2-6 a; session: $F(8,160) = 37.89$; $p < 0.0001$; treatment: $F(8,160) = 4.877$; $p = 0.0391$). VPA-exposed rats generated higher magazine entry rates, consistent with previous reports concerning impulsive

behavior. Overall, magazine entry rates during the inactive periods on day 1 were higher than all other sessions (with Bonferroni correction, $p<0.05$), evidence that rats in both groups were able to learn that inactive period head entries were penalized (conditioning days 1-7) or that food was not being delivered (extinction days 8-9).

Elevated theta band oscillatory strength during non-active periods in extinction testing

Analysis of LFPs in the NAc and BLA during inactive periods revealed certain effects of trials phase. While theta $Coherence_{BLA\text{-}NAc}$ during inactive periods did not change across sessions (Fig. 2-6 b; $F(8,152) = 0.3995$, $p=0.9195$), oscillatory strength in the theta band in both the BLA and NAc was higher during inactive periods during the extinction sessions, compared to CS-US conditioning sessions (Fig. 2-6 c-d; BLA: $F(8,152) = 9.187$, $p<0.0001$; NAc: $F(8,152) = 7.100$, $p<0.0001$). Thus, oscillatory strength in the theta band in the BLA and NAc during the inactive periods was sensitive to CS-US *vs.* extinction conditions.

Enhanced oscillatory strength in theta and gamma bands during social interaction

We next examined the oscillatory activities of the BLA and NAc during a social preference test consisting of 3 phases: baseline, interaction and retention. During the baseline phase, rats were allowed to interact with two empty cages. Rats did not exhibit a preference for the to-be-rat or to-be-empty cages (Fig. 2-7 a; $F(1,20) = 0.5113$, $p=0.4828$). In the interaction phase, a juvenile probe rat was contained within one cage. Experimental rats demonstrated a strong preference for the rat-containing cage (Fig. 2-7 b; $F(1,20) = 151.0$, $p<0.0001$). Short-term social memory was examined by comparing time spent in the previously-rat and previously-empty sides of the chamber in the retention phase. All rats preferred the previously-rat side (Fig.

7c; $F(1,20) = 12.94$, $p=0.0018$). Thus, rats displayed social preference and intact short-term social memory.

The power spectra of the BLA and NAc during the social preference test revealed two distinct peaks in theta (5-8 Hz) and gamma (50-110 Hz) bands (Fig. 2-7 d-e). We analyzed the oscillatory power of both brain regions and $\text{coherence}_{\text{BLA-NAc}}$ in the theta and gamma bands. A main effect of test phase on oscillatory power of the theta band in both the BLA and NAc was detected (Fig. 2-7 f-g; BLA: $F(2,36) = 6.231$, $p=0.0047$; NAc: $F(2,36) = 4.005$, $p=0.0269$). Post-hoc multiple comparisons indicated that oscillatory strength of the theta band in the NAc during the *interaction* phase was higher than at baseline (with Bonferroni correction, $p<0.05$). Meanwhile, the oscillatory power of the theta band in the BLA during the *retention* phase was higher than at baseline (with Bonferroni correction, $p<0.05$).

A main effect of test phase was also detected for the oscillatory power of the gamma band in both the BLA and NAc (Fig. 2-7 h-i; BLA: $F(2,36) = 13.70$, $p=<0.0001$; NAc: $F(2,36) = 11.34$, $p=0.0002$). Post-hoc multiple comparisons indicated that the oscillatory power of the gamma band during the *interaction* and *retention* phases was higher than at baseline in both the BLA and NAc (with Bonferroni correction, $p<0.05$). Changes in oscillatory power and spectral coherence were not attributable to interactions with specific cages, but rather, with the testing phase (all $p>0.05$; not shown). No effects were detected for $\text{coherence}_{\text{BLA-NAc}}$ in either frequency band (Fig. 2-7 j-k).

DISCUSSION

Here we recorded LFPs from the BLA and NAc during the presentation of reward-related cues (termed CS's), revealing that as rats learn to associate a CS with food, coherence between the BLA and NAc increases. Moreover, the modulatory strength of theta-high gamma PAC in the

NAc decreases. Importantly, enhanced theta coherence, as well as decreased theta-high gamma PAC strength, disappeared upon extinction, evidence that these neural patterns are associated with adaptive CS-food coupling. Interestingly, theta power in both the BLA and NAc increased during certain phases of extinction training, a possible mechanism by which reward-seeking behavior is inhibited when familiar cues fail to predict food availability. In a social preference test – another situation with presumed appetitive value – we observed no modifications in BLA-NAc theta coherence. On the other hand, theta and gamma power *within* the BLA and NAc increased upon social interaction and remained elevated when rats were allowed to investigate a cage that previously contained a social conspecific. Thus, coherent theta oscillations may coordinate amygdalo-accumbens interactions during reward-related cue presentation and might serve as a mechanism by which the BLA communicates with the NAc.

Reward-related BLA-NAc theta coherence

Synchronized neural activity can be viewed as rhythmic changes in neuronal excitability, with functional implications depending on the frequency range (Fries, 2005). Long-range theta coherence is thought to be involved in inter-regional information transfer (Mizuseki et al., 2009; Benchenane et al., 2010; Colgin, 2013; O'Neill et al., 2013). Thus, we interpret enhanced BLA-NAc theta coherence upon CS-US pairing as evidence of adaptive transfer of information regarding the predictive relationship between a CS and food. Inter-regional theta coherence is also associated with memory formation (Jones and Wilson, 2005; Kay, 2005; Hyman, 2010), anxiety (Adhikari et al., 2010b), and reward-related decision making (DeCoteau et al., 2007; Benchenane et al., 2010; Kim et al., 2011b). Also, Popescu and colleagues reported enhanced gamma coherence between BLA and ventral putamen (adjacent to the NAc) in response to reward-associated cues (Popescu et al., 2009). Additionally, gamma coupling strength increased

as learning progressed and correlated with behavioral accuracy. To determine the functionality of BLA-NAc coherence in reward processing, closed-loop optogenetic strategies (Grosenick et al., 2015; Kilias et al., 2018) could be ideal, because they allow for selective disruption of coherence between brain regions.

Importantly, the 5-8 Hz theta coherence frequency range that correlated with reward-related information processing here is distinct from previously reported fear-related oscillatory activities in the BLA, which lie between 2-5 Hz in rats (Lesting et al., 2011; Madsen and Rainnie, 2012; Karalis et al., 2016). Tye and colleagues recently characterized two distinct BLA neuron populations with divergent routing to the NAc and the central amygdala (CeA) and differential reactivity to "positive" and "negative" stimuli (Namburi et al., 2015; Beyeler et al., 2016). Future studies should simultaneously record field potentials from the BLA, NAc and CeA during the presentation of stimuli with positive and negative valence in order to identify specific neuron populations and oscillatory patterns that respond to positive and negative stimuli.

