Computational Modeling of the Fear Circuit: A System Approach to Understand Anxiety and Stress Disorder

A Dissertation

presented to

the Faculty of the Graduate School

University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

by GUOSHI LI

Dr. Satish S Nair, Dissertation Supervisor

Dr. Gregory J Quirk, Co-Advisor

DECEMBER 2009
The undersigned, appointed by the dean of the Graduate School, have examined the
dissertation entitled

**Computational Modeling of the Fear Circuit:**
**A System Approach to Understand Anxiety and Stress Disorder**

presented by Guoshi Li,
a candidate for the degree of doctor of philosophy
and hereby certify that, in their opinion, it is worthy of acceptance.

Dr. Sarah S Nair
Dr. Gregory J Quirk
Dr. Dominique Do
Dr. Guilherme DeSouza
Dr. Yosha Nair
ACKNOWLEDGEMENTS

First, I would like to thank my dissertation advisor, Dr. Satish Nair, for his invaluable guidance, insightful advices, and continuous encouragement. It is his direction and support that motivates me to overcome various challenges, improve and excel. He is not only a remarkable advisor but also a good friend.

I would also like to thank my co-advisor, Dr. Gregory Quirk, for his generous support and great contribution to this computational work. His amazing expertise in fear learning provides essential help in this dissertation. My gratitude is extended to Dr. Dominic Ho, Dr. Guilherme DeSouza and Dr. Jyotsna Nair for their suggestions and serving on my committee.

I also greatly appreciate Dr. Dennis Pare for his wonderful collaboration and profound knowledge in neurophysiology and emotional learning.

Many thanks go to my lab fellows, Ashwin Mohan, Sandeep Pendyam, John Ball and Charles Franklin for their help and friendship.

My parents, sisters and my wife Huanjue Fu deserve my most special thanks. I am forever indebted to their love and support.
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ABSTRACT

Computational models are becoming increasingly relevant to systems neuroscience. Models serve as proofs of concepts, tests of sufficiency, and as quantitative embodiments of working hypotheses and are important for understanding and interpreting complex data sets. In fear learning, although there have been a few attempts at modeling emotional learning and memory in the past, most were limited to simplified connectionist or artificial neural network models which did not incorporate current knowledge about the biophysical properties of accurate neurons. This research focused on extending our understanding of the neural mechanisms underlying fear learning and extinction using biophysically realistic network models. Since disruption of the fear circuit is thought to underlie the pathology of post traumatic stress (PTSD) and other anxiety disorders, such models could potentially provide ideas and approaches for the development of new medications.

We initiated modeling of the overall fear circuit starting with the most critical component, the lateral amygdala (LA), and attempted to describe how a single structure (i.e., LA) can encode both acquisition and extinction memories learned during auditory fear conditioning. A conditioning protocol consisting of unpaired/paired tone and shock stimuli was used to train the LA network and synaptic plasticity was determined by intracellular calcium levels, according to the calcium control hypothesis. The LA model provided several important insights including the prediction that extinction, which has been thought to be due to a Hebbian unlearning process, is critically dependent on the potentiation of GABAergic synapses that serve to inhibit the “fear memory” stored in the pyramidal cells.
Next, we developed a biophysical model of another critical element of the fear circuit, the ITC. (intercalated cells) to understand how strongly adaptive BLA (basolateral amygdala) signal can lead to sustained fear expression and to study the impact of infralimbic (IL) vmPFC input in suppressing fear. Model experiments showed that persistent network activity can be maintained in ITC neurons and strongly adaptive fear/extinction signal from BLA can be transformed into more sustained output due to the slow deactivation of an unusual potassium current. They also showed that activation of the IL input effectively increases the responses of ITC neurons which inhibit the central amygdala (CE) output, regardless of the inter-ITC inhibition.

After successful development of component model for the LA and ITC network, an overall amygdala network model was developed to investigate how conditioning-induced potentiation of LA response leads to activation of the CE output, by inclusion of another important unit of the circuit – basal amygdala (BA). The amygdala model is trained by the same conditioning protocol used for the LA network and synaptic plasticity was implemented into all excitatory/inhibitory connections. Results showed that expression of fear was regulated by both the LA-BA-CE and the LA-ITC-CE pathways and interruption of each pathway resulted in impaired fear acquisition or extinction. The model also suggested sites for distributed storage for fear and extinction memories in the amygdala circuit. Furthermore, the model provided insights into neural mechanisms underlying acquisition and extinction, and about the specific role of each amygdala component in fear learning by simulating a series of lesioning experiments.
Chapter 1

Introduction

Anxiety and fear are normal human emotional states that serve an adaptive function. The capacity to efficiently learn about and appropriately respond to cues and contexts that predict or signal danger is critical for survival across species (Rauch et al., 2006). The last decade has witnessed an extraordinary interest in studying the neurobiology of fear conditioning. The relative tangibility of the circuit and the important clinical implications of its study are key factors. The result is an incredibly beautiful picture of different brain areas working in concert to mediate behavioral expressions of fear memory. As the gateway to understand the circuit, researchers used the fear conditioning paradigm, based on Pavlovian classical conditioning, which involves pairing an emotionally-neutral conditioned stimulus (CS) with an innately aversive unconditioned stimulus (US). Animals appear to respond to the US with a constellation of physiological changes collectively known as the unconditioned response (UR). Following pairing of the CS in presentation with the US, the CS comes to elicit a conditional response (CR), which is generally similar to the UR (Pavlov, 1927). Pavlov also noted that after successful
conditioning, repeated presentation of the CS in the absence of US causes conditioned fear responses to rapidly diminish, a phenomenon termed fear extinction (Rescorla 2002, Myers and Davis, 2007). Following extinction training with a series of unpaired CS presentations, the animal would no longer respond to a CS that no longer predicts aversive stimuli (Pavlov, 1927).

Understanding the neural circuitry underlying fear learning and extinction is of great importance to understand the pathophysiology of fear and anxiety disorders, such as post traumatic stress disorder (PTSD). PTSD develops after exposure to a terrifying event or ordeal in which grave physical harm occurred or was threatened. The cardinal triad of symptoms include avoidance e.g., avoiding situations that remind the individual of the traumatic event, reexperiencing (e.g., flashbacks) and hypervigilance (e.g., exaggerated startle response) (Rauch et al., 2006). People with PTSD may startle easily, become emotionally numb (especially in relation to people with whom they used to be close), lose interest in things they used to enjoy, have trouble feeling affectionate, be irritable, become more aggressive, or even become violent. PTSD affects about 7.7 million American adults, but it can occur at any age, including childhood (Davidson, 2000). Studies indicate that patients with PTSD demonstrate delayed extinction learning as compared to controls (Orr et al., 2000; Milad et al., 2008). Disruption of the fear circuit, which includes thalamus, amygdala, prefrontal cortex, and the locus coeruleus, is thought to underlie the pathology of PTSD and other anxiety disorders (Phelps et al., 2004; Corcoran and Quirk, 2007). While much of the neural data originated in rodent studies, recent brain imaging studies in humans show that homologous areas of ventral mPFC show both morphological and functional abnormalities, suggesting that extinction circuits
are compromised in PTSD (Quirk et al., 2006). A computational model of the fear circuit would enable the integration of such findings and provide valuable insights into the pathology of PTSD, and also provide ideas for potential treatments (Anderson et al., 2004). Our goal, therefore, is to integrate diverse neurophysiology data into a biophysical computational framework and analyze possible neural mechanisms from a systems perspective. The ultimate goal of this computational study is to model pathologies associated with the fear circuit (e.g., PTSD) and assist in the development of new treatments.

![Neural circuitry of fear conditioning and extinction.](image)

Fig. 1.1. Neural circuitry of fear conditioning and extinction. The sensory information is delivered to LA via the medial division of medial genicular body (MGm) or via posterior intralaminar nucleus (PIN). The auditory cortex (Te3) also receives sensory inputs from the thalamus and projects to BL. Output from the LA projects to the central nucleus (CE) through inter-calated cells (ITC) or BL neurons. The medial part of ITC neurons (ITC2) receives excitatory inputs from the BL and gating inputs from the mPFC. The hippocampus holds context information and projects to LA and mPFC.

### 1.1 Neural circuitry of fear conditioning

The amygdaloid complex is an essential component of the fear circuit. It is located within the medial temporal lobe and plays a critical role in the acquisition and expression of learned fear associations (LeDoux, 2000; Maren and Quirk, 2004). The amygdala is
subdivided into different parts including the lateral nucleus (LA), the basal nucleus (BL), and the central nucleus (CE) (Price et al., 1997). Lateral nucleus of the amygdala is the sensory-receptive region, and is particularly important in mediating fear conditioning (LeDoux, 1995). In auditory fear conditioning, the tone (CS, auditory information) and foot-shock (US, somatosensory information) inputs are delivered to LA from the auditory cortex and auditory thalamus (Bordi and LeDoux, 1994, Quirk et al., 1997). Individual neurons within the LA respond to both auditory and somatosensory stimuli, suggesting convergence of CS and US inputs at the cellular level (Sigurdsson et al., 2007). The potentiated impulses from LA then project to the CE, both directly and indirectly, via the BL and intercalated (ITC) cells, and then to the brain stem and hypothalamic sites, eliciting the fear response (Fig. 1.1; Paré et al., 2004). Consistent with the anatomical data, lesioning or functionally inactivating the LA prior to training leads to deficits in fear conditioning (LeDoux et al., 1990; Goosens and Maren, 2001), suggesting that the LA is critically involved in the formation and storage of conditioned fear memories (LeDoux, 2000; Maren, 2001; Sigurdsson et al., 2007).

1.2 Neurobiology of fear conditioning

An important goal of the neurobiological analysis of fear conditioning is to identify the essential substrate for the encoding and storage of fear memories (i.e., CS-US associations) (Maren, 2001). As mentioned earlier, the amygdaloid complex (LA in particular) is a widely accepted site for mediating fear learning and expression. Below we briefly review the recent experimental findings in cellular neurophysiology, synaptic
plasticity and neuronal activity in the LA during training. Then we describe the cellular hypothesis proposed for fear conditioning.

1.2.1 Electrophysiological properties of LA neurons

There are two main types of neurons within the LA and the BL: pyramidal-like glutamatergic projection neurons, and local circuit GABAergic interneurons (Faber and Sah, 2002). Principal neurons in the LA exhibit a range of firing properties in response to prolonged current injection (Fig. 1.2; Faber et al., 2001). Most of these pyramidal cells (~60%) show full frequency adaptation, firing only a few spikes in response to a sustained depolarization. About 30% of LA pyramidal cells show clear spike frequency adaptation during the first 5-10 action potentials, and ~10% fire repetitively with little accommodation in response to a depolarizing current step (Faber et al., 2001). This difference in firing patterns is largely due to the amplitude of the after-hyper-polarization (AHP) following spike trains (Faber et al., 2001).

![Fig. 1.2. Previously reported responses of LA pyramidal cells to prolonged current injection (600 ms). Rows 1, 2, and 3 show a large depolarization, small depolarization, and hyperpolarization, respectively. A-C: Firing patterns for three cells that show the extremes (A and C) and middle (B) of the range of spike frequency adaptation reported in LA (adapted from Faber et al., 2001).](image-url)
About 25% of the LA neurons are inhibitory interneurons (McDonald and Augustine, 1993). Compared to pyramidal cells, the interneurons have faster action potentials with a half-width of 0.76 ± 0.04 ms (1.2 ± 0.1 ms for pyramidal cells) and do not show appreciable frequency adaptation (Mahanty and Sah, 1998). The resting potential is about -69.4 mV and the steady-state firing frequency at 0.4 nA current injection is about 80 Hz (Szinyei et al., 2003).

1.2.2 Synaptic transmission in the LA

The afferent projections from the auditory thalamus and cortex to the amygdala form excitatory synapses on principal neurons and on inhibitory interneurons (Mahanty and Sah, 1998; Weisskopf and LeDoux, 1999). Glutamatergic synaptic transmission to pyramidal cells and interneurons is mediated by both AMPA and NMDA receptors (Weisskopf and LeDoux, 1999; Szinyei et al., 2003) (but see Mahanty and Sah, 1998 for interneurons). Excitatory AMPA- or NMDA- synaptic conductance is involved in both the pyramidal-to-pyramidal and pyramidal-to-GABAergic cell connections (Smith et al., 2000; Szinyei et al., 2000). GABAergic interneurons send inhibitory GABA_A recurrent collaterals onto pyramidal cells, which tightly control their activity (Li, et al., 1996; Lang and Paré, 1997). Though there are far fewer interneurons than pyramidal cells, one interneuron can inhibit many pyramidal cells through divergent projections (Mahanty and Sah, 1998). The processing and transmission of excitatory inputs to the principal cells along neural pathways in the amygdala are determined by both feedback and feed-forward GABAergic inhibition (Wang et al., 2001).
1.2.3 Fear conditioning increases the CS responses in the LA

The important role of the LA in encoding and storing fear associations has been supported by electrophysiological recordings of amygdaloid neuronal activity during fear conditioning. Quirk et al. (1995) described the experimental tone responses of pyramidal neurons in the LA recorded simultaneously at different points during the training process. The main finding in that study was that conditioning significantly increased the number of tone-elicited spikes, with the greatest effects at the shortest latency following tone onset. Furthermore, these conditioned responses were reversed in late extinction (Fig. 1.3). In a subsequent study (Quirk et al., 1997), it was shown that LA neurons exhibit plasticity earlier than auditory cortical neurons in training, further suggesting that the direct thalamo-amygdala pathway, rather than the cortico-amygdala pathway, mediate neuronal plasticity in the LA.

1.2.4 Fear conditioning induces LTP in the LA

Learning of conditioned fear is accompanied by changes in synaptic strengths in the neural circuitry of LA. Long-term potentiation (LTP) has been demonstrated in slice preparations for both the cortico-amygdala pathway (Huang and Kandel, 1998; Tsvetkov et al., 2002) and the thalamo-amygdala pathways (Huang et al., 2000; Tsvekov et al., 2002, 2004). Induction of LTP in both pathways depends on postsynaptic processes, because postsynaptic depolarization is needed to trigger influx of Ca$^{2+}$ ions via NMDA receptors (Bauer et al., 2002; Tsvekov et al., 2002) or voltage-dependent L-type Ca$^{2+}$ channels (Weisskopf et al., 1999; Bauer et al., 2002). In vivo, LTP can be induced in both thalamic and cortical inputs when tone and footshock are paired (Rogan and LeDoux,
1995), but not when they are unpaired (Rogan et al., 1997). Excitatory glutamatergic input to interneurons from the thalamus or cortex can also express LTP and this is NMDA-receptor-dependent (Szinyei et al., 2003; Bauer and LeDoux, 2004). It was further shown that inhibitory inputs onto pyramidal cells are also modifiable in a Ca²⁺-dependent manner (Mahanty and Sah, 1998; Bauer and LeDoux, 2004). In addition to LTP, long-term depression (LTD) can be readily induced in excitatory amygdala synapses as shown by low-frequency stimulation of the lateral nucleus at 1 Hz for 15 min (Wang and Gean, 1999).

1.2.5 A cellular hypothesis of fear conditioning

Based on the fear conditioning circuitry and experimental evidence, a specific cellular hypothesis has been proposed that fear conditioning is mediated by an increase in the

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**Fig. 1.3.** The tone responses of simultaneously recorded neurons in LA at different points during training (Quirk et al., 1995) (A) Representative time histograms showing 5 neurons from LAd simultaneous recorded prior to conditioning (sensitization), 1 hr after conditioning (early extinction), and following 30 extinction trials (late extinction). Bin width is 10 ms and 10 trials are summed at each phase. (B) A difference time histogram showing the conditioned responses of the 5 cells. Spike counts during sensitization were subtracted from counts during early extinction.
strength of synapses that transmit CS information to principal neurons in the LA (Rogan and LeDoux, 1995; Blair et al., 2001; Maren, 2001; Sigurdsson et al., 2007). Prior to conditioning, the CS inputs are relatively weak and unable to elicit fear response. However, when CS and US are paired during conditioning, strong depolarization caused by the US strengthens the CS inputs according to Hebbian learning so that the CS is capable of driving the LA projection neurons effectively to produce fear.

1.3 Cellular mechanisms of fear extinction

Unlike fear conditioning, the neural mechanisms of fear extinction are not well understood, and a neural analysis of extinction and inhibition is still in its infancy (Quirk and Mueller, 2008). Some psychological theories described extinction as an “unlearning” process due to a violation of the CS-US association established in fear acquisition (Rescorla and Wagner, 1972). For example, an early hypothesis is that extinction could result from a depression of potentiated synapses and LTD may be the cellular mechanism for extinction (Teyler and Discenna, 1984; Goldman et al., 1990). This “unlearning” view has been challenged by the observation that fear spontaneously recovers with the passage of time after extinction (Bouton and King, 1983; Quirk et al., 2002). In addition, exposure to US alone or other stressful stimuli following extinction leads to the "reinstatement" of the CS-US association expression (Pavlov, 1927; Bouton and Bolles, 1979). These observations have led to an alternative theory which proposes that extinction does not erase the CS-US association, but instead forms a new memory that inhibits conditioned responding (Pavlov, 1927; Bouton and King, 1983; Quirk et al., 2002). In such a case, a different site would store the new extinction memory; it could
reside in excitatory neurons in the vicinity of the amygdale which inhibit the principal cells in LA via an extra-amygdaloid circuit, or in the inhibitory interneurons in the LA (Falls et al., 1992).

1.3.1 LA is also a site of plasticity for fear extinction

Given the central role of the LA in the acquisition and expression of fear memory, it is not surprising that that this structure may be a site of inhibition in extinction (Myers and Davis, 2002; Hobin et al., 2003; Corcoran and Quirk, 2007). As shown in Fig. 1.3, the firing activity of LA pyramidal cells returns back to pre-conditioning levels with extinction training (Quirk et al., 1995). Falls et al. (1992) showed that extinction of fear was blocked by infusion of an NMDA antagonist (AP5) into the amygdala. In a more recent study, Sotres-Bayon et al. (2007) demonstrated that both initial acquisition and retention of fear extinction memories requires activation of NR2B-containing NMDA receptors in the LA. Furthermore, fear extinction requires intracellular signaling cascade leading to new protein synthesis in the BLA (LA & BL) (Lu et al., 2001; Herry et al., 2006). One such synthesized protein, gephyrin, clusters GABA receptors near the synapse, thereby increasing their inhibitory effect. The level of gephyrin goes down during fear conditioning, and then increases to baseline values with extinction learning (Chhatwal et al., 2005). The return to baseline level of gephyrin is associated with an increase in the surface expression of GABA_A receptors, corresponding to more inhibitory neurotransmission in the amygdala (Harris and Westbrook, 1998).

1.3.2 vmPFC is required for extinction recall
Besides LA, the vmPFC is identified to be a strong candidate for the storage of extinction memory. Morgan et al. (1993) showed that rats with vmPFC lesions were able to acquire fear conditioning normally, and were able to extinguish normally early after the extinction training. However, they showed deficits in recalling extinction 24 hours later. A similar finding was also reported by Quirk et al. (2000), suggesting that vmPFC plays a crucial role in the consolidation of across-sessions extinction recall. Following initial lesion studies, convincing evidence came from electrophysiological recordings of neuronal activity. Recordings from single neurons of the infra-limbic part of the rat vmPFC showed that these neurons responded to CS presentation 24 hours after initial extinction training, but not during acquisition or extinction training (Milad and Quirk, 2002). Moreover, electrical stimulation of the vmPFC in rats decreased the expression of fear response (Milad and Quirk, 2002). Revisiting the intra-amygdalar circuits, it is noted that vmPFC sends projections that heavily innervate the ITC of the amygdala (Fig. 1.1). This organization places the vmPFC in a suitable position to signal extinction memory through its excitatory projections to the ITC cells; and by modulating the activity of the inhibitory ITC neurons, the transmission of information from LA to CE can be tightly controlled (Quirk et al. 2003).

1.4 Contextual modulation of fear and extinction memory

Two interesting phenomena shed light on contextual-modulation of the fear response. One is the context-shift effect that causes a decrease in CR when the animal encounters the CS in a context other than the context of the original conditioning training (Gordon et al., 1981). The other phenomenon is renewal, the expression of a CR to a CS in a context other than the context of initial extinction training (Bouton and King, 1983; Harris et al.,
An interesting observation was that the CR renewed by changing context is not as strong as the CR exhibited by subjects that have never received extinction training (Bouton and King, 1983), which strongly suggests that extinction is only partly context-specific (Bouton et al. 2006). Several behavioral studies of renewal facilitated understanding the nature of context modulation. The initial thoughts were that context is just a second CS that acquires excitatory or inhibitory associations with fear response. Current evidence provides a more complex picture where direct association between context and US is neither sufficient nor necessary to modulate fear response. The context appears to influence the meaning of the CS, whereas the CS-US association is activated in one context and the CS-no US is activated in another (Bouton, 1993).

In contrast to the discrete CS information (e.g., tone) which reaches the amygdala through the sensory thalamus and cortex, contextual information is transmitted to the amygdala via multisensory brain areas. It has been shown that the hippocampus is responsible for assembling contextual representations and transmitting these representations to the amygdala for association with US (Fig. 1.1; Maren, 2001). The hippocampus also sends dense projections to vmPFC (Fig. 1.1; Conde et al., 1995; Tierney et al., 2004), and the hippocampus-vmPFC connection may be a site of LTP for extinction recall (Farinelli et al., 2006). Consistent with this, inactivation of the hippocampus prior to extinction results in deficient recall of extinction the following day (Corcoran et al., 2005), an effect similar to vmPFC inactivation. Besides, electrolytic lesions of the dorsal hippocampus prior or after extinction training impaired the renewal of conditioned response when the context was changed (Ji and Maren 2005). All this suggests a more complex interplay between the amygdala and the vmPFC along with the
hippocampus as an extended circuit that regulates maintenance and expression of extinction memory (Fig. 1.1). A current hypothesis is that these three areas work in concert to suppress the expression of fear memory to extinguished stimuli in the right context (Corcoran and Quirk, 2007; Milad et al., 2007).

1.5 Computational modeling of fear: A review

Computational models have long been used to study emotional learning and memory. Earlier models attempted to explain a wide range of different complex behavioral responses with a post-hoc identification of the underlying neural circuit subserving them and were not constrained well by neuroanatomical findings (e.g. Grossberg & Schmajuk, 1987). Some researchers also attempted to apply artificial neural network models to building intelligent control systems by associating the CS and US stimuli occurring at different time points (e.g. Grossberg and Schmajuk 1989). However, the behavior responses of these models offered little insights for the development of useful control systems due to a lack of biological inspiration (Chester, 1990).

Armony and co-workers developed an anatomically constrained neural network model of fear conditioning based on known physiological data (Armony et al., 1995). They started from a very specific and well known circuit involved in fear conditioning and attempted to provide a link between the circuit level description and observed physiological and behavioral changes as a result of learning. Similar to the previous connectionist models, this model also focused on areas of convergence of CS and US pathways, but specifically examined information processing via the two parallel sensory pathways to the amygdala from the auditory thalamus and the auditory cortex. The model considered tone input with a specific frequency (CS) associated with a mild foot-shock
(US) and was trained by a modified Hebbian-type learning rule. The model was able to reproduce data related to frequency-specific changes of the receptive fields known to exist in the auditory thalamus and amygdala. Extinction and other related phenomena were not included in the model. In a following study (Armony et al., 1997), the model was used to simulate processing capacity of the thalamo-amygdala pathway by making lesions of the auditory cortex. The model predicted that lesions of the cortical pathway would not affect the specificity of the behavioral response to a range of frequencies centered on the training frequency, and was consistent with experimental observations.

Balkenius and Morén (2001) proposed a model for emotional learning dependent on classical conditioning which relied on crude representations and mathematically oriented circuits. The neural network model focused on the amygdala and the orbitofrontal cortex and their interactions. The amygdala was the locus of conditioning acquisition and the orbitofrontal cortex was the site for extinction learning. The model was successful in demonstrating basic phenomena related to emotional conditioning including acquisition, extinction, blocking, and habituation. More recently, Burgos and Murillo-Rodríguez (2007) used a neural-network model to simulate two context-dependent phenomena in Pavlovian conditioning: context specificity and renewal. Prior to that, the computational framework was used to simulate a wide range of conditioning phenomena such as reacquisition savings, reinforcement reevaluation, and latent inhibition (Burgos 2003).

A common criticism of the connectionist models described above is that the elementary units are too simplistic in their input-out function to represent fully the complex behavior of actual cells, though they captures some of the response
characteristics of real neurons (Armony et al., 1997). To fully investigate the neural mechanisms of emotional learning and memory, biologically realistic models are required.

1.6 Specific objectives and structure of the dissertation

The dissertation research aims to develop a biophysical computational framework for the simulation of fear acquisition, extinction and recovery. The framework will include all the important structures involved in fear learning and regulation (Fig. 1.1) and allows easy simulation of experimental manipulations such as lesioning and investigating the effects of new medications. An important objective is to investigate and analyze the underlying neural mechanisms of fear acquisition and expression from a systems perspective including adaptation, learning and control features. The ultimate goal of this computational study is to model pathologies associated with fear.
with the fear circuit (e.g., PTSD) and assist in the development of new treatments.

The dissertation includes 6 chapters (Fig. 1.4). The overall problem with specific objectives is introduced in Chapter 1. In Chapter 2, we discuss how computational models help to understand the underlying principals of the brain in the intra-cellular, cellular and network levels. A case study of the fear circuit modeling is given to illustrate how model predictions give new insights into drug development. The development of the computational framework employs a bottom-up approach, starting with modeling of the core unit of the fear circuit, the LA. Chapter 3 describes the LA network model and its predictions. The LA model address three critical issues of relevance to the fear learning community: (1) how can fear and extinction memory be encoded in a single structure (i.e., LA); (2) what is the neural mechanism of extinction? (3) how can fear spontaneously recover? Recent data indicates that the intercalated (ITC) neurons play an important role in expression of fear and extinction by controlling the impulse traffic between the input (BLA) and output (CE) of the amygdala. Also, experiments find that activation of the infra-limbic (IL) vmPFC inputs dramatically reduced the responsiveness of CE, presumably through ITC cells, yet the underlying mechanisms remain elusive. To shed light on the role of IL in fear inhibition, we develop a biophysical ITC network model in Chapter 4 and study how the intrinsic properties and synaptic mechanisms act together to shape the network responses to BLA and IL inputs. After the LA and ITC networks are completed, we develop another important component of the fear circuit – BL and connect all these three components together with the CE output in Chapter 5. In the overall amygdala circuit, we specifically investigate three critical problems: (1) how do plasticity in ITC and CE regulate conditioned fear? (2) the role of BL in acquisition and expression
of fear and extinction; and (3) how are fear/extinction memories distributed stored in the amygdala circuit? Chapter 6 summarizes the main findings of the computational model and discusses future work. The appendix has copy of my other publication not directly related to the work here. A copy of my CV is attached at the end of this dissertation.

1.7 References


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Chapter 2

Computational modeling: A tool for new psychiatric medication development*

Computational models are being used in a variety of medical applications including drug discovery research where genomic and proteomic software tools facilitate modeling complex intracellular pathways. Increasing understanding of brain functioning due to advances in basic neuroscience techniques and imaging modalities has led to the emergence of computational modeling as an important tool for studying brain mechanisms and circuits. Recent advances in the areas of cellular neurophysiology and synaptic plasticity permit the development of biophysically realistic models that more closely approximate learning, both at the network and membrane levels. Such models have the potential to enhance a psychiatrist’s understanding of brain circuits with applicability to a range of phenomena from the mechanism of action of drugs to the neural basis of mental illness. An example case is provided in the fear circuit to illustrate the ability of a model to provide insights into possible sites for the storage of auditory fear and extinction memories. Since disruption of the fear circuit is thought to underlie the pathology of post traumatic stress and other anxiety disorders, such a model could potentially provide ideas and approaches for the development of new medications.

* This work was done with J Nair, GJ. Quirk, and SS Nair and published in Psychiatric Annals 38(4): 296-305, 2008.
2.1 Introduction

The human brain is a dynamic system made up of about 100 billion neurons connected in complex circuits and synapses, humming with continuous electrical and chemical activity. It is divided into different areas including the evolutionary older parts of the central nervous system and the newer parts such as the cortex. Most of the normal and pathological changes in brain processes can be attributed to changes in transmission and excitability of neurons in specific circuits connecting these areas. Treatment modalities such as psychiatric medications lead to changes in the excitability of neurons either through the direct action of neurotransmitters on membrane channels, or through indirect action via receptors triggering a cascade of intracellular reactions. Such reactions typically modulate release of neurotransmitters by the presynaptic neuron, or modulate the excitability of the postsynaptic neuron, and ultimately control the target symptoms (Fig. 2.1; [1]).

Rapid advances in basic neuroscience techniques including single cell recordings and microdialysis continue to add to our understanding of brain function at intracellular and cellular (membrane) levels. Structural neuroimaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) have, at the same time, made possible high resolution visualization at the network/systems level of brain regions in patients. Functional imaging techniques provide more detailed understanding of brain function with real time images using techniques such as functional Magnetic Resonance Imaging fMRI, Positron Emission Tomography (PET) and single photon-emission computed tomography (SPECT). Major advances such as these, both in basic neuroscience and in neuroimaging techniques, continue to enhance our understanding of
the neural basis of mental illness and of treatment modalities. In most cases, however, findings are limited to specific mechanisms/processes/cells, and fail to generalize to higher brain circuits and regions.

Figure 2.1. Major pathways for the modulation of synaptic transmission (adapted from [1]).

2.2 Modeling

A computational model can combine different types of information related to a system using mathematical equations, and then describes the system’s response to prescribed inputs. In neuroscience, such models are typically of two types: phenomenological models using connectionist (e.g., artificial neural network) and statistical schemes, and biophysical models which attempt to model the underlying biological mechanisms directly. Biophysical models are typically either at the intracellular level (e.g., of gene
interactions, pathways, [2]), cellular level (e.g., of cell firing patterns, effect of blockers/drugs on channel conductances, etc.), or network/systems level (e.g., of interconnected neurons in the fear circuit, the case study reported in this paper), or may include several of these levels. The most powerful approach is to combine the cellular and network levels, to model regional interactions with biological realism.

Computational modeling is a tool that has been effectively used to integrate information related to different aspects of a problem, and to provide testable predictions, in a variety of disciplines. For instance, computational modeling is presently an indispensable part of the design of airplanes, e.g., Boeing 777. For an airplane, such a model would integrate the complex mathematical equations describing different types of dynamics such as the air flow, vibrations, and response of the control surfaces, and then predict their effect on responses such as ride quality. Computational models have now become indispensable for the airplane designer because they enable rapid and inexpensive evaluation of a variety of ‘what if’ scenarios, including the effect of design changes. It is argued that increased understanding of the functional organization of the brain requires integration of similar mathematical/statistical equations from molecular, cellular and network level studies, something that can be facilitated by computational models [3]. For instance, recent technical advances have resulted in a rapid accumulation of information on intracellular signaling pathways and relationships to long-term neuronal changes [4]. Computational techniques and tools are being developed to model such mechanisms with increasing accuracy and are found to be essential to generate an understanding of the underlying functions in such cases [3,5]. Indeed, drug discovery and development teams now include computational techniques as an important tool in their repertoire. The term
'computational neuropharmacology' has recently been proposed for the application of computational modeling to drug development, drug discovery and the modeling of the mechanisms of action of psychiatric drugs [6].

2.3 Software

Software exists presently to model systems in neuroscience at typically only one of the levels, either molecular, cellular, or network/systems level. One reason for this is the large difference in both temporal and spatial complexities between the levels. The reader is referred to [2] for a discussion of software related to molecular level modeling. Here we briefly discuss software relevant to cellular and network level modeling, followed by an illustration of their usage in modeling fear learning.

Once the mathematical equations/model for the neuron or network are developed (case study below provides more details), the values of the biophysical parameters in the model have to be determined systematically. In addition to research articles, sources for such information include databases such as CellPropDB, NeuronDB and ModelDB (http://neuron.duke.edu/). After collecting information pertaining to biophysical parameters, one can make use of public domain software packages available for modeling neurons and networks.

Computational modeling platforms at the cellular and network levels range from general purpose software such as Matlab (http://www.mathworks.com/) which directly model the mathematical equations, to special purpose public domain ones such as GENESIS [7] and NEURON [8] which are being designed for biologists, and require minimal understanding of the underlying mathematics. Figure 2.2 shows the hierarchical
structure used for modeling in GENESIS. The packages can perform simulations of models ranging from single neurons to complex networks representing brain circuits. For example, Leblois et al. [9] use a systems level NEURON model to explain the pathology in the basal ganglia circuit with Parkinson’s disease.

![Network Diagram]

Figure 2.2. Elements in GENESIS are organized in a tree structure [7]. The symbol ‘I’ represents current, e.g., $I_{Na}$ is the sodium current. The network comprises cells (pyramidal and interneurons here), which in turn consist of soma and dendrites populated with the various current channels. The software developers aim to provide these as ‘LEGO’ blocks facilitating the process of model development.

### 2.4 Case Study: Modeling acquisition and extinction of fear

Post Traumatic Stress Disorder (PTSD) is a condition that involves exposure to trauma followed by symptoms of avoidance, reexperiencing and hypervigilance. Studies indicate that patients with PTSD demonstrate delayed extinction learning as compared to controls. Disruption of the fear circuit, which includes thalamus, amygdala, prefrontal cortex, and the locus coeruleus, is thought to underlie the pathology of PTSD and other anxiety disorders [10]. While much of the neural data originated in rodent studies, recent brain
imaging studies in humans show that homologous areas of ventral mPFC show both morphological and functional abnormalities, suggesting that extinction circuits are compromised in PTSD [11]. A computational model of the fear circuit would enable the integration of such findings and provide insights into the pathology of PTSD, and also provide ideas for potential treatments [12].

