Chapter 6

Auditory Fear Circuits in the Amygdala – Insights from Computational Models

Satish S. Nair

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/47814

1. Introduction

The human brain has 100 billion neurons that are constantly humming with electrical and chemical activity. These individual neurons are networked into complex local and inter-region circuits that are thought to implement functions that support life. One such circuit that is critical for survival is the fear circuit, the key elements of which are thought to include the amygdala, prefrontal cortex, and the hippocampus. Amygdala is an important site of plasticity in auditory fear conditioning and plays a key role in both the acquisition and storage of fear and extinction memories (Blair et al., 2001; LeDoux, 2000; Malkani and Rosen, 2000; Maren, 2001). The role of the amygdala in fear has been studied using fear conditioning, a training paradigm in which an organism learns to predict aversive events. Typically, a relatively neutral stimulus (conditioning stimulus, CS), such as a tone, light or an odor, is paired with an aversive one (unconditioned stimulus, US), such as a footshock. After only a few pairings, the previously neutral stimulus becomes aversive and can itself evoke an emotional reaction typically resulting in a freezing behavior. The learning processes underlying conditioning develop rapidly and the memory of this association persists for long periods of time, reflecting the biological significance of the learning experience for the organism. Even though there is consensus that the amygdala is a critical component of the mammalian fear circuit, the relevant interconnections among the amygdalar nuclei and their contributions to the acquisition and storage of fear and extinction memories are not well understood presently.

Disruption of the fear circuit is thought to underlie the pathology of post-traumatic stress and of other anxiety disorders (Corcoran and Quirk, 2007). Such disruptions are also manifested as changes in excitability of individual neurons, as well as changes in synaptic strengths between neurons in specific sub-circuits, within these areas. 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 such changes. Progress in the areas of cellular neurophysiology and synaptic plasticity permit the development of biologically realistic computational models that more closely approximate learning, both at the membrane and network levels. *Such biologically realistic computational models have the potential to enhance our understanding of brain circuits with potential applicability to a range of phenomena from the neural basis of mental illness to the mechanism of action of drugs.*

What is a computational model? A computational model combines different types of information related to a system using mathematical equations, and then describes the system's response to prescribed inputs. In neuroscience, such computational models are typically of two types: (i) phenomenological models using connectionist (e.g., artificial neural network) and statistical schemes, and (ii) biophysical models which attempt to model the underlying biological mechanisms directly. Biophysical models are typically either at the intracellular level (e.g., gene interactions, pathways), cellular level (e.g., cell firing patterns, effect of blockers/drugs on channel conductances, etc.), or network/systems level (e.g., interconnected neurons in the fear circuit, the subject of this chapter), or may include a combination of several of these levels.

Computational modeling is a tool that has been effectively used in a variety of disciplines to integrate information related to different aspects of a problem, and to provide testable predictions. For instance, computational modeling is presently an indispensable part of the design of airplanes, e.g., Boeing 777 was claimed as being the 'first entirely computer-designed commercial aircraft' (Boeing 777, 2012). For an airplane, such a model would integrate the complex mathematical equations for air flow, engine dynamics, frame vibrations, and responses of the control surfaces, and then predict their effect on outputs 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 levels , something that can be facilitated by computational models (Koch and Segev, 2011). For instance, recent technical advances have resulted in a rapid accumulation of information on intracellular signaling pathways and their relationships to long-term neuronal changes (Byrne and Roberts, 2004). 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 (Koch and Segev, 2001; Mauk, 2000). 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 (Aradi and Erdi, 2006).

In this chapter, we review the preliminary insights related to the amygdalar fear circuit provided by biologically realistic computational models. Specifically, we investigate how sensory information might be associated within the amygdala, and how the various amygdalar nuclei interact to *acquire and store* both fear and extinction memories via long

term potentiation and depression of synapses. The 'higher' level structures such as the prefrontal cortex and the hippocampus are known to influence the amygdala to *modulate* such memories. However, not much is known about the underlying mechanisms presently and so this modulatory effect is discussed only briefly. This is followed by a discussion of the unique insights that computational models might add to what is already known about the fear circuit, and about the potential of such models to contribute to reverse engineering the mammalian fear circuit. This ability of computational models derives from the fact that they can 'integrate' different types of information into a self-consistent and coherent portrait of how fear might be learned, and, in the process, reveal presently unknown mechanisms and interactions associated with such learning.

2. Auditory fear and the amygdala

Fear is one of the few emotions that can be observed in non-primate mammalian organisms (LeDoux, 2000). After fear conditioning, a number of physiological manifestations can be observed upon re-exposure to the conditioning stimulus, including increased autonomic arousal, increased stress hormone release, reflex potentiation, and defensive behaviors (LeDoux, 2000). Extensive studies indicate that freezing is a defensive behavior and serves as a reliable index of fear in rodents (Blanchard and Blanchard, 1972).

The mammalian circuit related to auditory fear and extinction has been studied by several researchers and, although not fully understood, consensus is emerging about the specific roles of the amygdalar nuclei in this circuit. In rodents, such studies typically use fear conditioning, which is a form of Pavlovian learning where the stimulus parameters can be regulated by the experimenter. Fear conditioning is a highly conserved form of behavior that is exhibited in both laboratory situations and in normal environments (LeDoux, 1994). Animals do not need to be food- or water-deprived to demonstrate fear conditioning.

Fear conditioning protocol. A typical auditory fear conditioning session for a rodent (see fig 1) in a cage starts with a habituation phase involving the presentation of several CS tones (e.g., 5 trials, with each trial consisting of a 2 sec 5 kHz pure tone (80 dB) + a varying 1-3 min inter-tone interval), followed by a conditioning phase where the tone is paired with shock US (e.g., 5 trials, where the tone co-terminates with a 0.5 mA, 0.5 s, foot shock). Testing is typically performed the next day using 2 CS tones. For studies that involve extinction, the paradigm continues to day 3 where pure tones are delivered during an extinction session (e.g., 30 trials with pure tones). Testing for extinction in that case takes place on day 4 (e.g., 2 trials with pure tones). Freezing, defined as the total lack of movement except for respiration, is used as the measure of learning in this task (Blanchard and Blanchard, 1972). After conditioning, a robust and long-lasting behavioral change is produced which is particularly amenable to genetic and pharmacological studies. It also affords the advantage that detailed time courses of the sequence of events that may occur after conditioning can be easily generated. Thus, fear conditioning serves as a model for elucidating the crucial electrophysiological and biochemical mechanisms underlying learning (Wehner and Radcliffe, 2004).