We also discovered decreased NAc theta-gamma PAC during the CS presentations. PAC is a neural mechanism for integrating information between or within distinct networks proving a framework for hierarchical neural computation (Canolty and Knight, 2010b). Theta-gamma PAC is a commonly observed phenomenon occurring during movement, memory formation, fear expression and social bonding (Axmacher et al., 2010; Li et al., 2012; Stujenske et al., 2014b; Lega et al., 2016; Amadei et al., 2017). The NAc maintains significant theta-gamma PAC during resting (Malhotra et al., 2015). Thus, decreased theta-gamma PAC strength during the CS here may reflect reduced intrinsic activity that allows the NAc to receive information from the BLA and other brain regions. In humans, theta-gamma PAC in the NAc is associated with movement (Dürschmid et al., 2013). Therefore, alternatively, reduced theta-gamma PAC during the CS presentation may be explained by reduced activity during this time.

When we withheld food delivery (in extinction), BLA-NAc theta coherence and NAc PAC normalized, while theta power *within* the BLA and NAc increased. This pattern can be appreciated in fig. 2-4g and 2-4h, showing no change in baseline-normalized theta power during cue presentation throughout CS-US conditioning and extinction sessions in the BLA and NAc, as well as fig. 2-6c and 2-6d, showing increased baseline theta power in the BLA and NAc in extinction sessions, indicating increased theta power during cue presentation in extinction sessions. Increased theta power during extinction may be a mechanism by which the BLA and NAc "learn" that a CS no longer predicts food. Interestingly, the loss of coherence suggests that these structures operate independently in this context, potentially receiving inputs from key regions involved in extinction such as the medial prefrontal cortex (mPFC) (Gourley and Taylor, 2016).

Social interaction stimulates oscillatory activities in the BLA and NAc

Stimuli associated with social interaction – as opposed to food – also modify oscillatory activities. For instance, social interaction, but not fear conditioning, increases theta power in the high-theta range (Tendler and Wagner, 2015). In humans, social images increase power in the theta band (Wiggert et al., 2017). Moreover, tasks requiring participants to infer others' mental state based on social cues increase power in the gamma band (Cohen et al., 2009b). Here, access to a novel conspecific enhanced theta power in the NAc and gamma power in both the BLA and NAc. Meanwhile, BLA-NAc theta coherence was unaffected, unlike in our Pavlovian approach procedure. While we might have anticipated that approaching food *vs.* a novel conspecific would elicit similar neuronal patterns, one important distinction is that the social interaction task lacks a discrete Pavlovian stimulus, while stimulus-outcome (S-O) associations guide behavior in

Pavlovian approach procedures. Thus, BLA-NAc theta coherence may help rats use S-O associations to generate abstract representations of likely outcomes that guide adaptive behavior.

Both the BLA and NAc play important roles in social interaction. BLA or NAc lesions reduce social play (Meaney et al., 1981; Wolterink et al., 2001; Daenen et al., 2002); however, social play stimulates the immediate-early gene c-fos only in the NAc (van Kerkhof et al., 2013), suggesting different roles in social behavior that may be attributable to projections to NAc from several brain regions other than the BLA, including the mPFC, ventral tegmental area, and hippocampus (Gunaydin et al., 2014; Okuyama et al., 2016; Amadei et al., 2017). Indeed, our findings suggest that BLA→NAc projections are not a dominate input, at least under our testing conditions.

Prenatal VPA exposure induces impulsive-like behavior

Throughout, we included rats that had been exposed prenatally to VPA as a method by which to potentially disrupt decision making and social behavior and identify neural correlates. We were motivated by evidence that prenatal VPA exposure induces sensory hyper-sensitivity, deficits in social behavior, and impulsive-like phenotypes in both human and rodents (Schneider and Przewłocki, 2005; Christensen et al., 2013; Tyzio et al., 2014). Here, VPA-exposed rats entered the food magazine more during inactive periods, despite a penalty for premature responses – impulsive-like behavior. Interestingly, theta power in the BLA gradually increased across conditioning sessions in the same rats, consistent with excitatory/inhibitory imbalance in the amygdala following VPA (Lin et al., 2013) and elevated theta power in the hippocampus (Tyzio et al., 2014), but apparently unrelated to impulsive-like behavior that instead occurred stably across sessions.

Deficits in social behavior have been reported in many studies using prenatal VPA-exposed rodents (Roullet et al., 2013). To our surprise, we observed no deficits in social approach or memory. Three potential factors may explain differences relative to previous studies: First, many prior rats were tested during juvenile and adolescent period (Kim et al., 2011c; Lin et al., 2013; Barrett et al., 2017), whereas we tested our animals during adulthood. Similarly, one study showed no difference in total social interaction duration in adult rats with prenatal VPA exposure (Schneider and Przewłocki, 2005). Another study showed VPA-exposed adult rats only showed deficits in some, but not all, social behaviors (Markram et al., 2008). Thus, some VPA-induced neurobehavioral abnormalities may recover as rats mature. Secondly, in the present study, rats were singly-housed for 7 days prior to social preference testing to prevent damage to the implant. Social isolation can increase social interaction (Goodell et al., 2017), potentially masking any VPA-induced deficits. Lastly, most prior studies administered VPA via intraperitoneal administration, whereas we used oral gavage, which mimics the exposure route in humans. Thus, route of administration could have unexpected consequences.

Conclusion

In summary, this study revealed a distinct pattern of neural synchrony between the BLA and NAc when processing reward-related cues, specifically, identifying cues that predict food. The synchrony was not observed when social cues was presented. The present findings present new evidence supporting the importance of BLA→NAc coordination in reward seeking. Moreover, we provide a potential neural mechanism of sensing food and foraging, which is crucial for survival of an individual. Future studies that record from multiple brain regions, combined with viral-mediated tools to activate or inhibit the activity of specific circuits, will provide a more complete picture of the reward-processing networks in the brain.