The amygdaloid complex is an important component of the fear circuit. It is located within the medial temporal lobe and is critical in the acquisition and expression of learned fear [13,14]. The amygdala is subdivided into different parts including the lateral nucleus (LA), the basal nucleus, and the central nucleus (CE) [15]. Lateral nucleus of the amygdala is the sensory-receptive region, and is particularly important in mediating fear conditioning [16]. In auditory fear conditioning, the tone (conditioned stimulus, CS) and foot-shock (unconditioned stimulus, US) inputs are paired in experiments. The impulses from thalamus and cortex converge in LA, which then projects to the central nucleus via intercalated (ITC) cells, and then to the brain stem and hypothalamic sites, eliciting the fear response (Fig. 2.3, [17]). Following conditioning, repeated presentation of tone without shock leads to an extinction of the fear response [18,19]. Although the neural mechanisms of fear extinction are not well understood, it is now generally accepted that extinction does not erase the CS-US association, but instead forms a new memory that inhibits the original fear conditioned response [20,21]. The lateral nucleus of the amygdala has been proposed as a site of inhibition in extinction [22]. In addition, recent studies have identified the medial prefrontal cortex (mPFC) as an important part of the neural circuit for fear extinction [11,23]. Since deficits in extinction learning are thought to underlie PTSD [24,25], a model that could integrate these findings would be an important tool for studying such circuits.
We initiated model development of the overall fear circuit using a bottom-up approach starting with the core unit, the LA, as a first step. Our future goal is to use the overall model to determine potential sites in the fear circuit for the storage of fear and extinction memories in PTSD and to elucidate the underlying mechanisms. We illustrate below the potential of a computational model to provide insights into fear acquisition and extinction mechanisms, using a simple two cell model.

**Figure 2.3. Auditory fear conditioning.** The tone information is delivered to LA via the medial division of medial geniculate body (MGm) and the shock information is delivered to LA via posterior intralaminar nucleus (PIN). The tone input to LA is potentiated when tone and shock are paired. Output from the LA projects to the central nucleus (CE) through inter-calcated cells (ITC) or BL neurons, eliciting a fear response.

**2.4.1 Single cell characteristics**

There are two main types of neurons within the LA and the basal nucleus: pyramidal-like glutamatergic projection neurons, and local circuit GABAergic interneurons [15]. Principal neurons in the LA exhibit a range of firing properties in response to prolonged current injection [26]; accordingly two types of pyramidal cells were modeled, type A with strong frequency adaptation and type B with medium frequency adaptation. The interneuron was modeled as a basket-type, fast-spiking, aspiny cell with each compartment containing a fast Na⁺ current and a delayed rectifier K⁺ current with different kinetics from those of pyramidal cells to reproduce the much shorter spike
duration [27]. Similar to pyramidal cells, interneurons can also receive excitatory glutamatergic inputs from the thalamus and/or the cortex, and inhibitory inputs from other local interneurons. For each cell, the excitatory \(\alpha\)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and the N-methyl D-aspartate (NMDA) channels were placed in the dendrite compartment, and the inhibitory \(\text{GABA}_A\) channels were placed on the soma. Figure 2.4 provides details of the pyramidal cell and interneuron models with the various ionic and synaptic channels.

Figure 2.4. Two-cell model of pyramidal cell and interneuron with ionic and synaptic channels. Each cell model has soma (spherical) and dendrite (cylindrical) compartments with each having the specific current channels shown. The \(\text{Ca}^{2+}\) pools involved in the learning algorithm implemented are also depicted. Both cells receive afferent inputs (tone and shock) via AMPA/NMDA synapses. In addition, the interneuron receives excitatory input from the pyramidal cell and provides feed-forward/feedback inhibition to the pyramidal cell.

2.4.2 Using GENESIS to model a single cell

Single cell models were developed [28] for the pyramidal cell and the interneuron (Fig. 2.4) using the GENESIS software package. The modeling process involves several steps
where different ‘LEGO blocks’ are defined such as the soma or cell body of the neuron, the dendrite, the axons and the membrane channels (Fig. 2.2). The software provides a virtual environment, making most of the mathematical details transparent to the user. The model parameters are then iteratively adjusted, within biophysical bounds, to match certain baseline characteristics. The membrane potential responses to different levels of current of the pyramidal cell and interneuron models (Fig. 2.5) showed characteristics consistent with experimental observations, for both depolarizing and hyperpolarizing current cases [26].

Figure 2.5. A: Membrane potential responses of pyramidal cell model to three 600 ms current injections starting at 200 ms (top: 400 pA; middle: 300 pA; bottom: 100 pA); B: Responses of interneuron cell model to 400 pA (top) and 200 pA (bottom) current injections. These estimates matched experimental recordings [27].

2.4.3 Synaptic connections are sites of plasticity

Learning of conditioned fear leads to changes in synaptic strength in the neural circuitry in LA. Three different excitatory glutamatergic LA synapses that are capable of
strengthening (long term potentiation, LTP) or weakening (long term depression, LTD) with training [30,31] are the following: (i) thalamic/cortical auditory tone synapses to pyramidal cells or interneurons, (ii) synapses between the pyramidal cells themselves, and (iii) pyramidal cell to interneuron synapses. In addition, LTP can be induced in GABAergic inhibitory synapses from interneurons to pyramidal cells [32].

After modeling single cells as described above, a network model consisting of multiple cells, synaptic connections, and inputs, can be developed using GENESIS. We illustrate such a development using the two-cell model schematic in Fig. 2.4. In the model, both the pyramidal cell and the interneuron received direct afferent tone/shock inputs via synaptic connections. The pyramidal cell was inhibited by the interneuron via a GABAergic synapse. The pyramidal cell, on the other hand, excited the interneuron via excitatory AMPA synapses. Both the cells received random background inputs that represent afferent connections from other brain areas such as prefrontal cortex and hippocampus. The frequency and strength of the random inputs was adjusted to obtain pyramidal cell spontaneous firing rates of less than 1 Hz [29].

2.4.4 Model training and predictions

We implemented LTP/LTD in these synapses [26] using an NMDA-based learning rule, and then ‘trained’ the model with the fear conditioning protocol used in experiments [33]. After training the model was probed to determine the synaptic sites for the storage of fear and extinction memories.

Figure 2.6A shows the membrane potential responses of a pyramidal cell and interneuron for a segment of the training cycle. This segment consisted of two tones (500
ms each) and two shocks (100 ms each) with the second shock present in the last 100 ms of the second tone. Both tone and shock excited the cells, with shock input having a stronger effect. Embedded in a more complex system, this unit of two cells illustrates how conditioning and extinction can be learned. Due to Hebbian strengthening between tone inputs and shock inputs, the tone input to the pyramidal cell strengthens during conditioning and is maintained throughout extinction. In the interneuron, on the other hand, tone inputs strengthen during extinction phase, due to Hebbian pairing between different sets of tone inputs. This causes inhibition of pyramidal excitation and reduction in fear behavior. Consistent with behavioral findings, the fear memory is not lost during extinction, but is suppressed by LTP like potentiation of the interneuron. This is illustrated schematically in Fig. 2.6B.

![Fig. 2.6A. Membrane potential responses for pyramidal cell (top panel) and interneuron (lower panel) to a segment of the training trial. In the segment the input consists of a series of two tones (green bars) and two shocks (red bars) with the second tone paired with the second shock.](image)
2.4.5 Overall fear circuit model

The authors are presently extending the model to include other regions including the ITC, CE, pre-frontal cortex and hippocampus which could also have sites for LTP/LTD in fear acquisition and extinction. Once completed, such an overall model would be useful for studying disruptions associated with the fear circuit, leading to PTSD and anxiety disorders. For instance, studies have shown that in humans with PTSD there is a delay in acquisition of extinction as compared to controls [34]. With the model, one can modify parameters to predict changes in the fear circuit that could be correlated with a delay in acquisition of extinction. These parameters would then point to the changes in the circuit in PTSD and provide insights into the pathology of the illness. The model would also shed light on therapeutic approaches such as cognitive restructuring that could provide a new emotional significance to a negative cognition and reducing physiological arousal [35].

Figure 2.6B. Schematic showing the connections between synaptic strengthening/weakening and behavior. The training protocol had four phases: SENS- unpaired tone/shock; COND – paired tone/shock; a gap with no tone or shock; and EXT – tone alone.
2.4.6 Potential application to drug development

Another application of such a model would be in the development of psychiatric medications for PTSD. The computational model predicted that three types of glutamatergic synapses (including NMDA type) and one type of GABAergic synapse could be involved in storing fear and extinction memories. These predictions seem to be consistent with recent experimental findings, two of which are listed here: (i) A partial NMDA agonist D-cycloserine has been shown to facilitate extinction of fear conditioning in rats [36]. D-Cycloserine was also effective in treating Social Anxiety Disorder and acrophobia in combination with psychotherapy [37,38]. The mechanism of action of D-Cycloserine and other drugs acting on the glutamatergic system can now be modeled at the receptor and at the cellular level in the specific LA neurons indicated by the model; (ii) NMDA receptors in the amygdala activate an intracellular signaling cascade leading to new protein synthesis [39-41]. One such synthesized protein, gephyrin, clusters GABA receptors near the synapse, thereby increasing their inhibitory effect. The level of gephyrin goes down during fear conditioning, and then increases to baseline values with extinction learning [21]. The return to baseline level of gephyrin is associated with an increase in the surface expression of GABA_A receptors, corresponding to more inhibitory neurotransmission in the amygdala [42]. Drugs that impact these mechanisms would have potential for the treatment of PTSD and anxiety disorders. Finally, the cannabinoid receptor CB1 has been shown to modulate GABAergic neurons in the amygdala and facilitate extinction [43]. A similar strategy could be used in humans. This is consistent with the model prediction that the inhibitory synapse from the interneuron to the pyramidal cell could be a site for the storage of extinction memory.
2.5 Conclusion

Computational models can predict how a complex system evolves with experience. A new class of models incorporates biophysical realism with known synaptic connectivity, to more effectively model the learning brain. Such models integrate the intracellular and cellular levels of neuroscience with the network/systems level to provide a coherent picture of the higher level functions in health and disease (e.g., behavior, symptom). A case study of auditory fear conditioning was used to illustrate the ability of a computational model to provide insights into the neural causes of disruptions in the fear circuit thought to underlie symptoms of PTSD and anxiety disorders. Such insights have the potential to aid in drug discovery research, by allowing scientists to test predictions about the cellular and behavioral effects of new drugs.

2.6 References


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Chapter 3

A biologically realistic network model of acquisition and extinction of conditioned fear associations in lateral amygdala neurons*

The basolateral amygdala plays an important role in the acquisition and expression of both fear conditioning and fear extinction. To understand how a single structure could encode these “opposite” memories, we developed a biophysical network model of the lateral amygdala (LA) neurons during auditory fear conditioning and extinction. Membrane channel properties were selected in order to match waveforms and firing properties of pyramidal cells and interneurons in LA, from published in vitro studies. Hebbian plasticity was implemented in excitatory AMPA and inhibitory GABAA receptor-mediated synapses to model learning. The occurrence of synaptic potentiation vs. depression was determined by intracellular calcium levels, according to the calcium control hypothesis. The model was able to replicate conditioning- and extinction-induced changes in tone responses of LA neurons in behaving rats. Our main finding is that LA activity during both acquisition and extinction can be controlled by a balance between pyramidal cell and interneuron activations. Extinction training depressed conditioned synapses and also potentiated local interneurons, thereby inhibiting the responses of pyramidal cells to auditory input. Both long-term depression and potentiation of inhibition were required to initiate and maintain extinction. The model provides insights into the sites of plasticity in conditioning and extinction, the mechanism of spontaneous recovery, and the role of amygdala NMDA receptors in extinction learning.

* This work was done with SS Nair and GJ Quirk. It was published in Journal of Neurophysiology 101: 1629-1646, 2009.
3.1 Introduction

It is well established that the amygdaloid complex plays an important role in the acquisition and expression of learned fear associations (Davis 2006; LeDoux 2000; Maren and Quirk 2004). Recent data indicate that it also plays a key role in extinction of those memories (Falls et al. 1992; Herry et al. 2006; Laurent et al. 2008; Sotres-Bayon et al. 2007). Given that extinction is itself a learning process, an important goal is to understand how a single structure can encode both acquisition and extinction memories. To address this, we employed a computational modeling approach, incorporating known biophysical and connectivity properties of lateral amygdala neurons, to predict learning-induced changes in the responses of single-units to conditioned stimuli. Our overall goal is to bridge biophysical and network modeling approaches in order to gain insight into how the amygdala solves the “problem” of extinction, and ultimately how it interacts with other structures to regulate fear expression.

The components of the amygdala that are critical for fear conditioning are the lateral nucleus (LA), the basal nucleus (BL), and the central nucleus (CE) (Maren 2001). LA is widely accepted to be a key site of synaptic events that contribute to fear learning (Paré et al. 2004; Sigurdsson et al. 2007). There are two main types of neurons within the LA and the BL: pyramidal-like glutamatergic projection neurons, and local circuit γ-aminobutyric acid (GABA)-ergic interneurons (McDonald 1984). In auditory fear conditioning, convergence of tone (conditioned stimulus, CS) and foot-shock (unconditioned stimulus, US) inputs in LA leads to potentiation of CS inputs, resulting in larger tone responses in LA (Blair et al. 2001). Increased LA responses are relayed to the CE via the basal nuclei (Pitkanen 2000), and the intercalated (ITC) cell masses (Paré et al. 2004), eliciting fear
responses via successive projections to brain stem and hypothalamic sites (LeDoux 2000). As a result, rats learn to freeze to tones that predict foot-shock.

Once acquired, conditioned fear associations are not always expressed. Repeated presentation of the tone CS in the absence of the US causes conditioned fear responses to diminish rapidly, a phenomenon termed fear extinction (Myers and Davis 2007; Rescorla 2002). The neural mechanisms of fear extinction are not well understood, and a neural analysis of extinction and inhibition is still in its infancy (Delamater 2004; Quirk and Mueller 2008). Some psychological theories described extinction as an “unlearning” process due to a violation of the CS-US association established in fear acquisition (Rescorla and Wagner 1972). This unlearning view has been challenged by the observation that fear recovers spontaneously after extinction. An alternative theory proposes that extinction does not erase the CS-US association, but instead forms a new memory that inhibits conditioned responding (Bouton and King 1983; Quirk 2002).

Given the central role of the LA in the acquisition and expression of fear memory, it has been proposed that this structure may be a site of inhibition in extinction (Hobin et al. 2003; Myers and Davis 2002; Sotres-Bayon et al. 2004).

Computational models have long been used to understand emotional learning and memory, and to explain a wide range of behavioral responses (e.g., Grossberg and Schmajuk 1987). Armony et al. (1995) developed an anatomically constrained neural network (connectionist) model of fear conditioning based on single-unit recording data. Focusing on areas of convergence of CS and US pathways, tone inputs with a specific frequency (CS) were associated with a mild foot-shock (US). Using simplified computational units, a neural network model of the thalamo-cortico-amygdala circuitry
was constructed and trained using a modified Hebbian-type learning rule (Armony et al. 1995). The model was able to reproduce data related to frequency-specific changes of the receptive fields known to exist in the auditory thalamus and amygdala, but extinction and other related phenomena were not simulated. Balkenius and Morén (2001) proposed a neural network model for emotional conditioning. The model focused on the amygdala and the orbitofrontal cortex and their interaction; the amygdala was the locus of acquisition and the orbitofrontal cortex was the site for extinction learning. The model simulated basic phenomena related to emotional conditioning including acquisition, extinction, blocking, and habituation. The main drawback of such connectionist models is that the elementary units for cells are not biophysically realistic, and therefore cannot model the underlying neural processes responsible for learning. What is needed is a model that incorporates recent advances in cellular neurophysiology and synaptic plasticity.

The electrophysiological and morphological properties of LA/BL neurons have been characterized in a number of studies (Faber et al. 2001; Faber and Sah 2002, 2005; Lang and Paré 1998; Paré et al. 1995; Washburn and Moises 1992a, b; Womble and Moises 1993). There are several in vitro and in vivo recording studies of LA neurons during fear conditioning and extinction (McKernan and Shinnick-Gallagher 1997; Ono et al. 1995; Paré and Collins 2000; Quirk et al. 1995, 1997; Repa et al. 2001). Our goal is to integrate diverse neurophysiology data into a biophysical computational framework and analyze possible neural plasticity mechanisms from a systems perspective.

Starting with experimentally validated biophysical single cell models, we developed an LA network consisting of eight pyramidal cells and two GABAergic interneurons.
Hebbian-type plasticity was implemented in the AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and the GABA<sub>A</sub> receptor synapses involved in fear learning. We showed that LA can learn both conditioning and extinction. Fear memory is not erased fully by extinction, but is inhibited by interneurons which undergo synaptic plasticity during extinction training. We also evaluated the contribution of depotentiation in pyramidal excitatory synapses, and of N-methyl-D-aspartic acid (NMDA) receptors to extinction learning. The model predicts that fear memory is stored in the pyramidal cells, and extinction memory is stored in both interneurons and pyramidal cells.

### 3.2 Methods

#### 3.2.1 LA pyramidal cell model

Principal neurons in the LA exhibit a range of firing properties in response to prolonged current injection (Faber et al. 2001). Most of these pyramidal cells (~60%) show full frequency adaptation, firing only a few spikes in response to a sustained depolarization. About 30% of LA pyramidal cells show clear spike frequency adaptation during the first 5-10 action potentials, and ~10% fire repetitively with little accommodation in response to a depolarizing current step (Faber et al. 2001). This difference in firing patterns is largely due to the amplitude of the afterhyperpolarization (AHP) following spike trains (Faber et al. 2001). To represent the range of firing properties, three different types of pyramidal cells were modeled: type A with full adaptation, type B with medium adaptation, and type C with weak adaptation. All cells had the same ionic currents but differed in maximal conductance densities for some currents responsible for frequency accommodation (Table 1 in the Appendix).
The LA pyramidal cell model had two compartments representing a soma (diameter of 15 µm; length of 15 µm) and a dominant apical dendrite (diameter of 5 µm; length of 400 µm) (Faber et al. 2001; Washburn and Moises 1992b). Both compartments had a leakage current ($I_L$), a spike-generating sodium current ($I_{Na}$), a potassium delayed rectifier ($I_{DR}$) and a high-voltage activated Ca$^{2+}$ current ($I_{Ca}$). Three calcium-activated potassium currents exist in LA principal neurons: (i) fast BK Ca$^{2+}$- and voltage-dependent C-type current ($I_C$), (ii) apamin-sensitive, small-conductance (SK) current ($I_{AHP}$), and (iii) a slow apamin-insensitive, voltage-independent afterhyperpolarization current ($I_{sAHP}$), contributing to fast, medium and slow AHP, respectively (Faber and Sah 2002). The current $I_{AHP}$ is not included in this model since blocking the medium AHP current has no effect on discharge frequency or on spike frequency adaptation (Faber and Sah 2002). The slow AHP current plays a key role in spike frequency adaptation (Sah 1996) and is thought to localize to the proximal apical dendrite (within ≈ 200 µm of the soma) to interact with the inhibitory postsynaptic potentials (IPSPs) (Sah and Bekkers 1996). In addition, an $\alpha$-dendrotoxin ($\alpha$-DTX)-sensitive, slowly inactivating voltage-gated K$^+$ current ($I_D$) mediated by Kv1.2-containing channels also controls spike frequency adaptation in LA pyramidal cells and is selectively expressed in the apical dendrite rather than the soma (Faber and Sah 2004, 2005). $I_D$ differs from $I_{sAHP}$ in that it determines the initial frequency, while the main effect of $I_{sAHP}$ occurs only after a few action potentials (Faber and Sah 2005). Basolateral amygdala (BLA, which includes LA and BL) neurons also exhibit a pronounced and slow depolarizing sag in response to hyperpolarizing current pulses and this is mediated by two hyperpolarization-activated currents ($I_{H}$ and $I_{IR}$) (Womble and Moises 1993). Since $I_{IR}$ has a threshold for activation near -85 mV, it
does not contribute to the resting potential of pyramidal cells (Womble and Moises 1993) and thus is not included in the model. The $I_H$ current is included only in the dendrite compartment because its density in the soma is much lower than in the apical dendrite (Magee 1998). Activation of muscarinic cholinergic receptors produces a long-lasting depolarization and enhanced excitability of BLA neurons, demonstrating the existence of a voltage-gated persistent muscarinic current ($I_M$) (Washburn and Moises 1992a). $I_M$ also contributes to medium AHP (Storm 1989) and to spike frequency adaptation in BLA neurons (Washburn and Moises 1992a). Eqs. 1 and 2 represent the membrane voltage equation for the two compartments (Fig. 1),

$$C_s \frac{dV_s}{dt} = -g_{Ls}(V_s - E_{Ls}) - g_v(V_s - V_d) - \sum I^\text{int}_s - \sum I^\text{syn}_s + I_s$$

(1)

$$C_d \frac{dV_d}{dt} = -g_{Ld}(V_d - E_{Ld}) - g_v(V_d - V_s) - \sum I^\text{int}_d - \sum I^\text{syn}_d + I_d$$

(2)

where $V_s$ and $V_d$ are the somatic and dendritic membrane potentials (mV), $I^\text{int}_s$ ($I^\text{int}_d$) and $I^\text{syn}_s$ ($I^\text{syn}_d$) are the intrinsic currents and the synaptic currents in the soma/dendritic compartments respectively, $I_s$ ($I_d$) is the electrode current applied to the soma (dendrite), $C$ is the membrane capacitance (in the model $C_d = C_s$, and $g_{Ld} = g_{Ls}$) and $g_c$ is the coupling conductance between the soma and the dendrite. The passive properties of the model were adjusted to reproduce the input resistance ($R_{IN}$), membrane time constant ($\tau_m$), and resting potential ($V_{rest}$) of LA pyramidal neurons recorded in vitro. The values for the specific membrane resistance, membrane capacity and cytoplasmic (axial) resistivity were, respectively, $R_m = 30$ K$\Omega$-cm$^2$, $C_m = 1.0$ $\mu$F/cm$^2$, and $R_a = 150$ $\Omega$-cm. The leakage reversal potential ($E_L$) was set to -75 mV. The resulting $V_{rest}$ is about – 69.5 mV (empirically measured mean is – 69.5 mV; Washburn and Moises 1992b), $R_{IN} \sim 150$ M$\Omega$
(matching the empirically measured mean of ~ 150 MΩ; Faber et al. 2001), and \( \tau_m = R_mC_m = 30 \text{ ms} \) (~ 29 ms empirically; Faber et al. 2001).

CURRENT KINETICS. Pyramidal neurons in the BLA are remarkably similar in morphological and electrophysiological characteristics to pyramidal neurons in the hippocampus and cerebral cortex (Washburn and Moises 1992a, 1992b). Thus, most of the conductance kinetics in our model were adapted from computational studies on CA1 hippocampal neurons by Warman et al. (1994) and from prefrontal cortex (PFC) models by Durstewitz et al. (2000): \( I_{Na}, I_{DR}, I_{Ca} \), and \( I_C \) kinetics were adapted from Durstewitz et al. (2000), and those for \( I_M \) and \( I_SAHP \) from Warman et al. (1994). The mathematical description of \( I_H \) was based on a study of the current in the rat BLA neurons (Womble and Moises 1993) and the current \( I_D \) was taken from Locke and Nerbonne (1997) who studied this current in rat visual cortical cells with the activation kinetics adjusted to fit LA pyramidal cells (Faber and Sah 2004). The ionic current for channel \( i \), \( I_i \), was modeled as \( I_i = g_i m^p h^q (V - E_i) \), where \( g_i \) was its maximal conductance, \( m \) its activation variable (with exponent \( p \)), \( h \) its inactivation variable (with exponent \( q \)), and \( E_i \) its reversal potential. The kinetic equation for each of the gating variables \( x \) \((m \text{ or } h)\) takes the form

\[
\frac{dx}{dt} = \frac{x_i(V,[Ca^{2+]]) - x}{\tau_x(V,[Ca^{2+]])}
\]

where \( x_i \) is the voltage- and/or \( Ca^{2+} \)- dependent steady state and \( \tau_x \) is the voltage- and/or \( Ca^{2+} \)- dependent time constant. The maximal conductances for all ionic currents and the expressions of \( x_i \) and \( \tau_x \) for each gating variable are listed in Tables 1 and 2 in the appendix. The reversal potentials were: \( E_{Na} = 45 \text{ mV} \), \( E_K = -80 \text{ mV} \), \( E_{Ca} = 120 \text{ mV} \), and \( E_H = -43 \text{ mV} \) (Huguenard and McCormick 1992).
CALCIUM DYNAMICS. Different Ca\(^{2+}\) ionic pools (Warman et al. 1994) were modeled for ionic and synaptic currents (Fig. 1). For instance, there were two in the dendrite of the pyramidal cell for ionic currents, one mediating the activation of \(I_c\) ([\(Ca^{2+}\)]\(_1\)) and the other mediating the activation of \(I_{sAHP}\) ([\(Ca^{2+}\)]\(_2\)). This is because \(I_c\) must deactivate rapidly following an action potential to generate the fast AHP, while \(I_{sAHP}\) should activate gradually after each spike for the slow afterhyperpolarization. The intracellular calcium for each pool is regulated by a simple first-order differential equation (Warman et al. 1994) of the form

\[
\frac{d[Ca^{2+}]_i}{dt} = -f_i \frac{I'_{Ca}}{zFV} + \frac{[Ca^{2+}]_{rest} - [Ca^{2+}]_i}{\tau_i}
\]

(4)

where \(i = 1\) or \(2\), representing the first or second calcium pool, \(f_i\) is the fraction of the Ca\(^{2+}\) influx via calcium current \(I'_{Ca}\) (\(f_1 = 0.7, f_2 = 0.024\), \(I'_{Ca} = I_{Ca}\) for ionic currents), \(z = 2\) is the valence of the Ca\(^{2+}\) ion, \(F\) is the Faraday constant; volume \(V = wA\) with \(w\) as the shell thickness (1\(\mu\)m) and \(A\) the dendritic surface area, and \(\tau_i\) represents the Ca\(^{2+}\) removal rate of the \(i^{th}\) pool. The amplitude of the slow AHPs in cells showing full spike frequency adaptation is much larger than in cells which discharge tonically (Faber and Sah 2001). Also, there is evidence that Ca\(^{2+}\) ions, as opposed to channel kinetics, determine the time course of AHP currents (Mainen and Sejnowski 1998). Accordingly, \(\tau_1 = 1\) ms for all three types of pyramidal cells, while \(\tau_2 = 1000\) ms for type A cell, 500 ms for type B cell, and 120 ms for type C cell. The resting Ca\(^{2+}\) concentration was [\(Ca^{2+}\)]\(_{rest} = 50\) nmol/l, which was the same as the initial concentration (Durstewitz et al. 2000). The Ca\(^{2+}\) pools for synaptic currents were involved in learning and are described later.
3.2.2 LA GABAergic interneuron model

A quarter of the LA neurons are inhibitory interneurons (McDonald and Augustine 1993). Recent data indicate that four subtypes of parvalbumin-positive interneurons exist in the BLA: fast spiking (FS), delayed firing (DF), accommodating (AC), and stuttering (ST) (Rainnie et al. 2006; Woodruff and Sah 2007). Our network used only the FS cell type since it is the most common cell type and the vast majority of synaptic connections between principal neurons and parvalbumin-positive interneurons are formed by the FS and DF interneuron subpopulations (Woodruff and Sah 2007). Compared to pyramidal cells, the FS interneurons have faster action potentials with a half-width of 0.76 ± 0.04 ms (1.2 ± 0.1 ms for pyramidal cells) and do not show appreciable frequency adaptation (Lang and Paré 1998; Mahanty and Sah 1998; Rainnie et al. 2006; Woodruff and Sah 2007). The resting potential is about -69.4 mV and the steady-state firing frequency at 0.4 nA current injection is about 80 Hz (Szinyei et al. 2003). The interneuron model also consisted of two compartments, a soma (diameter of 15 µm; length of 15 µm) and a dendrite (diameter of 10 µm; length of 150 µm). Each compartment contained a fast Na$^+$ ($I_{Na}$) and a delayed rectifier K$^+$ ($I_{DR}$) currents with kinetics that reproduced the much shorter spike duration (Durstewitz et al. 2000). The passive membrane properties were as follows: $R_m = 20 \text{ K}\Omega \cdot \text{cm}^2$, $C_m = 1.0 \mu\text{F/cm}^2$, $R_a = 150 \Omega \cdot \text{cm}$, and $E_L = -70 \text{ mV}$.

3.2.3 Network structure and synaptic interactions

The afferent projections from the auditory thalamus and cortex to the amygdala form excitatory synapses on both principal neurons and inhibitory interneurons (Mahanty and Sah 1998; Weisskopf and LeDoux 1999). Glutamatergic synaptic transmission to
pyramidal cells and interneurons is mediated by both AMPA and NMDA receptors (Szinyei et al. 2003; Weisskopf and LeDoux 1999). Excitatory AMPA- or NMDA-synaptic conductance is involved in both the pyramidal-to-pyramidal and pyramidal-to-interneuron connections (Smith et al. 2000; Szinyei et al. 2000). GABAergic interneurons send inhibitory $\text{GABA}_A$ recurrent collateralas onto pyramidal cells, and tightly control their activity (Lang and Paré 1997; Li et al. 1996). Although there are far fewer interneurons than pyramidal cells, one interneuron can inhibit many pyramidal cells through divergent projections (Mahanty and Sah 1998). The processing and transmission of excitatory inputs to the principal cells along neural pathways in the amygdala are determined by both feedback and feedforward GABAergic inhibition (Wang et al. 2001).

Utilizing the experimental information cited above, we developed an LA network model consisting of eight pyramidal cells and two GABAergic interneurons (see Fig. 2A) with all-to-all connectivity (Durstewitz et al. 2000; Wang 1999). Among the eight pyramidal cells, five were type A (P1-P5); two were type B (P6-P7); and one was type C (P8). In the network model, we were particularly interested in information processing in the sensory-receptive region – the dorsal part of LA (LAd). Three of the pyramidal cells (P5, P7 & P8) and both the interneurons received direct tone/shock inputs; P3 received only tone input, and P1 & P4 received only shock input; and P2 & P6 received no direct afferent inputs. In this fully connected architecture, each pyramidal neuron received excitatory inputs from all other pyramidal cells (excluding itself), as well as inhibitory inputs from both the interneurons. Both interneurons received excitatory inputs from all pyramidal cells, and thus provided both feedforward and feedback inhibition to pyramidal cells. Also, the two interneurons inhibited each other (Woodruff and Sah 2007). The
synaptic delays for tone and shock inputs were set to 8 ms, to represent the transmission delay between the start of tone and the arrival of information in the LA (Li et al. 1996). The synaptic delays for all intrinsic transmission were set to 2 ms.

The AMPA/NMDA receptors were placed in the dendrite compartment, while the GABA_A receptors were located in the somata of both the pyramidal cell and interneuron models (Fig. 1). The synaptic conductance induced by the arrival of presynaptic spikes was summed at each synapse with saturation. On glutamate binding, both AMPA and NMDA receptors become permeable to a mixture of ions including Na^+, K^+, and Ca^{2+}, and binding of GABA to GABA_A receptors leads to the opening of channels selective to chloride ions (Koch 1999). The summed response of these ionic channels to transmitter binding can be treated as a time-varying change in the membrane conductance in series with the synaptic reversal potential (Koch 1999). Accordingly, the AMPA, NMDA and GABA_A synaptic transmission currents were all modeled by dual exponential functions as listed in Eqs. 5-7 (Durstewitz et al. 2000),

\[ I_{\text{AMPA}} = G_{\text{AMPA}}(V - E_{\text{AMPA}}) = \overline{A}w(t)g_{\text{AMPA,max}}\frac{\tau_1\tau_2}{\tau_2 - \tau_1} \{\exp(-t/\tau_2) - \exp(-t/\tau_1)\}(V - E_{\text{AMPA}}) \]  

\[ I_{\text{NMDA}} = G_{\text{NMDA}}(V - E_{\text{NMDA}}) = \overline{A}wg_{\text{NMDA,max}}s(V)\frac{\tau_1\tau_2}{\tau_2 - \tau_1} \{\exp(-t/\tau_2) - \exp(-t/\tau_1)\}(V - E_{\text{NMDA}}) \]  

\[ I_{\text{GABA}} = G_{\text{GABA}}(V - E_{\text{GABA}}) = \overline{A}w(t)g_{\text{GABA,max}}\frac{\tau_1\tau_2}{\tau_2 - \tau_1} \{\exp(-t/\tau_2) - \exp(-t/\tau_1)\}(V - E_{\text{GABA}}) \]  

where \( w(t) \) is the adjustable synaptic weight for AMPA and GABA_A synapses (\( w \) was held fixed for the NMDA synapses); \( \overline{A} \) is a normalization constant chosen so that \( g_{\text{AMPA,max}}, g_{\text{NMDA,max}} \) and \( g_{\text{GABA,max}} \) assume their maximum values; and \( \tau_1 \) and \( \tau_2 \) are the rise and decay time constants respectively. sEPSCs were always significantly faster in interneurons than in pyramidal cells (Mahanty and Sah 1998). So, for AMPA receptor
channels, $\tau_1 = 0.5$ ms and $\tau_2 = 7$ ms for pyramidal cells, and $\tau_1 = 0.3$ ms and $\tau_2 = 2.4$ ms for interneurons (Mahanty and Sah 1998). For NMDA receptor channels, $\tau_1 = 5$ ms, $\tau_2 = 125.0$ ms for both pyramidal cells and interneurons (Weisskopf and LeDoux 1999). The voltage-dependent variable $s$ which implements the Mg$^{2+}$ block was defined as: $s(V) = [1 + 0.33 \exp(-0.06 V)]^{-1}$ (Zador et al. 1990). The maximal conductances were chosen as $g_{\text{AMPA, max}} = 1$ nS, and $g_{\text{NMDA, max}} = 0.5$ nS, so that the relative contributions of AMPA and NMDA components to excitatory postsynaptic currents matched data reported by Weisskopf and LeDoux (1999; total charge transfer is 1:4.6 at $V_m = -75$ and +45 mV, respectively). The synaptic reversal potentials were set as $E_{\text{AMPA}} = E_{\text{NMDA}} = 0$ mV (Durstewitz et al. 2000). For the GABA$_A$ synaptic current, $\tau_1 = 0.25$ ms, $\tau_2 = 3.75$ ms and $g_{\text{GABA}_A, \text{max}} = 0.6$ nS (Wolf et al. 2005), $E_{\text{GABA}_A} = -75$ mV for the pyramidal cells and $E_{\text{GABA}_A} = -60$ mV for the interneurons (Martina et al. 2001).