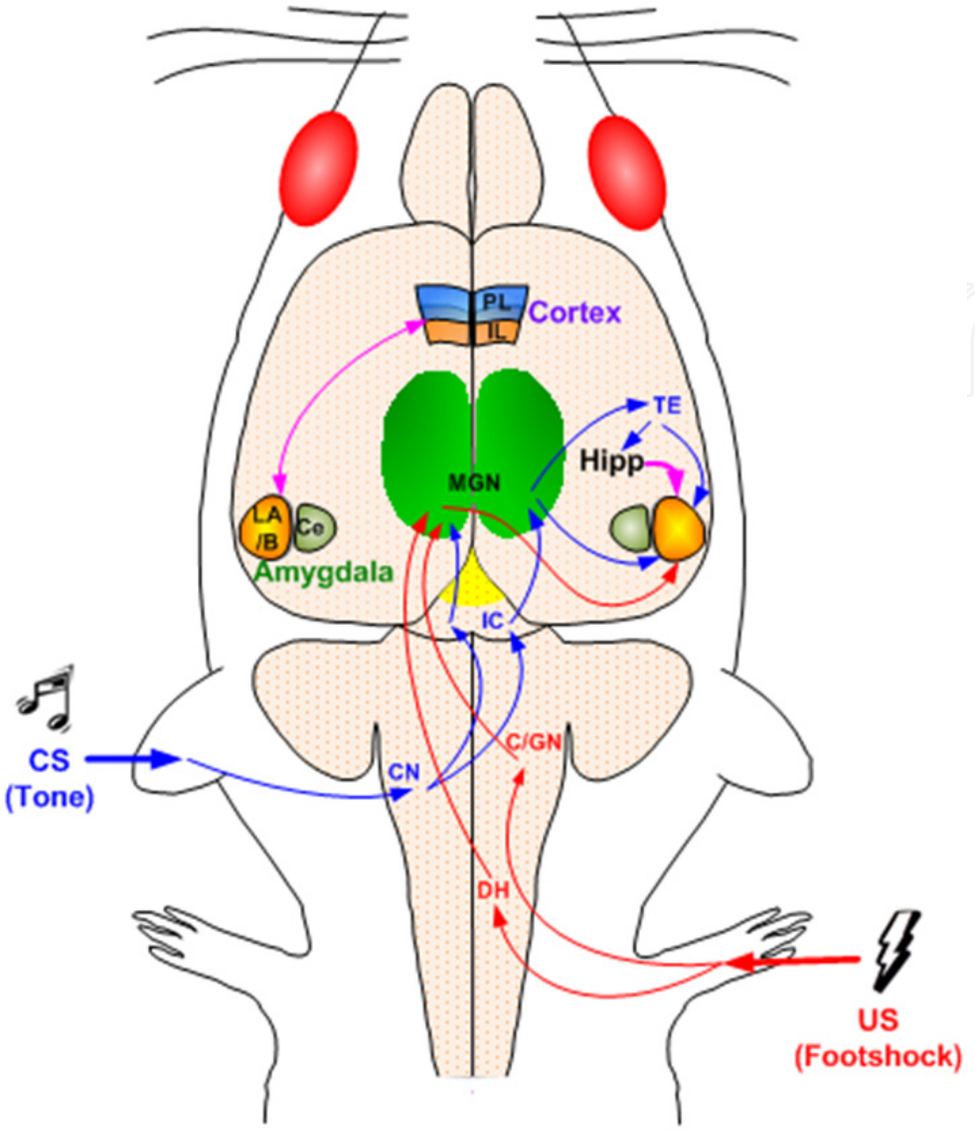

Figure 1. Pathways involved in auditory fear conditioning. The tone information is delivered to LA via the medial division of medial geniculate body (MGN) and the shock information is delivered to LA via posterior intralaminar nucleus (PIN). The tone input to LA is potentiated when tone and shock are paired. Output from the LA projects to the central nucleus (Ce) through inter-calated cells (ITC, not show in the figure) and BA neurons, eliciting a fear response. LA, lateral nucleus; BA, basal nucleus; CN, cochlear nucleus; DH, dorsal horn of spinal cord; IC, inferior colliculus; PL: prelimbic medial prefrontal cortex, IL: infralimbic medial prefrontal cortex. (adapted from figure provided by J. Kim)

Role of the amygdala. The amygdala is located within the medial temporal lobe and is recognized as being critical for Pavlovian fear learning. In their review article, Paré et al. (2004) note that identification of pathways that mediate the expression of conditioned responses by way of amygdala outputs and pathways that transmit CS information from sensory systems to the amygdala greatly increased interest in the intra-amygdaloid substrates of Pavlovian fear learning. Multiple experimental modalities including field potential response to high frequency stimulation, patch clamp recordings, single unit recordings, pharmacological manipulations and transgenic approaches all implicated the

amygdala in the acquisition of learned fear. These findings have also been confirmed in humans by functional magnetic resonance imaging techniques (e.g., Buchel et al., 1998).

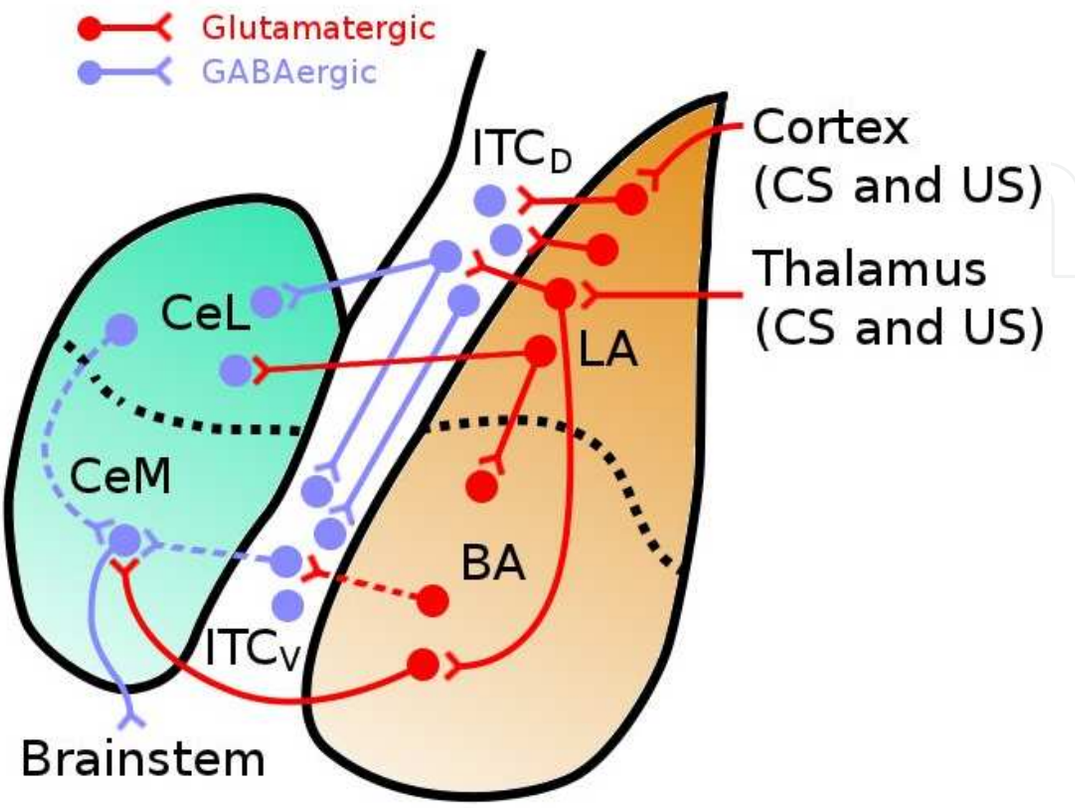

Figure 2. Amygdalar pathways relevant to auditory fear. Tone and shock information arrive at LA via thalamic and cortical routes. LA projects to BA and also to ITC$_D$ and Ce$_L$. Based on our present understanding (Amano et al., 2011), LA projects to BA, ITC$_D$ and CeL. BA fear neurons project to Ce$_M$ and BA extinction neurons project to ITC$_V$ (fear recall circuit in bold and extinction recall in dashed line type). Ce represents the amygdalar output which projects to the brainstem and other regions eliciting fear. ITC: inter-calated cells (subscripts D - dorsal, V-ventral); Ce$_L$/Ce$_M$: lateral/medial part of the central nucleus of the amygdala.

The components of the amygdala that are critical for fear conditioning are the lateral nucleus (LA), the basal nucleus (BA), intercalated cells (ITC) and the central nucleus (Ce) (Maren, 2001). Thalamic inputs conveying information about the auditory tone (conditioned stimulus, CS) and foot shock (unconditioned stimulus, US) arrive first at the lateral nucleus. LA is widely accepted to be a key site of synaptic events that contribute to fear learning (LeDoux, 1995; Paré et al., 2004; Sigurdsson et al., 2007). There are two main types of neurons within the LA and the BA: pyramidal glutamatergic projection neurons and local circuit γ-aminobutyric acid (GABA) releasing interneurons. The amygdalar nuclei LA, BA, Ce and the ITC clusters act in concert to store auditory fear and extinction memories, and these nuclei are in turn modulated by external structures such as the prefrontal cortex and hippocampus. 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 subsequent LA tone responses being larger (Quirk et al., 1995; Blair et al.,

2001). These increased LA responses are relayed to the Ce via BA (Amano et al., 2011), 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 CS that predict foot shock US.