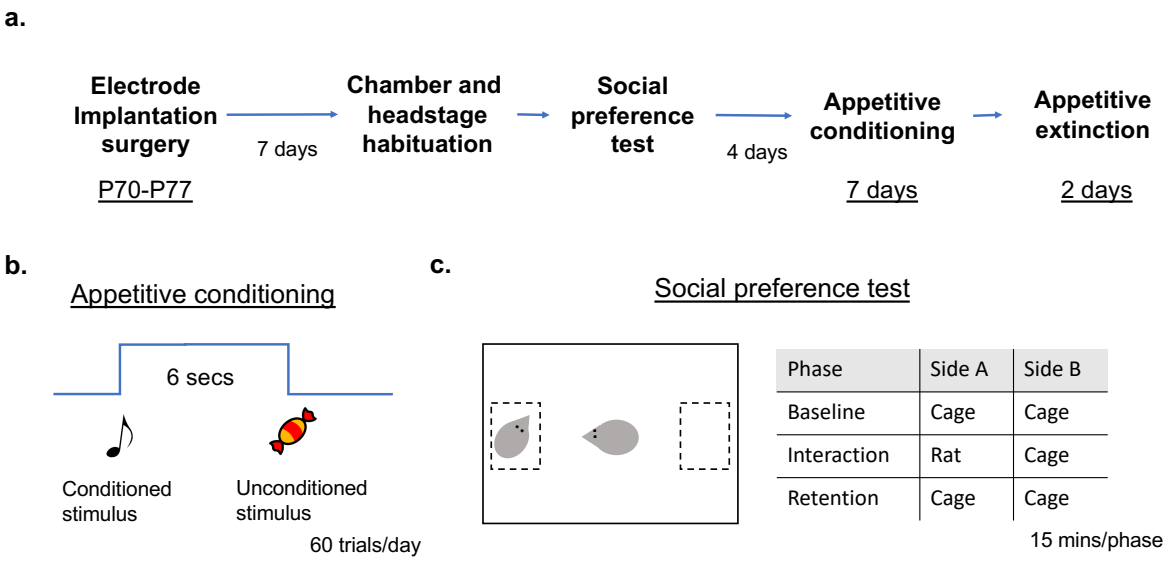

Figure 2-1. Timeline and schematics of appetitive conditioning and social preference testing. (a) Experimental timeline. Control and VPA-exposed rats received electrode implantation surgery targeting the NAc and BLA between postnatal day (P) 70-77. After 7 days of post-surgical recovery, rats underwent social preference testing. Rats were then food restricted to 90% of baseline body weight prior to 7 days of CS-US conditioning and 2 days of extinction training, with 1 session per day. (b) A schematic of an appetitive CS-US conditioning trial. A 6-second tone at 6kHz was used as CS. A food pellet was delivered into a food magazine at the end of a tone, the US. During extinction training, the food dispenser was activated, but food pellets were withheld. A session comprised 60 trials. (c) Schematic of the social preference test. Social preference was assessed in an acrylic chamber with two small wire cages placed at the opposite ends of the chamber. Social preference testing consisted of 3x15-minute phases. In the baseline phase, rats were allowed to interact with two empty wire cages. In the interaction phase, wire cages were replaced with a cage containing a novel adolescent rat and a clean wire cage. In the retention phase, cages were replaced again with two empty wire cages.

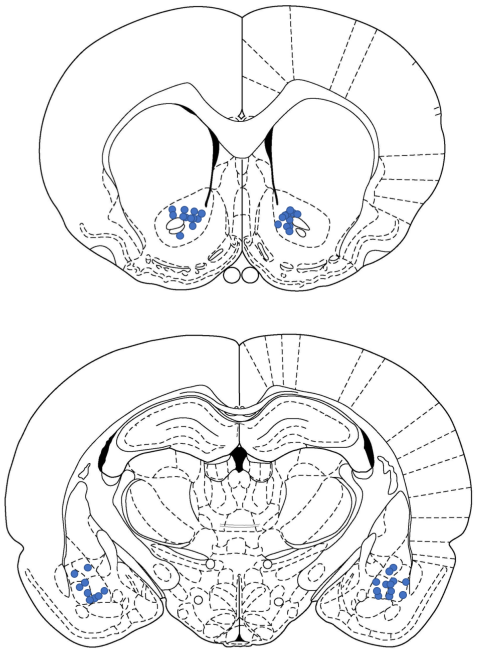

Figure 2-2. Electrode placements in the NAc and BLA. Electrodes were targeted to the NAc (top) and the BLA (bottom) (graph modified from Paxinos and Watson, 2013).