### 3.2.4 Background inputs and specific afferent inputs

LA projection neurons show low rates of spontaneous activity in control conditions (Gaudreau and Paré 1996; Paré and Collins 2000). To achieve the low average spontaneous firing rate of around 1 Hz in the experiments modeled (Quirk et al. 1995), independent, Poisson-distributed, random excitatory background inputs were delivered to all the pyramidal cells. These inputs represent unmodeled synaptic connections from other brain areas such as prefrontal cortex and hippocampus. Similar background inputs were provided to the interneurons to generate reported spontaneous firing rates of around 8 Hz (Paré and Gaudreau 1996).

In fear conditioning, specific afferent excitatory (AMPA and NMDA) synapses
encoding the CS (tone) and US (shock) information are delivered to LA from the auditory cortex and auditory thalamus (Bordi and LeDoux 1994, Quirk et al. 1997). The specific tone and shock inputs were represented by two separate regular spike trains delivered to the AMPA/NMDA channels in the cells. The firing frequency for the tone and shock inputs was set at 200 Hz, to model the summed activity of multiple inputs in vivo. The tone inputs also included noise represented by random Poisson spikes with an average frequency of 2 Hz. Given that the tone starts out as neutral and the shock as noxious, the conductance strength encoding the shock information was set much higher than that representing the tone inputs (see Table 3 in the Appendix).

### 3.2.5 Hebbian learning in LA

Learning of conditioned fear is accompanied by changes in synaptic strengths in the neural circuitry of LA. Long-term potentiation (LTP) has been demonstrated in slice preparations for both cortico-amygdalar (Huang and Kandel 1998; Humeau et al. 2003, 2005; Tsvetkov et al. 2002) and thalamo-amygdalar pathways (Huang et al. 2000; Humeau et al. 2005; Tsvekov et al. 2004). Induction of LTP in both pathways depends on postsynaptic processes, since postsynaptic depolarization is needed to trigger influx of Ca$^{2+}$ ions via NMDA receptors (Bauer et al. 2002; Tsvekov et al. 2002) or voltage-dependent L-type Ca$^{2+}$ channels (Bauer et al. 2002; Humeau et al. 2005; Weisskopf et al. 1999). In vivo, LTP can be induced in both thalamic and cortical inputs when tone and footshock are paired (Rogan and LeDoux 1995), but not when they are unpaired (Rogan et al. 1997). Excitatory glutamatergic synapses from the thalamus or cortex onto interneurons exhibit NMDA-receptor-dependent potentiation (Bauer and LeDoux 2004).
This potentiation is also AMPA-receptor-dependent because AMPA receptors on inhibitory neurons lack the GluR2 subunit, making them calcium-permeable (Mahanty and Sah 1998). It was further shown that inhibitory inputs onto pyramidal cells are modifiable via a Ca$^{2+}$-dependent mechanism (Bauer and LeDoux 2004; Mahanty and Sah 1998). In addition to LTP, long term depression (LTD) can be readily induced in excitatory amygdala synapses by low-frequency stimulation of the lateral nucleus at 1 Hz for 15 min (Wang and Gean 1999).

Following the experimentally determined locations for plasticity described above, all the excitatory AMPA synapses in the model were set to be adjustable, except for those delivering shock or background inputs. Similarly, the inhibitory synapses from interneurons onto pyramidal cells were modifiable, but the strength of the NMDA synapses was held fixed. We used a biophysical Hebbian rule termed ‘calcium control hypothesis’ (Gerstner and Kistler 2002; Shouval et al. 2002a, b) to implement learning by adjusting the synaptic weight $w_j$ in Eqs. 5 and 7 as

$$\Delta w_j = \eta([Ca]_j)\Delta t(\lambda_1\Omega([Ca]_j) - \lambda_2 w_j)$$

where $\eta$ is the Ca$^{2+}$-dependent learning rate and $\Omega$ is a Ca$^{2+}$-dependent function with two thresholds ($\theta_d$ and $\theta_p$; $\theta_d \leq \theta_p$) (see Fig. 9 in the Appendix); $\lambda_1$ and $\lambda_2$ represent scaling and decay factors respectively; the local calcium level at synapse $j$ is denoted by $[Ca]_j$ and $\Delta t$ is the simulation time step. With this learning rule, the synaptic weight decreases when $\theta_d < [Ca]_j < \theta_p$, and increases (with modulation by the decay term $\lambda_2 w_j$) when $[Ca]_j > \theta_p$.

One of the key assumptions of this learning rule is that the dominant source of calcium influx in the postsynaptic cell is through NMDA receptors. This calcium influx was calculated as $I_{Ca}^N = P_0 w^{-1}G_{NMDA}(V - E_{Ca})$ (Shouval et al. 2002b) where $G_{NMDA}$ is the
NMDA conductance in Eq. 6 (the term $w^{-1}$ ensures that it is calculated per synapse). $P_0$ was selected to be 0.015 so that the fraction of the NMDA current carried by Ca$^{2+}$ ions averaged to 7% at negative potentials (Koch 1999).

As mentioned earlier, the calcium influx (used for learning) at the glutamatergic synapses on interneurons occurs through both NMDA and AMPA receptors. The calcium influx through AMPA receptors was calculated as $I_{Ca}^{AMPA} = P_0 w^{-1}(0) G_{AMPA}(V - E_{Ca})$ with $P_0 = 0.001$. $G_{AMPA}$ is the AMPA conductance in Eq (5) and $w(0)$ is the initial AMPA synaptic weight. The Ca$^{2+}$ current through the AMPA/NMDA receptors was separated from the total AMPA/NMDA current in this manner and used for implementation of the learning rule (Kitajima and Hara 1997, Shouval et al. 2002b).

Potentiation of the GABA synapse between the interneuron and pyramidal cell was demonstrated by directly stimulating inhibitory neurons within the LA in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and (2R)-amino-5-phosphonopentanoic acid (APV) which block glutamatergic transmission (Bauer and LeDoux 2004). This potentiation was Ca$^{2+}$-dependent and expressed postsynaptically, but the exact underlying mechanism is presently unknown (Bauer and LeDoux 2004; Sigurdsson et al. 2007). Several different mechanisms have been reported for potentiation at GABAergic synapses in other brain regions (Gaiarsa et al. 2002). In all of the mechanisms, a postsynaptic rise in intracellular Ca$^{2+}$ concentration is required to trigger long-term plasticity. In the neonatal rat hippocampus, potentiation could be induced by Ca$^{2+}$ influx through the voltage-dependent Ca$^{2+}$ channels (VDCCs), whereas in the cortex and cerebellum, this process requires Ca$^{2+}$ release from postsynaptic internal stores on GABA receptor activation.
(Gaiarsa et al. 2002). Thus, both pre-synaptic activity (GABA receptor activation or interneuron firing) and postsynaptic activity (activation of VDCCs by membrane depolarization) contribute to the potentiation of GABA synapses. The process from GABA receptor stimulation to internal Ca\(^{2+}\) release involves activating a cascade of complex intracellular reactions (Komatsu, 1996). Such a complex process can be simplified by assuming that the Ca\(^{2+}\) release is proportional to the stimulation frequency or GABA\(_A\) conductance. Hence, we modeled this simplified process by considering Ca\(^{2+}\) release from the internal stores into a separate Ca\(^{2+}\) pool (Fig. 1), using an equation similar to that for the AMPA/NMDA case: 

\[
I_{Ca}^{G} = P_0 w^{-1}(t) G_{GABA}(V-E_{Ca})
\]

with \(P_0 = 0.01\), and \(G_{GABA}\) as the GABA\(_A\) conductance in Eq. 7. Note that \(I_{Ca}^{G}\) models the dependence of Ca\(^{2+}\) release on GABA\(_A\) stimulation frequency, but not Ca\(^{2+}\) influx through the GABA\(_A\) channel. Post-synaptic activity contributed Ca\(^{2+}\) to the same pool from VDCCs \((I_{Ca})\) in the soma compartment (Fig. 1).

The concentration of the calcium pool at synapse \(j\) followed the same dynamics as in Eq (4), with \(f_j = 0.024\) (Warman et al. 1994), \(\tau_j = 50\) ms (Shouval et al. 2002b), \(V\) is the volume of a local Ca\(^{2+}\) pool with a diameter of 2 \(\mu m\) (similar to a spine; Kitajima and Hara 1997); 

\[
I'_{Ca} = I_{Ca}^{N} + I_{Ca}^{A}
\]

for the excitatory synapses on interneurons; 

\[
I'_{Ca} = I_{Ca}^{G} + kI_{Ca}
\]

\((k = 0.01)\) for the inhibitory GABA\(_A\) synapses onto pyramidal cells, and 

\[
I'_{Ca} = I_{Ca}^{N}
\]

for all other modifiable synapses (see Fig. 1).

All the synaptic weights were constrained by upper \((W_{max})\) and lower \((W_{min})\) limits (Hasselmo and Barkai 1995). Maximum \((f_{max})\) and minimum \((f_{min})\) folds were specified for each modified synapse so that \(W_{max} = f_{max} \times w(0)\) and \(W_{min} = f_{min} \times w(0)\). For each adjustable synapse, the following parameters were selected iteratively (Table 3 in the
Appendix) to match the neuronal behaviors recorded \textit{in vivo} for LA cells (Quirk et al. 1995): scaling factor, decay factor, two thresholds and the initial weight. It was found that the qualitative results reported were similar even with different initial weights.

\textbf{3.2.6 Simulation design}

The schedule of tone and shock inputs in the simulation was based on \textit{in vivo} studies (Quirk et al. 1995, 1997). We scaled down the timing of the auditory fear conditioning protocol by approximately two orders of magnitude, so that it would be suitable for computational study. The simulation included a sensitization phase, conditioning phase, and two extinction phases (Fig. 2B). Each tone lasted 500 ms and each shock lasted 100 ms, and the interval between two tones was 3.5 sec. During the sensitization phase, 10 unpaired tones and shocks were presented to the network, with the shocks occurring randomly between the tones. Following sensitization, 10 paired tones and shocks were provided in the conditioning phase, with shock present during the last 100 ms of the tone. In extinction, 30 tones were delivered to the neurons without any shock (pure tones). The gap between conditioning and extinction phases was 40 sec and the model was tested for spontaneous recovery after a delay of 840 sec. The second extinction phase also used 30 pure tones. The entire schedule lasted 1200 sec. Simulations were performed on a personal computer using the software package GENESIS (Bower and Beeman 2003) with the Crank-Nicholson integration method, and a time step of 10 µs.
3.3 Results

3.3.1 Firing properties of single neurons

Waveforms and firing properties of model neurons closely matched data obtained from in vitro studies (Faber et al. 2001; Faber and Sah 2002). Voltage responses of three types of pyramidal cell (A, B and C) models in response to three different levels of current injections are shown in Fig. 3A. With a 600 ms, 400 pA depolarizing current step, cell A fired only 4 spikes, cell B showed clear frequency adaptation during the first 11 spikes, and cell C fired repetitively. A slow depolarizing sag was observed in response to a hyperpolarizing current injection (lower panel in Fig. 3A), a phenomenon termed “anomalous rectification” due to the activation of the $I_H$ current (Womble and Moises 1993). The interneuron model showed no frequency adaptation and fired at relatively high frequency in response to depolarizing current steps (Fig. 3B), consistent with experimental observations in vitro (Mahanthy and Sah 1998; Martina et al. 2001; Washburn and Moises 1992b) and in vivo (Lang and Paré 1998).

3.3.2 Fear learning and extinction

FIRING RATE OF LA UNITS. Quirk et al. (1995) described the tone responses of simultaneously recorded LA pyramidal neurons at different points during the training process. The main finding in that study was that conditioning significantly increased the number of tone-elicited spikes, with the greatest effects at the shortest latency following tone onset. These conditioned responses were reversed by extinction training. The peri-event time histograms (PETHs) of all pyramidal cells and one interneuron (I1) in the LA network during sensitization are shown in Fig. 4A. All pyramidal neurons showed clear
frequency adaptation with the tone responses concentrated in the first 100 ms, indicating a good match with the experimental recordings (Quirk et al. 1995). Also, the tone response latency for pyramidal cells was in the 10-20 ms range, consistent with experimental data (Quirk et al. 1997). The magnitude of the tone responses varied across units, depending on the cell type and whether or not the unit received a direct tone input. For example, the type A neuron P1 showed little tone responsiveness because it received no direct tone input. Although both P5 and P7 received direct tone inputs, P7 was more responsive than P5 since P7 is a type B cell, while P5 is type A. The interneuron (I1) fired continuously in response to tone input with an average firing frequency of around 50 Hz.

The PETHs of LA cells during early extinction (tones 1-10) and late extinction (tones 20-30) are shown in Fig. 4B-C. Conditioning significantly increased the magnitude of tone responses of all the cells receiving direct shock input (Fig. 4D). The effects of conditioning were most pronounced at the shortest response latencies. The latency of the earliest significant plasticity (the first bin showing an increase of two or more spikes with conditioning) was 10-20 ms for P1, P4, P7 and P8, and 20-30 ms for P5. Extinction training brought the tone responses of the conditioned pyramidal cells back to pre-conditioning levels, or even lower (Fig. 4C). All these results were consistent with experimental findings for LA pyramidal cells (Quirk et al. 1995). Though conditioning also enhanced the interneuron tone responses, extinction further increased its responding (Fig. 4C). Fig. 4E shows very good match between experimental (Fig. 4, Quirk et al. 1997) and model conditioned tone responses for the last block of five trials in sensitization and successive five-trial blocks during extinction.
To test the importance of GABAergic inhibition on extinction, we blocked GABA transmission by setting $g_{GABA, \text{max}} = 0$ immediately before extinction training. Fig. 4F shows the average change in pyramidal tone responses across the experiment, relative to the sensitization phase, for both control and GABA-blocked cases. In the control case, the average tone response increased by 120% after conditioning, and returned to sensitization levels in late extinction. However, when GABA receptors were blocked, tone responses were greatly augmented and could not be extinguished. Thus, similar to experimental reports (Chhatwal et al. 2005, Harris and Westbrook 1998), GABA transmission in LA is essential for extinction learning.

SYNAPTIC WEIGHTS. An examination of synaptic weights provides insights into the functioning of the model. Fig. 5A shows the time courses of synaptic weights for the tone-to-pyramidal (tone-pyr) and tone-to-interneuron (tone-inter) synapses. All tone synaptic weights decreased slightly during the sensitization phase when tone and footshock were unpaired, and increased during conditioning (except P3). The synapse showing greater potentiation (e.g., Tone-P8) underwent greater depression in extinction. This is because the synapse with greater potentiation has a relatively high level of $Ca^{2+}$ for faster and larger plasticity. In contrast to the tone-pyr weights, the tone-inter weights continued to increase as extinction proceeded. Three factors account for this extinction-induced increase: first, interneurons have no frequency adaptation and so continued to fire at high rates during extinction, allowing more $Ca^{2+}$ influx via removal of the $Mg^+$ block from NMDA receptors; second, the AMPA receptors on interneurons are $Ca^{2+}$-permeable (Mahanty and Sah 1998); third, the shock inputs to interneurons have been effectively replaced in extinction by the excitatory drive from pyramidal cells which were
potentiated during conditioning. Representative pyramidal-to-pyramidal (pyr-pyr) coupling strengths are shown in Fig. 5B. The strength between two pyramidal cells depended on whether one or both cells received direct shock input. If the postsynaptic neuron did not receive direct shock input, the connection remained unchanged throughout training (e.g., P5-P6). If the postsynaptic neuron received direct shock input, and the presynaptic neuron received direct tone input (no shock), then the connection strength potentiated more during conditioning than sensitization (e.g., P3-P4). If both pre- and postsynaptic neurons received direct shock input, the connection potentiated during both sensitization and conditioning phases to a larger degree than when only the postsynaptic neuron receives direct shock input, (e.g., P1-P4 vs. P3-P4). Similar to the tone-pyr synapses, the pyr-pyr synapse which showed greater potentiation also showed deeper depotentiation during the extinction phase (e.g., P7-P8).

As with tone synapses onto interneurons, potentiation of pyramidal-to-interneuron (pyr-inter) synapses depends on both AMPA and NMDA receptors. Fig. 5C shows the time courses of synaptic strengths for the P1-I1, P3-I1 and P8-I1 synapses. The potentiation of the P1-I1 and P8-I1 synapses was much larger than that of the P3-I1 synapse during sensitization and conditioning phases since P3 received no direct shock input. During extinction, the P8-I1 synapse potentiated to the maximum level and then decreased slightly, while the P1-I1 strength showed a slow but consistent decay because P8 had a higher firing rate compared to P1. Time courses of the inhibitory synaptic strengths from I1 to each of P1, P6, and P8 are shown in Fig. 5D. These interneuron-to-pyramidal (inter-pyr) connections are important in that they control both feedforward and feedback inhibition on the pyramidal cells. All the inter-pyr synaptic strengths had very
similar time courses: they potentiated in sensitization, conditioning and extinction phases, and showed appreciable decay during the two gaps. Plasticity at this GABAergic synapse depends on the interneuron firing rate. As cited in the Methods section (‘Hebbian Learning in LA’), GABA receptor stimulation is hypothesized to trigger internal Ca\(^{2+}\) release proportionally to the GABA\(_A\) conductance. Furthermore, this has been modeled using an equation similar to that for the AMPA/NMDA receptors, with the Ca\(^{2+}\) release from internal stores replacing Ca\(^{2+}\) through the receptor model. The postsynaptic Ca\(^{2+}\) also comes from VDCCs. After conditioning, the interneuron firing increased significantly (Fig. 4B), enabling the inhibitory synapse to continue to strengthen in extinction. This plasticity also depends on Ca\(^{2+}\) influx through voltage-gated calcium channels (on pyramidal cells), explaining why the synapse with more post-synaptic activity (e.g., I1-P8) showed stronger strengthening. Even though P3 and P6 did not show conditioned responses, they received increasing inhibition from the interneurons. Thus, our model predicts that a neuron can show the effects of extinction, even if it does not exhibit a conditioned response.

To summarize, this model provides insights into the mechanisms of fear acquisition and extinction. The large increase in tone synaptic strengths of pyramidal cells, together with the potentiation of pyr-pyr synapses, resulted in elevated tone responses in early extinction. Although the inhibition from interneurons also increased with conditioning, it was overcome by potentiated excitation at this stage. During extinction, the excitatory inputs to pyramidal cells (tone-pyr and pyr-pyr inputs) underwent differing degrees of depression with the more active synapses losing a greater percentage of the strength gained in conditioning. At the same time, excitatory inputs to interneurons (tone-inter and
some pyr-inter synapses) continued to strengthen in extinction, leading to stronger potentiation of the inhibitory GABA synapses onto pyramidal cells. The model showed that this increase in inhibition, combined with LTD at the excitatory pyramidal synapses, ultimately caused tone responses of pyramidal cells to decrease to pre-conditioning levels. This predicts that LTD at excitatory synapses serves to accelerate and/or strengthen extinction.

3.3.3 Persistence of fear memory and spontaneous recovery

Although extinction training reversed some of the conditioning-induced increases in tone-pyr and pyr-pyr synapses, the strengths of these synapses were still well above pre-conditioning levels at the end of extinction, indicating a persistence of fear memory. Following extinction, we inserted a long gap (840 sec) followed by re-extinction, in order to model spontaneous recovery of fear. During the gap, tone inputs were never activated, however, activity fluctuated randomly with the noise present. Accordingly, the strengths of tone-inter and some pyr-inter synapses (e.g., P8-I1) decayed considerably (Fig. 5A & Fig. 5C) during the gap due to Hebbian weakening, whereas little decay was seen in the tone-pyr and pyr-pyr synapses (Fig. 5A & Fig. 5B). This was due to the fact that, with only background inputs, pyramidal cells fire at much lower frequencies compared to the interneurons, limiting $\text{Ca}^{2+}$ influx and resulting plasticity. $\text{Ca}^{2+}$ entry into pyramidal cells was also limited by the fact that AMPA receptors on pyramidal cells are not $\text{Ca}^{2+}$ permeable, while, as cited earlier, those in interneurons are (Bauer and LeDoux 2004; Mahanty and Sah 1998). Thus, the model predicts that the low spontaneous firing rate of pyramidal cells acts to preserve the original fear memory.
The strength of inter-pyr synapses also decreased considerably during the long gap due to relative high spontaneous firing rates of interneurons (Fig. 5D), causing extinguished tone responses in pyramidal neurons to recover spontaneously (Fig. 5E). During subsequent re-extinction, excitatory synapses onto pyramidal cells were again depressed (Fig. 5A & Fig. 5B), and the tone synapses onto interneurons exhibited potentiation (Fig. 5A). As in initial extinction, the inhibitory synapses onto pyramidal cells potentiated (Fig. 5D), permitting re-extinction (Fig. 5E). Figure 5E shows the modulation of tone responses by trials in conditioned pyramidal cells (n = 5) during the entire training course. The recovered response of the units was higher than that in sensitization or late extinction, but was lower than that in early extinction, consistent with some loss of conditioning memory during both the first extinction and the gap. Following re-extinction, the response was the lowest among all phases.

Some other observations with respect to the long gap can be made. First, the decay in the excitatory synapses onto pyramidal cells in re-extinction was smaller than that during the first extinction (compare phases E2 and E1 in Figs. 5A & B), due to a decrease in the pyramidal cell firing rate in re-extinction. Second, during re-extinction, some pyr-inter coupling strengths (see black line in Fig. 5C) were depressed. Thus, the inhibition on pyramidal cells could be relieved briefly at the beginning of re-extinction, before the tone-inter and inter-pyr synapses re-strengthened. This may cause tone responses to increase slightly during the early re-extinction trials (Fig. 5E), and may explain the paradoxical increase in conditioned freezing observed in experiments at the start of extinction (see Mueller et al. 2008, Fig 1C; Quirk 2002, Fig 2B). This provides additional confirmation for the model. Lastly, the amount of decay at interneuron and inter-pyr
3.3.4 Some predictions from the model

1. Importance of low spontaneous firing rate for maintaining plasticity

Pyramidal cells in LA have very low spontaneous firing rates (Gaudreau and Paré 1996; Likhtik et al. 2006; Paré and Collins 2000), but the implications of this low firing rate for learning are not clear. Our model predicts that a low rate of spontaneous firing in LA may act to preserve the fear memory (due to decreased incidence of Hebbian weakening). To test this, we increased the spontaneous firing rate of pyramidal cells by a factor of 4.7 (from 1.5 to 7 Hz, by increasing both the strength and firing frequency of background inputs) during the gap between extinction and re-extinction. This led to a corresponding increase in the spontaneous firing rates of the interneurons. The time courses of representative synaptic weights are shown in Fig. 6A-D. Tone inputs to pyramidal cells and interneurons showed a small increase in their rate of decay across the gap (Fig 6A). In contrast, the internal synaptic connections showed much faster decay. This was true for excitatory (pyr-pyr) as well as inhibitory (pyr-inter and inter-pyr) connections (Fig. 6B-D), consistent with our prediction that increased spontaneous firing results in faster decay of plasticity. In fact, the strength of inhibitory connections (but not tone-inputs) decayed to pre-sensitization levels. The loss of inhibitory plasticity apparently compensated for the loss of excitatory plasticity, because conditioned tone responses were still apparent during recovery (Fig. 6E). Thus, increasing the spontaneous activity of cells during the long gap did cause faster decay of both excitatory and inhibitory
plasticity, but did not alter the expression of either conditioning or extinction memory.

This suggests a degree of robustness in the network: despite large fluctuations in spontaneous firing rates, the output of tone responses is preserved.

2. NMDA currents are required for extinction learning

Consistent evidence shows that NMDA-type glutamate receptors are required for the formation of extinction memory (Falls et al. 1992; Santini et al. 2001; Sotres-Bayon et al. 2007; Suzuki et al. 2001). To test the effect of blocking NMDA receptors on the acquisition of extinction, we reduced $g_{\text{NMDA, max}}$ from 0.5 to 0 nS for all the NMDA channels in the model, just prior to extinction training. The time courses of representative synaptic strengths in the network are shown in Fig. 7A-D. Both potentiation and depression require adequate postsynaptic Ca$^{2+}$ concentration. Depending on the synapse, this comes from NMDA, AMPA, internal Ca$^{2+}$ stores or VDCC channels (Fig. 1) as cited earlier. With NMDA receptors blocked, LTD was blocked at the excitatory synapses onto pyramidal cells (Fig. 7A-B), and potentiation was blocked at the tone-inter, pyr-inter and inter-pyr synapses (Fig. 7A, C & D) due to low Ca$^{2+}$ levels. Though the Ca$^{2+}$ level at the inter-pyr synapse was not NMDA-dependent, it was dependent on the pre-synaptic firing and went down as the interneuron firing rate decreased due to NMDA blockade (results not shown). As shown in Fig. 7E, the loss of NMDA-mediated inhibitory plasticity completely prevented extinction of tone responses. However, if NMDA receptors were blocked only in pyramidal cells, extinction proceeded normally (green line in Fig. 7E), because NMDA-mediated inhibition in interneurons continued to potentiate. Thus, this suggests that LA interneurons may be a critical site of NMDA-mediated plasticity in extinction.
3. LTD is necessary for complete extinction

As shown above, blocking NMDA receptors prevented depotentiation of the excitatory synapses onto pyramidal cells, but at the same time blocked potentiation of inhibitory connections. To evaluate the contribution of LTD, independent of potentiation of inhibition, we selectively blocked LTD only at the tone-pyr and pyr-pyr synapses by preventing $\text{Ca}^{2+}$ influx via the NMDA channels. This blockade of LTD was induced during the two extinction sessions. The time courses of representative synaptic weights are shown in Fig. 8A-D. During extinction, the decay of weights at the tone-pyr and pyr-pyr synapses was blocked as expected, but potentiation of tone-inter, pyr-inter, and inter-pyr connections was maintained. Under these conditions, tone responses still extinguished, but more slowly than controls, as evidenced by higher tone responses in late-extinction and re-extinction (Fig. 8E). In addition to being slower, extinction in the absence of LTD was not as complete as controls (note that tone responses reached a “floor” of 6 spikes during extinction in Fig. 8E). This is because the extinction-induced decrease in pyramidal cell firing rate reduced the excitatory drive onto the interneurons. As a result, the degree of inhibition of pyramidal cells reached a steady value. Thus, LTD allows for faster and deeper extinction.

3.4 Discussion

Our network represents a first attempt to incorporate cellular neurophysiology and synaptic plasticity mechanisms into a biophysical model to investigate the underlying mechanisms of fear learning. The model provides a plausible mechanism as to how memory for conditioning and extinction develop and co-exist in LA, and how they can
regulate fear expression.

3.4.1 Both acquisition and extinction can be learned within the LA

Our model predicts that fear expression is determined by a balance between pyramidal cell and interneuron excitations, and that cells in LA can learn both conditioning and extinction. The large potentiation of the excitatory inputs onto pyramidal cells caused by conditioning leads to elevated responses in early extinction. As extinction progresses, increasing feedforward and feedback inhibition from the interneurons, combined with depotentiation at excitatory synapses onto pyramidal cells, ultimately bring the responses of the pyramidal cells back to pre-conditioning levels, or even lower.

The key to extinction in this model is that tone-inter and inter-pyr synapses exhibit strengthening during extinction, even in the absence of shock. This occurs because the shock-induced excitation of the interneurons is replaced by pyramidal cell inputs that have been potentiated during conditioning. Thus, in the absence of pyramidal to interneuron connections, there would be no strengthening of the tone-inter and inter-pyr inputs, and therefore no extinction. From a functional point of view, this suggests that the foundation for extinction is laid in conditioning, and that strong conditioning enables strong extinction (homeostatic control). Since the excitatory inputs to interneurons exhibit potentiation during extinction, our model predicts that the interneurons within LA may increase their tone responses as extinction progresses. A recent experimental finding indicated the presence of such “extinction cells” within the basal amygdala (Herry et al. 2008), but the particular neuron type has yet to be determined. Nevertheless, an appreciable increase in interneuron firing rate across extinction training is not necessary for extinction learning. This is because the conditioning-induced increase in interneuron
firing rate was sufficiently high to enable potentiation at the inhibitory GABA synapses onto pyramidal cells. Indeed, Fig. 4A-C shows that there was a significant increase in the interneuron firing rate immediately after conditioning, but little further increase as extinction progressed.

3.4.2 Both LTD and potentiation of inhibition contribute to extinction

The cellular mechanism of fear extinction has long been an issue of debate. An early hypothesis was that extinction could result from a depression of potentiated synapses and depotentiation may be the cellular mechanism for extinction (Goldman et al. 1990; Teyler and Discenna 1984). However, this was challenged when spontaneous recovery of fear was demonstrated, implying the persistence of fear memory after extinction (Bouton and King 1983; Pavlov 1927). This led to an alternative hypothesis where extinction does not erase the fear memory, but instead creates a new memory that inhibits the fear response (Bouton and King 1983; Quirk et al. 2002). Such an inhibitory memory could reside in excitatory neurons in the vicinity of the amygdala which inhibit the principal cells in LA via an extra-amygdaloid circuit, or in the inhibitory interneurons in the LA (Falls et al. 1992).

Our model suggests a co-existence of these two mechanisms. Extinction did cause depression in potentiated synapses, but did not completely reverse conditioning-induced changes. Furthermore, the degree of depotentiation varied from synapse to synapse. Even if potentiation at one synapse (such as the tone-pyr synapse) is completely reversed by extinction, fear could still be stored at other synapses (such as the pyr-pyr synapse). Thus, a proportion of the fear memory is erased by extinction, while the rest is inhibited by potentiation at local GABAergic synapses. This agrees with previous findings supporting
extinction-induced depotentiation (Lin et al. 2003) and augmentation of inhibition (Chhatwal et al. 2005) in the BLA. A recent study (Kim et al. 2007) reported a unique form of depotentiation during extinction that reversed conditioning-induced potentiation at thalamic input synapses onto the LA *ex vivo*. Moreover, extinction reversed conditioning-induced enhancement of surface expression of AMPA receptor subunits in LA synaptosomal preparations (Kim et al. 2007). This new experimental finding supports our prediction that multiple mechanisms underlie extinction of consolidated memory (also see Myers et al 2006). Indeed, our simulation shows that potentiation of inhibition alone is not sufficient for complete extinction.

### 3.4.3 The location of storage sites within LA

There are two possible sites for fear memory storage in LA: the tone synapses from the auditory thalamus (or cortex) onto the pyramidal cells, or the synapses between pyramidal cells. While both are capable of storing fear memory, there is an important difference. The synaptic coupling between two pyramidal cells will be strengthened as long as both receive strong inputs such as shocks. In contrast, the tone synaptic weight increases only when tone and shock are paired, and deceases when tone and shock are unpaired. Hence, the model predicts that tone synapses will only store specific tone-shock associations, while the pyr-pyr synapses can store a generalized fear memory related to the occurrence of shock (which could be related to contextual fear conditioning). Also, the pyr-pyr synapses decayed less on average in extinction compared to the tone-pyr synapses as a result of frequency adaptation of pyramidal cells (Fig. 5A, B). Together, these findings suggest that the pyr-pyr synapse is well suited to encode long-term fear memory. In support of this, Repa et al. (2001) showed that neurons in the ventral part of
LA (which receive input from the dorsal part) serve to store extinction-resistant long-term fear memories.

LA contains different types of principal neurons which have different degrees of frequency adaptation (Faber et al. 2001). The model suggests that these have different functional roles. The cells with stronger adaptation are slower to learn fear, but are able to maintain fear memory for a long time, whereas the cells with weaker adaptation learn fear faster, but also extinguish faster (Figs 5A, B). Therefore, pyramidal cells with weaker adaptation are important for fear expression, while those with stronger adaptation are important for long-term storage of fear associations. Since the majority of pyramidal cells in LA are strongly adapting, LA is well suited for storing long-term fear memory.