In the rodent brain, estimates of the numbers of cells (unilateral) in the amygdalar nuclei are as follows: LA – 60,000; BA – 47,000; ITC – 19,000; and Ce – 37,000 (Tuunanen and Pitkanen, 2000). The principal cell to GABAergic interneuron ratio in BLA is 80:20. The amygdalar nuclei are themselves not homogeneous. LA has distinct dorsal and ventral regions which seem to store fear memories in different ways (Repa et al., 2001). Herry et al. (2008) reported three subpopulations of neurons in BA whose CS responsiveness varied with fear training and they termed these as 'fear,' 'extinction' and 'extinction-resistant' cells. Fear cells acquire CS responses as a result of fear conditioning, but lose them following extinction training; extinction cells become CS responsive only following extinction training, and extinction-resistant cells acquire CS responses during conditioning and remain CS responsive even after extinction training. Also, Amano et al. (2011) have shown that the two sub-regions within BA, the lateral part (BL), and the medial part (BM), act in concert to express fear but possess a certain amount of redundancy between themselves. Similarly, there are two different ITC cell clusters and they are thought to contribute differentially to the expression of fear and extinction memories (Royer et al., 2000; Pape and Paré, 2010). The output nucleus Ce also has distinct sub-circuits with different functions in fear learning (Coicci et al., 2010; Haubensak et al., 2010). Even with these advances in understanding, a clear portrait of how the various amygdalar nuclei interact to acquire and store fear is still lacking.

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 as fear extinction (Myers and Davis, 2007). 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 describe extinction as an "unlearning" process due to a violation of the CS-US association established during acquisition of fear (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).

Modulation by cortical structures. Fear is thought to be expressed via projections from LA to BA, ITC and Ce (see fig 2), and expression of this fear memory has been shown to be influenced by cortical structures. For instance, although LA responds transiently to conditioned tones, the animal continues to freeze throughout the period of a 30 second tone. Quirk and colleagues investigated whether the prelimbic (PL) region of the medial prefrontal cortex (mPFC) might be involved in sustaining freezing. In a series of experiments they showed how PL was critical for the expression of fear over the duration of the tone: Pharmacological inactivation of PL was found to abolish the expression of conditioned fear (Blum et al., 2006; Corcoran and Quirk, 2007), and micro-stimulation of PL

was found to augment conditioned fear (Vidal-Gonzalez et al., 2006); and, importantly, that the time course of PL tone responses parallels the time course of conditioned fear (Burgos-Robles et al., 2009). This finding is supported by studies examining neuronal activity with cFos which showed that PL activation is correlated with fear expression and extinction failure.

What are the structures that might modulate the memory of auditory fear extinction? Again, several studies by Quirk and others reveal that the infralimbic (IL) region of mPFC modulates the amygdala during recall of extinction memory: activity in IL, which is adjacent to PL, was found to facilitate recall of extinction (Quirk et al., 2006; Quirk and Mueller, 2008), and deficient IL activity results in failure to recall extinction (e.g., Milad and Quirk, 2002). Burgos-Robles et al. (2009) also noted that a higher percentage of PL neurons responded to tones in rats showing poor recall of extinction, suggesting that these rats had excessive consolidation of fear memory. This led the authors to suggest that extinction failure might be caused by excessive activity in PL, combined with deficient activity in the IL, and that recall of fear and extinction memories may depend on the optimal balance of activity between PL and IL.

What is the role of context in auditory fear? Since fear conditioning takes place in a chamber (Fig. 1b; with its own flooring, color, odor, lighting, etc. – the 'context'), the rat subsequently learns to fear not only the tone but also the context. That is, after fear conditioning, it will express fear by freezing in the trained context, even in the absence of tone. Acquisition of contextual fear may involve configural or spatial learning and many lines of evidence support hippocampal involvement in contextual fear conditioning (Anagnostaras et al., 1999). It is well established that contextual information gates behavioral response to conditioned stimuli, especially following extinction (e.g., Bouton, 2004). Contextual information is processed in the hippocampal formation (HPC), which plays a critical role in gating the response of rats to extinguished tone stimuli (Corcoran et al., 2005). The route by which the HPC exerts its effects is thought to be through the mPFC (Hobin et al., 2003; Maren and Quirk, 2004). The HPC (especially the ventral HPC) projects strongly to both PL and IL (e.g., Hoover and Vertes, 2007). This pathway has been hypothesized to serve a 'teaching' role for IL neurons, by generating Ca-dependent bursting in IL neurons. Also, it has been shown that contextual fear memories formed in the absence of the baso-lateral amygdala (BLA which includes BA and LA; Poulos et al., 2009) or the dorsal hippocampus (DH; Zelikowsky et al., 2012) do not persist across time, suggesting that both the DH and BLA are essential components of the circuitry required for a contextual fear memory to become permanent (Zelikowsky et al., 2012).

3. Modeling fear memories - A simple computational model

Computational models have been used in the field of emotional learning and memory to explain behavioral responses (e.g., Grossberg and Schmajuk, 1987). Single unit recording data were used by Armony et al. (1995) to develop an anatomically constrained thalamo-cortico-amygdala connectionist model of fear conditioning which associated tone inputs

with a specific frequency (CS) with foot shock (US). The model was trained using a modified Hebbian-type learning rule and was able to reproduce data related to frequency-specific changes of the receptive fields known to exist in the auditory thalamus and amygdala. However, extinction and other related phenomena were not simulated. Balkenius and Morén (2001) proposed a neural network model for emotional conditioning focusing on the amygdala and the orbitofrontal cortex and their interaction. 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. Vlachos et al. (2011) reported a neural network model that reproduced the differential recruitment of two distinct subpopulations of basal amygdala neurons as seen in experiments. The model revealed how the two populations might encode contextual specificity of fear and extinction memories. Krasne et al. (2011) report a model of the amygdala and hippocampus where fear conditioning and extinction memories are the result of neuromodulation-controlled LTP at synapses of thalamic, cortical, and hippocampal afferents on principal cells and inhibitory interneurons of lateral and basal amygdala. The model was developed using a firing rate framework and was able to reproduce several known features of fear learning and make testable predictions. Although connectionist and reduced order models provide very useful information from a top-down systems perspective, they do not fully incorporate the neurobiological information related to individual current channels and their effect on intrinsic excitability, or related to synaptic plasticity mechanisms, and so may be not be able to shed light on the underlying mechanisms to any significant level of detail.

This chapter focuses on a class of computational models that incorporate biological realism (i.e., they include membrane channels, synapses and receptors) to more effectively model the learning brain. Such models integrate information from 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). 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. We focus largely on cellular and network level modeling in this chapter.

Computational modeling platforms at the cellular and network levels include public domain software such as NEURON (Carnevale and Hines, 2006) and GENESIS (Bower and Beeman, 2003) which are being designed for biologists, and require minimal understanding of the underlying mathematics. Figure 3 shows the hierarchical structure used for modeling. Such packages can perform simulations of models ranging from single neurons to complex networks representing brain circuits. Sources for biological information to develop such models include research articles, and databases such as CellPropDB, NeuronDB and ModelDB (http://neuron.duke.edu/). For example, Leblois et al. (2006) used a mathematical model to explain the pathology in the basal ganglia circuit with Parkinson's disease.