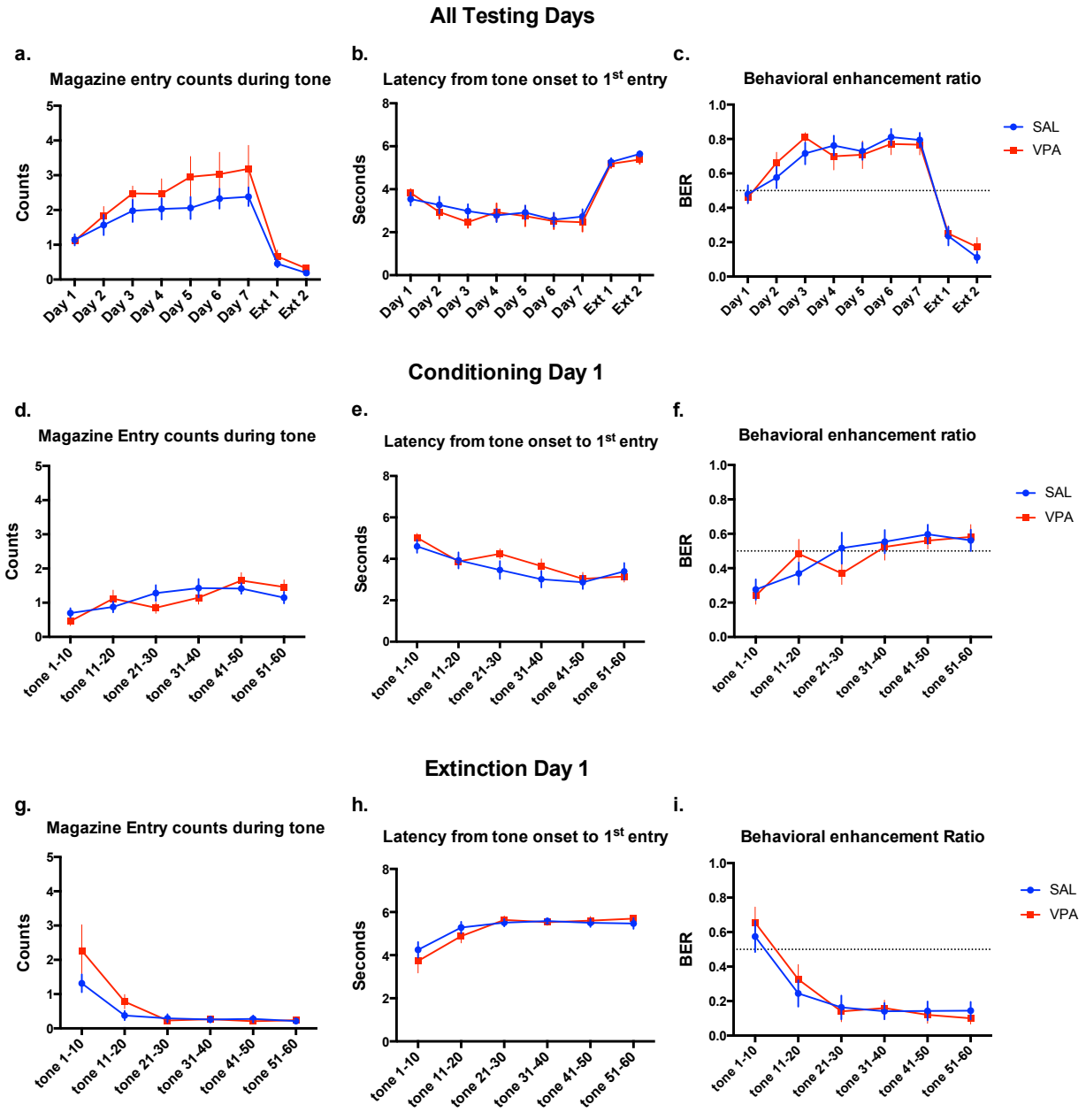

Figure 2-3. Rats learned CS-US associations and extinguished approach when the US was withheld. (a) Rats entered the food magazine progressively more during CS-US pairings and decreased entries during extinction training. (b) Latency of the first magazine entry following tone onset decreased during CS-US training, and it increased in extinction conditions. (c) Meanwhile, BER increased during CS-US pairings and decreased during extinction. (d-f) Within-session plasticity was also observed as early as CS-US conditioning day 1 and (g-h)

extinction day 1. No group differences were observed throughout. Symbols represent means ± SEMs, *$p \leq 0.05$. n = 10-11 per group. “Ext” refers to extinction conditioning session.

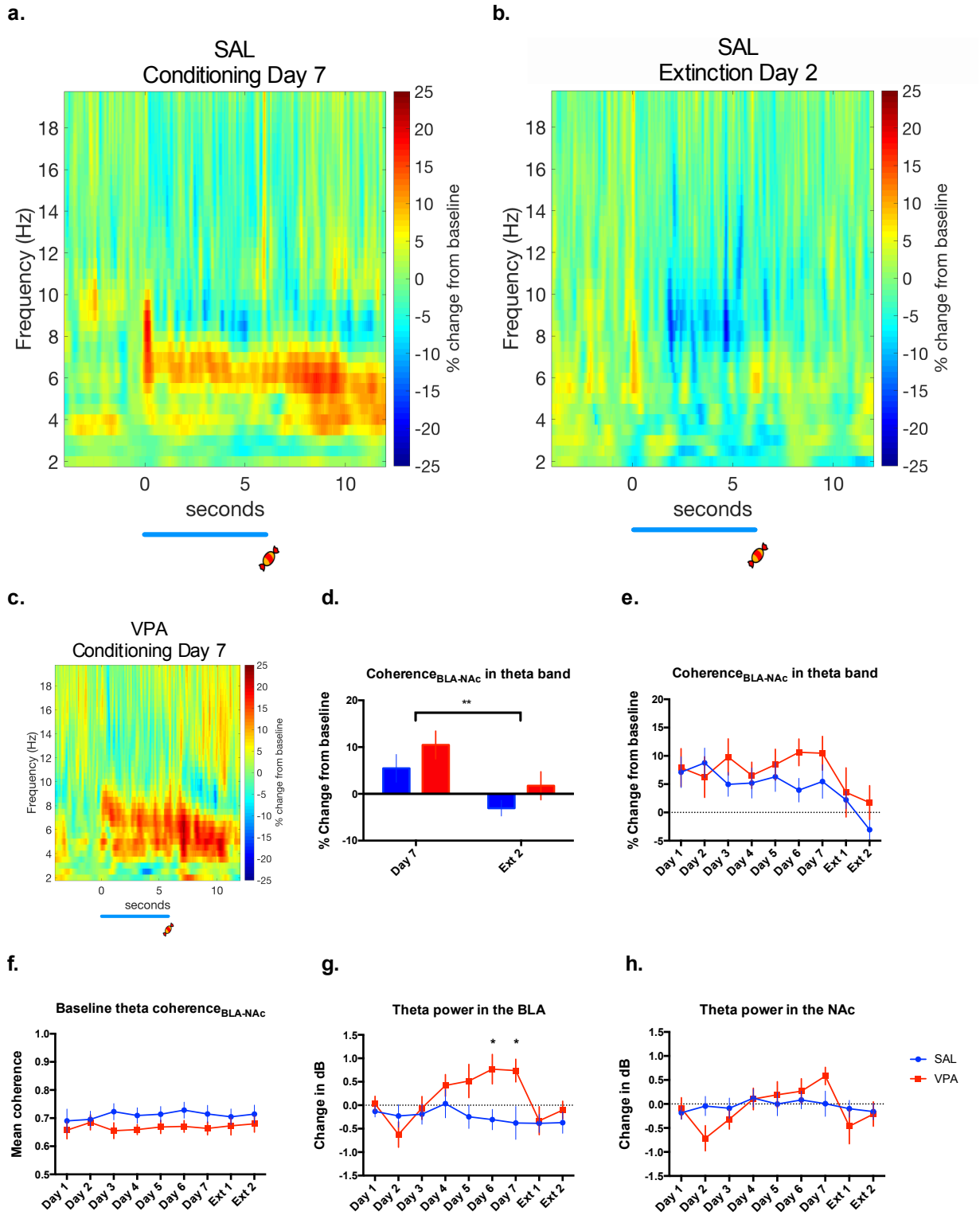
a.
SAL
Conditioning Day 7
Frequency (Hz)
seconds
% change from baseline
b.
SAL
Extinction Day 2
Frequency (Hz)
seconds
% change from baseline
c.
VPA
Conditioning Day 7
Frequency (Hz)
seconds
% change from baseline
d.
Coherence BLA-NAc in theta band
% Change from baseline
**
Day 7
Ext 2
e.
Coherence BLA-NAc in theta band
% Change from baseline
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Ext 1
Ext 2
f.
Baseline theta coherence BLA-NAc
Mean coherence
g.
Theta power in the BLA
Change in dB
h.
Theta power in the NAc
Change in dB
SAL
VPA

Figure 2-4. Enhanced theta coherence between the BLA and NAc during reward-related CS presentations. (a-b) Trial- and subject-averaged coherogram between the BLA and NAc of control rats on (a) CS-US conditioning day 7 and (b) extinction day 2. An increase in the theta band (5-8 Hz) during the CS presentation was observed on CS-US conditioning day 7 but not extinction day 2. (c) Trial- and subject-averaged coherogram of VPA-exposed rats on CS-US conditioning day 7. The same CS-elicited elevation in theta coherence between the BLA and NAc was observed. (d) Quantification of these data further confirms that the change in theta coherence between the BLA and NAc during the CS presentation on CS-US conditioning day 7 was significantly higher than on extinction day 2, with no group differences. (e) When all sessions were analyzed, enhanced theta coherence was observed throughout CS-US conditioning, but not during extinction training, again with no group difference. (f) Baseline theta conference did not change over time. (g-h) By contrast, the strength of theta oscillations in the BLA and NAc changed significantly across sessions. Specifically, theta power in the BLA of VPA-exposed rats on CS-US conditioning days 6 and 7 were significantly higher than in control rats. Symbols and bars represent means ± SEMs, *$p \leq 0.05$, **$p \leq 0.01$. n = 10-11 per group. “Ext” refers to extinction conditioning session.