For extinction memory, there are three possible sites of plasticity: the tone synapse at the interneuron, the inhibitory synapse from interneuron to pyramidal cell, and the excitatory synapse from pyramidal cell to interneuron. A comparison of the decay rates of these three synapses (Fig. 5) suggests that the first two may mediate short-term extinction memory (large and uniform decay rates during the gap), while the last could store long-term extinction memory (e.g., P1-I1 in Fig. 5C). Nevertheless, the tone-inter and inter-pyr synapses exhibited substantial potentiation during both extinction sessions, while the pyr-inter synapses did not. Long-term storage of extinction in LA pyramidal cells suggests that many of the molecular mechanisms of extinction storage in the LA are similar to storage of conditioning, as has been observed (Myers and Davis, 2007; Quirk and Mueller, 2008).
3.4.4 Maintenance of conditioning and extinction memories

Our model suggests that, with a sufficiently long gap after conditioning, conditioned fear memory would be lost due to Hebbian weakening. This suggests the necessity of some active process that maintains fear memory through rehearsal or replay. In support of this, NMDA receptors are needed days and weeks after training to maintain conditioned fear memory (Wang et al. 2006), and reactivation of memory triggers an NMDA-dependent reconsolidation processes within the amygdala (Nader et al. 2000; Tronson and Taylor 2007). Thus, long-term consolidation and reconsolidation processes may serve to counteract depotentiation of synapses that would occur over an extended period of time. With respect to extinction memory, high spontaneous activity during the long gap was sufficient to completely eliminate inhibitory plasticity at the inter-pyr, and pyr-inter connections, but re-extinction was not impaired. Interneuron activity depends on the pyramidal cell firing rate and the strength of the pyr-inter connection. Although the pyr-inter plasticity vanished, the pyramidal cell firing was high at the start of re-extinction (recovery), causing the interneuron to re-establish the inhibition plasticity. However, if the pyr-inter connection is removed (no pyramidal input to the interneuron), extinction will fail. This underscores an important insight from this model, namely, that a critical amount of the plasticity necessary for extinction is accrued during conditioning.

3.4.5 Limitations

There are a number of limitations of our model which should be acknowledged: (i) The size of this LA network was intentionally small in order to facilitate the study of the underlying neural plasticity in detail, and is typical of previous biophysical modeling studies (Durstewitz et al. 2000). It remains to be tested whether the qualitative
conclusions and predictions from the model will hold for a larger network; (ii) The model parameters in Table 3 (Appendix) were selected to match experimental data as closely as possible. Improved understanding of connectivity and learning mechanisms in LA will help refine these estimates; (iii) As mentioned earlier, there is considerable heterogeneity in the firing properties of GABAergic interneurons in BLA (Rainnie et al. 2006; Woodruff and Sah 2007). We only modeled the most common cell type – fast spiking (FS) interneurons. This is justifiable because the second most common cell type – delay firing (DF) cells also fire non-accommodating trains of high frequency spikes after an initial delay in response to depolarizing current injection, and FS and DF cells together account for 70% of the Parvalbumin-positive interneurons in BLA (Woodruff and Sah 2007); (iv) Although potentiation of inhibitory synapses in LA is dependent on postsynaptic calcium, the molecular induction and expression mechanisms are currently unknown (Bauer and LeDoux 2004; Sigurdsson et al. 2007). We modeled this plasticity based on inhibitory LTP mechanisms reported in the rat hippocampus, cortex and cerebellum (Gaiarsa et al. 2002). Specifically, we used an equation similar to that for the AMPA/NMDA receptors, with the Ca\(^{2+}\) release from internal stores replacing the Ca\(^{2+}\) through the receptor model. A better understanding of the Ca\(^{2+}\) mechanisms underlying potentiation of inhibitory synapses in LA will help improve this model; (v) As mentioned earlier, the present model investigates information processing primarily in LAd neurons receiving direct thalamic inputs. Extensions to include neurons showing persistence of potentiated tone responses observed in the ventral part of LA (LAv) (Repa et al. 2001) will be the topic of a separate study; and (vi) Our model focused only on plasticity in the LA. Recent physiological studies have shown that, in addition to LA, plasticity is also seen in other amygdaloid...
nuclei (ITC and CE) (Royer and Paré 2002; Samson and Paré 2005; Wilesky et al 2006). Also, expression of fear and extinction are regulated by contextual and temporal factors that are processed by other structures that influence the amygdala, such as the hippocampus and medial prefrontal cortex (Corcoran and Maren 2004; Milad and Quirk 2002). Thus, additional modules will be needed to model the processes that regulate fear expression in real-life conditions.

In conclusion, we have shown that realistic LA neurons, incorporating known conductances, connectivity, and synaptic plasticity mechanisms, can learn fear conditioning and extinction. The biophysical realism of the model allowed us to test the importance of basal firing rates, NMDA receptors and depotentiation. Furthermore, our results suggest specific storage sites within the LA for conditioning vs. extinction, and factors that can affect the persistence of these memories. The ultimate goal of this computational study is to model pathologies associated with the fear circuit (e.g., post traumatic stress disorder) and assist in the development of new treatments.
3.5 References


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FIG. 3.1. Two-compartment pyramidal cell and interneuron models with ionic and synaptic currents. For the pyramidal cell, two different Ca$^{2+}$ pools mediated by voltage-dependent calcium channels (VDCCs) regulated $I_C$ and $I_{AHP}$ currents in the dendrite. The plasticity at AMPA receptors was a function of NMDA Ca$^{2+}$, and plasticity at GABA$_A$ synapses was a function of Ca$^{2+}$ from two sources described in the text. For the interneuron, the Ca$^{2+}$ underlying learning at the AMPA synapse came from both AMPA and NMDA receptors.
FIG. 3.2. Architecture of the LA network and conditioning protocol. A: triangles represent pyramidal cells and circles representing interneurons. Among the eight pyramidal cells, five were type A (P1-P5); two type B (P6-P7) and one type C (P8). Each pyramidal cell excited all the other cells including interneurons. Each interneuron inhibited all the pyramidal cells and the other interneuron. Three pyramidal cells (P5, P7 & P8) and two interneurons received direct tone/shock inputs; P3 received tone input only; P1 & P4 received shock input only; and P2 & P6 did not receive direct tone/shock inputs.

B: simulation schedule showing tone (green) and shock (red) inputs during sensitization, conditioning and the two extinction phases. There was a short gap between conditioning and extinction, and a longer gap before re-extinction.
FIG. 3.3. Responses of units to intracellular current pulses. A: voltage responses of the three types of LA pyramidal cell models (A, B and C) to three 600 ms current injections starting from 100 ms (top row: 400 pA; middle row: 300 pA; lower row: -100 pA). Compare to Fig. 2 of Faber et al. (2001) (reproduced as supplementary figure 1). B: voltage responses of the interneuron model to 200 ms current injections of the same magnitude (top row: 400 pA; middle row: 300 pA; lower row: -100 pA).
FIG. 3.4. The effects of training on the tone responses of individual neurons. Tone responses of pyramidal cells (P1-P8) and one interneuron (I1) during A: sensitization, B: early extinction and C: late extinction phases. D: differences in tone responses between early extinction and sensitization. Tone started at t = 0; bin width was 10 ms and spike counts during 10 trials were summed together. In the upper right hand of each box in the sensitization phase, “T” indicates direct tone input and “S” indicates direct shock input. Compare with Fig. 2 of Quirk et al. 1995 (reproduced as supplementary figure 2). E: comparison of the experimental data (Fig. 4, Quirk et al. 1997) and the model tone responses for the last block of five trials in sensitization and successive five-trial blocks during extinction. The total spikes (0-50 ms) in each block of 5 trials were
normalized to the responses in the first block of extinction for each cell and the mean ratio (with standard error) among four significant conditioned pyramidal cells calculated. $F$: comparison of the tone responses in conditioned pyramidal neurons ($n = 5$) for the control and GABA-block cases. The tone responses (total spikes in 0-200 ms) for each cell in each phase were normalized to the responses during sensitization and the mean ratio (with standard error) among all cells calculated. Same for Fig. 6E and Fig. 7E below.

FIG. 3.5. Time courses of representative synaptic strengths in the LA network during acquisition and extinction of fear. $A$: tone synapses; $B$: pyr-pyr synapses; $C$: pyr-inter synapses; and $D$: inter-pyr synapses. $S$ = Sensitization; $C$ = Conditioning; $E_1$ = First extinction; $E_2$ = Second extinction. $E$: spontaneous recovery of fear and re-extinction. Average tone responses (spikes in 0-200 ms) by block of 2 trials of conditioned pyramidal cells ($n = 5$) for the entire schedule.
FIG. 3.6. Effect of increasing spontaneous firing rate on memory storage. Fear memory stored in the tone-pyr and pyr-pyr synapses decayed faster when the spontaneous firing rate of pyramidal cells was increased near five-fold during the long recovery gap; Extinction memory stored in the tone-inter, pyr-inter and inter-pyr synapses also decayed faster. Time courses of A: representative tone synaptic strengths; B: representative pyr-pyr synaptic strengths; C: representative pyr-inter synaptic strengths; and D: representative inter-pyr synaptic strengths. Red arrows indicate the time when the spontaneous rate was increased. E: comparison of the tone responses in conditioned pyramidal neurons (n = 5) in the control and spontaneous rate-increased cases.
FIG. 3.7. Effect of blocking all NMDA receptors. Extinction failed when NMDA receptors were blocked immediately before extinction. Time courses of A: representative tone synaptic strengths; B: representative pyr-pyr synaptic strengths; C: representative pyr-inter synaptic strengths; and D: representative inter-pyr synaptic strengths. Red arrows indicate the instant when the block was implemented. E: comparison of the tone responses in conditioned pyramidal neurons (n = 5) for three cases: control, NMDA-blocked in all synapses, and NMDA-blocked in pyramidal cells. Pyramidal cells continued to have significantly elevated responses in late extinction when all NMDA receptors were blocked, but the tone responses decreased if NMDA receptors were blocked only in the pyramidal synapses. Sens: last 5 trials in sensitization; E. Ext: first 5 trials in extinction; L. Ext: last 5 trials in extinction.
FIG. 3.8. Effect of blocking LTD. Extinction slowed down when LTD at the excitatory synapses on pyramidal cells was blocked during extinction. Shown are time courses of A: representative tone synaptic strengths; B: representative pyr-pyr synaptic strengths; C: representative pyr-inter synaptic strengths; and D: representative inter-pyr synaptic strengths. Red arrows indicate the time when LTD blockade was implemented. E: comparison of tone responses (0-200 ms) in the control and LTD-blocked cases by block of 2 trials for conditioned pyramidal cells (n = 5) during extinction and re-extinction.
FIG. 3.9 (Appendix). Functions used in the calcium control hypothesis of Eq. 8. A: $\Omega$ function. B: learning rate $\eta$; adapted from Shouval et al. (2002b).
TABLE 3.1. Maximal conductance densities (in mS/cm$^2$) and Ca$^{2+}$ time constants for the $I_{sAHP}$ current (in ms)

<table>
<thead>
<tr>
<th></th>
<th>$I_{Na}$</th>
<th>$I_{DR}$</th>
<th>$I_{M}$</th>
<th>$I_{H}$</th>
<th>$I_{D}$</th>
<th>$I_{Ca}$</th>
<th>$I_{C}$</th>
<th>$I_{sAHP}$</th>
<th>$\tau_{Ca}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pyramid A</strong></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Soma</td>
<td>120</td>
<td>12</td>
<td>0.30</td>
<td>–</td>
<td>–</td>
<td>0.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dendrite</td>
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<td>0.10</td>
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<tr>
<td>Soma</td>
<td>120</td>
<td>12</td>
<td>0.20</td>
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<td>–</td>
<td>0.1</td>
<td>–</td>
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<tr>
<td>Dendrite</td>
<td>40</td>
<td>3</td>
<td>0.20</td>
<td>0.1</td>
<td>0.4</td>
<td>0.2</td>
<td>0.5</td>
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<tr>
<td><strong>Pyramid C</strong></td>
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</tr>
<tr>
<td>Soma</td>
<td>120</td>
<td>12</td>
<td>0.25</td>
<td>–</td>
<td>–</td>
<td>0.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dendrite</td>
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<td>3</td>
<td>0.25</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.5</td>
<td>0.50</td>
<td>120</td>
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<td>Soma</td>
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<td>–</td>
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</tbody>
</table>
TABLE 3.2. Gating variables for ion channels used in the network model

(a) LA pyramidal cell model

<table>
<thead>
<tr>
<th>Current Type</th>
<th>Gating Variable</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$x_\infty$</th>
<th>$\tau_x$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{Na}$</td>
<td>$p=3$</td>
<td>$-0.2816(V + 25)$ (\exp(-V + 25)/9.3 - 1)</td>
<td>$0.2464(V - 2)$ (\exp(V - 2)/6 - 1)</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
</tr>
<tr>
<td></td>
<td>$q=1$</td>
<td>$0.098\times \exp(-(V + 40.1)/20)$</td>
<td>$1.4\exp(-(V + 10.1)/10 + 1)$</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
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<tr>
<td>$I_{DR}$</td>
<td>$p=4$</td>
<td>$-0.036(V - 13)$ (\exp(-(V - 13)/25 - 1)</td>
<td>$0.0108(V - 23)$ (\exp(V - 23)/12 - 1)</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
</tr>
<tr>
<td>$I_M$</td>
<td>$p=2$</td>
<td>$0.016\exp(-(V + 52.7)/23)$</td>
<td>$0.016\exp((V + 52.7)/18.8)$</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
</tr>
<tr>
<td>$I_H$</td>
<td>$p=1$</td>
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<td>—</td>
<td>—</td>
<td>$1/(\exp(V + 89.2)/9.5 + 1)$</td>
</tr>
<tr>
<td>$I_D$</td>
<td>$p=1$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>$1/(\exp((V + 8.6)/11.1 + 1)$</td>
</tr>
<tr>
<td></td>
<td>$q=1$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>$1/(\exp((V + 21)/9 + 1)$</td>
</tr>
<tr>
<td>$I_{Ca}$</td>
<td>$p=2$</td>
<td>$-0.00642V_m - 0.1152$ (\exp(-(V_m + 18)/12 - 1)$ with (V_m = V + 40\log_{10}([Ca]_o))</td>
<td>$1.7\exp(-(V_m + 152)/30)$</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>(\max(1/(\alpha + \beta), 1.1)$</td>
</tr>
<tr>
<td></td>
<td>$q=1$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>$1/(\exp((V + 12.6)/18.9 + 1)$</td>
</tr>
<tr>
<td>$I_{AHP}$</td>
<td>$p=1$</td>
<td>$0.0048\exp(-5\log_{10}([Ca]_o^2) + 17.5)$</td>
<td>$0.012\exp(2\log_{10}([Ca]_o^2) + 20)$</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>$48$</td>
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</table>

(b) LA interneuron model

<table>
<thead>
<tr>
<th>Current Type</th>
<th>Gating Variable</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$x_\infty$</th>
<th>$\tau_x$</th>
</tr>
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<tbody>
<tr>
<td>$I_{Na}$</td>
<td>$p=3$</td>
<td>$2.1\exp((V + 18.5)/11.57)$</td>
<td>$2.1\exp(-(V + 18.5)/27)$</td>
<td>$\alpha/(\alpha + \beta)$</td>
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<tr>
<td></td>
<td>$q=1$</td>
<td>$0.045\times \exp(-(V + 29)/33)$</td>
<td>$0.045\times \exp((V + 29)/12.2)$</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
</tr>
<tr>
<td>$I_{DR}$</td>
<td>$p=4$</td>
<td>$0.15\times \exp((V + 19)/10.67)$</td>
<td>$0.15\times \exp(-(V + 19)/42.68)$</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
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TABLE 3.3. Model parameters for network connections and inputs

<table>
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<tr>
<th>Connection</th>
<th>Initial weight</th>
<th>$f_{\text{max}}$ ($f_{\text{min}}=0.8$ for all)</th>
<th>Learning factor</th>
<th>Ca$^{2+}$ threshold (µmol)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>scaling</td>
<td>decay</td>
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<tr>
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<td>3</td>
<td>15.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Pyr to Pyr</td>
<td>1.5</td>
<td>3</td>
<td>2.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Inter to Pyr</td>
<td>5.0</td>
<td>4</td>
<td>2.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Tone to Inter</td>
<td>3.0</td>
<td>3</td>
<td>1.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Pyr to Inter</td>
<td>1.0</td>
<td>3</td>
<td>2.0</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Pyr – Pyramidal cell; Inter – Interneuron
* The shock synapse (weight = 40 for pyramidal cell and 20 for interneuron) and the interneuron-to-interneuron synapse (weight = 3) do not potentiate
3.7 Supplemental Figures

FIG. S1. A-C: firing patterns for 3 cells that show the extremes (A and C) and middle (B) of the range of responses observed in LA (Faber et al. 2001). Responses to a large current injection (600 ms, 400 pA), a current injection near the threshold and a hyperpolarizing current injection of (100 pA) are shown in row 1, 2 and 3 respectively.
FIG. S2. The tone responses of simultaneously recorded neurons in LA at different points during training (Quirk et al. 1995). A: representative time histograms showing 5 neurons from LAd simultaneously recorded prior to conditioning (sensitization), 1 hr after conditioning (early extinction), and following 30 extinction trials (late extinction). Bin width is 10 ms and 10 trials are summed at each phase. B: a difference time histogram showing the conditioned responses of the 5 cells. Spike counts during sensitization were subtracted from counts during early extinction. Bin width is 4 ms and 10 trials are summed.
FIG. S3. Synaptic properties of pyramidal cells. 

A: AMPA and NMDA synaptic conductance in response to one single spike input (w = 1). 

B: AMPA and NMDA synaptic currents in response to one single spike input (compare to Fig. 3A, Weisskopf and LeDoux 1999). The ratio (total charge transfer) of AMPA to NMDA current is 1:4.6. 

C: the Mg-block function for NMDA current. 

D: top panel: voltage response of a single type A pyramidal cell to tone input (200 Hz from 500 to 1000 ms; w = 20); middle panel: AMPA current; low panel: NMDA current.
FIG. S4. Spontaneous recovery of fear and re-extinction. A: tone responses of all pyramidal cells and one interneuron during the early part of re-extinction (first ten tones) showing recovery. B: tone responses during the late part of re-extinction (last ten tones). C: difference between recovered responses and the responses during the late part of first extinction. Tone started at t = 0; bin width was 10 ms and spike counts during 10 trials were summed together.
FIG. S5. The tone/shock evoked membrane potential fluctuations with A: a slow (entire tone) and B: fast (tone onset) time base for three representative pyramidal cells (P4, P5 & P7) and one interneuron (I1) at various phases of the training. S1: tone 1 in sensitization; C1: tone 1 in conditioning; C10: tone 10 in conditioning; E1: tone 1 in extinction; E30: tone 30 in extinction. Tone started at t = 0 and black arrows indicate the start of shock input.
Chapter 4

Impact of infralimbic inputs on intercalated amygdala neurons: a biophysical modeling study*

Several recent studies indicate that the intercalated (ITC) neurons play an important role in the expression of fear and extinction by controlling the impulse traffic between the input (BLA) and output (Ce) of the amygdala. Indeed, experiments find that activation of the infra-limbic (IL) vmPFC inputs dramatically reduced the responsiveness of Ce to BLA input, presumably through ITC cells, although the underlying mechanisms remain elusive. To shed light on the role of IL in inhibition of the expression of fear, we developed a biophysical ITC network model with dorsal and ventral modules, and realistic BLA input. Model experiments showed that (1) persistent network activity can be maintained in ITC neurons and the strongly adaptive fear/extinction signal from BLA can be transformed into a more sustained output through the ITC cells; (2) the slow deinactivation of the $I_{SD}$ current is necessary for persistent network activity which facilitates Ce inhibition; (3) for a range of IL synaptic weights, activation of the IL inputs effectively increases the responses of both dorsal and ventral ITC neurons inhibiting Ce output, regardless of the inter-ITC inhibition; and (4) the inhibitory inter-ITC coupling strength needs to be limited to make IL effective in suppressing the expression of fear.

* This work was done with D Pare and SS Nair. It is ready to submit to the Journal of Neurophysiology.
4.1 Introduction

Intercalated (ITC) amygdala neurons occur as multiple densely-packed cell clusters distributed along the lateral and medial aspects of the basolateral amygdaloid (BLA) complex. Medial ITC clusters are thought to constitute critical regulators of classically conditioned fear responses (Pare et al., 2004) because they are in a strategic position to control impulse traffic between the sensory input and fear output stations of the amygdala: the BLA and central nucleus (Ce), respectively. Indeed, ITC cells receive glutamatergic afferents from the BLA, and send GABAergic projections to Ce (Pare and Smith 1993a,b; Royer et al. 1999; Jungling et al., 2008). In addition, ITC neurons located dorsally (ITC_D) at the BLA-Ce border inhibit more ventral ones (ITC_V; Royer et al. 2000a), thereby allowing for a spatiotemporally differentiated gating of impulse traffic between BLA and Ce (Fig. 1A; Royer et al. 1999).

Much evidence indicates that medial ITC cell clusters participate in the extinction of conditioned fear responses (Royer and Pare, 2002; Likhtik et al. 2008; Jungling et al., 2008). It is currently believed (Pare et al., 2004; Quirk and Mueller, 2008) that extinguished conditioned stimuli (CS) activate infralimbic (IL) neurons with glutamatergic projections to ITC cells. In turn, ITC cells would reduce conditioned fear responses by generating feedforward inhibition in fear output Ce neurons (Pare et al. 2004). Consistent with this, IL stimulation dramatically reduces the responsiveness of Ce neurons to BLA inputs (Quirk et al. 2003). However, IL axons target all medially-located ITC cells clusters (McDonald et al. 1996). Since there are inhibitory connections between (Royer et al. 2000a; see Fig. 1A) as well as within ITC cells clusters (Geracitano et al. 2007), it is not immediately clear how IL inputs could overcome the inter-ITC inhibition.
Unfortunately, it is currently impossible to address this question experimentally because we lack criteria to identify ITC cells on the basis of their extracellularly recorded activity. Thus, we developed a realistic biophysical conductance-based model of the ITC network (Fig. 1B) to study how inter-ITC inhibitory connections affect their responses to IL inputs. A second objective of our study was to examine how the peculiar electroresponsive properties of ITC cells shape their responsiveness to BLA inputs. Indeed, ITC cells express an unusual voltage-dependent K+ conductance whose slow-deactivation kinetics allows them to produce prolonged depolarizing plateaus after a transient depolarization (Royer et al. 2000b). The ability of ITC neurons to transform transient excitatory inputs into a prolonged state of increased excitability may have important consequences for how they regulate conditioned fear. In keeping with this idea, during prolonged auditory CSs, BLA principal neurons fire transiently due to their strong frequency adaptation (Quirk et al. 1995, 1997; Repa et al. 2001; Herry et al. 2008). At present, it is not clear how such transient responses are converted into a sustained behavioral output. We therefore tested the hypothesis that the bistable electroresponsive properties of ITC cells allow them to transform transient BLA signals into a more sustained output.

4.2 Methods

4.2.1 Overview of the model

We developed a biophysical conductance-based model of the medial ITC network (Fig. 1B) with dorsal and ventral ITC modules. We first elaborated a single cell ITC model that could replicate the bistable behavior of ITC cells, as seen experimentally (Royer et al.,
Short-term facilitation and depression were implemented in the GABAergic ITC-ITC and ITC-Ce connections and the learning parameters were adjusted to reproduce stimulation frequency-dependent changes in release probability observed experimentally (Geracitano et al. 2007). We first investigated whether the sustained activity seen in a single ITC cell can be maintained in the ITC network with inhibitory connectivity. The model was then used to study the impact of a global increase in network excitability by IL inputs on the distribution of activity among ITC neurons located at different dorsoventral levels along the BLA-Ce border.

4.2.2 ITC single cell model

The ITC cell was modeled using a Hodgkin-Huxley type formulation (Byrne and Roberts, 2004). It had two compartments representing a soma (diameter of 8 µm; length of 8 µm) and a dendrite (diameter of 4 µm; length of 200 µm) (Marowsky et al. 2005). The current balance equations were of the form given in Eqns. 1 and 2,

\[ C_m \frac{dV_s}{dt} = -g_L(V_s - E_L) - g_c(V_s - V_d) - \sum I^\text{int}_s - \sum I^\text{syn}_s + I_s \]  
\[ C_m \frac{dV_d}{dt} = -g_L(V_d - E_L) - g_c(V_d - V_s) - \sum I^\text{int}_d - \sum I^\text{syn}_d + I_d \]  

where \( V_s \) and \( V_d \) were the somatic and dendritic membrane potentials (mV), \( I^\text{int}_s \) (\( I^\text{int}_d \)) were the intrinsic currents and the synaptic currents in the soma (dendritic) compartments respectively, \( I_s \) (\( I_d \)) was the electrode current applied to the soma (or dendrite), \( C_m \) was the membrane capacitance and \( g_c \) was the coupling conductance between the soma and the dendrite. The values for the specific membrane resistance, membrane capacitance and cytoplasmic (axial) resistivity were, respectively,
$R_m = 30 \text{ K}\Omega\text{-cm}^2$, $C_m = 1.0 \mu\text{F/cm}^2$, and $R_a = 150 \Omega\text{-cm}$. The leakage reversal potential ($E_L$) was set to -93 mV. The resulting $V_{\text{rest}}$ was about – 85 mV (Geracitano et al. 2007), $R_{\text{IN}}$ was 700 M$\Omega$ when measured from rest, and $\tau_m = R_mC_m$ was 30 ms, consistent with experimental observations (Royer et al., 2000b). The ITC model contained several ionic currents including a leakage current $I_L$, a spike-generating sodium current $I_{\text{Na}}$, a potassium delayed rectifier $I_{\text{DR}}$, a slow deinactivating current $I_{\text{SD}}$, a voltage-gated persistent muscarinic current $I_M$, a hyperpolarization-activated current $I_H$, a high-voltage activated Ca$^{2+}$ current $I_{\text{Ca}}$ and a slow Ca$^{2+}$-dependent afterhyperpolarization current $I_{\text{sAHP}}$.

The ionic current for channel $i$, $I_i$, was modeled as $I_i = g_i m^p h^q (V - E_i)$, where $g_i$ was its maximal conductance, $m$ its activation variable (with exponent $p$), $h$ its inactivation variable (with exponent $q$), and $E_i$ its reversal potential. The kinetic equation for each of the gating variables $x$ ($m$ or $h$) had the form

$$\frac{dx}{dt} = \frac{x_{\infty}(V,[Ca^{2+}]_i) - x}{\tau_x(V,[Ca^{2+}]_i)}$$

where $x_{\infty}$ was the voltage- or Ca$^{2+}$- dependent steady state and $\tau_x$ was the voltage- or Ca$^{2+}$- dependent time constant. The maximal conductance densities for all ionic currents and the expressions of $x_{\infty}$ and $\tau_x$ for each gating variable are listed in Tables A1 and A2 in the appendix.

**Kinetics of $I_{\text{SD}}$ current.** The “A” current model in Huguenard and McCormick (1992) was modified to obtain the slow deinactivating conductance of the $I_{\text{SD}}$ current. The half-activation/inactivation and the activation/inactivation time constants were adjusted to replicate the experimental observations of Royer et al. (2000b), and the current was modeled as $I_{\text{SD}} = g_{\text{SD}} m^4 h(V - E_K)$. The steady state functions $m_{\infty}(V)$ and $h_{\infty}(V)$ are
shown in Fig. 2A, and the inactivation time constant function $\tau_h(V)$ is shown in Fig. 2B.

We note that the half-activation and half-inactivation voltages were about -80 mV and -50 mV respectively, and $I_{SD}$ reached its maximum at around -60 mV ($m^4h$ has the largest value), consistent with the observations of Royer et al. (2000b). The time constant of the inactivation variable was 80 ms for $V > -30$ mV and it increased considerably for $V < -50$ mV. Hence, $I_{SD}$ activated fully in the subthreshold range ($V \approx -60$ mV), inactivated slowly in response to suprathreshold depolarization ($V > -40$ mV), and deinactivated very slowly ($V < -50$ mV).

**Calcium dynamics.** Intracellular calcium was regulated by a simple first-order differential equation of the form (Warman et al. 1994):

$$\frac{d[Ca^{2+}]}{dt} = -f \frac{I_{Ca}}{VFA} + \frac{[Ca^{2+}]_{rest} - [Ca^{2+}]_i}{\tau_{Ca}}$$

(4)

where $f$ is the fraction of the Ca$^{2+}$ influx ($f = 0.024$), $V = wA$ with $w$ the shell thickness (1 µm), $A$ the cell surface area, $F$ is the Faraday constant, $\tau_{Ca}$ is the Ca$^{2+}$ removal rate ($\tau_{Ca} = 80$ ms). The resting Ca$^{2+}$ concentration $[Ca^{2+}]_{rest} = .05$ µm/l, which was the same as the initial concentration, as determined experimentally (Durstewitz et al. 2000).

**4.2.3 Ce single cell model**

The Ce cell model also contained two compartments: a soma (diameter of 15 µm; length of 15 µm) and a dendrite (diameter of 5 µm; length of 300 µm). Previously, Ce cells have been classified into three subtypes, based on their responses to depolarizing current injections: adapting, late-firing and regular spiking (Lopez De Armentia and Sah,
2004; Dumont et al. 2002). Since Ce was the output station of this fear circuit, we only modeled the prevalent Ce cell type (regular spiking). The Ce model included a leakage current $I_L$, a sodium current $I_{Na}$, a delayed rectifier $I_{DR}$, a muscarinic current $I_M$, a hyperpolarization-activated current $I_H$, a high-voltage activated Ca$^{2+}$ current $I_{Ca}$ and a slow Ca$^{2+}$-dependent afterhyperpolarization current $I_{SAHP}$. The passive membrane properties were as follows: $R_m = 30 \text{ K}\Omega \cdot \text{cm}^2$, $C_m = 1.0 \mu\text{F/cm}^2$, $R_a = 150 \Omega\cdot\text{cm}$, and $E_L = -70 \text{ mV}$.

4.2.4 Network structure and inputs

The ITC network included 10 ITC$_D$ cells and 10 ITC$_V$ neurons, plus one Ce output neuron (Fig 1B). Based on experimental findings (Geracitano et al. 2007), the synaptic connections between the ITC neurons was split equally into facilitating, depressing, and constant types (each neuron formed only one type of synapse with all its targets, as seen experimentally; Geracitano et al. 2007). Each ITC neuron inhibited three other randomly selected neurons within the same cluster. This connectivity ratio (30%) is about twice higher than reported in Geracitano et al. (2007), and was needed to compensate for the fewer number of cells in our reduced network (Dyhrfjeld-Johnsen et al. 2007). Also, each ITC$_D$ neuron inhibited three randomly selected ITC$_V$ neurons (the inhibitory connectivity among ITC$_D$ and ITC$_V$ cell clusters is shown in Table A3). LA inputs projected to ITC$_D$ while BA and IL inputs projected to both ITC$_D$ and ITC$_V$ neurons, as observed experimentally (McDonald et al. 1996; Royer et al. 1999; Royer et al. 2000a; Fig. 1B). The Ce neuron received excitatory inputs from the BA, and inhibitory inputs from ITC$_V$ neurons (Pare and Smith 1993; Royer et al. 1999; Pare et al. 2004). The network received
10 LA, 10 BA and 10 IL inputs. Each LA input projected to 5 randomly selected ITC_D neurons, and each BA or IL input projected to 5 ITC_D as well as 5 ITC_V neurons, both again chosen randomly. The Ce neuron received excitation from all 10 BA inputs, as well as inhibition from all 10 ITC_V neurons. Random background inputs were delivered to all ITC neurons and to the Ce neuron. Based on experimental data (Faber et al. 2001; Quirk et al. 95, 97; Herry et al. 2008), the 10 LA/BA inputs were modeled with different degrees of frequency adaptation (see Appendix for details). The IL inputs were modeled as Poisson-distributed random spike trains.

In the model, excitatory synaptic transmission was mediated by glutamate acting at AMPA/NMDA receptors, while the inhibitory synapses were mediated by GABA acting at GABA_A receptors (Sah et al., 2003 in Physiol Rev). The AMPA/NMDA and GABA_A synaptic currents were modeled by dual exponential functions, as follows (Li et al. 2009):

\[ I_{AMP} = wA g_{AMP,max} \frac{\tau_1 \tau_2}{\tau_2 - \tau_1} [\exp(-t/\tau_2) - \exp(-t/\tau_1)](V - E_{AMP}) \]  
\[ I_{NMDA} = wA g_{NMDA,max} s(V) \frac{\tau_1 \tau_2}{\tau_2 - \tau_1} [\exp(-t/\tau_2) - \exp(-t/\tau_1)](V - E_{NMDA}) \]  
\[ I_{GABA} = wA g_{GABA,max} \frac{\tau_1 \tau_2}{\tau_2 - \tau_1} [\exp(-t/\tau_2) - \exp(-t/\tau_1)](V - E_{GABA}) \]

where \( w \) was the synaptic weight, \( A \) was a normalization constant chosen so that \( g_{AMP,max}, g_{NMDA,max} \) and \( g_{GABA,max} \) assumed maximum values of the conductances; \( \tau_1 \) and \( \tau_2 \) were the rise and decay time constants, respectively. For AMPA receptor channels, \( \tau_1 = 0.5 \) ms and \( \tau_2 = 7 \) ms for ITC cells, and \( \tau_1 = 1.8 \) ms and \( \tau_2 = 4.4 \) ms for Ce neurons (Lopez De Armentia and Sah. 2003). For NMDA receptor channels, \( \tau_1 = 5 \) ms and \( \tau_2 = 125 \) ms for ITC cells, and \( \tau_1 = 5 \) ms and \( \tau_2 = 162 \) ms for Ce neurons (Lopez De Armentia and Sah.
The voltage-dependent variable $s(V)$ which implements the Mg$^{2+}$ block of NMDA receptors was defined as: $s(V) = [1 + 0.33 \exp(-0.06 V)]^{-1}$ (Zador et al. 1990). For the GABA$_A$ synaptic current, $\tau_1 = 0.8$ ms, $\tau_2 = 13$ ms for ITC cells (Geracitano et al. 2007) and $\tau_1 = 0.8$ ms, $\tau_2 = 40$ ms for Ce cells (Esmaeili et al. 2009). The maximal conductances were chosen as: $g_{\text{AMPA}, \text{max}} = 1$ nS, $g_{\text{NMDA}, \text{max}} = 0.5$ nS (Li et al. 2009), and $g_{\text{GABAA}, \text{max}} = 0.6$ nS (Geracitano et al. 2007). Synaptic reversal potentials were set as $E_{\text{AMPA}} = E_{\text{NMDA}} = 0$ mV, $E_{\text{GABAA}} = -75$ mV (Li et al. 2009).