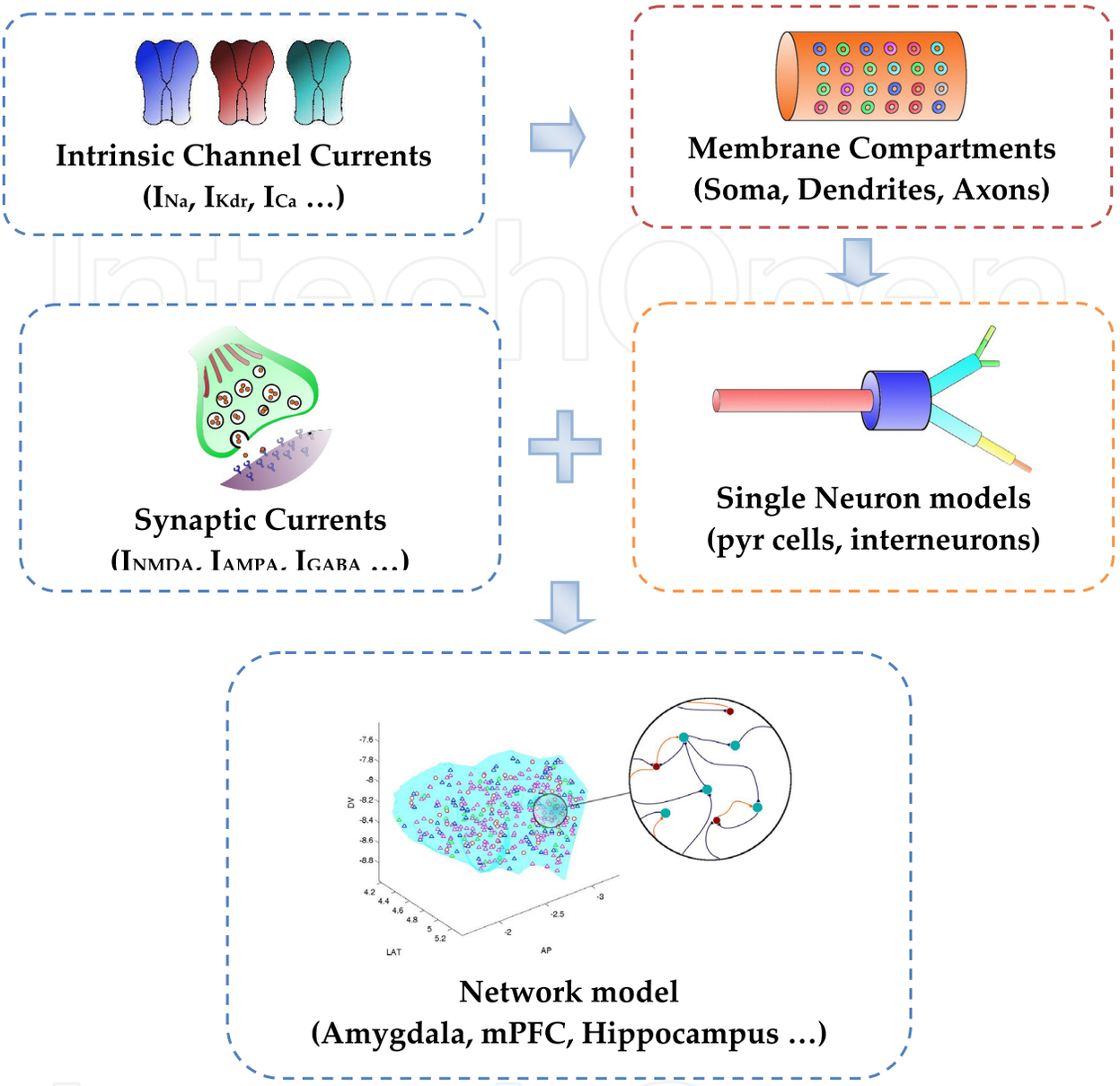

Figure 3. Elements of a biologically realistic neuronal network. The symbol 'I' represents current, e.g., I_{Na} is the sodium current. The network comprises cells (e.g., pyramidal and interneurons), which in turn consist of soma and dendrites populated with the various current channels.

Illustrative example case. Single cell models can be developed for the pyramidal cell and the interneuron using the software cited. The modeling process involves several steps where the software requires the user to define 'LEGO' blocks for the neuron, such as the soma or cell body, dendrite and axon, 'insert' into them specific suites of membrane channels, and then connect them to form networks (fig 4). Such software aim to provide an easy-to-use interface, making most of the mathematical details transparent to the user. The model parameters within all these blocks are then iteratively adjusted, within biophysical bounds, to match biological data such as resting potential, input resistance, and membrane potential responses to various current injections. After reliable single cell models are developed, they can be embedded into network models of regions and circuits.

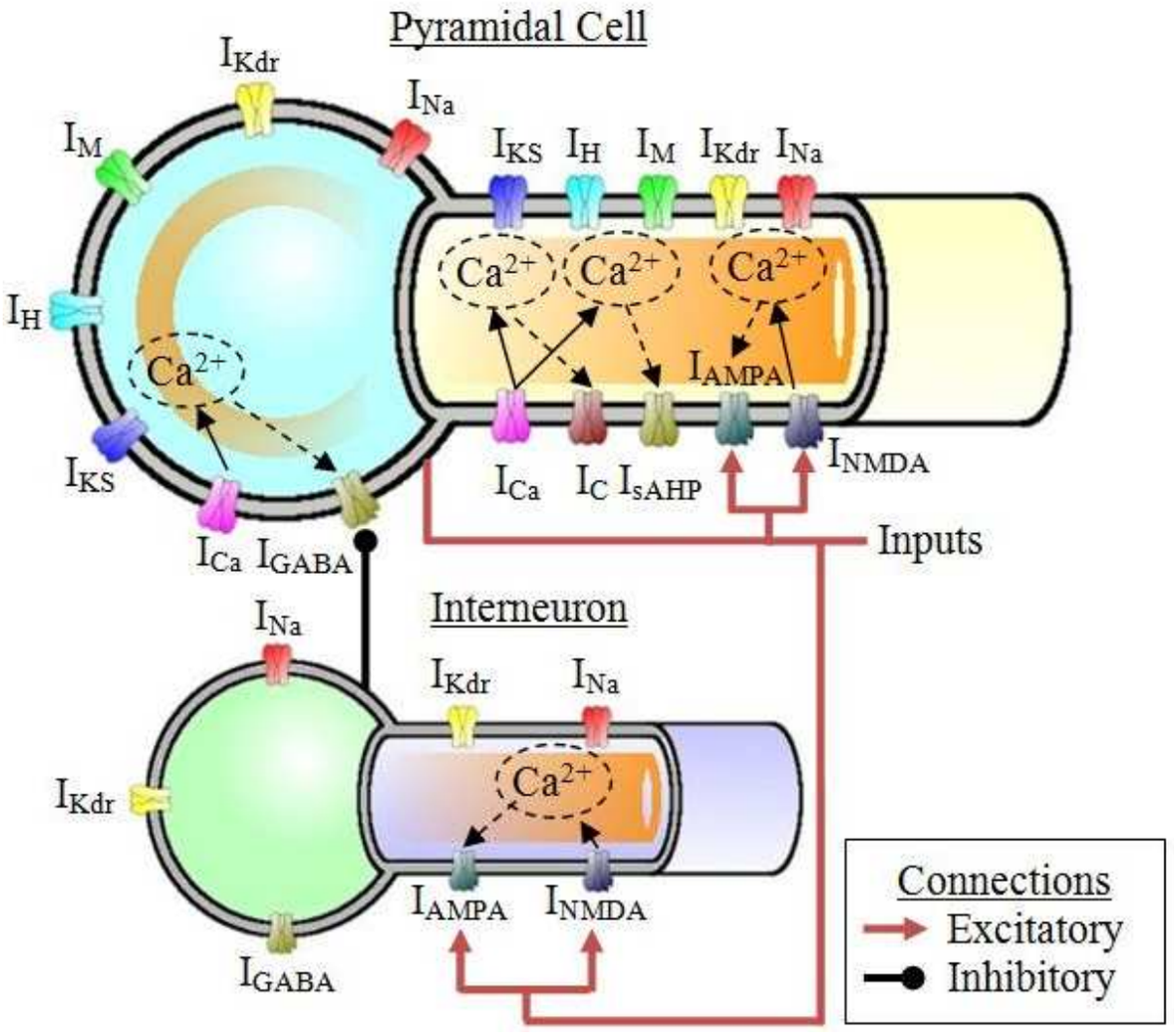

Figure 4. Two-cell model of pyramidal cell and interneuron with ionic and synaptic channels. Each cell model has soma (spherical) and dendrite (cylindrical) compartments with each having the specific current channels shown. The Ca^{2+} pools involved in the learning algorithm implemented are also depicted. Both cells receive afferent inputs (tone CS and shock US) 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.