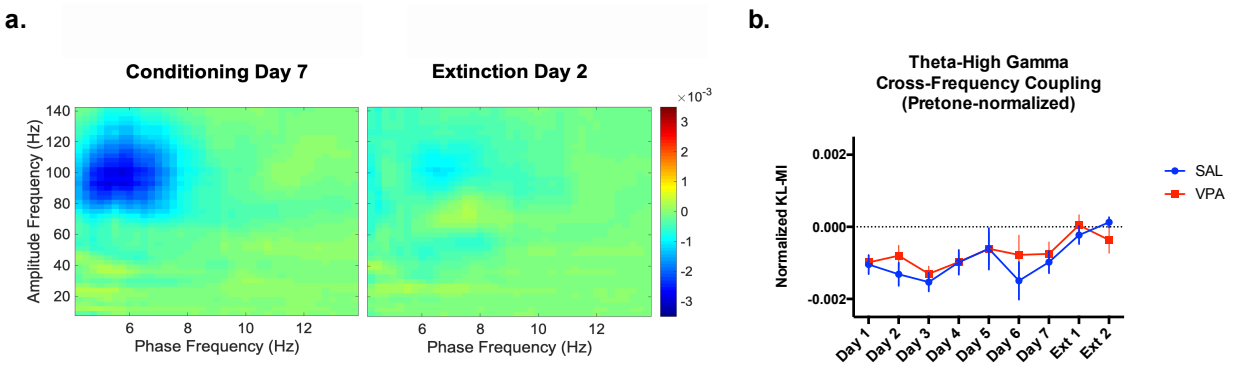

Figure 2-5. Decreased strength of theta-high gamma phase amplitude cross-frequency coupling in the NAc during appetitive conditioning. (a) Trial- and subject-averaged phase-amplitude comodugrams of control rats during the CS presentation on CS-US conditioning day 7 (left) and extinction day 2 (right). The y-axes of the comodugrams represent the power of high frequency, while x-axes represent the phase of low-frequency oscillations. Cool color indicates weaker modulation. Kullback-Leibler modulation indices (KL-MI) were pretone-normalized. Note the theta (5-8 Hz)-high gamma (70-110 Hz) phase amplitude cross-frequency coupling on conditioning day 7, which was not observed on extinction day 2. (b) Normalized KL-MI of theta-high gamma phase amplitude cross-frequency coupling in the NAc during the initial conditioning and then extinction sessions. Post-hoc multiple comparisons (with Bonferroni corrections) revealed that KL-MI on CS-US conditioning day 1 is significantly different from both extinction days 1 and 2, but not other CS-US conditioning days. Symbols represent means ± SEMs, *$p \leq 0.05$. n = 10-11 per group. “Ext” refers to extinction conditioning session.

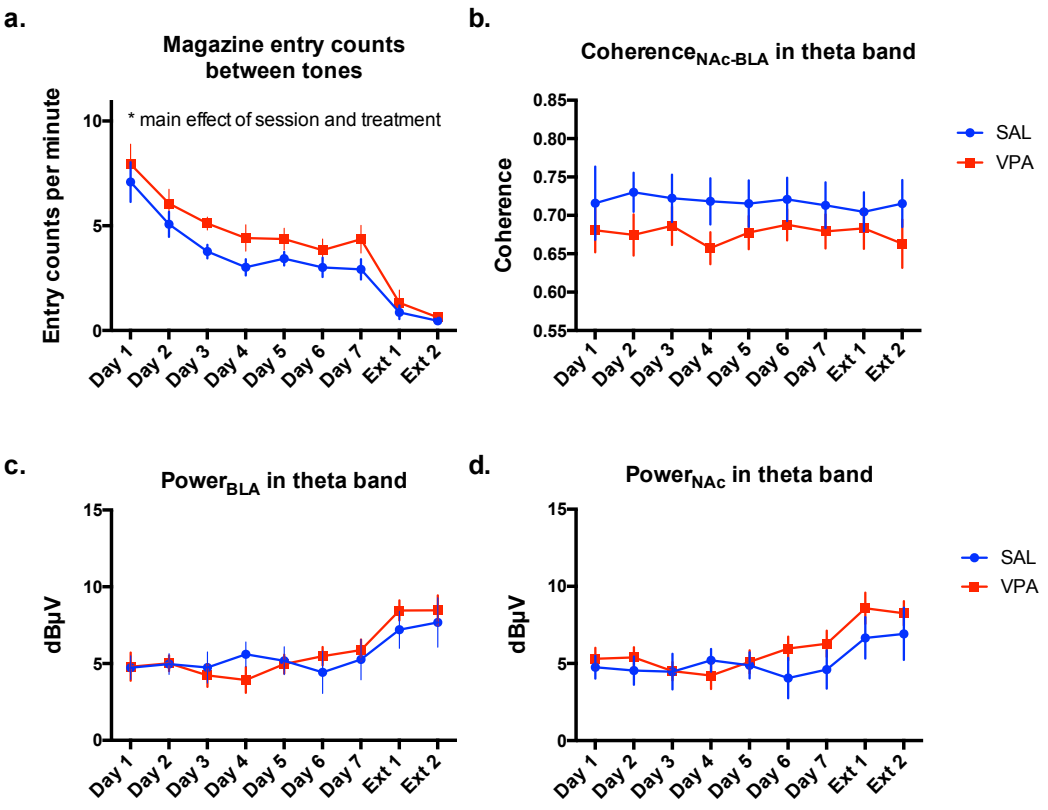

Figure 2-6. Impulsive-like behavior of VPA-exposed rats does not correlate with theta band coherence or power. (a) Magazine entry rates during inactive periods. Rats were penalized for entering the food magazine between tones. Nevertheless, VPA-exposed rats generated higher magazine entry rates compared to control rats, impulsive-like behavior. (b) Theta coherence between the BLA and NAc during the inactive periods was calculated. Unlike during the CS presentation (see again, Fig. 4), theta coherence did not change across training sessions. (c-d) Theta power in the BLA (c) and NAc (d) during inactive periods is also represented. Theta power in both the BLA and NAc during extinction training sessions was higher than during CS-US conditioning. Symbols represent means ± SEMs, *$p \leq 0.05$. n = 10-11 per group. "Ext" refers to extinction conditioning session.