### 4.2.5 Presynaptic release probability

As observed experimentally (Geracitano et al. 2007), short-term synaptic plasticity in ITC-ITC connections was heterogeneous and depended on presynaptic firing rates. In the absence of specific information, such heterogeneous plasticity was also assumed to exist in the ITC$_V$-Ce connections. Presynaptic release probability was updated according to the following learning rule:

$$\frac{dp}{dt} = \frac{\Phi(f) - p}{\tau}$$

(8)

where the time constant $\tau$ was selected to be 100 ms (Wang 1999), $\Phi$ was a function of the presynaptic firing frequency $f$, determined by non-linear regression modeling of the experimental data (Geracitano et al. 2007; Fig. A2 in the appendix). When a presynaptic spike occurred, the presynaptic frequency was updated as $f = 1/\Delta T$, where $\Delta T$ was the interval between the previous and current spikes. Between spikes, $f$ decayed back towards zero with a time constant $\tau_f$ of 1 sec. When a presynaptic spike occurred, a uniform random number $P_r$ (between 0 and 1) was generated. If $P_r$ was less than the current...
release probability $p$ then neurotransmitter release was successful, otherwise it was not. The initial release probability was 0.3 and 0.8 for the facilitating and depressing synapses, respectively, and the release probability of constant synapses was fixed at 0.75 (Geracitano et al. 2007).

4.2.6 Other model details

In the absence of detailed knowledge, the model incorporated the following assumptions: (1) BA and LA inputs were modeled in the same manner; (2) LA and BA inputs projected equally to the ITCD neurons; (3) BA projected equally to ITCD and ITCV, i.e., the average number of BA inputs per ITCD/ITCV neuron was the same; (4) IL projected equally to ITCD and ITCV.

We simulated network responses to a 2 sec auditory tone input during expression of fear. The steady-state firing frequency was measured during the last 1 sec of the tone, while the estimate of average frequency was based on the entire 2 sec tone period. The instantaneous firing frequency was calculated by dividing tone period into 100 ms bins. Spontaneous firing rate was measured during the 1 sec period prior to tone onset. Most model experiments considered two scenarios, a weakly coupled network (all ITC-ITC synaptic weights set to 1, i.e., $W_{ITC-ITC} = 1$) and a strongly coupled network ($W_{ITC-ITC} = 5$). All other synaptic weights used are shown in Table A4. Model runs were performed on a personal computer using the software package GENESIS (Bower and Beeman 2003) with the Crank-Nicholson integration method, and a time step of 20 $\mu$s.
4.3 Results

4.3.1 Single cell firing properties

*ITC model neuron.* The responses of the ITC model neuron to current injections from rest reproduced the behavior of ITC cells observed experimentally (Fig. 2C-D). In response to low amplitude depolarizing current injections (Fig. 2C2), there was a delay to the first spike and the firing frequency gradually increased with time, due to the inactivation of the $I_{SD}$ current (green curve). With higher current injections (Fig. 2C1), the delay to first spike was reduced and the neuron fired tonically at a higher rate. Also, the $I_{SD}$ current inactivated faster compared to low amplitude injected currents. In response to a hyperpolarizing current injection (Fig. 2C3), a slow depolarizing sag was observed, due to the activation of the $I_h$ current.

The impact of $I_{SD}$ on the repetitive firing behavior of the ITC model neuron is shown in figure 2D that plots instantaneous firing frequency evoked by depolarizing current injections with (solid lines) and without (dashed lines) the $I_{SD}$ current. In the presence of $I_{SD}$ and with low amplitude depolarizing current injections (100 pA, bottom), the firing frequency gradually increased. However, when the $I_{SD}$ current was removed from the model, the firing frequency started at a higher rate and slowed down to about 70 Hz due to the activation of the $I_{sAHP}$ current. Thus, the inclusion of the $I_{SD}$ current changed regular frequency adaptation to reverse frequency adaptation. When the applied current increased, the firing frequency with $I_{SD}$ current no longer increased monotonically with time, but showed a tri-phasic profile: it increased for about 100 ms at the beginning of the current pulse, then decreased for about 100 ms, and then increased slightly thereafter. This indicates that with high current injections, the $I_{sAHP}$ current activated rapidly, which
overcame the effect of the $I_{SD}$ current inactivation after 100 ms. Without the $I_{SD}$ current, the frequency curve also showed a similar trend, with a larger second-phase adaptation decrease due to the loss of the opposing influence from the $I_{SD}$ current inactivation.

A characteristic feature of ITC cells is that due to inactivation of the $I_{SD}$ current, following spike trains, they generate after-depolarizations (ADPs) that increase in amplitude with the amount of depolarizing current (Royer et al. 2000b). To test whether our model could reproduce this behavior, transient suprathreshold current injections of various amplitudes were applied from a constant pre-pulse membrane potential (Fig. 3A). With low current injections (Fig. 3A1), the ADP had a low amplitude, and a characteristic “hump and sag” developed with increased current (Fig. 3A2). With an even larger current, the ADP reached spike threshold (Fig. 3A3). Time-dependent variations in the activation ($m$) and inactivation ($h$) gating variables of the $I_{SD}$ current are shown below each voltage trace in Fig. 3A. As the current injection increased, the variable $h$ was inactivated to a lower level, i.e., the current was more efficiently closed, thereby causing a larger ADP. To test that the ADP was due to the $I_{SD}$ current, voltage responses to depolarizing inputs were compared under TTX condition ($g_{Na} = 0$) for the whole (Fig. 3B1) and partial model without $I_{SD}$ current ($g_{SD} = 0$) (Fig. 3B2). The ADP was completely abolished when $I_{SD}$ was removed from the model (only AHP was shown), demonstrating that $I_{SD}$ was necessary for ADP generation.

Experimentally, the ADP amplitude was reported to increase with the duration of suprathreshold depolarizing current pulses (Royer et al. 2000b). To test whether our model could reproduce this behavior, current injections of constant intensity but variable duration were applied from the same prepulse $V_m$ (Fig. 3C). In keeping with experimental
observations, the ADP amplitude increased with current duration, eventually leading to tonic firing (Fig. 3C3). Examination of gating variables shows that longer current injections inactivated \( I_{SD} \) current more effectively, giving rise to larger ADPs. In the sustained firing case (Fig. 3C3), the \( I_{SD} \) current remained inactivated for a long time.

Last, we examined the effect of the prepulse \( V_m \) on the ADP amplitude. Depolarizing current pulses adjusted to elicit approximately the same number of spikes were delivered at different prepulse voltages (Fig. 3D). When \( V_m \) was low (-74 mV), little ADP was produced (Fig. 3D3). An ADP was seen when \( V_m \) was depolarized to -66 mV (Fig. 3D2). With a more depolarized \( V_m \) (-62 mV), the ADP could reach the spike threshold after the current pulse (Fig. 3D1), consistent with experimental observations (Royer et al. 2000b). This was because \( I_{SD} \) reached its maximal activation at around -60 mV, as mentioned earlier.

**CE. Model neuron.** The responses of the Ce model neuron to current injections from rest reproduced the behavior of regular spiking Ce cells observed experimentally (Lopez De Lopez De Armentia and Sah, 2004; Dumont et al. 2002). As shown in figure 4, with low amplitude depolarizing current injections, only one spike was elicited whereas current pulses of higher amplitude evoked repetitive firing with modest spike frequency adaptation. Also characteristic of regular spiking Ce neurons, negative current injection elicited a hyperpolarizing response with a slowly developing depolarizing sag, produced by the activation of \( I_H \).

**4.3.2 Persistent network activity**

The above indicates that because of the slow deinactivation kinetics of \( I_{SD} \), single ITC model neurons can generate sustained depolarizations in response to transient current
injections (Figs. 3A, C). However, it remained unclear whether transient inputs would have the same effect in a realistic network where ITC cells are reciprocally connected by inhibitory synapses. For instance, BLA cells are known to fire transiently at CS onset (Quirk et al. 1995; 1997), yet conditioned fear responses last for the entire duration of the CS. Could $I_{SD}$ allow ITC cells to transform the transient signals arising from the BLA into a more persistent output? To test this, we injected a brief current pulse (300ms; 160 pA; red lines in Fig. 5) into all ITC cells, mimicking the transient BLA signal, while the Ce neuron was driven by actual BA inputs for 2 sec. In figure 5 as well as in most subsequent figures, we show the voltage responses of several $ITC_D$ (D3, D8) and $ITC_V$ (V1,V4) neurons, together with the Ce output for simulations with weak (Fig. 5A) and strong (Fig. 5B) inter-ITC coupling.

Prior to current injection, ITC neurons fired at about 1.5 Hz (ITC$_D$: 2.3 Hz; ITC$_V$: 1 Hz) due to random background inputs. In the weak coupling case (Fig. 5A), current injection produced high frequency discharges in most ITC cells. However, at the end of the current pulse, most ITC cells continued to fire at higher rates than baseline (D3, D8 > 15 Hz; V1, V4 > 10 Hz). Insights into the mechanisms underlying these phenomena can be obtained by considering time-dependent fluctuations in the $I_{SD}$ inactivation variable (thin lines superimposed on the voltage responses of Fig. 5AB). This reveals that $I_{SD}$ inactivated rapidly during the current injection, but that it remained inactivated thereafter. Thus, inactivation of $I_{SD}$ enabled most $ITC_D$ neurons as well as $ITC_V$ cells to fire continuously beyond the current injection, which in turn kept the $I_{SD}$ current inactivated for a long time. In keeping with the idea that $I_{SD}$ plays a critical role in generating
persistent activity, accelerating the de-inactivation kinetics of $I_{SD}$ abolished the persistent activity of ITC cells (Supplementary figure S1).

Expectedly, the impact of $I_{SD}$ inactivation on persistent activity varied depending on the strength of inhibitory connections between ITC cells. Comparing the weak (Fig. 5A) and strong (Fig. 5B) inter-ITC coupling cases revealed that increasing inhibition strength reduced sustained activity in V1 and V4, while little change was seen in D3 and D8. That was because V1 and V4 received more inhibitory inputs than D1 and D8 (see Table A3 in the appendix).

As a group, ITC$_V$ cells exhibited less persistent activity than ITC$_D$ cells due to the unidirectional inhibitory connections from ITC$_D$ to ITC$_V$ neurons. This can be appreciated by examining the average instantaneous firing rates of all ITC$_D$ and ITC$_V$ cells (Fig. 5C) or their steady-state firing rate (measured in the last 1 sec) (Fig. 5D). In both, the weak and strong inter-ITC coupling cases, the persistent activity of ITC$_V$ cells was markedly lower than in ITC$_D$ cells (weak coupling, 9.9 vs. 15.5 Hz; strong coupling, 4.2 vs. 8.9 Hz, respectively; Fig. 6D). As a result, the Ce output neuron fired at a higher frequency when the inter-ITC inhibitory strength increased because this caused an overall decrease in the firing rate of ITC$_V$ neurons and a consequent disinhibition of Ce.

4.3.3 Impact of IL inputs in network conditions reproducing high fear state

Prior to studying the impact of IL inputs on ITC cells, it is important to characterize the model’s behavior in response to LA and BA inputs. Indeed, unit recordings have revealed that after fear conditioning, the responses of LA and BA neurons to CS
presentations consist of an early phase of strongly increased firing frequency that quickly
decays to levels slightly over baseline for the remainder of the stimulus (Quirk et al. 1995;
Herry et al. 2008). Thus, we first consider the impact of this CS-evoked activity on ITC
cells and then examine how gradually increasing the frequency of IL inputs modulate
network behavior.

Figure 6 shows the activity of LA, ITC_D (D1, D8) and ITC_V (V1,V4) neurons, together
with the Ce output, for weak (Fig. 6A,C) and strong inter-ITC coupling (Fig. 6B,D) cases.
When a 2 sec CS is presented (red arrow in Fig. 6A,B), it triggers an adapting train of
action potentials in BLA neurons. In turn, CS-evoked BLA firing activates ITC_D and
ITC_V cells. However, the firing rates of ITC_D cells are higher than those of ITC_V due to
the unidirectional inhibitory connections from ITC_D to ITC_V neurons. For the weak inter-
ITC coupling case (Fig. 6A,C), ITC_D cells displayed continuous discharges throughout
the 2 sec period. When the inhibition strength is increased, D1 and D8 displayed episodic,
almost anti-phase firing (Fig. 6B) because these two ITC cells inhibited each other (Table
A3). In addition, the increased strength of inhibition between ITC cells reduced the
activity of ITC_V cells (Fig. 6B,D). As to the Ce neuron, because it received adaptive BA
inputs, it also showed frequency adaptation, but fired continuously at a higher frequency
(about 20 Hz) than baseline (8 Hz) during the CS, signaling a high “fear” state. With
stronger coupling between ITC neurons, Ce firing rate further increased because ITC_V
cells were inhibited by ITC_D neurons, disinhibiting Ce. Figure 6C,D shows the average
instantaneous firing rates of all BLA inputs, all ITC_D and ITC_V neurons, as well as of the
Ce output.
To compare the amount of spike frequency adaptation in BLA and ITC neurons, we computed the quantity $F_{\text{adap}}$, proposed by Wang (1998): 

$$F_{\text{adap}} = \frac{f_0 - f_{\text{ss}}}{f_0},$$

where $f_0$ is the initial firing rate and $f_{\text{ss}}$ is the steady-state firing rate. This revealed that adaptation was much higher in BLA cells (88%) than in ITC cells (range of 45% to 61% for weak or strong inter-ITC coupling cases), due to the slow de-inactivation of $I_{SD}$. Thus, the strongly adapting signal from BLA neurons can be transformed into a more sustained, less adaptive output by ITC cells. The Ce output also exhibited less frequency adaptation compared to BLA inputs (70% for weak and 67% for strong inter-ITC coupling cases).

Figure 7A shows the CS-related firing rates (integrated over 0-2 sec) of individual ITC$_D$ (Fig. 7A1) and ITC$_V$ neurons (Fig 7A2), and the average discharge frequencies of all cells (Fig. 7A3), for both the weak and strong inter-ITC coupling cases. As the inhibitory weight increased five-fold, the firing rate of ITC$_D$ neurons was reduced from 39.6 to 22.7 Hz, and that of ITC$_V$ neurons was reduced from 14 to 5 Hz, while the firing frequency of the Ce neuron increased from 18.5 to 24.5 Hz.

Figure 8 illustrates the impact of IL inputs on CS-evoked network activity in both the weak (Fig. 8A,C) and strong (Fig. 8B,D) inter-ITC coupling cases. Based on experimental observations (Milad and Quirk, 2002), IL inputs were introduced 100 ms after CS onset and activated at various frequencies for the duration of the CS. An IL stimulation frequency of 20 Hz is shown in the examples of Figure 8A-D whereas Fig. 7B shows the effects observed in a range of frequencies (0-20 Hz). Irrespective of inter-ITC coupling strength, activation of IL inputs caused a large increase in the discharge rates of ITC$_D$ and ITC$_V$ cells (compare Figs. 8A-D with Figs. 6A-D). Interestingly, the activation of IL inputs completely abolished frequency adaptation in ITC neurons,
leading to a sustained and persistent output (Fig. 8C,D). In the Ce neuron, the discharge rate 100 ms after CS onset was significantly reduced by IL inputs and there were only a few spikes for the weak coupling case, indicating a “low fear” state. It should be noted that this reduction in CS-related CE responses to BLA inputs is consistent with earlier experimental observations (Quirk et al. 2003).

The average firing frequency of ITC_D, ITC_V, and Ce neurons is shown in Figure 8B3. As the IL stimulation frequency increased, the discharge rate of ITC_D and ITC_V neurons augmented, resulting in decreased Ce firing. However, this effect of IL stimulation on Ce activity was more prominent in the weak than the strong inter-ITC coupling case (Fig. 8B). For the weakly coupled case, IL stimulation (20 Hz) caused a 125% increase in the firing rate for ITC_D neurons during the CS, compared to a 350% increase for ITC_V neurons, and a 70% decrease in Ce firing rate. In contrast, for the strongly coupled network, a 129% increase in firing rate was observed for ITC_D neurons, and a 371% increase for ITC_V cells, but IL stimulation caused only a 45% reduction in the Ce firing rate. This is because for the strong inter-ITC coupling case, the absolute ITC_V firing rate was much lower compared to the weak inter-ITC coupling case, although the % increase was similar for both cases. Importantly, qualitatively identical findings were obtained when the same tests were carried out using a large range of IL input strengths (supplementary figure X).

Interestingly, IL inputs caused a larger increase in the firing rate of ITC_V cells than ITC_D cells (% increase was larger). From the standpoint of the ITC_V neurons which inhibit Ce output neurons, an increase in IL activity also leads to augmented inhibitory inputs from other ITC_V neurons as well as from ITC_D cells. But, our results suggest that
the excitation by IL inputs can overwhelm the inhibitory impact of inter-ITC inhibitory connections, as suggested in previous experiments (Quirk et al. 2003). Thus, despite the inter-ITC inhibition, IL stimulation can still be effective in increasing ITCV responses, thus inhibiting Ce and consequently fear responses.

4.4 Discussion

The IL cortex is believed to play a critical role in extinction by inhibiting fear output Ce neurons via the activation of ITC cells (Pare et al. 2004; Quirk and Mueller 2008). However, previous physiological studies have revealed that ITC cells inhibit each other (Royer et al. 2000a; Geracitano et al. 2007) raising the following question: can IL inputs overcome the inter-ITC inhibition to regulate the CS-evoked activity Ce neurons? Because it is currently impossible to test this experimentally, we approached this problem using a biophysical network model. Our findings indicate that in a wide range of stimulation frequency and input strengths, IL activation can overwhelm inter-ITC inhibition and reduce the activity of fear output Ce neurons. In addition, we observed that ITC neurons could transform transient CS-related signals arising in the BLA into a persistent pattern of activity. Below, we consider the significance of these observations in light of previous work about the physiology of ITC neurons.

**IL control of ITC cells and the regulation of conditioned fear expression**

Mounting evidence implicates ITC neurons in the regulation of conditioned fear expression. For instance, ITC lesions (Likhtik et al. 2008) as well as pharmacological inhibition of BLA inputs to ITC cells (Jungling et al., 2008) interfere with the extinction
of conditioned fear responses. The inhibition of conditioned fear responses by ITC cells during extinction is thought to depend on their activation by glutamatergic inputs from the infralimbic cortex (Pare et al. 2004; Quirk and Mueller 2008). Several observations support this view. First, IL lesions interfere with the consolidation of extinction (Quirk et al. 2000). Second, IL sends a massive projection to ITC cells (McDonald et al. 1996; Freedman et al. 2000). Third, disinhibition of IL with local picrotoxin infusions lead to increased c-Fos expression in ITC cells (Berretta et al. 2005). Fourth, extinction testing is associated with marked increases in the number of c-Fos positive IL and ITC cells (Maren et al., 2009). Last, electrical stimulation of IL produces a reduction of conditioned fear (Milad and Quirk 2002; Vidal-Gonzalez et al. 2006) and an abolition of BLA-evoked responses in Ce neurons (Quirk et al. 2003). In principle, ITC cells could produce the latter two effects by generating feed-forward inhibition in Ce when activated by IL inputs (Pare and Smith 1993a,b; Royer et al. 1999).

However, other observations cast doubt on the validity of this interpretation. Indeed, ITC cells occur as densely packed clusters and there are connections between ITC cells located in the same (Geracitano et al. 2007) as well as different clusters (Royer et al. 2000a). The latter connections are directionally polarized, in the dorsoventral direction (Royer et al. 2000a). A further complication comes from the fact that inter-ITC connections show short-term plasticity (Geracitano et al. 2007). In a recent in vitro study, when presynaptic ITC cells were repeatedly activated with current injection in a range of frequencies, in an approximately equal proportion of cell pairs, transmitter release probability decreased, increased, or remained constant (Geracitano et al. 2007). As a result, it is not immediately clear whether IL inputs might overcome the inter-ITC
inhibition and how the short-term plasticity of inter-ITC synapses might alter the impact of IL activity over time.

To examine these issues, we first developed a compartmental model of a single ITC cell that could reproduce their unusual electroresponsive properties, as observed experimentally (Royer et al. 2000b). These included their very high input resistance, repetitive firing behavior, and ability to transform transient depolarizations into prolonged periods of increased intrinsic excitability via the inactivation of a potassium current with slow de-inactivation kinetics (I_{SD}). Next, we generated a larger ITC network that implemented the short-term synaptic plasticity of inter-ITC connections described above. The network behavior was then tested during patterns of BLA activation designed to simulate the rapidly adapting profile of activity evoked by CS presentations, as observed experimentally (Quirk et al. 1995; Pare and Collins 2000).

A first interesting observation to emerge from these tests was that despite the presence of recurrent inhibition between ITC cells, transient CS-related BLA inputs were transformed by ITC cells in a sustained state of increased activity via the inactivation of I_{SD}. Although the magnitude of this persistent activity was affected by the strength of inter-ITC inhibitory connections, it remained robust in a wide range of inhibitory synaptic weights (5-fold change). Therefore, these results suggest that ITC cells express a form of short-term memory, inscribed in their intrinsic properties, allowing for persistent alterations in fear responsiveness following transient sensory signals.

Finally, we examined the impact of IL inputs in a wide range of frequencies on the responsiveness of ITC cells to transient CS-related BLA inputs. IL inputs caused a marked increase in the firing rate ITC cells, associated with a near complete abolition of
frequency adaptation. Importantly, qualitatively identical results were obtained when the strength of inter-ITC inhibition was varied 5-fold. The net consequence of IL activity for Ce outputs was a markedly reduced CS responsiveness that increased with the IL stimulation frequency but was stable over time for a given frequency. The latter observation suggests that the short-term synaptic plasticity exhibited by a proportion of inter-ITC connections have a negligible impact on IL control of Ce responsiveness. However, it is likely that this short-term plasticity plays an important role in stabilizing ITC activity.

**Conclusions**

Overall, our results indicate that although the inhibitory connections between ITC cells tend to oppose excitatory influences onto ITC cells, both intrinsic and synaptic, their impact is limited. In particular, IL inputs do overcome the inhibition produced by inter-ITC connections, leading to an overall excitation of ITC cells and a persistent decrease in Ce fear output. These results support the notion that IL inputs are in strategic position to control extinction of conditioned fear via the activation of ITC neurons.
4.5 References


FIG. 4.1. A: schematic of the classical fear conditioning model (adapted from Pare et al. 2004). The LA receives conditioned stimulus (CS) and unconditioned stimulus (US) from the thalamus and projects to the ITC neurons located more dorsally (ITC_D), which in turn project to ITC cells located more ventrally (ITC_V), inhibiting CeM responses. The BA sends excitatory inputs to both ITC_D and ITC_V cells, and the CeM. The output from the CeM projects to the brain stem structures mediating fear responses. BS, brain stem; Glu, glutamate.

B: structure of the proposed ITC network. There are 10 neurons each in ITC_D and ITC_V regions, with random connectivity. Each neuron in ITC_D or ITC_V sends inhibition to three other randomly selected neurons in the same cluster (only one projection per neuron is shown in the figure). All ITC_D neurons send dorsal-to-ventral inhibition to ITC_V neurons. Each ITC_D neuron sends inhibition to three ITC_V neurons randomly selected (e.g., ITC_D2 inhibits ITC_V5). For clarity, the figure only shows partial connectivity between ITC_D and ITC_V (i.e., neurons 1, 3, 5, 7 & 9 in ITC_D also send inhibition to ITC_V neurons which are not shown). There is one Ce output neuron in the model which receives excitatory inputs from the BA, and inhibition from ITC_V neurons. ITC_D and ITC_V: Type A neurons (with facilitating synapses) are neurons 1, 2 & 3; type B neurons (with depressing synapses) are neurons 4, 5 & 6; and type C neurons (with constant synapses) are neurons 7, 8, 9 & 10.
FIG. 4.2. A-B: Kinetics of the $I_{SD}$ current. A: steady-state activation and inactivation curves; B: inactivation time constant function.

C: voltage responses of ITC neuron model to three 1 sec current injections from the resting potential (top: 200 pA; middle: 100 pA; low: -100 pA). Evolution of the inactivation variable $h$ of $I_{SD}$ current is shown in green in the top and middle plots. B: instantaneous firing frequency of ITC model to three different levels of current injections (100, 200 and 300 pA) with and without $I_{SD}$ current.
FIG. 4.3. Bistable properties of the ITC neuron model due to the $I_{SD}$ current. A: dependence of the ADP on the amplitude of current injection. Transient current injections (300 ms) were applied at the same prepulse $V_m (= -60 \text{ mV})$ with different intensity (left: 30 pA; middle: 40 pA; right: 50 pA). The prepulse $V_m$ was controlled by a baseline current injection, and $m$ & $h$ are the activation and inactivation gating variables of the $I_{SD}$ current respectively (same for below). B: comparison of the whole model with the partial model without $I_{SD}$ current in the presence of TTX; C: dependence of the ADP on the duration of current injection. Current injections were applied at the same pre-pulse $V_m (-60 \text{ mV})$ and had the same amplitude. The duration of the current injection was: left panel: 100 ms; middle panel: 200 ms and right panel: 300 ms. D: dependence of the ADP on the prepulse voltage $V_m$. Current injections (300 ms) adjusted to elicit approximately the same number of spikes were applied at different $V_m$ as indicated.
FIG. 4.4. Voltage responses of ITC neuron model to three 600 ms current injections from the resting potential (top: 300 pA; middle: 100 pA; low: -100 pA).
FIG. 4.5. Persistent network activity to transient BLA inputs. A & B: voltage responses of ITC-D3, ITC-D8, ITC-V1, ITC-V4 and Ce neurons for the weak (W_{ITC-ITC} = 1; A) and strong (W_{ITC-ITC} = 5; B) inter-ITC coupling cases. Evolution of the inactivation variable of the I_{SD} current is shown in green. The red bars indicate the duration of the transient input. C: instantaneous firing frequency of ITC_D and ITC_V neurons. Bin width is 100 ms. D: top two panels: steady-state firing frequency (from 1 to 2 sec) of individual ITC_D and ITC_V neurons; bottom panel: average steady-state firing frequency of all ITC_D, ITC_V and Ce neurons.
FIG. 4.6. Network behavior during expression of fear without IL inputs. A & B: voltage responses of ITC-D1, ITC-D8, ITC-V1, ITC-V4 and Ce neurons, together with the spike input of LA5, for the weak and strong inter-ITC coupling cases. BLA inputs were applied at t = 0. The red arrow indicates the start of tone input. C&D: instantaneous firing frequency of all BLA inputs, ITC_D and ITC_V cells with the Ce neuron, for the weak and strong inter-ITC coupling cases. Bin width is 100 ms. E: top two panels: average firing frequency (from 0 to 2 sec) of individual ITC_D and ITC_V neurons; bottom panel: average firing frequency of all ITC_D, ITC_V and Ce neurons.
FIG. 4.7. Network behavior during expression of fear with IL inputs. A&B: voltage responses of ITC-D1, ITC-D8, ITC-V1, ITC-V4 and Ce neurons for the weak and strong coupling cases when the IL stimulation frequency is 20 Hz. C&D: instantaneous firing frequency of all BLA inputs, ITC-D and ITC-V cells with the Ce neuron, for the weak and strong inter-ITC coupling cases. Bin width is 100 ms. E: average firing frequency of ITC_D, ITC_V and Ce neurons under different degrees of IL stimulation.
Appendix

Generation of adaptive BLA inputs
When subjected to an injected current pulse, the adaptation time course of cortical cells can be fitted empirically by an exponential time course, i.e., the instantaneous firing rate
\[ f(t) = f_{ss} + (f_0 - f_{ss}) \exp(-t/\tau_{adap}), \]
where \( f_0 \) is the initial firing rate, \( f_{ss} \) is the steady-state firing rate, and \( \tau_{adap} \) is the adaptation time constant (Wang, 1998). Thus this time course is characterized by two quantities: \( \tau_{adap} \) and the percentage adaptation of firing frequency \( F_{adap} = (f_0 - f_{ss})/f_0 \). In response to a depolarizing current pulse, BLA pyramidal cells showed different degrees of frequency adaptation (Faber et al. 2001). Based on the actual firing frequency of LA pyramidal cells in vivo during auditory fear conditioning (Quirk et al. 1995), the 10 BLA inputs were modeled with different adaptation time constant and percentage adaptation. The adaptation time constant ranged from 50 ms to 95 ms, with 5 ms interval, i.e., \{50, 55, 60… 95\}. The initial frequency ranged from 50 to 95 Hz, with 5 Hz increment, \{50, 55, 60… 95\}, and the steady-state frequency ranged from 0 to 9 Hz, i.e., \{0, 1, 2, … 9\}. Thus, the instantaneous firing rate of the first input is \( f_1(t) = 50 \exp(-t/50) \) and that of the last input is \( f_{10}(t) = 9 + 86 \exp(-t/95) \). The 10 firing rate functions are then converted into 10 spike inputs to the network model. The instantaneous firing rates of three BLA inputs (2, 5 and 8) together with the spike inputs are shown in Fig. A1.

Table 4.1. Maximal conductance densities (ms/cm²) for the two-compartment ITC and Ce model

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<th>( I_{Na} )</th>
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<th>( I_M )</th>
<th>( I_H )</th>
<th>( I_{SD} )</th>
<th>( I_{Ca} )</th>
<th>( I_{sAHP} )</th>
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</thead>
<tbody>
<tr>
<td>ITC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soma</td>
<td>120</td>
<td>10</td>
<td>0.01</td>
<td>–</td>
<td>0.1</td>
<td>0.02</td>
<td>–</td>
</tr>
<tr>
<td>Dend</td>
<td>40</td>
<td>4</td>
<td>0.01</td>
<td>0.02</td>
<td>0.1</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>Ce</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soma</td>
<td>120</td>
<td>12</td>
<td>0.2</td>
<td>–</td>
<td>–</td>
<td>0.1</td>
<td>–</td>
</tr>
<tr>
<td>Dend</td>
<td>40</td>
<td>5</td>
<td>0.2</td>
<td>0.03</td>
<td>–</td>
<td>0.2</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 4.2. Gating variables for all ion channels used in the ITC and Ce model

<table>
<thead>
<tr>
<th>Current Type</th>
<th>Gating Variable</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$\infty_x$</th>
<th>$\tau_x$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{Na}$</td>
<td>$p=3$</td>
<td>$-0.2816(V + 21)$</td>
<td>$0.2464(V - 6)$</td>
<td>$\alpha(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
</tr>
<tr>
<td></td>
<td>$q=1$</td>
<td>$0.098 \times \exp(-(V + 36.1)/20)$</td>
<td>$1.4 \exp(-(V + 6.1)/10) + 1$</td>
<td>$\alpha(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
</tr>
<tr>
<td>$I_{DR}$</td>
<td>$p=4$</td>
<td>$-0.018(V + 13)$</td>
<td>$0.0054(V - 23)$</td>
<td>$\alpha(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
</tr>
<tr>
<td>$I_{M}$</td>
<td>$p=2$</td>
<td>$0.016 \exp(-(V + 52.7)/23)$</td>
<td>$0.016 \exp((V + 52.7)/18.8)$</td>
<td>$\alpha(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
</tr>
<tr>
<td>$I_{H}$</td>
<td>$p=1$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>$1 \exp((V + 89.2)/9.5) + 1$</td>
</tr>
<tr>
<td>$I_{SD}$</td>
<td>$p=4$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>$1 \exp(-(V + 80)/8.5) + 1$</td>
</tr>
<tr>
<td></td>
<td>$q=1$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>$1 \exp((V + 53)/6) + 1$</td>
</tr>
<tr>
<td>$I_{Ca}$</td>
<td>$p=2$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>$1 \exp(-(V + 24.6)/11.3) + 1$</td>
</tr>
<tr>
<td></td>
<td>$q=1$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>$1 \exp((V + 12.6)/18.9) + 1$</td>
</tr>
<tr>
<td>$I_{sAHP}$</td>
<td>$p=1$</td>
<td>$0.0048 \exp(-5 \log_{10}([Ca]_2) - 17.5)$</td>
<td>$0.012 \exp(2 \log_{10}([Ca]_2) + 20)$</td>
<td>$\alpha(\alpha + \beta)$</td>
<td>48</td>
</tr>
</tbody>
</table>

\[\tau_{sAHP}^{SD} = \frac{1}{\exp((V + 35.8)/19.7) + \exp(-(V + 79.7)/12.7)} + 0.37 \]
\[\tau_{sAHP}^{SD} = \frac{10}{\exp((V + 36)/5) + \exp(-(V + 228)/37.5)} + 200 \]
\[\tau_{Ca} = 1.25 \times sec h(-0.031(V + 37.1)) \]
Table 4.3-a. Inhibitory connectivity of ITCD neurons

<table>
<thead>
<tr>
<th></th>
<th>Facilitating inputs</th>
<th>Depressing inputs</th>
<th>Constant inputs</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>—</td>
<td>D5</td>
<td>D8</td>
</tr>
<tr>
<td>D2</td>
<td>D3</td>
<td>—</td>
<td>D7, D9</td>
</tr>
<tr>
<td>D3</td>
<td>—</td>
<td>D4</td>
<td>D9</td>
</tr>
<tr>
<td>D4</td>
<td>D2</td>
<td>D6</td>
<td>D7, D8, D9</td>
</tr>
<tr>
<td>D5</td>
<td>D1, D2</td>
<td>D4</td>
<td>D10</td>
</tr>
<tr>
<td>D6</td>
<td>D3</td>
<td>—</td>
<td>D7, D8</td>
</tr>
<tr>
<td>D7</td>
<td>D1</td>
<td>D4, D6</td>
<td>D10</td>
</tr>
<tr>
<td>D8</td>
<td>D1</td>
<td>D5</td>
<td>—</td>
</tr>
<tr>
<td>D9</td>
<td>D3</td>
<td>D5</td>
<td>D10</td>
</tr>
<tr>
<td>D10</td>
<td>D2</td>
<td>D6</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 4.3-b. Inhibitory connectivity of ITCV neurons

<table>
<thead>
<tr>
<th></th>
<th>Facilitating inputs</th>
<th>Depressing inputs</th>
<th>Constant inputs</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>M3</td>
<td>D5, D6, V4</td>
<td>D8, D9, V7</td>
</tr>
<tr>
<td>V2</td>
<td>D3</td>
<td>V5, V6</td>
<td>D9, D10, V8</td>
</tr>
<tr>
<td>V3</td>
<td>D2, V2</td>
<td>D6</td>
<td>D8, D10, V9, V10</td>
</tr>
<tr>
<td>V4</td>
<td>V1, V3</td>
<td>D4</td>
<td>D9, V9, V10</td>
</tr>
<tr>
<td>V5</td>
<td>D3, V1</td>
<td>D4, V4</td>
<td>D7, V8</td>
</tr>
<tr>
<td>V6</td>
<td>D1, V2</td>
<td>D5, D7</td>
<td>D10, V7, V8</td>
</tr>
<tr>
<td>V7</td>
<td>—</td>
<td>D5, V5</td>
<td>D7, D8, V10</td>
</tr>
<tr>
<td>V8</td>
<td>D1, V1, V2</td>
<td>D4, V6</td>
<td>—</td>
</tr>
<tr>
<td>V9⁺</td>
<td>D2, D3</td>
<td>V4, V5, V6</td>
<td>V7</td>
</tr>
<tr>
<td>V10</td>
<td>D1, D2, V3</td>
<td>D6</td>
<td>V9</td>
</tr>
</tbody>
</table>

Table 4.4. Synaptic weights used in the ITC network model

<table>
<thead>
<tr>
<th></th>
<th>LA→ITCD</th>
<th>BA→ITCD</th>
<th>IL→ITCD</th>
<th>ITCD→ITCV</th>
<th>ITCV→Ce</th>
<th>BA→Ce</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1 or 5</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>
FIG. A1. Instantaneous firing rates of three BLA inputs (2, 5 and 8) together with the spike inputs.
FIG. A2. A: release probability as a function of the stimulation frequency from Geracitano et al. J Physiol, 2007. Upper panel: facilitating synapse; lower panel: depressing synapse. B1: curve-fitting of the facilitating data points in (A) using a logarithmic equation. B2: curve-fitting of the depressing data points in (A) using a logarithmic equation. Note that the frequency range have been extended in B.