Two-cell network. Figure 4 illustrates the development of a two-cell network model showing how 'memories' can be stored in the synapses (Li et al., 2008). The first step is to develop single cell models using experimental data. In this example case, we use a lateral amygdala pyramidal cell model and a lateral amygdala interneuron model. As cited, the single cell properties should match those reported in biology, including the membrane potential responses to various current injections. Once such single cell models are developed (see Li et al., 2009), they can be embedded into networks. In our simple two-cell network model, both the pyramidal cell and the interneuron received direct afferent tone/shock (CS/US) inputs via synaptic connections. The pyramidal cell was inhibited by the interneuron via a GABA-ergic synapse. The pyramidal cell, on the other hand, excited the interneuron via an excitatory AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) synapse.

Both 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 seen in LA neurons. We implemented plasticity in all the synapses using a calcium-based learning rule, and then 'trained' the model with the fear conditioning protocol used in experiments (Quirk et al., 1995). Figure 5a 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 occurring during the last 100 ms of the second tone. Both tone and shock excited the cells, with the shock input having a stronger effect. 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 the 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 inhibition. This is illustrated

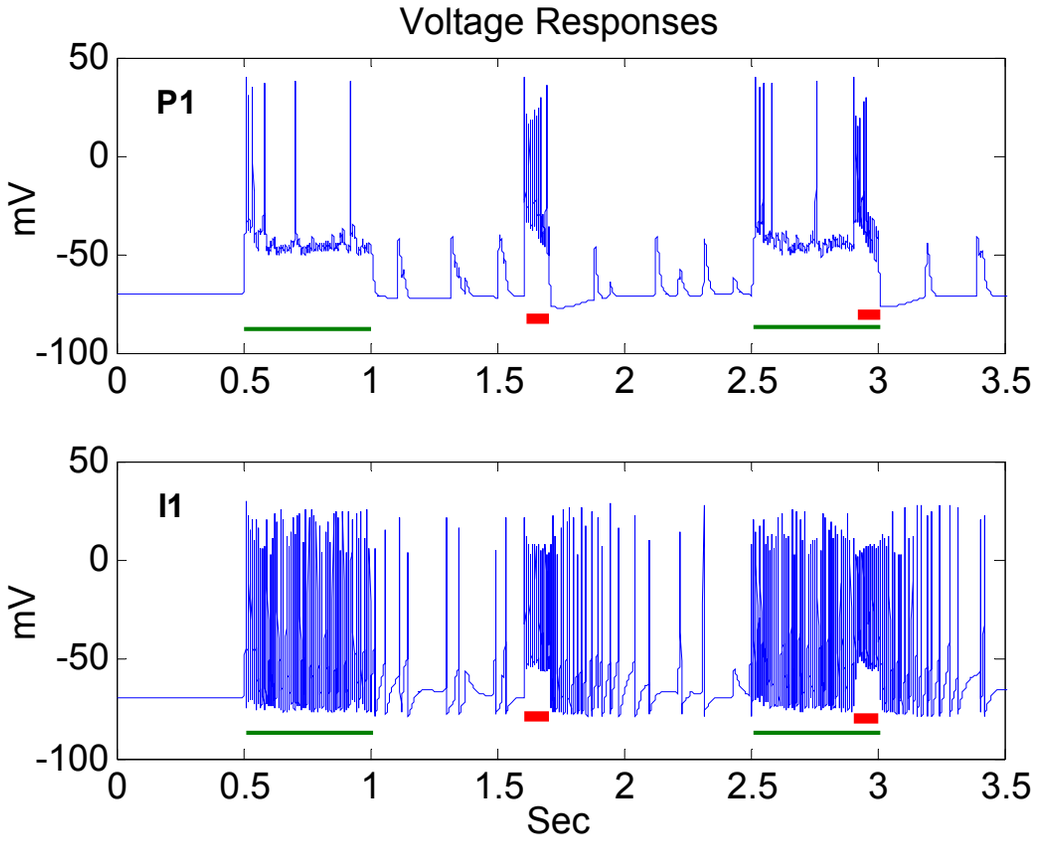

Figure 5. Response characteristics of the illustrative network model. 5a. Membrane potential responses for pyramidal cell (top panel) and interneuron (lower panel) to a segment of the training trial. In the segment the input consists of a series of two tones (green bars) and two shocks (red bars) with the second tone paired with the second shock. 5b. 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.

schematically in Fig. 5b. This unit of two cells illustrates how conditioning and extinction are learned in this network, i.e., conditioning is essentially the strengthening of the tone-pyramidal synapse which increases pyramidal cell activity, and extinction is the strengthening of tone-interneuron, interneuron-pyramidal cell and pyramidal cell-interneuron synapses, all of which decrease pyramidal cell activity. The concepts and insights illustrated by this simple two-cell network, such as potential storage sites for memory, translate directly to larger networks, as we discuss in the following.

4. Reverse engineering auditory fear circuits in the amygdala

The overall goal of the reverse engineering effort is to integrate diverse morphological and neurophysiological data into biologically realistic models of the various amygdalar nuclei (lateral nucleus, basal nucleus, intercalated cells, and the central nucleus) and then use the model to investigate how the different nuclei participate in the acquisition and extinction of auditory fear memories, and how they are modulated by cortical structures. We initiated the model development of the overall fear circuit using a bottom-up approach starting with the core unit: the lateral amygdala nucleus, LA. As the next step, we modeled the amygdala intercalated cell clusters, ITC. The LA and ITC models provided unique insights that are presently not possible to obtain via experiments.

A. Modeling the lateral amygdala (Li et al., 2009)

Motivation. LA is a key site of plasticity in auditory fear learning (Blair et al., 2001; LeDoux, 2000; Malkani and Rosen, 2000; Maren, 2001). Given the central role of LA in the acquisition and expression of fear memory, it has been proposed that this structure may be a site of inhibition in extinction (e.g., Hobin et al., 2003). The motivation of the Li et al. (2009) study was to determine how LA might acquire and store both conditioning and extinction memories related to auditory fear. After a review of the development of the model, and its validation, we discuss the unique insights provided by the model.

Single cell properties. There are two types of principal neurons within the LA: pyramidal-like glutamatergic projection neurons, and local circuit GABAergic interneurons (Faber and Sah, 2001). The electrophysiological and morphological properties of LA neurons have been characterized in a number of studies (e.g., Faber et al., 2001). Also, there are several in vitro and in vivo recordings of LA neurons during fear conditioning and extinction (e.g., Quirk et al., 1995, 1997; Repa et al., 2001).

Principal neurons in the LA exhibit a range of firing properties in response to prolonged current injection (Faber et al., 2001). Accordingly three types of pyramidal cells were modeled, types A, B, and C, where type A had strong, B had medium, and C had minimal 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 its much shorter spike duration (Durstweitz et al., 2000). 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 AMPA and the N-methyl D-aspartate (NMDA) channels were placed in the dendrite compartment, and the inhibitory $GABA_A$ channels were placed on the soma. Fig.4 provides details of typical pyramidal cell and interneuron models with the various ionic and synaptic channels.

Network model and synaptic connections. The LA network model consisted of eight pyramidal cells and two GABAergic interneurons (fig. 6) with full 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 dorsal sensory-receptive region of LA (LAd). Three of the pyramidal cells (P5, P7, and P8) and both the interneurons received direct tone/shock inputs; P3 received only tone input, and P1 and P4 received only shock input; and P2 and P6 received no direct afferent inputs. In this fully connected architecture, each pyramidal neuron received excitatory inputs from all other pyramidal cells as well as inhibitory inputs from the two interneurons. Both interneurons received excitatory inputs from all pyramidal cells and thus provided feedforward and feedback inhibition to pyramidal cells. Also the two interneurons inhibited each other. 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.