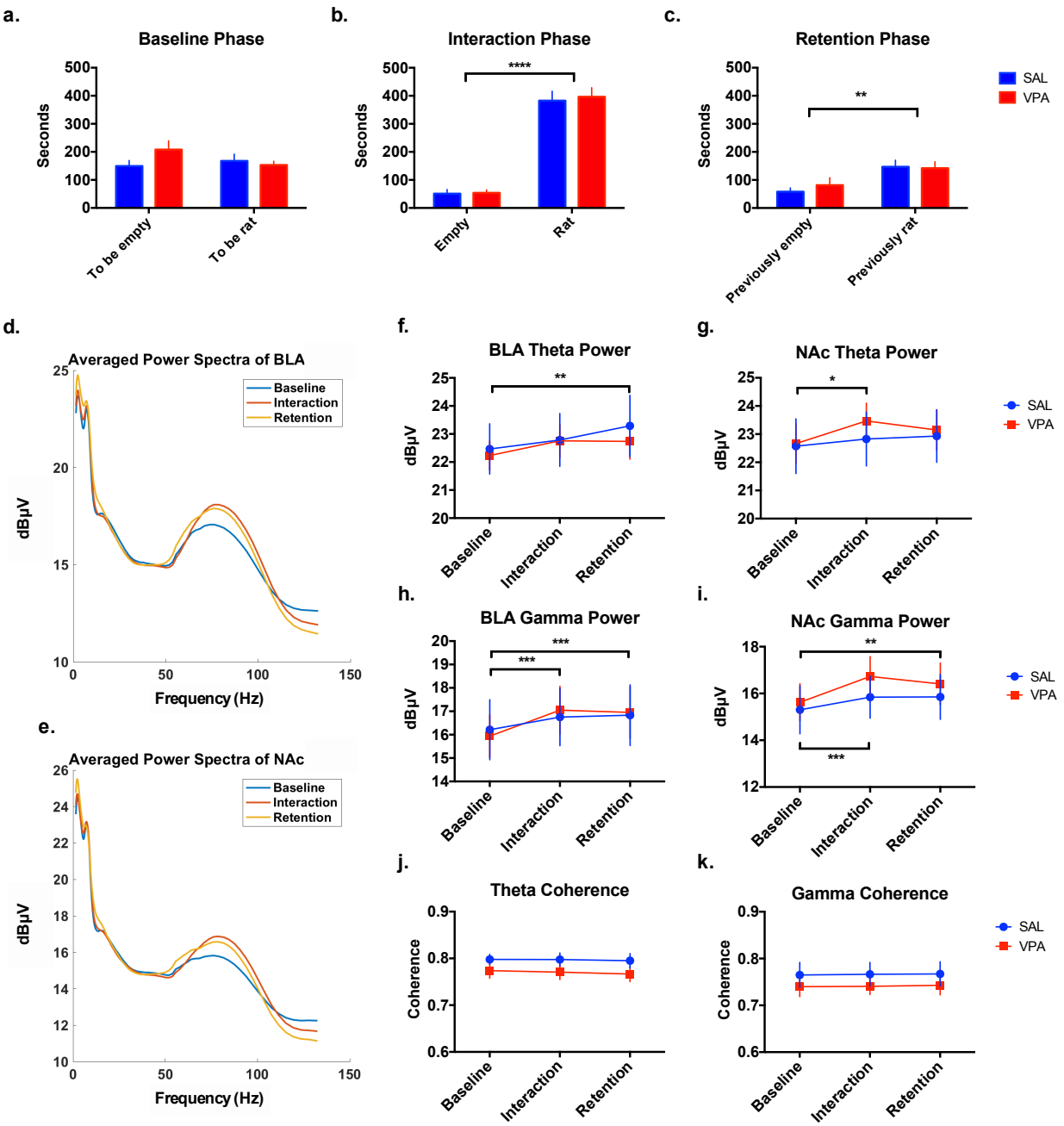

Figure 2-7. Enhanced theta power in, but not coherence between, the BLA and NAc during social preference testing. Rats underwent social preference testing, which consisted of 3 phases: baseline, interaction, and retention. (a) Direct interaction time with the locations that would ultimately contain an empty cage (empty-to-be) *vs.* a conspecific (rat-to-be) during the baseline phase. No side preference was observed. (b) Direct interaction time with empty and rat-

containing cages in the interaction phase. Both group of rats demonstrated a strong preference for the rat-containing cage. (c) Direct interaction time with the previously empty and previously rat-containing sides of the testing chamber in the retention phase. Both group of rats demonstrated a preference for the side of the chamber that had previously contained the rat. (d-e) Power spectra from the BLA and NAc during the 3 phases of the social preference test. Frequency peaks at theta band and gamma band were detected. (f-g) Theta power in the BLA and NAc during social preference test. Theta power in the BLA was higher in the retention phase, and theta power in the NAc was higher in the interaction phase, compared to baseline. (h-i) Gamma power in the BLA and NAc during the social preference test. Gamma power was higher in the interaction and retention phases in both the BLA and NAc, compared to baseline. (j-k) Spectral coherence between the BLA and NAc in the theta and gamma band during social preference testing. We detected no differences. Symbols and bars represent means ± SEMs, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$. n = 10-11 per group.

Chapter 3: Conclusions and Future Directions

SUMMARY AND INTEGRATION OF FINDINGS WITH THE CURRENT LITERATURE

Broadly, the findings described in Chapter 2 provide significant insights into neural synchrony between the basolateral amygdala (BLA) and nucleus accumbens (NAc) when processing reward-related cues. We revealed enhanced coherent neural activity in the theta frequency range (5-8 Hz) between the BLA and NAc, as well as decreased strength in theta-high gamma (50-110 Hz) phase-amplitude cross-frequency coupling (PAC) in the NAc during the presentation of food-associated cues in a Pavlovian approach procedure. Importantly, neither neural activity "signature" was observed upon extinction. Interestingly, theta power in both the BLA and NAc were higher during extinction sessions, suggesting a potential neural mechanism of extinction learning – *i.e.*, that a previously reward-associated cue no longer predicts reward. On the other hand, in social preference tests, theta and gamma power within the BLA and NAc increased when interacting with a social conspecific and stayed elevated after the social conspecific was removed, but the theta synchrony was not observed.

As reviewed in Chapter 1, the BLA integrates sensory information and sends projections to downstream targets, including the NAc, to modulate emotional responses. My discovery of coherent theta oscillation between the BLA and NAc in conjunction with food (unconditioned stimulus; US)-associated tone (conditioned stimulus; CS) presentation is in agreement with considerable evidence showing the BLA→NAc projection plays a critical role in processing positive emotional valance (Ambroggi et al., 2008; Stuber et al., 2011b; Namburi et al., 2015; Beyeler et al., 2016). Furthermore, despite decreased latency enter the food magazine across training sessions (evidence of progressively increasing task proficiency), the degree of theta coherence did not change across sessions, suggesting that theta coherence may correlate solely with the presence and valence of reward, but not between-session learning and memory, motor

function or response efficiency. In contrast, Popescu and colleagues reported enhanced gamma coherence between the BLA and putamen that increased as responding to food-associated cue presentation became more efficient (Popescu et al., 2009), which might reflect the level of reward expectation (or learning – *i.e.*, confidence in reward delivery) or the execution of a reward-motivated motor behavior.