FIG. A3. Time constants of a slowly deinactivating current $I_{SD}$ and a fast deinactivating current $I_{FD}$. 
4.7 Supplementary figures

Role of the $I_{SD}$ current

To investigate the role of the $I_{SD}$ current in sustaining the activity of ITC neurons, we reduced the recovery time of the $I_{SD}$ current so that it inactivated faster in all ITC neurons (see Fig. A3 in the appendix). Figure S1A shows the voltage responses of the same representative neurons for the weakly coupled case. With a fast deinactivating current $I_{FD}$, spontaneous firing activity was increased in both ITCD (from 2.3 to 4.8 Hz) and ITCV neurons (from 1 to 2.9 Hz). After the burst firing to transient inputs, the inactivation variable $h$ recovered rapidly from inactivation and all four ITC neurons only fired a few action potentials. Figure S1B compares the instantaneous firing frequency of ITCD and ITCV neurons in the control and $I_{FD}$ cases. Replacement of the $I_{SD}$ current by an $I_{FD}$ current almost completely abolished the sustained activity of both ITCD and ITCV neurons as the steady-state frequency (ITCD: 5.8 Hz; ITCV: 4.6 Hz) was almost the same as the spontaneous firing frequency. Thus the model showed that the slow deinactivation of the $I_{SD}$ current was necessary for persistent ITC network activity initiated by a transient input. Figure S1C compares the steady-state frequency of individual ITCD and ITCV neurons for the control and $I_{FD}$ current cases (top two panels), and the average steady-state frequency of all cells (bottom panel) in the weakly coupled network. Alteration of the $I_{SD}$ current significantly reduced the firing rates of all ITCD and ITCV neurons. The average steady-state frequency was reduced from 15.5 to 5.8 Hz for ITCD neurons, and from 9.9 to 4.6 Hz for ITCV neurons. As a result of decreased inhibition from ITCV, the steady-state Ce firing rate was increased from 19 to 20 Hz. Thus, the model suggests that a reduction of the recovery time of the $I_{SD}$ current would reduce both ITCD and ITCV firing rates, and the slow $I_{SD}$ current helps in sustaining the inhibition on Ce for a longer time.
FIG. S1. Persistent activity in ITC cells was lost when the slowly deinactivating current $I_{SD}$ is replaced by a fast deinactivating current $I_{FD}$. A: voltage responses of ITC-D3, ITC-D8, ITC-V1, ITC-V4 and Ce neurons for the weak coupling case. Evolution of the inactivation variable of the $I_{FD}$ current is shown in green. B: comparison of the instantaneous firing frequency of ITC_D and ITC_V neurons for the control and fast recovery cases. C: top two panels: steady-state firing frequency (from 1 to 2 sec) of individual ITC_D and ITC_V neurons; bottom panel: average steady-state firing frequency of all ITC_D, ITC_V and Ce neurons.
Chapter 5

Biophysical modeling of the amygdala network in acquisition and extinction of conditioned fear*

It is well established that the amygdaloid complex plays an important role in the acquisition and expression of learned fear associations. Our group has previously modeled the sensory-receptive region of the complex – lateral amygdala (LA) and the result showed that LA can learn both acquisition and extinction due to local plasticity. To investigate how conditioning-induced potentiation of LA response lead to activation of the central nucleus (CE), the output station of the amygdala, we extended the LA model into a more complete amygdala circuit by adding the basal nucleus (BA), intercalated (ITC) cell mass together with the CE nucleus. Hodgkin-Huxley type single cell models were developed first to match the firing properties of neurons in each extended region. Biophysical Hebbian learning rule was implemented in plastic AMPA receptor-mediated synapses to model learning, according to the calcium control hypothesis. The model was able to simulate the amygdala responses during acquisition, extinction, and recall of extinction in auditory fear conditioning in mammals. Results showed that expression of fear was regulated by both the LA-BA-CE and the LA-ITC-CE pathways and interruption of each pathway resulted in impaired fear acquisition or extinction. The model also suggested additional possible storage sites for the storage fear and extinction memories, thus supporting the theory of distributed memory storage in the amygdala circuit. Besides, the model provides insights into neural mechanisms underlying acquisition and extinction and the specific role of each amygdala component in fear learning by simulating a series of lesioning experiments.

* This work was with D Pare, GJ Quirk and SS Nair. It is currently under revision.
5.1 Introduction

The brain structures involved in fear learning exhibit abnormal activity patterns in clinically anxious subjects (Shin et al. 2006; Bremner et al. 2008). Thus, studying aversive learning might help us understand human anxiety disorders. The laboratory model most commonly used to study this process is Pavlovian fear conditioning where a neutral sensory stimulus (conditioned stimulus, CS) comes to elicit fear responses after pairing with a noxious unconditioned stimulus (US). Previous studies have revealed that the amygdala is critical for this form of learning and that the entry point for cortical and thalamic afferents about the CS and US to the amygdala is its lateral nucleus (LA; LeDoux 2000; Maren and Quirk 2004). During fear conditioning, CS and US convergence in LA is thought to cause a potentiation of synapses conveying CS information, resulting in larger CS-evoked responses in LA neurons (Quirk et al. 1995; Repa et al. 2001; Collins and Paré 2000). In turn, LA would trigger conditioned fear via its projections to the central nucleus of the amygdala (CE; Kapp et al. 1979; reviewed in Davis 2000).

How does LA influence CE? CE is comprised of several subnuclei, only one of which (central medial – CEm) projects to the brainstem sites mediating fear responses (Hopkins and Holstege 1978; Schwaber et al. 1982; Veening et al. 1984). However, LA has no direct projections to CEm (Krettek and Price 1978; Smith and Paré 1994; Pitkanen et al. 1995). It was therefore concluded that LA influences CEm indirectly, via the basal nuclei (BA) or intercalated (ITC) amygdala neurons (Pare et al. 2004). In support of the first possibility, Anglada-Figueroa and Quirk (2005) showed that post-training BA lesions block the expression of conditioned fear responses. However, pre-training BA
lesions do not prevent fear conditioning (Amoranpath et al. 2000; Holohan and White 2002; Nader et al. 2001) suggesting that another route exists between LA and CEm. It was proposed that ITC cells constitute this alternate route (Pare et al., 2004). ITC cells are GABAergic neurons located at the CE/LA-BA border (McDonald and Augustine 1993; Nitecka and Ben-Ari 1987; Pare et al. 1993a). ITC cells are ideally positioned to control CE excitability because they receive glutamatergic inputs from principal LA and BA neurons and in turn generate feedforward inhibition in CE cells (Marowsky et al. 2005; Pare et al. 1993b; Royer et al. 1999). Moreover, there is a lateromedial correspondence between the position of ITC cells, their projection site in CE, and the source of their excitatory afferents in the LA-BA nuclei (Royer et al. 1999). However, because there are lateromedial inhibitory connections between ITC cell clusters, the feed-forward inhibition they generate in CE varies depending on the distribution of activity in LA and BA (Royer et al. 1999, 2000). Thus, it is possible that CS driven LA activity causes a disinhibition of CEm neurons via the directionally polarized connections that exist between ITC cell clusters.

Besides their potential role in the transmission of CS-related LA activity to CEm, strong evidence implicates ITC cells in the extinction of conditioned fear responses (Jungling et al. 2008; Likhtik et al. 2008), a phenomenon that occurs when the CS is presented repeatedly in the absence of US. However, because ITC cells occur as small distributed cell clusters, studying their role in fear acquisition and extinction constitutes a major experimental challenge. Thus, to circumvent this problem, we developed a simplified, yet biophysically-realistic computer model of the amygdala that allowed us to test various predictions regarding the role of ITC cells in fear conditioning.


5.2 Materials and Methods

5.2.1 Overall structure of the amygdala circuit

In a recent study (Li et al. 2008), we developed a model of LA that could reproduce learning-induced changes in the tone responses of principal LA neurons, as recorded experimentally during fear conditioning (Quirk et al, 1995). The same model is used here, but with additional nuclei of the amygdala: BA, ITC and CE nuclei. The overall amygdala circuit is shown in Fig. 1A.

LA model. In keeping with previous tracing studies (LeDoux et al., 1987; Linke et al., 2001; Turner and Herkenham, 1991), our LA model received tone and shock inputs from the posterior thalamus. A biophysical Hebbian learning rule (see below) was used to reproduce the changes in CS responsiveness induced by fear conditioning (Quirk et al, 1995). The reader is referred to our previous study for a detailed justification of the LA model structure (Li et al. 2008).

BA model. To constrain the BA model, we sought a network design that could reproduce the findings of a recent study on the behavior of BA cells during fear conditioning and extinction (Herry et al. 2008). This study emphasized the opposite behavior of two groups of BA cells. The first (termed “fear cells”) increased their tone responsiveness following fear conditioning but lost it as a result of extinction training. The second (termed “extinction cells”) maintained increased responses to the CS as a result of extinction training. Pilot simulations with various network configurations revealed that the most parsimonious way to reproduce these findings while generating realistic patterns of CE activation was to assume the following: two subsets of principal BA cells with identical intrinsic properties (see below) but with a different connectivity


on the input and output side. The first group of BA cells receives inputs from principal LA cells and projects to CE. Since firing of these cells activates CE, we named them “fear cells” (BAf). To explain the extinction-resistant tone responsiveness of the second group of BA cells (extinction cells – BAe), we assumed that they received inputs from the cortical auditory area Te3, where neurons with extinction-resistant tone responses have been observed (Quirk et al., 1997). On the output side, to account for the fact that fear expression is reduced in extinction despite the activity of BAe cells, we assumed that they project to the medial ITC neurons (ITCm); not to CE. Finally, because previous tracing studies have provided evidence of strong interconnections between principal BA neurons (Pare et al. 1995; Pitkanen et al. 1995), the model had to assume that the two cell types could also inhibit each other via local-circuit GABAergic neurons.

**ITC model.** On the basis of previous tract-tracing (Pare and Smith 1993b), and electrophysiological studies (Royer et al. 1999, 2000), ITC neurons were assigned glutamatergic inputs from LA or BA, depending on whether they had a lateral (ITCl) or medial (ITCm) position, respectively. Moreover, because lateral ITC cells were shown to project to medial ones (Royer et al. 1999, 2000), the model included GABAergic projections from ITCl to ITCm neurons. As a result, activation of LA leads to the inhibition of ITCm, via ITCl. Finally, ITC cells were assigned glutamatergic inputs from IL, as indicated by previous tract-tracing studies (Cassell and Wright 1986; McDonald et al. 1996).

**CE model.** In keeping with previous anatomical findings, CE neurons received glutamatergic inputs from BAf neurons (Pare et al. 1995; Pitkanen et al. 1995), GABAergic inputs from ITCm cells (Pare and Smith 1993ab; Royer et al. 1999), as well
as tone and shock inputs from the posterior thalamus (LeDoux et al., 1987; Linke et al., 2001; Turner and Herkenham, 1991).

**Number of neurons.** To accelerate the simulations, our model included few cells. In LA, there were eight principal cells and two GABAergic interneurons with all-to-all connectivity. In BA, there were two BAf, two BAe, and one local-circuit cell. In addition, ITCI, ITCm, and CE were represented by one neuron each.

5.2.2 Models for each cell type

Neurons were modeled using the Hodgkin-Huxley-type formulation (Byrnes and Roberts, 2004), adjusting the relative importance of the different conductances to reproduce the known electroresponsive properties of the various cell types. Each cell model has two compartments representing the soma and dendrites. The current balance equations are of the form given in Eqns. 1 and 2,

\[
C_m \frac{dV_s}{dt} = -g_L(V_s - E_L) - g_L(V_s - V_d) - \sum I_{s\text{int}} - \sum I_{s\text{syn}} + I_s
\]

\[
C_m \frac{dV_d}{dt} = -g_L(V_d - E_L) - g_c(V_d - V_s) - \sum I_{d\text{int}} - \sum I_{d\text{syn}} + I_d
\]

where \(V_s\) and \(V_d\) are the somatic and dendritic membrane potentials (mV), \(I_{s\text{int}}\) (\(I_{d\text{int}}\)) and \(I_{s\text{syn}}\) (\(I_{d\text{syn}}\)) are the intrinsic currents and the synaptic currents in the soma/dendritic compartments respectively, \(I_s\) (\(I_d\)) is the electrode current applied to the soma (dendrite), \(C_m\) is the membrane capacitance and \(g_c\) is the coupling conductance between the soma and the dendrite. The ionic current for channel \(i\), \(I_i\), was modeled as \(I_i = g_i m^p h^q (V - E_i)\), where \(g_i\) was its maximal conductance, \(m\) its activation variable (with exponent \(p\)), \(h\) its inactivation variable (with exponent \(q\)), and \(E_i\) its reversal potential.
potential. The kinetic equation for each of the gating variables $x$ ($m$ or $h$) takes the form

$$\frac{dx}{dt} = \frac{x_\infty(V, [Ca^{2+}]) - x}{\tau_x(V, [Ca^{2+}])}$$  \hspace{1cm} (3)$$

where $x_\infty$ is the voltage- and/or Ca$^{2+}$- dependent steady state and $\tau_x$ is the voltage- and/or Ca$^{2+}$- dependent time constant. The maximal conductance densities for all ionic currents and the expressions of $x_\infty$ and $\tau_x$ for each gating variable are listed in Tables 1 and 2 in the Appendix.

**LA/BA model.** Principal cells in LA are known to exhibit a continuum of spike frequency adaptation ranging from neurons that fire only a few spikes at the onset of the depolarizing current pulses to cells that fire throughout depolarizing current injections (Faber et al. 2001; Duvarci and Pare 2007). To reproduce this variability, three different types of principal LA cells were developed: type A with full adaptation; type B with intermediate adaptation, and type C with weak adaptation (Li et al., 2008). LA also contained local-circuit GABAergic neurons which were modeled as fast spiking cells (Land and Pare 1998; Rainnie et al. 2006; Woodruff and Sah 2007). BA neurons were modeled as for LA cells. Among the two fear or extinction neurons, one belongs to type A and the other one belongs to type B. The firing properties of the LA/BA model neurons (principal cell and interneuron) are shown in Fig. 2A and 2B respectively.

**CE model.** Previously, CE neurons have been classified into three subtypes, based on their responses to depolarizing current injections: adapting, late-firing and regular spiking (Armentia and Sah, 2004; Dumont et al. 2002). Since CE is the output station of this fear circuit, we only modeled the prevalent CE cell type (regular spiking). The voltage responses of the model CE neuron are shown in Fig. 2C.
ITC model. Using whole-cell recordings in vitro, it was reported that ITC neurons exhibit a bistable electroresponsive behavior Royer et al. (2000b). That is, the activity of ITC cells is a function of their recent firing history because they express an unusual voltage-dependent K\(^+\) conductance (termed \(I_{SD}\) for slowly deinactivating). The \(I_{SD}\) current was adapted from an “A” current model (Huguenard and McCormick 1992) with the inactivation kinetics adjusted to replicate the experimental characteristics described in Royer et al. (2000b). The current was modeled as \(I_{SD} = g_{SD} m^4 h(V - E_K)\). The steady state functions of \(m_a(V)\) and \(h_a(V)\) are shown in Fig. 3A, and the inactivation time constant function \(\tau_h(V)\) is shown in Fig. 3B. The half-activation and half-inactivation voltages are at -80 mV and -55 mV respectively, so that \(I_{SD}\) reaches its maximum at around -60 mV (\(m h\) has the largest value). The time constant of inactivation was 200 ms for \(V > -30\) mV and greatly increases for \(V < -50\) mV. Hence, \(I_{SD}\) activates fully in the subthreshold range (\(V \approx -60\) mV), inactivates slowly in response to suprathreshold depolarization (\(V > -40\) mV), and deinactivates very slowly (\(V < -50\) mV).

The model could reproduce the unusual electroresponsive behavior of ITC cells, including time- and voltage-dependent inward rectification in the hyperpolarizing direction (Fig. 3C, bottom), repetitive firing behavior (Fig. 3C, top two panels) and voltage-dependent bistability (Fig. 3E-F), as observed experimentally (Royer et al. 2000). With respect to bistability, with a constant pre-pulse \(V_{in}\) of -60 mV, suprathreshold current pulses elicited afterdepolarizations (ADPs) that increased in amplitude with the amount of injected current (Fig. 3D, arrows), closely matching experimental observations (Royer et al. 2000). This is due to the fact that larger current injections inactivate \(I_{SD}\) more effectively. Also, keeping the amount of injected current constant but varying the
pre-pulse potential, with sufficient depolarization (beyond –60 mV), the ADP could exceed spike threshold (Fig 3E, middle), and eventually lead to self-sustaining repetitive firing (Fig. 3E, bottom). Finally, as observed experimentally (Royer et al. 2000), due to the slowly developing inactivation of $I_{SD}$, the ADP amplitude in the model was also dependent on the duration of the current injection (Fig. 3F).

**Calcium Dynamics.** Intracellular calcium was regulated by a simple first-order differential equation of the form (Warman et al. 1994):

$$\frac{d[Ca^{2+}]}{dt} = -f \frac{I'_{Ca}}{VFA} + \frac{[Ca^{2+}]_{rest} - [Ca^{2+}]_{I}}{\tau_{Ca}}$$

where $I'_{Ca} = I_{Ca}$ for ionic $Ca^{2+}$ pool, $f$ is the fraction of the $Ca^{2+}$ influx ($f = 0.024$), $V = wA$ with $w$ being the shell thickness (1µm) and $A$ is the cell surface area, $F$ is the Faraday constant, $\tau_{Ca}$ is the $Ca^{2+}$ removal rate and varied with different cell type (see Table 1 in the Appendix). The resting $Ca^{2+}$ concentration is $[Ca^{2+}]_{rest} = 50$ nmol/l, which is the same as the initial concentration (Durstewitz et al. 2000). The unit of the $Ca^{2+}$ concentration is µm/l.

**5.2.3 Synaptic currents**

In keeping with previous experimental observations (reviewed in Sah et al. 2003), excitatory transmission in the modeled network was dependent on glutamate acting at AMPA and NMDA receptors whereas inhibitory transmission depended on GABA acting on GABA-A receptors. The AMPA/NMDA and GABA$_A$ synaptic currents were modeled by dual exponential functions (Durstewitz et al. 2000):
\[ I_{\text{AMPA}} = \mathcal{A} w(t) g_{\text{AMPA}, \text{max}} \frac{\tau_1 \tau_2}{\tau_2 - \tau_1} \left[ \exp(-t/\tau_2) - \exp(-t/\tau_1) \right] (V - E_{\text{AMPA}}) \]  

(5)

\[ I_{\text{NMDA}} = \mathcal{A} w g_{\text{NMDA}, \text{max}} s(V) \frac{\tau_1 \tau_2}{\tau_2 - \tau_1} \left[ \exp(-t/\tau_2) - \exp(-t/\tau_1) \right] (V - E_{\text{NMDA}}) \]  

(6)

\[ I_{\text{GABA}} = \mathcal{A} w g_{\text{GABA}, \text{max}} \frac{\tau_1 \tau_2}{\tau_2 - \tau_1} \left[ \exp(-t/\tau_2) - \exp(-t/\tau_1) \right] (V - E_{\text{GABA}}) \]  

(7)

where \( w(t) \) is the adjustable synaptic weight for certain AMPA synapses (see below; \( w \) was held fixed for all NMDA synapses); \( \mathcal{A} \) is a normalization constant chosen so \( g_{\text{AMPA}, \text{max}}, g_{\text{NMDA}, \text{max}} \) and \( g_{\text{GABA}, \text{max}} \) assume maximum values of the conductances; \( \tau_1 \) and \( \tau_2 \) are the rise and decay time constants respectively. For AMPA receptor channels, \( \tau_1 = 0.55 \text{ ms} \) and \( \tau_2 = 2.2 \text{ ms} \) (except for BA interneuron \( \tau_1 = 0.3 \text{ ms} \) and \( \tau_2 = 2.4 \text{ ms} \)); for NMDA receptor channels, \( \tau_1 = 10.7 \text{ ms} \) and \( \tau_2 = 125.0 \text{ ms} \), and for GABA_A receptors, \( \tau_1 = 0.25 \text{ ms} \) and \( \tau_2 = 3.75 \text{ ms} \). The voltage-dependent variable \( s(V) \) which implements the \( \text{Mg}^{2+} \) block was defined as: \( s(V) = [1 + 0.33 \exp(-0.06 V)]^{-1} \) (Zador et al. 1990). The maximal conductances were chosen as: \( g_{\text{AMPA}, \text{max}} = 1 \text{ nS}, g_{\text{NMDA}, \text{max}} = 0.5 \text{ nS} \) and \( g_{\text{GABA}, \text{max}} = 1 \text{ nS} \). Synaptic reversal potentials were set as \( E_{\text{AMPA}} = E_{\text{NMDA}} = 0 \text{ mV} \) for all cell types. \( E_{\text{GABA}} \) was set to \(-75 \text{ mV} \) in all cell types except fast-spiking interneurons of LA and BA (-57 mV). This is based on a previous in vitro study where principal cells and fast-spiking interneurons were found to have different GABA-A reversals due to contrasting chloride homeostatic mechanisms (Martina et al. 2001).

**5.2.4 Background and specific afferent inputs**

Independent, Poisson-distributed, random excitatory background inputs were delivered to all the cells in the network to achieve reported spontaneous firing rates. The
specific tone/shock synaptic inputs represented by regular spike trains (200 Hz) were
delivered to LA, BAe and CE neurons with noise (Fig. 1A). Given that the tone starts out
as neutral and the shock as noxious, the conductance strength encoding the shock
information was set much higher than that representing the tone inputs (see Table 3 in the
Appendix). Since we used the same training protocol as in Li et al. (2008), the LA neuron
responses during the training were taken from the previous LA network model. The IL
input (regular spike train of 50 Hz for 100 ms) was present 100 ms right after the tone
onset and only during re-extinction (Milad and Quirk, 2002).

5.2.5 Hebbian Learning rule

In the LA network, the efficacy of all excitatory and inhibitory synapses on
principal cells were modifiable (Li et al. 2008). Similarly, in this larger fear circuit of Fig.
1A, the tone-BAe and the LA-BAf synapses were modifiable. As mentioned earlier, the
BA synapses onto intercalated neurons express NMDA-dependent LTP and LTD (Royer
and Paré, 2002). Besides, the thalamus input to CE was also modifiable (Samson and
Paré, 2005). Thus, additional plasticity was implemented in the tone-CE and BAe-ITCm
synapses. We used a biophysical Hebbian rule termed ‘calcium control hypothesis’
(Gerstner and Kistler 2002; Shouval et al. 2002a and 2002b) to implement learning by
adjusting the synaptic weight $w_j$ in Eq. 5 as,

$$\Delta w_j = \eta ([Ca]_j) \Delta t (\lambda_1 \Omega ([Ca]_j) - \lambda_2 w_j)$$

where $\eta$ is the Ca$^{2+}$-dependent learning rate and $\Omega$ is a Ca$^{2+}$-dependent function with two
thresholds ($\theta_d$ and $\theta_p$; $\theta_d \leq \theta_p$) (see Fig. 12 in Appendix); $\lambda_1$ and $\lambda_2$ represent scaling and
decay factors respectively; the local calcium level at synapse $j$ is denoted by $[Ca]_j$ and $\Delta t$
is the simulation time step. With this learning rule, the synaptic weight decreases when \( \theta_d < [Ca]_j < \theta_p \), and increases (with modulation by the decay term \( \lambda_2 w_j \)) when \([Ca]_j > \theta_p\).

One of the key assumptions of this learning rule is that the dominant source of calcium influx in the postsynaptic cell is through NMDA receptors. This calcium influx was calculated as \( I_{Ca}^N = P_0 w^{-1} G_{NMDA}(V - E_{Ca}) \) (Shouval et al. 2002b) where \( G_{NMDA} \) is the NMDA conductance in Eq. 6 (the term \( w^{-1} \) ensures that it is calculated per synapse). \( P_0 \) was selected to be 0.015 so that the fraction of the NMDA current carried by Ca\(^{2+}\) ions averaged to 7% at negative potentials (Koch 1999). The concentration of the calcium pool at synapse \( j \) followed the same dynamics as in Eq (4), with \( I'_{Ca} = I_{Ca}^N, f_j = 0.024 \) (Warman et al. 1994), \( \tau_j = 50 \text{ ms} \) (Shouval et al. 2002b), \( V \) is the volume of a spine head with a diameter of 2 \( \mu \text{m} \) (Kitajima and Hara 1997). All the synaptic weights were constrained by upper \( (W_{\text{max}}) \) and lower \( (W_{\text{min}}) \) limits (Hasselmo and Barkai 1995).

Maximum \( (f_{\text{max}}) \) and minimum \( (f_{\text{min}}) \) folds were specified for each modified synapse so that \( W_{\text{max}} = f_{\text{max}} \times w(0) \) and \( W_{\text{min}} = f_{\text{min}} \times w(0) \). The initial synaptic weights with learning parameters are shown in Table 3 in the Appendix.

### 5.2.6 Training protocol

The schedule of tone and shock inputs in the simulation was based on in vivo studies (Quirk et al. 1995, 1997). As in our previous modeling study with the restricted LA network (Li et al. 2008), we scaled down the duration of the auditory fear conditioning protocol by approximately two orders of magnitude, so that it would be suitable for a computational study. The simulation included sensitization, conditioning, and two extinction phases (Fig. 1B). Each tone lasted 500 ms and each shock lasted 100
ms. The interval between two tones was 3.5 sec. During the sensitization phase, 10 unpaired tones and shocks were presented to the network, with the shocks occurring randomly between the tones. In the conditioning phase, 10 paired tones and shocks were provided, with the tone and shock co-terminating. In the extinction phase, 30 tones were delivered without shocks. The gap between conditioning and extinction phases was 40 sec and the model was tested for extinction recall after a delay of 840 sec. The second extinction phase also used 30 pure tones. The entire schedule lasted 1200 sec. Simulations were performed on a personal computer using the software package GENESIS (Bower and Beeman 2003) with the Crank-Nicholson integration method, and a time step of 20 µs.

5.3 Results

The results section is organized as follows. First, we describe the overall behavior of the model. Second, to test its validity, we perform simulations where particular components of the network are “lesioned” to determine whether the model reproduces the lesion effects previously seen experimentally. Finally, we describe simulations modeling ITC lesion experiments for which there is no data because such experiments are virtually impossible.

5.3.1 Overall behavior of the model during fear conditioning and extinction

Figure 4A-C plots peri-event histograms (PEHs) of neuronal discharges around CS onset (10 ms bins). The CS responsiveness of the different cell types (top to bottom)
is shown at different stages of training (left to right: sensitization, tones 1-10 of extinction, tones 10-20 of extinction). As a result of CS-US pairings during fear conditioning, most units in the model increased their tone responsiveness but this effect was particularly marked for LA, BAd, ITCl, and CE neurons. Although the latency of tone responses increased from LA (10-20 ms) to BA (20-30 ms), to CE (30-40 ms), in all cell types the increase in tone responsiveness was most pronounced during the first 100 ms of the CS. Because of the inhibitory input from ITCl to ITCm, the latter unit showed little conditioning-induced change in CS responsiveness. In most units, extinction training retuned tone-evoked responses to pre-conditioning levels with two exceptions: B Ae and ITCm units. The pattern of training induced changes in tone responsiveness seen in LA and BA units is consistent with the results of previous single-unit recording studies (Quirk et al. 1995; Herry et al. 2008). No experimental data is available on the behavior of ITC cells.

Training-induced changes in tone responsiveness resulted from complex interactions between (a) the particular intrinsic properties of the units, (b) the varying levels of tone-evoked responses in their inputs, and (c) activity-dependent alterations in synaptic weights. We exemplify the impact of each variable with a few select examples.

(a) The impact of the units’ intrinsic membrane properties is best illustrated by comparing the time course of tone evoked responses in B Ai and B Ae units. Both receive identical tone information. Yet, the B Ai unit responds to the CS input for a longer duration than the B Ae unit. The difference is due to the higher input resistance and membrane excitability of the B Ai unit.
Although the presence of a slow AHP current in the CE unit acted to reduced the duration of tone-evoked responses, the main contributing factors were the firing adaptation in the inputs the CE unit received from BA and the temporal profile of activity in the inhibitory input it receives from the ITCm unit (low early in the tone and higher toward the end).

Figure 5 illustrates how training altered synaptic weights at a few connections. The weights of tone-BAe (Fig. 5A) and tone-CE (Fig. 5B) synapses fluctuated with a similar time course: slight decay in sensitization, large potentiation in conditioning and small depotentiation during the extinction sessions. This is similar to the impact of training on tone-LA synapses we described previously (Li et al. 2008). In contrast, LA-BAf synaptic strengths (Fig. 5C) potentiated not only during conditioning, but also during sensitization due to random shock inputs. Importantly, the magnitude of the potentiation was synapse-specific. For example, since LA8 was a type C cell and BAf2 was type B, the potentiation of the LA8-BLf2 synapse was much larger than at the other two synapses. As a result of extinction training, there was slight decay at the LA-BAf synapses (Fig. 5C), similar to the LA (pyr)-LA (pyr) connections (Li et al. 2008). In contrast, the BAe-ITCm connection potentiated only during the two extinction sessions (Fig. 5D). Two factors contributed to this. First, the ITCm unit receives a potent inhibitory input from ITCl during conditioning, which is reduced during extinction. Second, the excitatory drive from BAe increases during conditioning, and this effect persists during extinction. The combined effect of increased excitatory drive from BAe coupled with decreased inhibitory drive from ITCl causes ITCm to potentiate in extinction.
Figure 6 illustrates the overall impact of these three interacting variables by plotting CS-evoked responses in blocks of five trials for principal LA (Fig. 6A) and BA (Fig. 6B) units, as well as ITC (Fig. 6C) and CE (Fig. 6D) neurons. Because BAf and ITCl units receive inputs from the LA principal cells, their responses to the CS (Fig. 6B) closely matched those of principal LA neurons (Fig. 6A). The situation is different for BAe and ITCm cells.

To understand why the tone responsiveness of BAe cells diverges from that of principal LA and BAf cells, we must consider how training affects their various inputs. Indeed, BAe cells receive extinction resistant inputs from Te3 and inhibitory inputs from BAi, both of which are potentiated during conditioning. During extinction, although the tone-BAe synaptic strength decreased (Fig. 5A), so did the tone-BAf inputs, causing a reduced excitatory drive to BAi, and thus less inhibition onto BAe cells. This reduced inhibitory pressure, combined with extinction resistant TE3 inputs, caused a shift in favor of excitation, allowing BAe units to maintain an increased tone responsiveness throughout extinction (Fig. 6B). In contrast, the main factor explaining the peculiar behavior of the ITCm unit is the training-induced shifts in the inhibitory inputs it receives from ITCl. As a result, the behavior of ITCm tends to be opposite to that of ITCl (Fig. 6C). However, another contributing factor is the potentiation of BAe inputs during extinction training (Fig5D).