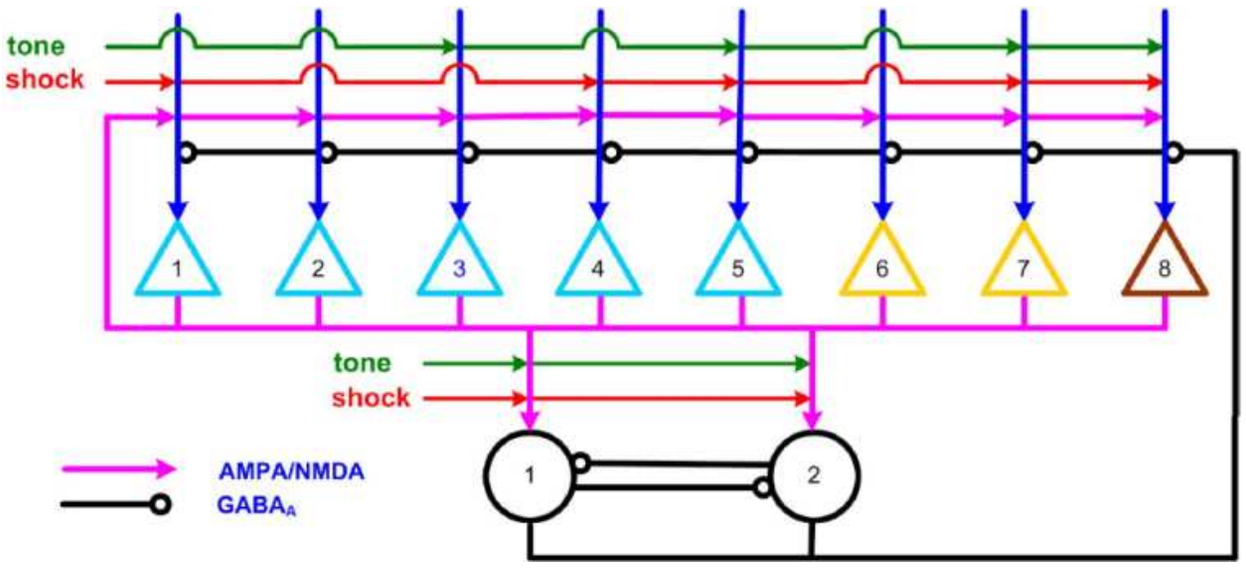

Figure 6. Biologically realistic LA network model of Li et al. (2009). Triangles 18 are pyramidal cells (5 of type A (1-5), 2 of type B (6-7), and 1 of type C (8)); circles are interneurons (1-2). Pyramidal cells excited all other pyramidal cells and interneurons, but not themselves. Interneurons inhibited one another and every pyramidal cell. Pyramidal cells 3, 5, 7, and 8, received direct tone, while pyramidal cells 1, 4, 5, 7, and 8 received direct shock input. Both interneurons received direct tone and shock inputs.

Excitatory glutamatergic AMPA synapses capable of strengthening (long term potentiation, LTP) or weakening (long term depression, LTD) with training were placed on the following

synapses of each cell: (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, plasticity was also modeled in GABAergic inhibitory synapses from interneurons to pyramidal cells. The occurrence of synaptic potentiation versus depression was determined by intracellular calcium levels, according to the calcium control hypothesis. Details related to the equations can be found in Li et al. (2009). Learning of conditioned fear leads to changes in synaptic strength in the neural circuitry and the magnitude and sign of these variations are unique insights that a computational model can provide.

Training protocol and background inputs. The schedule of tone and shock inputs to the model was based on 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, a conditioning phase, and two extinction phases. Each tone lasted 500 ms and each shock lasted 100 ms, and the interval between two tones was 3.5 s. 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 s and the model was tested for spontaneous recovery after a delay of 840 s. The second extinction phase also used 30 pure tones. The entire schedule lasted 1,200 s. 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.

To achieve the low average spontaneous firing rate of ~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 the reported spontaneous firing rates of ~8 Hz (Paré and Gaudreau, 1996). Simulations were performed on a personal computer using the software package GENESIS with the Crank-Nicholson integration method, and a time step of 10 μs.

Model validation. In addition to matching unit responses in the model to unit experimental data, the model of the 'network' should also reproduce experimentally observed behavior. Unit tone responses from the lateral amygdala of behaving animals have been reported by Quirk et al. (1995). Their main finding 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. With tuning of the

plasticity parameters in the model, the LA network model unit tone responses successfully reproduced experimental data in Quirk et al. (1995). All pyramidal neurons in the model

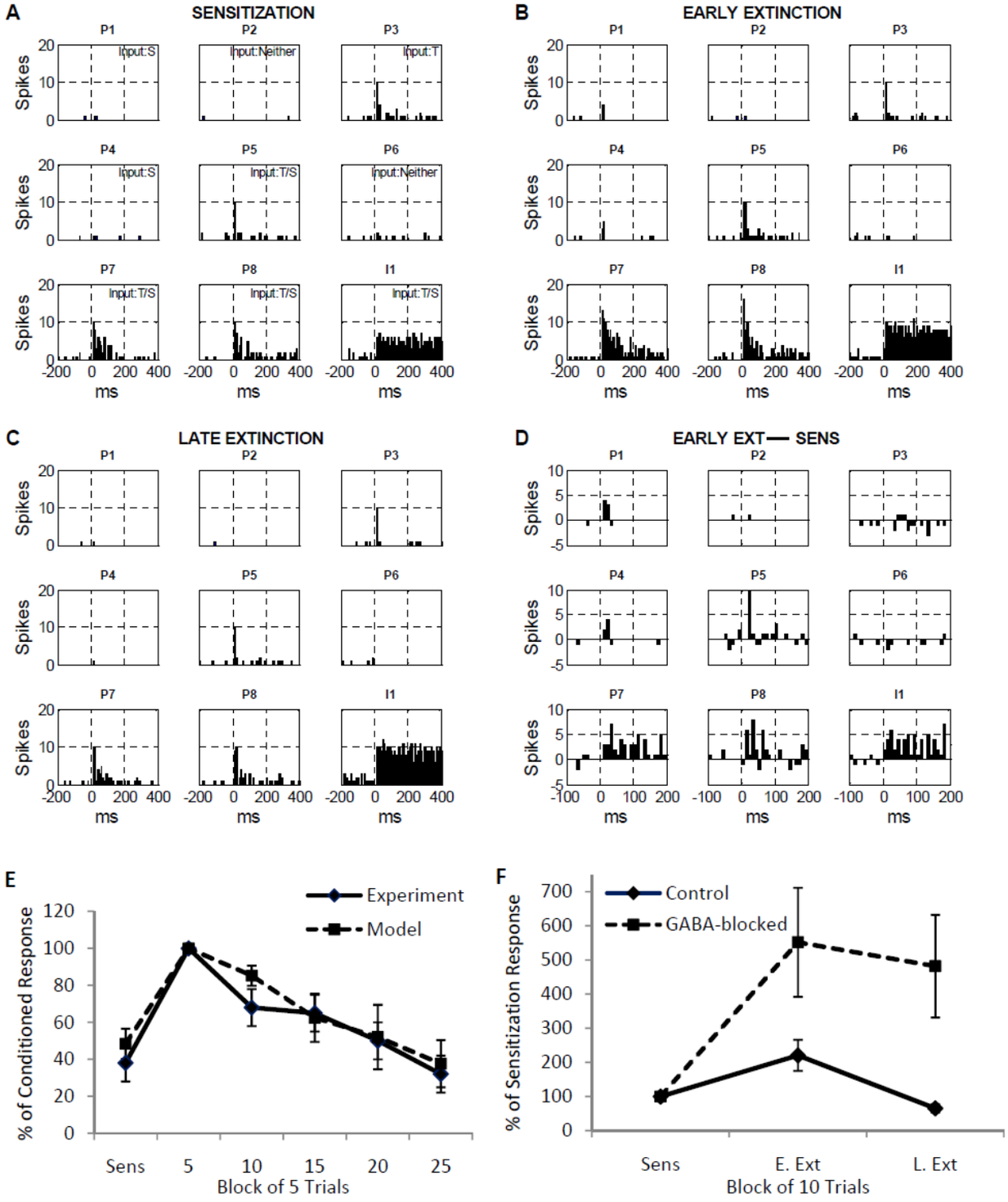

Figure 7. Comparison of the experimental data (Fig. 4, Quirk et al. 1997) and the model tone responses for the last block of 5 trials in sensitization and successive 5-trial blocks during extinction. The total spikes (0–50 ms) in each block of 5 trials were normalized to the responses in the 1st block of extinction for each cell and the mean ratio (with SE) among 4 significant conditioned pyramidal cells calculated.