Importantly, 5-8 Hz, the frequency range in which we observed enhanced coherence correlating to reward-related information processing, is distinct from previously reported 2-5 Hz oscillations during conditioned fear expression in the BLA (Lesting et al., 2011; Madsen and Rainnie, 2012; Karalis et al., 2016. Also see Chapter 1). This finding aligns with recent reports of Tye and colleagues characterizing two distinct groups of neurons in the BLA with divergent projections to the NAc and central amygdala (CeA), each having preference for "positive" and "negative" emotional valance, respectively (Namburi et al., 2015; Beyeler et al., 2016).

What might influence BLA and NAc neuron firing? The medial prefrontal cortex (mPFC) projects to the NAc and is bidirectionally connected with the BLA (Vertes, 2004; Hoover and Vertes, 2007). The dynamic connectivity between mPFC-BLA and mPFC-NAc are critical for conditioned fear expression, extinction and drug-seeking behavior (Peters et al., 2009; Herry et al., 2010; Courtin et al., 2014b; Likhtik et al., 2014; Stujenske et al., 2014b; Gourley and Taylor, 2016; Karalis et al., 2016; Goode and Maren, 2018). Both theta and gamma LFP power in the mPFC increase during reward "anticipation" (Donnelly et al., 2014), and activating mPFC→NAc neurons promotes reward-seeking behavior (Otis et al., 2017). The strength of theta-gamma PAC between the mPFC and NAc also predict durations of social interaction (Amadei et al., 2017). Moreover, disconnecting and inhibiting mPFC-NAc projections suppress drug-seeking behavior (Stefanik et al., 2013, 2016; McGlinchey et al., 2016). On the other hand, the entrainment from the mPFC to BLA signals safety (Likhtik et al., 2014; Stujenske et al., 2014b). Future

experiments aimed at exploring the dynamics and causal relationships between multiple brain structures will further clarify the network dynamics of reward processing.

Effects of valproic acid (VPA)

Prior studies utilized *in utero* VPA exposure in rodents to disrupt social behavior and decision making (Favre et al., 2013; Orczyk et al., 2014; Barrett et al., 2017). In Chapter 2, we adopted this model to potentially disrupt social interaction and reward processing. Interestingly, we did not observe deficits in social interaction or learning the CS-US association in an appetitive conditioning task in adult rats. Littermates of the same rats were used in another study, revealing delayed development and deficits in social behavior in when rates were juveniles (Barrett et al., 2017). Specific deficits included: delayed eye opening, decreased maternal approach, abnormal ultrasonic vocalization, and decreased social interaction with a novel conspecific. Taken together, these findings suggest that VPA causes developmental abnormalities detectable early in postnatal life, but they resolve as rats enter adulthood. The differences in behavioral deficits across ages is consistent with previous reports. Developmental and social deficits are commonly reported *before* adulthood in VPA-exposed animals (Vorhees, 1987; Kim et al., 2011c; Lin et al., 2013; Roullet et al., 2013; Tyzio et al., 2014), whereas studies testing VPA-exposed rats *in adulthood* found no or minimal differences in social interaction (Schneider and Przewłocki, 2005; Markram et al., 2008).

Prenatal VPA exposure can also enhance conditioned fear memory, assessed using traditional Pavlovian fear conditioning procedures (Markram et al., 2008; Sui and Chen, 2012). In Chapter 2, we trained rats in a complementary Pavlovian *appetitive* conditioning procedure and also examined head entry counts *during the first 10 tones* in an extinction test, serving as a potential indicator of "reward memory" (Figure 2-3 g). Although statistically non-significant, I

identified a trend, such that VPA-exposed rats entered the magazine more, compared to control rats. Furthermore, similar trends were also observed for head entry latency and behavioral enhanced ratio (BER), which is the ratio of head entries during the tone divided by all head entries. These trends suggest that, in addition to enhancing conditioned fear memory, *in utero* VPA exposure may also stimulate certain forms of Pavlovian appetitive memory. An alternative interpretation, however, is that VPA causes modest defects in the very early phases of extinction conditioning, also considered a form of learning and memory (and not just "forgetting").

IMPLICATIONS AND FUTURE DIRECTION

We are the first, to our knowledge, to report synchronized theta activity between the BLA and NAc in response to reward-related cues. This finding leads to several potential implications and interesting questions, specifically regarding the functional role of theta coherence between the BLA and NAc in processing reward-related information. In the last section of my dissertation, I lay out future directions and discuss potential implications and applications.

Functionality of the BLA-NAc theta coherence

To understand the functionality of BLA-NAc theta coherence in reward processing, I first need to determine whether BLA-NAc theta coherence is *required* for encoding positive emotional valance. The BLA→NAc projection promotes reward-seeking behavior (Stuber et al., 2011b) and is more active when reward-associated cues are presented (Namburi et al., 2015; Beyeler et al., 2016), thus a more specific method is required to disrupt only theta coherence between the BLA and NAc. Optogenetics is a technique that allows researchers to control the activity of specific group of neurons with high temporal precision. By adopting circuit-targeting

optogenetics, I could compare magazine entry behavior of rats during appetitive CS-US conditioning with different stimulation patterns. To target only NAc-projecting BLA neurons, I could deliver bilateral injections of Canine adenovirus type 2 (CAV2) viruses carrying Cre recombinase and Adeno-associated viruses (AAV) carrying a Double-floxed Inverted Orientation (DIO) sequence with channel rhodopsin 2 (ChR2) or control green fluorescence protein (GFP) into the NAc and BLA, respectively. The CAV2 virus would express Cre protein and would be transported retrogradely from the NAc (Junyent and Kremer, 2015) to regions that project to the NAc, including the BLA. Meanwhile, AAV-infected neurons in the BLA would require Cre protein to successfully express ChR2 or GFP (Saunders et al., 2012). Thereby, I would be able to target only NAc-projecting BLA neurons.