Now that we have characterized the behavior of all units converging onto CE, the impact of fear conditioning and extinction on the CE unit can be easily explained. During conditioning, the large increase in the tone responses of principal LA cells strongly excited ITCl, resulting in the inhibition of ITCm and the disinhibition of CE. In parallel,
the tone-CE and BAf-CE synapses were potentiated, causing an increased excitatory drive onto the CE unit. Thus, the “high fear” state seen in early extinction was due to a combination of decreased inhibition from ITCm and increased excitatory drive from BAf. As extinction proceeded, the decreasing LA responses relieved ITCm from the inhibitory influence of ITCl, resulting in a potentiation of the BAe-ITCm synapse. Both effects conspired to increase inhibitory pressures onto CE and this was combined with a depression of the tone-CE synapse and a decreased drive from BAf neurons. As a result, CE activation returns to sensitization level in late extinction. Thus, the ‘low fear’ state seen after extinction training resulted from largely increased inhibitory pressures from ITCm, a reduced excitatory drive from BAf, combined with a depression of tone inputs to CE.

5.3.2 Recovery and re-extinction

With the passage of time, LA tone responses spontaneously recovered (Fig. 6A). GQ: This partial return of tone responses was modeled in our earlier study (CITE LI ET AL) and is caused by Hebbian weakening that occurs when random activity in tone inputs occurs in the absence of shock. The return tone response in LA caused a similar return in BAf and ITCl units (Fig. 6B-C). Due to the relatively high spontaneous firing rate of the ITC neuron, the BAe-ITCm synaptic strength decayed considerable during the long gap (Fig. 5D). Although the excitatory drive from BAe decreased (Fig. 6B), ITCm received facilitating gating inputs from IL after the long gap so that ITCm tone responses were still high (Fig. 6C), despite the recovered inhibition from ITCl. However, the IL input was delivered to the ITCm 100 ms after tone onset so did not have a direct impact on the
CE response during 0-100 ms, where the ITCm response was low due to ITCl inhibition (similar to early extinction shown in Fig. 4B). Combined with the recovered excitation from BAf, the extinguished CE activation recovered (Fig. 6D). As re-extinction progressed, the LA responses extinguished again, followed by extinction of the BAf and ITCl responses (Fig. 6B & C). With the help of the IL input, the BAe-ITCm synapse had a stronger potentiation during re-extinction (Fig. 5D) and ultimately brought the recovered CE activation down to a lowest level during the entire training (Fig. 6D).

### 5.3.3 Effect of LA and BA “lesions”

The above indicates that the model can reproduce the training-induced changes in tone responsiveness observed in previous experimental studies (Quirk et al., 1995; Herry et al., 2008). To further test the validity of the model, we then performed simulations where particular components of the network were “lesioned” by setting the connection strengths of particular nuclei to zero. We then verified whether the model reproduced the lesion effects previously seen experimentally.

The first lesion experiment examined the impact of pre-training LA lesions. To this end, we set the LA-BAf and LA-ITCl connections to zero before sensitization (Fig. 7). In these conditions, conditioning did not increase CE responsiveness to the tone (Fig. 7F). This is consistent with experimental observations indicating the LA lesions or temporary inactivation of LA prevent the acquisition of conditioned fear responses (Amoranpath et al. 2000; LeDoux et al., 1990; Maren et al., 2001; Muller et al., 1997; Sachetti et al., 1999; Wilensky et al., 1999). However, since the CE unit still received
direct tone inputs from MGM/PIN, this raises the question as to what prevented the tone inputs from being potentiated during conditioning.

To understand this, we must consider the impact of the LA lesion on other components of the network. LA inputs set the tone responsiveness of BAf and ITCl units, the lesion abolished CS-evoked responses in these units. Consequently, the tone responses of BAe and ITCm were higher than in control simulations. In the case of BAe, this was due to a reduced excitation of BAi by BAf resulting in the disinhibition of BAe. In the case of ITCm, this was due to the reduced excitation of ITCl and the consequent disinhibition of ITCm. With little inhibition from ITCl to ITCm, the BAe-ITCm synapse potentiated when random shock inputs were delivered during the sensitization phase, reaching ceiling prior to conditioning. The evolutions of synaptic strengths are shown in Figs. 7A-D. As a result of the massively increased inhibitory drive from ITCm to CE, direct tone inputs to CE could not potentiate during conditioning, explaining the lack of conditioning-induced changes in the tone responsiveness of CE (Fig. 7F). GQ: Thus, the loss of conditioned activity in Ce (and consequently fear responses) following an LA lesion is due to increased inhibition, and not simply a loss of excitatory drive onto Ce.

The second lesion experiment tested the effect of pre-training BA lesions. To this end, we set all input and output connections of BA to zero before sensitization. As shown in figure 8, the BA lesion had two opposite effects on CE. On one hand, the excitatory drive from BAf to CE was abolished. On the other, the inhibition of CE by ITCm was greatly reduced as ITCm displayed reduced levels of activity when deprived of BAe input (Fig. 8A). Under these conditions, CE activity still increased after conditioning, but tone responses were lower than in control simulations (Fig. 8B). After BA lesions, the
conditioning effect was entirely due to the potentiation tone inputs to CE (Fig. 8C). This pattern of results is consistent with the experimental finding that pre-training BA lesion do not prevent fear conditioning (Amoranpath et al. 2000; Holohan and White 2002; Nader et al. 2001). However, note that because the inhibitory input from ITCm did not potentiate during extinction, the conditioned CE response was resistant to extinction (Fig. 8B). Thus, a non-trivial prediction of our model is that the conditioned fear responses observed after BA lesions are resistant to extinction. In fact, somewhat slower extinction has been observed in BA-lesioned rats (see figure 2A, Anglada-Figueroa and Quirk, 2005).

Since pre- and post-training BL lesions were reported to have different effects on conditioned fear responses (Anglada-Figueroa and Quirk, 2005), we next tested whether the model could reproduce these results. To this end, input and output connections of BA to zero after conditioning. Consistent with experimental observations (Anglada-Figueroa and Quirk, 2005), post-training BA lesions completely abolished the training-induced increases in the tone responsiveness of CE.

5.3.4 Impact of ITC lesions

Because ITC cells occur as small distributed cell clusters, studying their role in fear acquisition and extinction constitutes a major experimental challenge. In contrast, it is easy to run simulations where the activity of ITC cells is manipulated in various ways. To investigate the role of the directionally polarized inhibitory connections between ITC cell clusters, the synapses from ITCl to ITCm were set to zero prior to conditioning. As shown in figure 9, interfering with inter-ITC inhibition reduced but did not prevent the
training-induced increase in the strength on tone inputs to CE (Fig. 9A). However, as a result of the disinhibition of ITCm, the BAe-ITCm synapse started to potentiate during sensitization (Fig. 9B), as we observed following LA lesions. The resulting increase in the tone responsiveness of ITCm (Fig. 9C) caused a strong inhibition of CE that overwhelmed the excitatory drive CE receives from BAf and the tone input. As a consequence, CE was prevented from acquiring conditioned tone responses (Fig. 9D). Thus, this simulation suggests that although inter ITC connections do not transmit CS information to CE, they do play a permissive role in the development of conditioned tone responses in CE.

To test the impact of ITCm lesions on extinction, we next disconnected ITCm from the other components of the circuit before sensitization. As shown in figure 10, the absence of inhibitory inputs from ITCm had a marginal effect on the fate of the tone-CE synapse (Fig. 10A). Indeed, potentiation of the tone-CE synapse mostly depends on CS-US pairings that occur when ITCm activity is very low due to the inhibitory inputs it receives from ITCl. Thus, we predict that lesioning ITCm would not cause a major facilitation of conditioned fear responses, and this has been reported experimentally (Likhtik et al., 2008). However, during extinction training with ITCm removed, CE tone responses were higher than seen in control simulations and could not be extinguished completely (Fig. 10B).

The partial extinction was due to loss of excitatory drive from BAe neurons and the LTD at the tone-CE synapse. These results are consistent with experimental observations indicating that medial ITC cells plays a critical role in fear extinction (Likhtik et al., 2008; Jungling et al., 2008).
5.3.5 Removal of IL Input impaired extinction recall

As shown in Fig. 6D, recovery was somewhat low when the IL input was present during re-extinction, indicating good recall of extinction learning. Quirk et al. (2000) showed that vmPFC (particularly the IL nucleus) is necessary for the recall of extinction learning after a long delay. To test the effect of IL lesioning on extinction recall, we disconnected the IL input to ITCm during re-extinction. Fig. 11A compares the time courses of the synaptic strength from BAe-ITCm for both the control and IL lesioning cases. Although the synapse could still potentiate during re-extinction without IL input, it did so to a much lower level compared to controls. Notice that the BAe tone response in re-extinction was lower than that in the first extinction (Fig. 6B). Thus, the IL input could be thought of as replacing the excitatory drive onto ITCm lost by the extinction of tone responses in BAe (after the gap). The tone responses of ITCm and CE are shown in Fig. 9B & C respectively. Without the gating input from IL, the ITCm response decreased to a much lower level in re-extinction, resulting in a higher recovery in CE (Fig. 11C). Moreover, recovered CE tone responses with IL lesioning would extinguish slower than in the control case and could not be complete (Fig. 9C), consistent with the experimental finding that lesions of vmPFC delayed recall of fear extinction in rats (Lebron et al. 2004).

5.4 Discussion

Using a network model, we have shown that LA can learn both acquisition and extinction of conditioned fear association due to local plasticity (Li et al. 2008). However, the output station of the amygdala is CEm which mediates fear response by projecting to the brainstem sites. Recent physiological studies have also shown that, in addition to LA,
plasticity is also seen in other amygdaloid nuclei (ITC and CE) (Royer and Paré 2002; Samson and Paré 2005; Wilesky et al 2006). Thus, additional modules will be needed to model the processes that regulate fear expression. In this study, we extended to LA network into a more complete amygdala circuit by adding BA, ITC and CE components. The model provides plausible mechanisms and insights as to how the BA and ITC pathways act together to regulate fear and extinction memories and how these memories are distributed among the amygdala circuit. Taken all together, conditioned fear signals emanating from LA are modulated by subsequent stages of processing within the amygdala, for optimal regulation of fear behavior.

5.4.1 LA is essential for both fear acquisition and extinction

Numerous studies have confirmed the essential role of LA in fear acquisition. But how potentiated CS response in LA leads to CE activation is less clear. Our model suggests that LA contributes to CE activation via two pathways: (1) Elevated LA output excites BA fear neurons, which in turn excite CE; (2) Enhanced LA response disinhibits the CE via ITC cells so that the CS information from the thalamus to CE can be potentiated. In the LA lesion simulation, we have shown that without LA input, the medial ITC cell switched to a very excitable state which imposed great inhibition on the CE neuron. As a result, the tone-CE synapse could not be potentiated to the normal level and acquisition of fear failed. The progressively decreased LA response is also a key to extinction of the CE activity due to the same mechanism. If LA maintained its elevated response during extinction training, CE would continue to receive excitatory drive from BA fear neurons and the inhibition from ITCm would not potentiate due to large sustained lateral to
medial ITC inhibition. Thus, the lack of conditioning seen after LA lesions is due partly to loss of a conditioned memory, and partly to the increased ITC inhibition of Ce, which overwhelms plastic processes in Ce.

5.4.2 BA contributes to acquisition and is necessary for extinction

In this model, the roles of fear and extinction neurons in BA are different. While fear neurons relay the fear signal from LA to CE, extinction neurons relay extinction signal from auditory cortex to ITCm, then to CE. In simulation, the CS responses of fear neurons increased after conditioning and decreased back to sensitization level in late extinction. Though the CS responses of extinction neurons were also increased by conditioning, the elevated responding maintained throughout extinction phase. A recent experimental finding has confirmed the existence of these two subpopulations of neurons in BA (Herry et al. 2008). When BA was lesioned before training, acquisition was not prevented, but reduced to a lower level compared to controls. That was because the excitatory drive from fear neurons to CE was gone, but potentiation of the CS information proceeded normally. However, the conditioned CE response could not extinguish due to loss of excitation from BA extinction cells to ITCm. Actually when BA is lesioned, the LA can no longer influence CE as the ITCm has very little activity, regardless of the degree of inhibition from ITCl. So BA lesion implies LA is lesioned also (not effective), but not vice versa. The key to extinction in this model is that the BAe-ITCm synapse underwent potentiation during extinction, which was a result of increased activity of the BAe neurons by conditioning. Thus, similar to the LA model, the
The foundation of extinction is already laid during conditioning, and is expressed when the LA-driven ITCl inhibition released from ITCm.

### 5.4.3 ITC cells are important to fear acquisition as well as extinction

When the lateral-medial inhibition of ITC cells was interrupted, the conditioning effect of CE neuron was greatly impaired due to large inhibition from the medial ITC neuron. Thus, the later-medial connectivity is important to relay the disinhibition signal from LA to CE. On the other hand, if the medial ITC neuron was removed, fear expression would be augmented and conditioned CE tone response could not be extinguished completely. The results from the model were supported by experimental findings that the ITC neurons play an important role in controlling impulse traffic through the amygdala (Royer et al. 1999; Royer et al. 2000a). The model also supports the hypothesis that the basolateral synapses on ITC cells are critical sites for extinction memory (Royer and Paré, 2002).

### 5.4.4 A plausible mechanism for IL gating

In the simulation, the IL input was presented 100 ms after tone onset, as observed in experiment (Milad and Quirk, 2002). As mentioned earlier, most of CE responses concentrated on the first 100 ms. How can the IL input help to suppress the early CE response? The model showed that IL influenced the early CE responses indirectly by facilitating potentiation of the BAe-ITCm synapse (Fig. 11A). During the first 100 ms following tone onset, the BAe input depolarized the ITCm cell, which inactivated the $I_{SD}$ current and increased the excitability of ITC neurons. As a result, the IL input would trigger more action potentials, allowing increased $Ca^{2+}$ influx via NMDA receptors for
LTP. Such a mechanism emphasizes an important issue: timing. Since BA neurons fire the most in the first 100 ms, the IL input present 100 ms after tone onset is ideal to take advantage of the inactivated $I_{SD}$ current for stronger ITCm responses. The gating mechanism suggested by this model is supported by an experimental finding that IL stimulation only reduced fear if it was given 100 ms after tone onset (Milad et al. 2004).

5.4.5 Distributed storage of fear and extinction memories

It has been shown that LA has storage sites for both fear and extinction memories (Li et al. 2008). The present model suggested additional storage sites. Similar to the tone-LA synapse, the tone-CE synapse was potentiated during conditioning when tone and shock were paired and the increased strength was not reversed fully by extinction, suggesting a site for long-term fear memory. The LA-BAf connection was potentiated in both sensitization and conditioning phases due to shock inputs, and maintained elevated strengths throughout extinction. Thus, the LA-BAf connection is able to store a generalized fear memory related to the occurrence of noxious input such as shock. The model also suggested two possible storage sites for the extinction memory: the tone-CE synapse and the BAe-ITCm synapse. Similar to the tone-CE synapse, the tone-BAe synapse was potentiated only during conditioning. As mentioned earlier, potentiation of this synapse laid down the foundation for later extinction. The BAe-ITCm synapse, on the other hand, potentiated only during extinction sessions. This highlighted two important facts for the potentiation of this synapse in the first extinction: (1) it potentiated only after the activity of BAe neurons was increased by conditioning; and (2) it potentiated only when the inhibition from ITCm reduced. During the second extinction
session, although the excitatory drive from BAe decreased, the facilitating gating input from IL allowed the BAe-ITCm synapse to strengthen again. Due to the relative high spontaneous firing activity of ITC cells, this synapse decayed considerably during the long gap. Thus, the BAe-ITCm synapse encoded short-term extinction memory. Taken all these together, our model suggested that fear and extinction memories are distributed among the amygdala circuit. Such a distributed storage mechanism may increase the robustness of the system.

5.4.6 Limitations

Although our model captures short and long-term extinction, it did not model the contextual and temporal modulation of extinction recall. Recall of extinction is modulated by inputs from IL, which could communicate contextual information to the amygdala (Hobin et al; Corcoran & Quirk, Garcia). By virtue of its regulation of ITCm excitability and plasticity, IL can gate recall of extinction. Models incorporating hippocampal and prefrontal modules are currently being developed.

5.5 Conclusion

In conclusion, we have shown that a biologically realistic network model of the amygdala circuit with Hebbian plasticity, can model the regulation of fear by both the BA and ITC pathways with IL-mPFC modulation. The biophysical realism of the model allowed us to test the specific roles of LA, BA, ITC and IL in fear acquisition and extinction with plausible neural mechanisms. Furthermore, our results suggested additional specific storage sites within the fear circuit for conditioning and extinction
memories, thus supporting the theory of distributed memory storage. The long-term goal of this study is to model pathologies associated with the fear circuit, such as anxiety disorders.

5.6 References


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Figure 5.1. Proposed structure of the amygdala circuit of fear learning and conditioning protocol. 
A: The tone information is delivered to LA via the medial division of medial geniculate body (MGm) and the shock information is delivered to LA via posterior intralaminar nucleus (PIN). The LA output projects to CE indirectly via BA and ITC cells. The auditory cortex (Te3) receives sensory inputs from the thalamus and projects to BA extinction neurons which in turn project to the medial part of ITC cells (ITCm). CE also receives direct tone/shock inputs from the thalamus. The infralimic prefrontal cortex (IL) projects to ITCm mediating fear responses.

B: Simulation schedule showing tone (green) and shock (red) inputs during sensitization, conditioning and the two extinction phases. There was a short gap between conditioning and extinction, and a longer gap before re-extinction. The pink arrow indicates the start of the IL input.
Figure 5.2. Responses of units to intracellular current pulses. 
A: voltage responses of LA/BL principal cells to three 600 ms current injections (top: 400 pA; middle: 300 pA; low: -100 pA). B: voltage responses of the LA/BL interneuron model to 200 ms current injections of the same magnitude. C: voltage responses of CE neuron model to three 600 ms current injections (top: 300 pA; middle: 100 pA; low: -100 pA).
Figure 5.3. Kinetics of the slowly deinactivating current $I_{SD}$ and responses of ITC model to intracellular current pulses. 
A: steady-state activation and inactivation curves. B: inactivation time constant function. C: voltage responses of ITC neuron model to three 600 ms current injections from rest (top: 100 pA; middle: 75 pA; low: -100 pA). D: dependence of ADP on the amplitude of current injections (from 0.5 to 1 sec; left: 30 pA; middle: 40 pA; right: 60 pA). Current injections were applied at the same $V_m (= -60 \text{ mV})$. E: dependence of ADP on the pre-pulse $V_m$. Current injections were applied at different $V_m$ as indicated. F: dependence of ADP amplitude on the duration of current injection. The current injections (starting from 500 ms) were applied at the same pre-pulse $V_m$ (-60 mV) and had the same amplitude (60 pA). The durations of the current injections were: left panel: 300 ms; middle panel: 400 ms and right panel: 500 ms. $V_m$ is controlled by a baseline current injection. $m$ and $h$ are the activation and inactivation gating variables respectively.
Figure 5.4. The effects of training on the tone responses of individual neurons. The peri-event time histograms (PETHs) of one LA (principal cell), five BA (four principal cells and one interneuron), two ITC and one CE neurons during A: sensitization, B: early extinction, C: late extinction phases. D: difference in PETHs between early extinction and sensitization. Tone started at $t = 0$; bin width was 10 ms and spike counts during 10 trials were summed together.
Figure 5.5. Time courses of representative synaptic strengths in the fear circuit during acquisition and extinction of fear.
A: tone-BAe synapse; B: tone-CE synapse; C: LA-BAf synapse; and D: BAe-ITCm synapse. S = Sensitization; C = Conditioning; E1 = First extinction; E2 = Second extinction.
Figure 5.6. Tone responses of each component in the circuit by block of 5 trials during the entire training. A: LA principal cells; B: BA fear and extinction cells; C: lateral and medial ITC cells; D: CE neuron. For each component, the average spikes in 0-200 ms are calculated among all cells in each block of 5 trials. Same for below.
Figure 5.7. Effects of LA lesioning. 
A-D: comparison of evolutions of synaptic weights for the control and LA-lesioning cases. A: tone-BAe synapse; B: tone-CE synapse; C: LA-BAf synapse and D: BAe-ITCm synapse. E-F: comparison of tone responses by block of 5 trials for the control and LA-lesioning cases of E: medial ITC cell; and F: CE neuron.
Figure 5.8. Effects of BA lesioning. 
A-B: comparison of tone responses by block of 5 trials for the control and BA-lesioning cases of A: medial ITC cell; and B: CE neuron. C: comparison of evolution of the tone-CE synaptic weight for the control and BA-lesioning cases.
Figure 5.9. Effects of interrupting the lateral-medial connection. 

A-B: comparison of evolutions of synaptic weights for the control and L-M interruption cases. A: tone-CE synapse; and B: BAe-ITCm synapse. C-D: comparison of tone responses by block of 5 trials for the control and L-M interruption cases of C: medial ITC cell; and D: CE neuron.
Figure 5.10. Effects of removing the medial ITC neuron. 
A: comparison of evolution of the tone-CE synaptic weight for the control and ITCm removed cases. B: comparison of tone responses by block of 5 trials of the CE neuron for the control and ITCm removed cases.
Figure 5.11. Effects of removing IL input during re-extinction.  
A: comparison of evolution of the BAe-ITCm synaptic weight for the control and IL-lesioning cases.  
B-C: comparison of tone responses by block of 5 trials for the control and IL-lesioning cases of B: medial ITC cell; and C: CE neuron.
5.7 Appendix

Each cell model includes two compartments: a soma and a dominant apical dendrite which are modeled as cylinder shape. Each compartment includes several of the following currents: spike-generating sodium current ($I_{Na}$), potassium delayed rectifier ($I_{DR}$), voltage-gated persistent muscarinic current ($I_{M}$), slowly inactivating voltage-gated K$^+$ current ($I_{D}$), slowly deinactivating K$^+$ current ($I_{SD}$), hyperpolarization-activated current ($I_{H}$), high-voltage activated Ca$^{2+}$ current ($I_{Ca}$), and two Ca$^{2+}$-activated potassium currents: (i) fast BK Ca$^{2+}$- and voltage-dependent C-type current ($I_{C}$), and (ii) a slow apamin-insensitive, voltage-independent afterhyperpolarization current ($I_{sAHP}$).

The values for the specific membrane resistance, membrane capacity and cytoplasmic (axial) resistivity are, respectively, $R_m = 30\, \text{K}\Omega\cdot\text{cm}^2$, $C_m = 1.0\, \mu\text{F/cm}^2$, and $R_a = 150\, \Omega\cdot\text{cm}$. The reversal potentials are: $E_{Na} = 45\, \text{mV}$, $E_K = -90\, \text{mV}$, $E_{Ca} = 120\, \text{mV}$, and $E_H = -43\, \text{mV}$. The leakage reversal potentials ($E_{L}$) are set as: BA: -75 mV, ITC: -75 mV and CE: -73 mV.

Table 5.1. Compartment dimensions (µm), maximal conductance densities (ms/cm$^2$) and Ca$^{2+}$ time constants (ms) for each cell type

<table>
<thead>
<tr>
<th></th>
<th>$d$</th>
<th>$l$</th>
<th>$I_{Na}$</th>
<th>$I_{DR}$</th>
<th>$I_{M}$</th>
<th>$I_{H}$</th>
<th>$I_{D}$</th>
<th>$I_{SD}$</th>
<th>$I_{Ca}$</th>
<th>$I_{C}$</th>
<th>$I_{sAHP}$</th>
<th>$\tau_{Ca}$</th>
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<tbody>
<tr>
<td><strong>BA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Soma</td>
<td>15</td>
<td>15</td>
<td>120</td>
<td>10</td>
<td>0.15</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>0.15</td>
<td>0.1</td>
<td>0.1</td>
<td>–</td>
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<td>0.5</td>
<td>0.3</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Soma</td>
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<td>10</td>
<td>120</td>
<td>5</td>
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<td>0.1</td>
<td>0.02</td>
<td>–</td>
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<td>50</td>
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<tr>
<td><strong>CE</strong></td>
<td></td>
<td></td>
<td></td>
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<td>–</td>
<td>0.2</td>
<td>–</td>
<td>0.15</td>
<td>250</td>
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Table 5.2. Gating variables for all ion channels used in the cell model

<table>
<thead>
<tr>
<th>Current Type</th>
<th>Gating Variable</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$x_\infty$</th>
<th>$\tau_x$ (ms)</th>
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<td>$I_{Na}$</td>
<td>$p=3$</td>
<td>$-0.2816(V + 21)$</td>
<td>$0.2464(V - 6)$</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
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<tr>
<td></td>
<td>$q=1$</td>
<td>$0.098 \times \exp(-(V + 36.1)/20)$</td>
<td>$1.4 \exp(-(V + 6.1)/10)$</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
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<tr>
<td>$I_{DR}$</td>
<td>$p=4$</td>
<td>$-0.018(V - 13)$</td>
<td>$0.0054(V - 23)$</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
</tr>
<tr>
<td>$I_{M}$</td>
<td>$p=2$</td>
<td>$0.016 \exp(-(V + 52.7)/23)$</td>
<td>$0.016 \exp((V + 52.7)/18.8)$</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>$1/(\alpha + \beta)$</td>
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<tr>
<td>$I_H$</td>
<td>$p=1$</td>
<td>$\frac{1}{\exp((V + 89.2)/9.5) + 1}$</td>
<td>$1727 \times \exp(0.019V)$</td>
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</tr>
<tr>
<td>$I_D$</td>
<td>$p=1$</td>
<td>$\frac{1}{\exp((V + 43)/8) + 1}$</td>
<td>$1$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$q=1$</td>
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<tr>
<td>$I_{SD}$</td>
<td>$p=4$</td>
<td>$\frac{1}{\exp(-(V + 80)/8.5) + 1}$</td>
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<tr>
<td></td>
<td>$q=1$</td>
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<td>$\tau^SD_{\text{SD}}$</td>
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<tr>
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<tr>
<td></td>
<td>$q=1$</td>
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<td>$420.0$</td>
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<tr>
<td>$I_C$</td>
<td>$p=2$</td>
<td>$\frac{1.7 \exp(-(V_m + 152)/30)}{\exp(-(V_m + 18)/12) - 1}$</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>$\max(1/(\alpha + \beta), 1.1)$</td>
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<tr>
<td>$I_{sHPR}$</td>
<td>$p=1$</td>
<td>$\frac{0.0048}{\exp(-5 \log_{10}([Ca]_2) - 17.5)}$</td>
<td>$\frac{0.012}{\exp(2 \log_{10}([Ca]_2) + 20)}$</td>
<td>$\alpha/(\alpha + \beta)$</td>
<td>$48$</td>
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$$
\tau^SD_{\text{SD}} = \frac{1}{\exp((V + 35.8)/19.7) + \exp(-(V + 79.7)/12.7)} + 0.37
$$

$$
\tau^SD_{\text{SD}} = \frac{10}{\exp((V + 36)/5) + \exp(-(V + 228)/37.5)} + 200
$$

$$
\tau^CA_{\text{Ca}} = 1.25 \times \sec h(-0.031(V + 37.1))
$$
Table 5.3. Model parameters for network connections and inputs

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<tr>
<th>Connection</th>
<th>Initial weight</th>
<th>Synaptic delay (ms)</th>
<th>$f_{\text{max}}$ ($f_{\text{min}}=0.8$ for all)</th>
<th>Learning factor</th>
<th>Ca^{2+} threshold (μmol)</th>
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<td></td>
<td></td>
<td></td>
<td>scaling</td>
<td>decay</td>
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<td>10</td>
<td>0.01</td>
</tr>
<tr>
<td>Tone-CE</td>
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<td>3</td>
<td>8</td>
<td>0.01</td>
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<tr>
<td>LA-BAf</td>
<td>6</td>
<td>10</td>
<td>3</td>
<td>10</td>
<td>0.01</td>
</tr>
<tr>
<td>BAe-ITCm</td>
<td>5</td>
<td>10</td>
<td>3</td>
<td>15</td>
<td>0.03</td>
</tr>
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<td>LA-ITCI</td>
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<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>ITCI-ITCm</td>
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<td>5</td>
<td>—</td>
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</tr>
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<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>BAF-CE</td>
<td>5</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>IL-ITCm</td>
<td>5</td>
<td>10</td>
<td>—</td>
<td>—</td>
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</tr>
</tbody>
</table>
Figure 5.12 (Appendix). Functions used in the calcium control hypothesis of Eq.8. A: $\Omega$ function. B: learning rate $\eta$; adapted from Shouval et al. (2002b).
Chapter 6

Summary and Future Work

Computational models can predict how a complex system evolves with experience. A new class of models incorporates biophysical realism with known synaptic connectivity, to more effectively model the learning brain. Such models integrate the intracellular and cellular levels of neuroscience with the network/systems level to provide a coherent picture of the higher level functions in health and disease (e.g., behavior, symptom). A case study of auditory fear conditioning was used to illustrate the ability of a computational model to provide insights into the neural causes of disruptions in the fear circuit thought to underlie symptoms of PTSD and anxiety disorders. Such insights have the potential to aid in drug discovery research, by allowing scientists to test predictions about the cellular and behavioral effects of new drugs.

6.1 Contributions

LA network model: We have shown that realistic LA neurons, incorporating known conductances, connectivity, and synaptic plasticity mechanisms, can learn fear conditioning and extinction. The biophysical realism of the model allowed us to test the importance of basal firing rates, NMDA receptors and depotentiation. Furthermore, our results suggest specific storage sites within the LA for conditioning vs. extinction, and
factors that can affect the persistence of these memories. The ultimate goal of this computational study is to model pathologies associated with the fear circuit (e.g., post traumatic stress disorder) and assist in the development of new treatments.

**ITC network model:** Our results indicate that although the inhibitory connections between ITC cells tend to oppose excitatory influences onto ITC cells, both intrinsic and synaptic, their impact is limited. In particular, IL inputs do overcome the inhibition produced by inter-ITC connections, leading to an overall excitation of ITC cells and a persistent decrease in Ce fear output. These results support the notion that IL inputs are in strategic position to control extinction of conditioned fear via the activation of ITC neurons.

**LA-BA-ITC-Ce network model:** We have shown that a biologically realistic network model of the amygdala circuit with Hebbian plasticity, can model the regulation of fear by both the BA and ITC pathways with IL-mPFC modulation. The biophysical realism of the model allowed us to test the specific roles of LA, BA, ITC and IL in fear acquisition and extinction with plausible neural mechanisms. Furthermore, our results suggested additional specific storage sites within the fear circuit for conditioning and extinction memories, thus supporting the theory of distributed memory storage. The long-term goal of this study is to model pathologies associated with the fear circuit, such as anxiety disorders.
6.2 Future Research

- The size of the LA network was intentionally small in order to facilitate the study of the underlying neural plasticity in detail, and is typical of previous biophysical modeling studies (Durstewitz et al. 2000). To investigate whether the qualitative conclusions and predictions from the model holds in general, a larger network model with reduced connectivity needs to be developed. Also, the LA network will be extended to include neurons that show persistence of potentiated tone responses observed in the ventral part of LA (LAv) (Repa et al. 2001).

- In addition to increasing CS-induced spike firing in the LA, fear conditioning also enhances the anticipatory, pre-CS firing and synchronizes the neuronal activity through a modulation at the theta frequency in conscious cats (Pare and Collins, 2000). The appearance of coherent theta oscillations in the LA might be very important in that it is believed that the coincident neuronal activity has an essential role in synaptic plasticity in the LA (Fanselow and LeDoux, 1999). The LA model could be used to investigate the following issues: (1) why does the pre-CS firing in LA neurons increase? (2) what is the mechanism for theta frequency oscillations? (3) how does neuronal synchronization affects synaptic plasticity? (4) the role of frequency adaptation and inhibition in synchronization; (5) implications of neuronal oscillation in the storage of fear and extinction memory.

- Expression of fear and extinction are regulated by contextual and temporal factors that are processed by other structures that influence the amygdala, such as the hippocampus and medial prefrontal cortex (Corcoran and Maren 2004; Milad and
Quirk 2002). Thus, additional modules will be needed to model the processes that regulate fear expression in real-life conditions in the amgydala network.

- In this dissertation, we use biophysical network model to investigate the neural mechanisms of fear learning and extinction. The neuronal model is highly nonlinear and complex, making analytical studies intractable. To extract the important concepts and insights from the full model, reduced order models of the LA/ITC/Amygdala network using paradigms such as the simple leaky integrate-and-fire (LIF) type models need to be developed. The neural mechanism of fear leaning and extinction can then be analyzed using a systems perspective including adaptation, learning and control features.

6.3 References


Appendix A – Cell Cycle Modeling

This is a conference paper published in the 2005 ASME Mechanical Engineering Congress and Exposition, Orlando, Florida USA. The paper presented a G2/M cell cycle model for the mammalian cells and investigated the regulation of G2/M transition by oxidative stress. The model focused on two important proteins (p53 and p21) in cell cycle control and provided valuable insights into how oxidative stress may lead to genomic instability and predisposition to cancer.