showed clear frequency adaptation with the tone responses concentrated in the first 100 ms, indicating a good match with the experimental recordings. Figure 7 shows the match between experimental and model conditioned tone responses for the last block of five trials in sensitization and successive five-trial blocks during extinction. The large potentiation of the excitatory inputs onto pyramidal cells caused by conditioning, together with the potentiation of connections between several pyramidal cells leads to elevated tone responses in early extinction. As extinction progresses, increasing feedforward and feedback inhibition from the interneurons, combined with depotentiation at excitatory synapses onto pyramidal cells bring tone responses back to pre-conditioning levels or even lower. This provides an important validation for the network model. Once validated, a model can be used to provide insights into the underlying mechanisms, as described next.

Insights provided by the LA model

The Li et al. (2009) network model represents the first attempt to incorporate cellular neurophysiology and synaptic plasticity mechanisms into a biophysical model to investigate the underlying mechanisms of fear learning. The model was used to determine how the intrinsic and synaptic mechanisms interact in a network to shape unit tone responses. Computational models are unique in their ability to contribute to such insights.

How can LA can store both fear and extinction memories? After fear conditioning, the model was able to 'learn' both fear and extinction memories. In the process, the model predicted an important role for inhibition via interneurons. The model identified two possible sites for fear memory storage in LA: the tone synapses from the auditory thalamus (or cortex) onto the pyramidal cells and the synapses between pyramidal cells. Hebbian pairing indicates that 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 decreases when tone and shock are unpaired. This leads to the prediction that tone synapses only store specific tone-shock associations, while the pyr-pyr synapses are capable of storing a generalized fear memory related to the occurrence of shock, e.g., related to contextual fear conditioning. The model also showed that the pyr-pyr synapses decayed less on average in extinction compared to the tone-pyr synapses. This was because of frequency adaptation of pyramidal cells resulting in lower incidence of Hebbian weakening. Taken together, these findings suggest that the pyr-pyr synapses are well suited to encode long-term fear memory in LA neurons.

The model also suggested that the different types of principal neurons have different functional roles due to their distinct frequency adaptation characteristics (Faber et al., 2001). 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 (Fig. 5, *A* and *B* in Li et al., 2009). This suggests that pyramidal cells with weaker adaptation are important for fear expression, whereas those with stronger adaptation are important for long-term storage of fear associations. Since about 70% of pyramidal cells in LA are strongly adapting, LA is well suited for long-term fear memory storage.

Considering extinction memory, the model suggests 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. Model runs showed different decay rates of these three synapses suggesting that the first two, with large and uniform decay rates during the gap, may mediate short-term extinction memory, while the last, with smaller decay rate, could store long-term extinction memory (e.g., P1-I1 in Fig. 5*C* in Li et al., 2009). However, the tone-interneuron and inter-pyramidal cell synapses potentiated much larger during both extinction sessions, compared to the pyramidal-interneuron synapses.

How does the low spontaneous firing rate of pyramidal cells affect memory storage? LA cells signal fear and so it is logical that they have low spontaneous firing rates of around 1 Hz. But then, what are the implications of this low firing rate for learning? Experiments with the model revealed that the low rate of spontaneous firing in LA may act to preserve the fear memory due to decreased incidence of Hebbian weakening. The high spontaneous rates of interneurons, on the other hand, leads to a comparatively faster weakening of extinction memory stored in the interneurons synapses.

Does 'unlearning' of fear also occur with extinction? It is known that extinction involves the formation of a new and distinct memory. However, what is not clear is whether a part of the fear memory is also lost during extinction trials. Such fear memory would be stored in the tone-pyramidal synapses. Blocking NMDA receptors in experiments would prevent depotentiation of the excitatory synapses onto pyramidal cells (LTD) but at the same time it would also block potentiation of inhibitory connections. So, experiments cannot answer this question presently. A model, however, can implement selective blockade of LTD only at the tone-pyramidal and pyramidal-pyramidal synapses by preventing Ca^{2+} influx via the NMDA channels, to separate the effects of both these phenomena. To evaluate the contribution of LTD, which is independent of potentiation of inhibition, a selective blockade of LTD showed that extinction was not complete, i.e., potentiation of inhibition alone is not sufficient for complete extinction.

The LA model can be used as a test bed to investigate several other 'what if' scenarios that may be of interest but are difficult to test in experiments. Our study showed how such models are poised to complement experimental investigations by providing insights into how cellular and synaptic mechanisms contribute to implementing functions in brains. This is illustrated further by the model of intercalated cells discussed next.

B. Modeling the network of amygdala intercalated cells (Li et al., 2011)

Motivation. The amygdala intercalated cells (ITC; fig 2) are distributed along the lateral and medial parts of the basolateral amygdaloid complex. The more dorsally located ITC clusters receive glutamatergic excitatory input from LA and BA, while the medial ITC clusters receive GABAergic inhibition from the dorsal ITC clusters and excitation from BA; the medial clusters, in turn, inhibit Ce, the output station in the amygdala (Paré and Smith, 1993a,b; Royer et al., 1999; Royer et al., 2000; Jungling et al., 2008). This strategic location of the medial ITC clusters, between the sensory input (BLA) and fear output (Ce) stations of

the amygdala, is thought to be critical for regulating classically conditioned fear responses (Paré et al., 2004).

It is currently believed (Paré et al., 2004; Quirk and Mueller, 2008) that extinguished conditioned stimuli activate infralimbic (IL) neurons that have glutamatergic projections to ITC cells and ITC cells in turn reduce conditioned fear responses by generating feedforward inhibition in fear output Ce neurons (Paré et al., 2004). Consistent with this, IL stimulation was found to dramatically reduce the responsiveness of Ce neurons to BLA inputs (Quirk et al., 2003). IL axons are known to target ITC cells clusters located medially (McDonald et al., 1996), and there are inhibitory connections between (Royer et al., 2000) as well as within ITC cell clusters (Geracitano et al., 2007). Additionally, three different types of short-term synaptic plasticity have been observed in inter-ITC connections (Geracitano et al., 2007), but the role of such synaptic heterogeneity is not clear. How then might IL inputs overcome the inter-ITC inhibition and reduce the responsiveness of Ce? Again, it is currently difficult to address this question experimentally, because we lack criteria to identify ITC cells on the basis of their extracellularly recorded activity. So, in order to study how inter-ITC inhibitory connections affect their responses to IL inputs, we developed a biologically realistic model of the ITC network (Fig. 8). Another objective of the Li et al. (2011) study was to examine how the peculiar electroresponsive properties of ITC cells shape their responsiveness to BLA/IL inputs. ITC cells express an unusual voltage-dependent K^+ conductance whose slow-deinactivation kinetics allow them to produce a prolonged depolarizing plateau after a transient suprathreshold depolarization (Royer et al., 2000). This enables ITC neurons to transform transient excitatory inputs into a prolonged state of increased excitability with possibly important consequences for the regulation of conditioned fear.

During prolonged auditory CSs, BLA principal neurons show rapidly adapting responses (Quirk et al., 1995, 1997; Repa et al., 2001; Herry et al., 2008), but it is not clear how such transient responses are converted into sustained behavioral output, since rats freeze throughout the duration of the tone. Also, pairing CSs with brief (300 msec) electrical IL stimulation reduces conditioned freezing in a temporally specific manner (Milad and Quirk, 2002; Milad et al., 2004), again sustaining this transient input. We used the model to test whether bistable electroresponsive properties of ITC cells allow them to transform transient BLA/IL signals into a more sustained output.