In a next step of this hypothetical experiment, after two weeks, rats would receive another surgery implanting optrodes in the NAc and electrodes in the BLA. After a post-surgical recovery period, rats would undergo appetitive conditioning as described in Chapter 2. Optogenetic stimulation of axon terminals of NAc-projecting BLA neurons would start on the 5th session, given that I show in Chapter 2 that food port entry counts, food port entry latency and BER stabilized after the 4th session. Three stimulation protocols would be applied during cue presentation: 6 Hz, 6 Hz scrambled, and 20 Hz. The 6 Hz stimulation should induce "normal" theta coherence. A 6 Hz scrambled group would receive the same amount of light pulses as the 6 Hz stimulation group, but the pulses would be delivered randomly, serving as a control group for light exposure. On the other hand, the 20 Hz stimulation group, referring to a commonly used frequency for optogenetic experiments, would serve as another control group for stimulation frequency to determine whether the effect of stimulation is specific to the theta frequency range. Local field potentials (LFP) in both the BLA and NAc would be recorded simultaneously. I would expect to observe the 6 Hz stimulation group demonstrating stronger responses to the CS

than the 6 Hz scrambled and 20 Hz stimulation group, in the form of higher food port entry counts and decreased latency to enter the food port. Findings from this experiment would provide evidence regarding whether BLA-NAc theta coherence is *necessary* for Pavlovian approach.

I would also want to test whether BLA-NAc theta coherence is *sufficient* for the expression of positive emotional valance. Rats from the experiment described in the previous paragraphs would be used. I would is a conditioned place preference test. Rats would receive 6 Hz, 6 Hz scramble, or 20 Hz blue light stimulation on one side of the chamber, with no stimulation on the other side. I would expect to find that rats prefer the side with 6 Hz stimulation but not 6 Hz scrambled or 20 Hz stimulation.

Potential involvement of mPFC

As mentioned in the previous sections, it is possible that BLA-NAc theta coherence is driven by other brain regions, including the mPFC. Therefore, another path of future directions involves recording LFPs in the mPFC, BLA and NAc to determine whether the mPFC plays a role in reward-related BLA-NAc theta coherence and to crystalize the interactions between mPFC-BLA-NAc in Pavlovian appetitive conditioning. Experimental designs and methods could be mostly identical to those described in Chapter 2, except an extra electrode would be implanted into the mPFC. The analysis would examine theta coherence between mPFC-BLA and mPFC-NAc during CS presentations. I would expect to observe both enhanced theta mPFC-NAc and mPFC-BLA coherence, given that enhanced mPFC-NAc theta coherence was reported during mating in voles (Amadei et al., 2017), as well as enhanced mPFC-BLA low theta (4Hz) coherence during perceived threat with the directionality of mPFC→BLA (Karalis et al., 2016). Furthermore, I might also observe inter-regional theta-gamma PAC between mPFC-NAc and

mPFC-BLA. Directionality can be inferred using granger correlation analysis, computing spike timing and by computing the cross-correlation of instantaneous amplitudes of LFP oscillations (Adhikari et al., 2010a).

Social conditioning and naturalistic social interaction

The reason that we did not observe enhanced BLA-NAc theta coherence in social preference testing – in contrast to the Pavlovian approach procedure – might be attributable to the test itself. Social interaction involves in continuous interaction between stimuli, actions, and outcomes, whereas appetitive CS-US conditioning draws on more clearly delineated stimulus-outcome associations. Enhanced BLA-NAc theta coherence was observed during the stimulus period (CS), while there was no strictly defined equivalent stimulus period in social preference test. Furthermore, the strength of stimulus (sound and scent elicited from the probe animal) fluctuated over time, as we had no control over the vocalization of probe rat and the distance between probe rat and testing animal. Therefore, to further develop this line of research, I would need to create a comparable environment for social and appetitive stimuli. One strategy is social CS-US conditioning, a modified version of a social operant conditioning procedure from Martin and Iceberg (Martin and Iceberg, 2015). The testing chamber for social conditioning contains programmable guillotine door with a wire grid placed in the face of the doorframe. On the other side of the guillotine door is a wired cage that contains a probe rat. During social CS-US conditioning, I would train experimental rats to associate a tone (CS) with a social interaction opportunity (US). The guillotine door would open at the end of the CS (tone) for 10 seconds, allowing the experimental rat to interact with the probe rat through the wire grid. Then, the door would close. By recording LFPs during the CS presentation, I would be able to compare neural activity in the BLA and NAc when rats faced different types of reward-related stimuli and

examine whether theta coherence is also present when social cues are presented. The rats could undergo social isolation for 3 days prior to test to maximize effects. Furthermore, I could also add a food delivery module to the chamber and train rats to learn both a food-associated CS and a social CS. This experiment would enable us to conduct within-subject comparisons of neural activity when rats faced social and food-associated cues.

I would also be interested in recording neural activities during naturalistic social interaction. To achieve this goal, I can take advantage of the EnerCage, a wireless *in vivo* electrophysiology recording homecage system equipped with continuous video recording (Byunghun Lee et al., 2014; Mirbozorgi et al., 2016; Jia et al., 2017). The system was developed by Dr. Maysam Ghovanloo's group at Georgia Institute of Technology and tested at Emory University by Dr. Teresa Madsen and me (see Appendix). With the EnerCage system, I would be able to record neural activities wirelessly with simultaneous recording of naturalistic social interactions in a home-cage environment. Since LFP recording is wirelessly powered and transmitted, I could record LFPs and videos without interfering with rats for 2-3 days. I would analyze LFPs during social interactions in the home cage and compare the results to our findings from the social preference test.

Together, results from the experiments described above would strengthen our understanding of the functional impact of BLA-NAc theta coherence in reward-related processing. Furthermore, these findings would provide important insights into potentially utilizing BLA-NAc theta coherence as a diagnosis marker, as well as developing circuit-specific therapeutic methods, for certain psychiatric and neurodevelopmental disorders.

APPENDIX: PUBLICATIONS TO WHICH THE AUTHOR HAS CONTRIBUTED

1. **Hsu C-C**, Madsen TE, O'Gorman E, Rainnie DG, Gourley SL (in preparation). Reward-related coherence between the basolateral amygdala and nucleus accumbens.
2. Jia Y, Mirbozorgi SA, Wang Z, **Hsu C-C**, Madsen TE, Rainnie DG, Ghovanloo M (2017) Position and orientation insensitive wireless power transmission for EnerCage-Homecage system. IEEE Trans. Biomed. Eng. 64(10): 2439-2449.
3. Zimmermann KS, **Hsu C-C**, Gourley SL (2016) Strain commonalities and differences in response-outcome decision making in mice. Neurobiol. Learn. Mem. 131: 101-108.
4. Jia Y, Wang Z, Canales D, Tinkler D, **Hsu C-C**, Madsen TE, Mirbozorgi SA, Rainnie DG, and Ghovanloo M. (2016) A wirelessly-powered homecage with animal behavior analysis and closed-loop power control. Conf. Proc. IEEE Eng. Med. Biol. Soc. 6323-6326.
5. Rainnie DG, O'Flaherty B, **Hsu C-C**, Abbas A. (2016) "Cellular Physiology of the Basolateral Complex of the Amygdala and its Modulation by Stress." In: *Neurobiology of PTSD: From Brain to Mind*. Oxford University Press.

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