Regulation of G2/M transition in mammalian cells by oxidative stress

G. Li, and S. S. Nair, Engineering
S. Lees and F. Booth, Biomedical Sciences
University of Missouri-Columbia

Abstract: The regulation of the G2/M transition for the mammalian cell cycle has been modeled using 19 states to investigate the G2 checkpoint dynamics in response to oxidative stress. A detailed network model of G2/M regulation is presented and then a “core” subsystem is extracted from the full network. An existing model of Mitosis control is extended by adding two important pathways regulating G2/M transition in response to DNA damage induced by oxidative stress. Model predictions indicate that the p53 dependent pathway is not required for initial G2 arrest as the Chk1/Cdc25C pathway can arrest the cell in G2 right after DNA damage. However, p53 and p21 expression is important for a more sustained G2 arrest by inhibiting the Thr^{161} phosphorylation by CAK. By eliminating the phosphorylation effect of Chk1 on p53, two completely independent pathways are obtained and it is shown that it does not affect the G2 arrest much. So the p53/p21 pathway makes an important, independent contribution to G2 arrest in response to oxidative stress, and any defect in this pathway may lead to genomic instability and predisposition to cancer. Such strict control mechanisms probably provide protection for survival in the face of various environmental changes. The controversial issue related to the mechanism of inactivation of Cdc2 by p21 is addressed and simulation predictions indicate that G2 arrest would not be
affected much by considering the direct binding of p21 to Cdc2/Cyclin B given that the inhibition of CAK by p21 is already present if the binding efficiency is within a certain range. Lastly, we show that the G₂ arrest time in response to oxidative stress is sensitive to the p53 synthesis rate.

INTRODUCTION

The cell cycle is the sequence of events by which a growing cell duplicates all its components and then divides this material between two daughter cells [1]. It is the fundamental process underlying all biological growth and reproduction and consists of 4 phases: M-Mitosis phase, G₁-growth phase 1, S-synthesis phase, G₂-growth phase 2. M phase is the period of actual division where one copy of each DNA molecule is assigned to each sister cell. DNA is synthesized during S phase and G₁ and G₂ are the two “gaps” in the cell cycle where no DNA is replicated. For somatic animal cells, the division cycle repeats every 18-24 hours. G₁ phase takes most of the cycle time, varying from ~6 hours to ~ 12 hours [2]. The typical duration is 6-8 hours for the S phase. G₂ phase is usually the shortest part and M phase usually lasts <1 hour in duration [2]. The progression of the cell cycle is tightly regulated by control mechanisms called checkpoints which ensure the proper timing of cell cycle events by enforcing the dependency of late events on the completion of early events [3]. There exist G₁ and G₂ checkpoints which can result in cell cycle arrest, senescence or apoptosis in response to DNA damage so that cells cannot divide until DNA is repaired. Failure to be arrested in G₁ or G₂ after DNA damage leads to genomic instability and predisposition to cancer.

Oxidative stress is the accumulation of ROS (reactive oxygen species) above physiologic levels. ROS actively participate in a diverse array of biological processes, including proliferation and cellular senescence [4]. The vastly different biological outcomes likely reflect the types and amounts of ROS that are present, the duration of the oxidative burst, the cellular antioxidant systems, and the cellular context experiencing the oxidative stress [4]. Extrinsic stresses, such as oxidative stress, may affect intrinsic factors, such as DNA damage accumulation and telomere shortening rates [5]. It is thought that the production of ROS and
accumulation of oxidative damage contribute to decreased physiological function during aging (for reviews see ref. [6, 7]).

Oxidative stress can cause DNA damage and also accelerate telomere shortening rates [8, 9, 10]. The cell cycle regulation by oxidative stress is mediated primarily by p53 through the DNA damage pathways [5]. p53, the tumor suppressor protein, is a 393 amino acid nuclear phosphoprotein whose gene lies on the short arm of chromosome 12 in humans and contains 11 exons. Due to its critical role in the prevention of cancer, the p53 tumor suppressor protein has received a great deal of attention by researchers. It has been estimated that 80% or more of human cancers have defects in p53 signaling while roughly half of all such cancers display overt structural alterations of one or both p53 alleles [11]. Also, too much p53 can accelerate organismal aging [12]. In response to DNA damage, p53 has been shown to induce both G1 and G2 phase cell cycle arrests [13, 14]. This paper develops a model for the regulation of G2/M transition by oxidative stress through p53 mediation, and uses the model to highlight several issues related to the dynamics of the regulation. The G2/M transition mechanisms are reviewed first. A “core” subsystem [21] consisting of the two most important pathways responsible for the G2 cell cycle arrest is then extracted. It has been shown that this core subsystem can capture the major dynamics of the G2/M transition very well [21, 32]. Expression of p53 and p21 is not required for the initial G2 arrest in response to DNA damage induced by oxidative stress since well defined p53 independent pathways can arrest the cell promptly. However, the p53/p21 pathway is important for a more sustained G2 arrest, which ensures that the DNA damage is completely repaired before the cell enters mitosis. Any defect in this mechanism in mutant cells may lead to the development of cancer. Such strict control mechanisms probably provide protection for survival in the face of various environmental changes.

G2/M TRANSITION MECHANISMS
The fundamental components of M-phase control seem to be the same in all eukaryotic cells [15]. M phase division is triggered by M-phase promoting factor (MPF), which is a protein kinase consisting of a catalytic subunit (Cdc2 kinase) and a regulatory subunit (Cyclin B). MPF is activated by phosphorylation at threonine 161 by CDK-activating kinase (CAK) and inactivated by phosphorylation at tyrosine 15 and threonine 14 by Wee1 and Myt1.
respectively. Since the two inhibitory sites Tyr15 and Thr14 are dephosphorylated simultaneously by Cdc25 at the onset of mitosis, we need to keep track only of the phosphorylation state of Tyr15. Active MPF can further activate Cdc25 by phosphorylation and it is assumed that Wee1 is inhibited by active MPF, thus forming two positive feedback loops [15]. Once activated, MPF initiates M phase by phosphorylating a variety of target proteins involved in the major events of mitosis. Exit from the M phase is mediated by degradation of the Cyclins and active MPF stimulates its own destruction by a negative feedback loop with time delay. The M-phase control system is shown in Fig. 1.

p53 controls the G₂/M transition by regulating the cyclin dependent kinase Cdc2. A comprehensive review of the regulation of G₂/M transition by p53 is provided in [3] and the network is incorporated into Fig. 2a.

**p53 activation by ATM/ATR in response to oxidative stress**

As mentioned above, oxidative stress can cause DNA damage. The ATM and ATR proteins are phosphotidylinositol-3 kinases which are very important in transducing DNA damage signal to p53 [16]. DNA damage stimulates the kinases ATM and ATR, which in turn phosphorylates serines 15 and 37 of p53 [17]. The phosphorylation stabilizes p53 and causes it to accumulate, essential steps in activating p53 in response to oxidative stress.

**Mdm2 as the major regulator of p53**

The increased stability of activated p53 is in large part due to prevention of its degradation by Mdm2, the most important player in regulation of p53. In normal cells, p53 levels are kept at a very low value by Mdm2, while p53 becomes strongly deregulated to the extent that it can lead to apoptosis in the absence of Mdm2 [18]. It has been shown in [19] that loss of the p53 inhibitor Mdm2 gives rise to a p53-dependent embryonic lethal phenotype. Thus a cell can tolerate the absence of p53 function but not excess p53 function. Mdm2 ubiquitinates p53 for degradation and this function can be blocked by phosphorylation of p53. While Mdm2 inhibits the transcriptional function of p53 by binding to it, Mdm2 gene is transcriptionally activated by p53, which forms an important negative feedback loop [18].

**Role of p21**

p53 accumulation leads to transcriptional activation of p21. Four mechanisms have been postulated for how p21 participates in inhibiting Cdc2 activity to cause G₂ arrest. First, p21 inhibits CDK activity by binding directly to CDK/Cyclin complexes [20]. A second
mechanism for inhibiting Cdc2 is suggested by experiments in *Xenopus* showing that active Cdk2 is involved in generating active Cdc2. p21 may thus cause loss of the positive feedforward of Cdc2 activity by inhibiting Cdk2. Third, p21 can interfere with the activating phosphorylation of Cdc2 by CAK [21]. Last, p21 may inactivate PCNA, a processivity factor for DNA polymerases δ and ε, causing DNA damage during S phase, and thus leading to inhibition of Cdc2 and to G2 arrest by p53-independent mechanisms [3].

**Regulation of Cdc2/Cyclin subunit assembly by Gadd45**

Gadd45 is another transcriptional target of p53 involved in the G2/M transition. It may affect the CDK/Cyclin activity by dissociating complexes of Cycling B and Cdc2 [22]. The role of Gadd45 in regulating Cdc2 may be important in maintaining genomic stability and binding of Gadd45 to PCNA leads to the direct stimulation of DNA repair in vitro [23].

**Role of 14-3-3σ**

Cdc2 must enter the nucleus to induce mitosis and an additional mechanism by which p53 causes G2 arrest involves regulation of the subcellular localization of Cdc2. The protein 14-3-3σ, a direct transcriptional target of p53, can bind to Cdc2/Cyclin B and keep it in the cytoplasm, resulting in G2 arrest [3].

**Trans-repression of cdc2 and cyclin B1**

p53 not only activates the transcription of many genes that mediate its downstream functions but also can repress the transcription of different genes via several different mechanisms. Because of the transcriptional repression by p53, the level of Cyclin B1 in cells overexpressing p53 is reduced, which leads to inhibition of Cdc2 activity. It has been shown the loss of Cyclin B1 happened before most cells arrested in G2 [24]. Another part of the mechanism of G2 arrest by p53 is the reduction in the level of Cdc2 protein. It has been revealed that p53 repressed the cdc2 promoter leading to loss of the cdc2 mRNA, which in turn reduced the Cdc2 protein causing G2 arrest [24].

**p53-independent pathways**

The regulation of p53 in G2 arrest is complicated by p53-independent mechanisms that block entry into mitosis. ATM and ATR can also phosphorylate and activate the serine kinases Chk1 and Chk2 in response to DNA damage [25]. Chk1 and Chk2 then phosphorylates Cdc25C on the residue Ser216 [26], which causes it to bind to protein 14-3-3. Binding to 14-3-3 maintains Cdc25C in an inactive state that cannot dephosphorylate Cdc2 [27]. As a
result, the cell is arrested in G2 phase. However, these pathways are not completely independent because Chk1 and Chk2 can also phosphorylate serine 20 of p53 [28].

**Control block diagram for G2/M transition**

Based on the regulation mechanisms of G2/M transition, we can obtain the control block diagram, which is shown in Fig. 2b. In this diagram, p53 and Chk1 can be viewed as controllers, while p21, Gadd45, 14-3-3σ and Cdc25C can be viewed as actuators. The input of the system is DNA damage signal caused by oxidative stress and the output is the active Cdc2/Cyclin B level. The protein ATM is an important sensor in the system. Our goal is to investigate how p53 regulates the G2/M transition in response to oxidative stress by identifying the complex dynamics and control mechanisms involved in this system.

**MODEL DEVELOPMENT**

A series of mathematical models for cell cycle control have been developed and published by Novak et al. [15, 29-31]. Differential equations are employed to model the system based on the law of mass action and it has been shown those models did give new insights into cell cycle control. However, none of those models considered the effect of oxidative stress on cell cycle regulation. This paper adds p53 pathways into the cell cycle control system and investigate how G2/M transition can be regulated by oxidative stress. As a first step, we extract from the network model (shown in Fig. 2a) a “core” subsystem that consists of the two most important pathways that regulate G2/M transition in response to oxidative stress (Fig. 2c). The Chk1/Cdc25C pathway, the first one, blocks activation of Cdc2 through retainment of Thr14/Thr15 phosphorylation, while the p53/p21 pathway, the second one, inhibits Cdc2 by blocking the activating Thr161 phosphorylation [21]. Thus, these two pathways target different events at the G2 checkpoint. Based on the “core” network, we formulate a mathematical model for the regulation of G2/M transition by oxidative stress by adapting the M-phase control model reported by Novak and Tyson [15], by adding equations pertaining to the additional pathways into the final model shown in Table 1. We assume the model starts at the end of S phase.

In Eq. (2), the state $\text{DNA\_damage}$ represents the strength of the DNA damage signal modeled by first order dynamics with its initial condition determined by the level of oxidative stress. We assume linear dynamics for the ‘sensor’ ATM which transduces the
DNA damage signal to p53 and Chk1 linearly. Also, the DNA repair rate $k_{\text{repair}}$ is assumed to be constant since the DNA repair pathways has not been incorporated into the model. $[p53]$ and $[p53_p]$ denote the concentration levels of p53 and its phosphorylated form respectively. The same symbols are used for all other proteins. $d_1$ is the Mdm2-independent p53 degradation rate and $d_2$ is the Mdm2-dependent p53 degradation rate. $k_{\text{chk1}}$ represents the p53 phosphorylation rate by Chk1 which is dependent on the phosphorylation level of Chk1. The total amount of Chk1 is held for constant.

Equations (5-8) represent the p53 transcriptional effects on Mdm2 and p21. $[mdm2_{AA}]$ and $[p21_{AA}]$ denote two intermediate products which model the transcriptional delay in the p53-dependent induction of Mdm2 and p21. Eqs. (9-12) model the four different dimers of CDK and their transformation. $k_{11}$ is the synthesis rate of Cdc2/Cyclin B and the four dimmers are assumed to degrade with the same rate $d_7$. $k'_{\text{CAK}}$ represents the inhibition effect of p21 on Thr$^{161}$ phosphorylation of Cdc2. The rate constants $k_{\text{cdc25c}}, k_{\text{wee}}$ and $d_7$ are defined in terms of the concentrations of the alternative forms of Cdc25C, wee1 and UbE.

For the regulatory enzymes Cdc25C and Wee1, we use Michaelis-Menten rate laws as in [15]. Cdc25C is activated by hyperphosphorylation and $cdc25c_p$ denotes the active form of Cdc25C. Chk1 can phosphorylate Cdc25C on the residue Ser$^{216}$ as mentioned above, which creates a binding site for 14-3-3 family of proteins and inhibits the activation of Cdc2 by Cdc25C. We assume Chk1 only phosphorylates the active form of Cdc25C. $cdc25c_p'$ represents the inactive form of Cdc25C with residue Ser$^{216}$ phosphorylated. $k'_{\text{chk1}}$ is the Chk1 dependent Cdc25C phosphorylation rate on Ser$^{216}$. $K_a$, $K_b$, $K_c$ and $K_f$ are Michaelis constants. The total concentration levels of Wee1 and Cdc25C are taken as constants. The two enzymes IE and UbE are also modeled using Michaelis-Menten rate laws and their total concentrations are taken as constant.

**RESULTS AND DISCUSSION**

The parameter values and initial conditions for the model are shown in Tables 2 and 3 respectively. When the oxidative stress level is too low to induce DNA damage (which corresponds to $DNA_{\text{damage}}(0) = 0$ or $k_1 = 0$), the time courses of active MPF and active Cdc25C are shown in Fig. 3. Active MPF accumulates quickly with the help of Cdc25C and the cell enters mitosis at approximately 50 min. After reaching the peak at around 60 min,
active MPF begins to degrade and the cell exits from mitosis at around 80 min. Next, the DNA damage caused by high level of oxidative stress is introduced into the cell cycle \((DNA_{damage}(0) = 1)\). First we turn off the p53/p21 pathway by setting \(k_8 = 0\) to investigate the effect of the Chk1/Cdc25C pathway on cell cycle control. The Chk1 activation is shown in Fig. 4a and the time courses of active MPF and active Cdc25C are shown in Fig. 4b. Active Chk1 goes up very quickly and then decreases towards 0 with decreasing DNA damage signal. From Fig. 4b, we can see the switch-on time of active MPF is delayed to 170 min, indicating that the G2 arrest lasts for approximately 2 hours for this case. The active Cdc25C level is kept at a very low level as expected since active Chk1 phosphorylates Cdc25C on Ser216, causing it bind to the 14-3-3 family of proteins. However, active MPF can still switch on at 170 min, although active Cdc25C level is very low. This is because active MPF can accumulate from phosphorylation at Thr161 by CAK. Therefore, the G2 arrest induced by the Chk1/Cdc25C pathway is not a sustained one. With the p53/p21 pathway turned on, the active p53 and p21 levels are shown in Fig. 5a, while the active MPF and active Cdc25C levels are shown in Fig. 5b. Under the activation of p53, p21 accumulates quickly. At 300 min, the level of active p53 is very low, but the level of p21 is still quite high. Now the switch-on time of active MPF has been delayed to 330 min, almost doubling the G2 arrest time compared to the previous case. At 330 min, the rising active Cdc25C forces MPF to switch, driving the cell cycle into mitosis, regardless of the relatively high level of p21. All of these are consistent with the experimental observations that expression of p53 and p21 is not required for the initial arrest, but both proteins are essential to sustain the G2 arrest in response to DNA damage [32]. By plotting both the DNA damage signal and active MPF in Fig. 5c, it can be noted that there is almost no DNA damage signal after 150 min, but active MPF cannot switch till 330 min. Thus, the cell cannot move to mitosis right after DNA damage is repaired and a significant time lag exists. This phenomenon demonstrates that the cell cycle checkpoint is very strict, ensuring that the DNA damage is repaired completely.

As mentioned above, the Chk1/Cdc25C and p53/p21 pathways are not completely independent since active Chk1 can activate p53 by phosphorylation. To investigate how the G2 arrest would be affected without the coupling of these two pathways, the system model is simulated by taking off the \(k_{chk1}\) term from Eqn. (4). The time courses of active MPF with two correlated pathways and two uncorrelated pathways, in response to oxidative stress, are
shown in Fig 6, indicating little difference between the two active MPF profiles. As such, the p53/p21 pathway results in an important, independent contribution to inactivation of Cdc2, and lack of this response in certain tumor cells is expected to increase their sensitivity to DNA damaging agents [21]. Also, it has been shown that embryonic stem (ES) cells lacking Chk1 have a defective G2/M DNA damage checkpoint in [33]. We test this experimental finding by setting $c_1 = 0.1$ in Eqn (7) and the simulation result shown in Fig. 7 indicates that active MPF switches on at around 110 min, even though the DNA damage has not been completely repaired yet. So, we conclude that the p53/p21 pathway alone is unable to arrest the cell at G2 safely in response to oxidative stress.

In the “core” subsystem shown in Fig. 2c, p21 enforces G2 arrest by reducing CAK activity, thus inhibiting Thr$^{161}$ phosphorylation of Cdc2. It has also been postulated that p21 inhibits CDK activity by binding directly to CDK/Cyclin complexes (Fig. 2a) [20]. However, it was shown in [21] that though p21 is able to bind Cyclin B, this binding does not appear to be stoichiometric and the CDK activity is inhibited to an extent that cannot be explained by mere binding of p21 to these complexes. To investigate whether the G2 checkpoint would behave very differently given the two mechanisms are present at the same time, compared to only one being present, the following biochemical reaction is added to the “core” subsystem in Fig. 2c:

$$Cdc2/CyclinB + p21 \xrightarrow{k_{16}} Cdc2/CyclinB/p21$$

Eqns (11) and (12) now become:

11. $\frac{dp21}{dt} = k_{16}[p21]_{-AA} - d_{6}[p21] + k_{17}[cdc2 : cyclinB : p21] - k_{16}[p21][cdc2 : cyclinB]$

12. $\frac{d[cdc2 : cyclinB]}{dt} = k_{17} + k_{inh}[activeMPF] + k_{cdc25c}[p_ - cdc2 : cyclinB] - (k_{wee} + \frac{k_{C4k}}{1 + k_{C4k}[p21]} + d_{7})[cdc2 : cyclinB]$

+ $k_{17}[cdc2 : cyclinB : p21] - k_{16}[p21][cdc2 : cyclinB]$

Also a new equation needs to be added to the model:

31. $\frac{d[cdc2 : cyclinB : p21]}{dt} = k_{16}[p21][cdc2 : cyclinB] - k_{17}[cdc2 : cyclinB : p21]$

In the biochemical reaction shown above, the association and disassociation rates are $k_{16}$ and $k_{17}$ respectively, whose ratio is defined as $K = k_{16}/k_{17}$. In the model, we try different values of $K$ and the results are shown in Fig. 8. As expected, the larger the value of $K$, the more sustained is the G2 arrest. If in the biological system, this ratio is within a certain range (e.g.
less than 30), the $G_2$ arrest time won’t be affected much when the direct binding of p21 with Cdc2/Cyclin B is considered. So the contribution of direct binding of p21 with Cdc2/Cyclin B to the $G_2$ arrest is relatively small given that the mechanism that p21 inhibits Thr$^{161}$ phosphorylation of Cdc2 by reducing CAK activity is already present in the cell cycle in response to oxidative stress. But it should be noted that the amplitudes of active MPF have been reduced due to less Cdc2/Cyclin B and p-Cdc2-p/Cyclin B transformed to active MPF. Whether this reduction of active MPF would affect the $G_2$/M transition is an issue that needs further investigation.

Lastly, we vary an important parameter in the model: the synthesis rate $k_2$ of p53. The simulation results are shown in Fig. 9, where we can see the $G_2$ arrest time is quite sensitive to the p53 synthesis rate, especially when this value is greater than 0.1.

**CONTRIBUTIONS**

A new computational model for the regulation of $G_2$/M transition by oxidative stress through p53 mediation in mammalian cells is developed. The model did replicate behaviors found in physiology and resulted in the following additional findings: (i) expression of p53 and p21 is not required for the initial $G_2$ arrest, but both proteins are essential to sustain $G_2$ arrest in response to DNA damage; (ii) p53/p21 pathway alone may not be able to arrest cells in $G_2$ safely with a defective Chk1/Cdc25C pathway, in response to DNA damage; (iii) a significant time lag exists between the completion of DNA damage repair and the entry of mitosis; (iv) $G_2$ arrest time is sensitive to p53 synthesis rate; and (v) contribution of direct binding of p21 with Cdc2/Cyclin B to the $G_2$ arrest is relatively small given that the mechanism that p21 inhibits Thr$^{161}$ phosphorylation of Cdc2 by reducing CAK activity is already present in the cell cycle. These findings need to be experimentally validated and provide directions for experimental investigations.

**FUTURE WORK**

In the current “core” subsystem model, we neglected the effect of Gadd45 and 14-3-3σ proteins in the $G_2$/M transition. Also, the trans-repression of Cdc2 and Cyclin B by p53 and the subcellular localization of Cdc2 and Cdc25C has not been modeled. We now plan to
model the full network system shown in Fig. 2a and compare the behavior of the full system with that of the “core” subsystem. The current model is nonlinear with a number of differential equations. We will consider linearizing the model and reducing the model order to determine the components and interactions that underlie a specific behavior in the complex cellular network. Variation in the level of oxidative stress leads to a suite of possible outcomes including (a) no adverse effects; (b) cell survival after checkpoint arrest; (c) cell senescence during G2 checkpoint arrest; (d) cell death (apoptosis) in G2 phase. With the application of nonlinear dynamics concepts, response to oxidative stress via checkpoint arrest or apoptosis can be studied further by phase plane plots and bifurcation diagrams [34, 35]. It is still not clear how cells choose between senescence and apoptosis upon DNA damage-induced activation of p53 [5]. We may be able to identify the relevant mechanisms for selection between senescence and apoptosis using bifurcation concepts. In the G2/M model, we have shown that the p53-independent and dependent pathways contribute to a sustained G2 arrest in response to DNA damage. p21, Gadd45 and 14-3-3σ, the transcriptional targets of p53, can all block cells at the G2 checkpoint by inhibiting Cdc2 in response to oxidative stress. From a system point of view, multiple control pathways increase the robustness of the system, which needs to be analyzed using robustness measures.

References


regulated by Atr and required for the G2/M DNA damage checkpoint,” *Genes &

[34] Hatzimanikatis V, Lee H, Bailey JE. “A mathematical description of regulation of

Table 1.

Equations for p53 and Chk1 activation

\[
\begin{align*}
\frac{d}{dt}[\text{DNA}_\text{damage}] &= -k_\text{repair}[\text{DNA}_\text{damage}] \quad (1) \\
\frac{d}{dt}[p53] &= k_2 + k_3[p53] - (k_\text{ATM} + k_\text{chkl})[p53] - d_1[p53] - d_2[p53][mdm2] \quad (2) \\
\frac{d}{dt}[p53] &= (k_\text{ATM} + k_\text{chkl})[p53] - k_3[p53] \quad (3) \\
\frac{d}{dt}[\text{chk1}_\text{p}] &= k_\text{ATM}[\text{chk1}]-k_4[\text{chk1}_\text{p}] \quad (4)
\end{align*}
\]

Equations for p53 transcriptional effects

\[
\begin{align*}
\frac{d}{dt}[mdm2] &= k_5 + k_6[p53] - d_5[mdm2] \quad (5) \\
\frac{d}{dt}[p21] &= k_7 + k_8[p53] - d_4[p21] \quad (6) \\
\frac{d}{dt}[mdm2] &= k_9[mdm2] - d_5[mdm2] \quad (7) \\
\frac{d}{dt}[p21] &= k_10[p21] - d_6[p21] \quad (8)
\end{align*}
\]

Equations governing cyclin-dependent kinases (CDKs)

\[
\begin{align*}
\frac{d}{dt}[\text{cd2}]:[\text{cyclinB}] &= k_{11} + k_{\text{INH}}[\text{activeMPF}] + k_{\text{cd2c}25c}[p_{-}\text{cd2} : \text{cyclinB}] - (k_{\text{wee}} + \frac{k_{\text{CAK}}}{1+k_{\text{CAK}}[p21]} + d_7)[\text{cd2} : \text{cyclinB}] \quad (9) \\
\frac{d}{dt}[p_{-}\text{cd2} : \text{cyclinB}] &= k_{\text{wee}}[\text{cd2} : \text{cyclinB}] + k_{\text{INH}}[p_{-}\text{cd2} : \text{p} : \text{cyclinB}] - (k_{\text{cd2c}25c} + \frac{k_{\text{CAK}}}{1+k_{\text{CAK}}[p21]} + d_7)[p_{-}\text{cd2} : \text{cyclinB}] \quad (10) \\
\frac{d}{dt}[p_{-}\text{cd2} : p : \text{cyclinB}] &= k_{\text{wee}}[\text{activeMPF}] + \frac{k_{\text{CAK}}}{1+k_{\text{CAK}}[p21]}[p_{-}\text{cd2} : \text{cyclinB}] - (k_{\text{INH}} + k_{\text{cd2c}25c} + d_7)[p_{-}\text{cd2} : p : \text{cyclinB}] \quad (11) \\
\frac{d}{dt}[\text{activeMPF}] &= \frac{k_{\text{CAK}}}{1+k_{\text{CAK}}[p21]}[\text{cd2} : \text{cyclinB}] + k_{\text{cd2c}25c}[p_{-}\text{cd2} : p : \text{cyclinB}] - (k_{\text{INH}} + k_{\text{wee}} + d_7)[\text{activeMPF}] \quad (12)
\end{align*}
\]

Equations governing the enzymes of CDKs

\[
\begin{align*}
\frac{d}{dt}[\text{cd2c}25c] &= k_9[\text{activeMPF}][\text{cd2c}25c] - k_8[\text{cd2c}25c] - k_12[\text{cd2c}25c] - k_{\text{chkl}}[\text{cd2c}25c] \quad (13) \\
\frac{d}{dt}[\text{cd2c}25c] &= k_9[\text{cd2c}25c] - k_9[\text{activeMPF}][\text{cd2c}25c] K_a + [\text{cd2c}25c] \quad (14) \\
\frac{d}{dt}[\text{cd2c}25c] &= k_9[\text{cd2c}25c] - k_9[\text{activeMPF}][\text{cd2c}25c] K_a + [\text{cd2c}25c] \quad (15) \\
\frac{d}{dt}[\text{wee}] &= k_5[\text{activeMPF}][\text{wee}] - k_5[\text{wee}] \quad (16)
\end{align*}
\]
\[ \frac{d[14 - 3 - 3]}{dt} = k_{15} + k_{13}[\text{cdc25c} \cdot p : 14 - 3 - 3] - k_{14}[14 - 3 - 3][\text{cdc25c} \cdot p] - d_{8}[14 - 3 - 3] \] (17)

**Equations for the enzymes governing CDKs degradation**

\[ \frac{d[IE \cdot p]}{dt} = \frac{k_{c}[\text{activeMPF}][IE]}{K_{g} + [IE]} - \frac{k_{h}[IE \cdot p]}{K_{h} + [IE \cdot p]} \] (18)

\[ \frac{d[\text{UbE}^+]}{dt} = \frac{k_{c}[IE \cdot p][\text{UbE}]}{K_{c} + [\text{UbE}]} - \frac{k_{d}[\text{UbE}^+]}{K_{d} + [\text{UbE}^+]} \] (19)

**Definitions**

\[ k_{\text{ATM}} = k_{1}[\text{DNA damage}] \] (20)

\[ k_{\text{chkl}} = V_{1}[\text{chk}1 \cdot p] \] (21)

\[ k_{\text{cdc25c}} = V_{2}[\text{cdc25c}] + V_{3}[\text{cdc25c} \cdot p] \] (22)

\[ k_{\text{wee}} = V_{4}[\text{wee1}] + V_{5}[\text{wee}1 \cdot p] \] (23)

\[ k'_{\text{chkl}} = V_{5}[\text{chk}1 \cdot p] \] (24)

\[ d_{7} = V_{6}[\text{UbE}] + V_{7}[\text{UbE}^+] \] (25)

\[ [\text{chk}1] + [\text{chk}1 \cdot p] = C_{1} \] (26)

\[ [\text{cdc25c}] + [\text{cdc25c} \cdot p] + [\text{cdc25c} \cdot p : 14 - 3 - 3] = C_{2} \] (27)

\[ [\text{wee1}] + [\text{wee}1 \cdot p] = C_{3} \] (28)

\[ [IE] + [IE \cdot p] = C_{4} \] (29)

\[ [\text{UbE}] + [\text{UbE}^+] = C_{5} \] (30)
**Table 2. Parameter values for the model**

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<th>$k_1$</th>
<th>$k_{11}$</th>
<th>$V_1$</th>
<th>$C_3$</th>
<th>$k_a$</th>
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**Table 3. Initial conditions for the model**

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<th>DNA damage(0) = 1</th>
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<th>14 - 3 - 3(0) = 0.01</th>
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<td>$weel_{p}(0) = 0$</td>
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<td>$cdc25'_{p}(0) = 0$</td>
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</tbody>
</table>
**Fig. 1 The M-phase control system [15].** (A) The four phosphorylation states of Cdc2/CyclinB. The grey ellipse represents Cyclin B and the Y-T box represents Cdc2, where Y is the inhibitory Tyr residue and T is the activating Thr residue. Cyclin B is synthesized from amino acids and binds with free Cdc2 to form Cdc2-cyclin dimer. The CAK positively phosphorylates the dimer at Thr residue and the Wee1 kinase negatively phosphorylates the dimer at Tyr residue. The phosphatases Cdc25 and INH remove the inhibitory phosphorylation (Tyr) and excitatory phosphorylation (Thr) respectively. The dimer in the upper right corner is active MPF which can drive the cell into mitosis and division.

(B) Positive feedback loops. Active MPF activates Cdc25 and inhibits Wee1 by facilitating the phosphorylation of the two phosphatases, which results in positive feedback loops. Also, unreplicated DNA is assumed to prevent the cell from entering mitosis by influencing the positive feedback loops.

(C) Degradation pathways. The Cyclin subunit is labeled for destruction by the ubiquitin-conjugating enzyme (UbE). By activating the intermediary enzyme (IE), active MPF stimulates its own degradation forming a negative feedback loop.
Fig. 2a Regulation of G2/M transition by oxidative stress [modified from ref. 3]

Fig. 2b Control block diagram for G2/M transition
Fig. 2c “Core” subsystem of G2/M transition by oxidative stress
Fig. 3 Time courses of active MPF and active Cdc25C without oxidative stress

Fig. 4a Chk1 activation in response to oxidative stress; 4b Active MPF and active Cdc25C in response to oxidative stress with the p53/p21 pathway off
Fig. 5a Time courses of active p53 and p21 in response to oxidative stress; 5b Active MPF and active Cdc25C in response to oxidative stress with both the Chk1/Cdc25C pathway and the p53/p21 pathway on; 5c, Plots of DNA damage signal and active MPF with both the Chk1/Cdc25C pathway and the p53/p21 pathway on
Fig. 6 Time courses of active MPF with two correlated pathways and two uncorrelated pathways in response to oxidative stress.

Fig. 7 Defective G2/M DNA damage checkpoint for cells lacking Chk1 in response to oxidative stress.

Fig. 8 G2 arrest when direct binding of p21 with Cdc2/Cyclin B is considered with different efficiencies in response to oxidative stress.

Fig. 9 G2 arrest with different p53 synthesis rate response to oxidative stress.
Appendix B – Publications

Book Chapter


Journal Papers

- Li G, Pare D, Quirk GJ, Nair SS. Biophysical modeling of the amygdala network in acquisition and extinction of conditioned fear. Complete draft under revision.
- Li G, Pare D, Nair SS. Impact of infralimbic inputs on intercalated amygdala neurons: a biophysical modeling study. Complete draft under revision.

Conference Papers

Hebbian learning. *ASME International Mechanical Engineering Congress and Exposition*, Nov 5-11, Chicago, IL.


**Posters**

- The 39th SFN Annual Meeting, October 17-21, 2009 Chicago, IL.
- The 38th SFN Annual Meeting, November 15-19, 2008 Washington, DC.
- The 36th SFN Annual Meeting, October 14-18, 2006 Atlanta, GA.
- First Biologically Accurate Modeling Meeting, Mar 30-Apr 2, 2005 San Antonio, TX.
Guoshi Li was born in Taishan, Guangdong, China. He earned his Bachelor’s degree in automatic control from Xiamen University in 2001. He attended the Master’s program in State University of New York at Buffalo in 2001 and obtained the Master of Science degree in mechanical engineering in 2003. He joined the PhD program in mechanical engineering at University of Missouri-Columbia in 2004 and transferred to the department of electrical and computer engineering in 2005. His PhD research focuses on computational modeling of the fear circuit and has published in Psychiatric Annals and Journal of Neurophysiology. Mr. Li is a student member of American Society of Mechanical Engineers (ASME) and Society for Neuroscience (SFN). He is expected to receive his PhD degree in electrical engineering in December 2009 and will join the Cornell University for a post-doc position after graduation.