Single cell properties. Each ITC cell had two compartments representing a soma (diameter of 8 μm; length of 8 μm) and a dendrite (diameter of 5 μm; length of 200 μm). The values for the specific membrane resistance, membrane capacitance, and cytoplasmic (axial) resistivity were, respectively, R_m = 30 KΩ-cm^2, C_m = 1.0 μF/cm^2, and R_a =150 Ω-cm. The leakage reversal potential was set to -93 mV to match experimental measurements of their resting potential (–85 mV). The resulting input resistance was about 600MΩ when measured from rest, consistent with experimental observations. The ITC model contained several ionic currents including a leakage current I_L, a spike-generating sodium current I_{Na}, a potassium delayed rectifier current I_{DR}, a slow deinactivating current I_{SD}, a voltage-gated persistent muscarinic current I_M, a hyperpolarization-activated current I_H, a high-voltage activated Ca^{2+} current I_{CaL}, and a slow Ca^{2+}- dependent after-hyperpolarization current I_{sAHP}. As cited, the

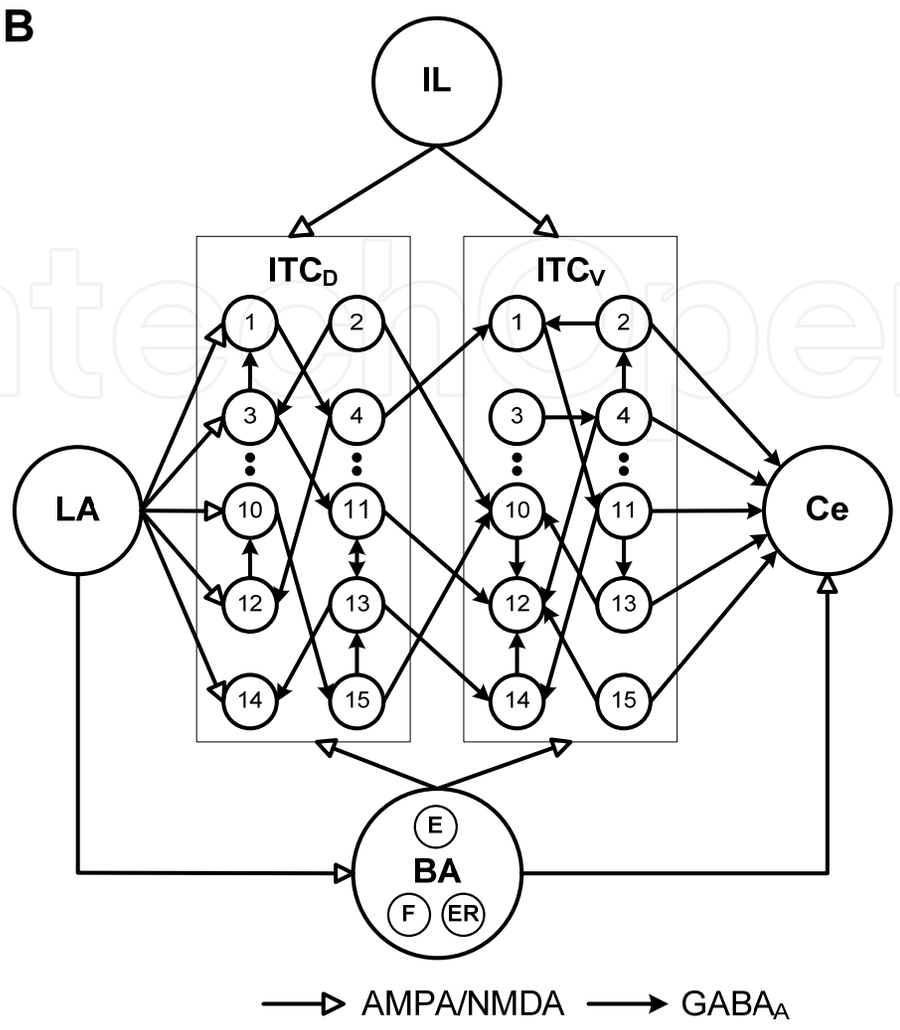

Figure 8. Structure of the model ITC network with 15 neurons each in ITC$_D$ and ITC$_V$ clusters (adapted from Li et al., 2011). For clarity and illustration purpose, the connectivity in the figure is partial and representative only. Each ITC neuron inhibits three randomly selected neurons in the same cluster. Each ITCD neuron also inhibits three randomly selected ITCV neurons (e.g., ITC$_{D2}$ inhibits ITC$_{V10}$). The network has five Ce output neurons that receive excitatory inputs from BA, and inhibitory inputs from ITC$_V$ neurons. ITC$_D$ and ITC$_V$: Neurons 1–5 facilitating output synapses; neurons 6 10 have depressing output synapses, and neurons 11–15 have constant synapses.

membrane potential and current dynamics were modeled using the standard Hodgkin-Huxley formulation (Li et al., 2011).

We modeled three different types of Ce neurons differing by their spike patterns, regular spiking, late firing, and low-threshold bursting. Each cell model had two compartments: a soma (diameter of 15 μm; length of 15 μm) and a dendrite (diameter of 5 μm; length of 300 μm), and the following currents: 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_{CaL}, and a slow Ca^{2+}-dependent after-hyperpolarization current I_{sAHP}. The late firing cell contained an additional inactivating transient K^+ current I_A known to delay the onset of the action potential (Storm 1986), while the low-threshold

bursting cell included an additional low-threshold inactivating calcium current I_{CaT} .The passive membrane properties were as follows: R_m = 30 KΩ-cm^2, C_m = 1.0 μF/cm^2, and R_a =150 Ω-cm.

Network model and synaptic connections. The ITC network model (Fig. 8) had dorsal and ventral ITC modules, and a Ce module. It had 15 ITC_D and 15 ITC_V neurons, and five Ce output cells. The network received inputs from LA, BA, and IL. LA inputs projected to ITC_D, while IL inputs projected equally to ITC_D and ITC_V (McDonald et al., 1996). BA inputs also projected to both ITC_D and ITC_V clusters, but with a lower density to ITC_D neurons (Royer et al., 1999, 2000; Pape and Paré, 2010). Based on the findings of Herry et al. (2008), the BA inputs were divided into fear, extinction, and extinction-resistant (ER) groups. The extinction inputs did not project to Ce because the activation profile of extinction cells was opposite to the expression of fear (Herry et al., 2008). Instead, both fear and ER inputs projected to Ce.

ITC neurons exhibit NMDA-dependent bidirectional synaptic plasticity (Royer and Paré, 2002) and in a recent experimental study, the BA inputs to ITC cells were reported to show a three-fold potentiation during extinction training (Fig. 4 in Amano et al., 2010). Given the fact that the firing rate of LA neurons is significantly increased after conditioning (Quirk et al., 1995), it is reasonable to assume that the LA–ITC_D connection is potentiated by conditioning. Hence, we used a threefold synaptic weight (compared with the habituation state) for the LA–ITC_D synapses in the fear state and a threefold synaptic weight for the BA–ITC synapses in the extinction state. For the LA–ITC_D connection, the potentiated synapses were assumed to be partially depotentiated in the extinction state (strength reduced from 3 to 2 for AMPA synapses only, Amano et al., 2010) based on results from a previous LA network model (Li et al., 2009). The BA–Ce, ITC–ITC, and ITC–Ce synaptic weights were assumed to be fixed. However, based on experimental findings (Geracitano et al., 2007), the presynaptic release probability of the ITC–ITC and ITC–Ce synapses was modifiable, and were split equally into facilitating, depressing, and constant types. The equations and specifics related to the plasticity mechanisms can be found in Li et al. (2011).

Model runs. We determined responses of the model to a 2-sec auditory tone input (CS) during three different network states: habituation, following fear conditioning, and after extinction training. The LA and BA inputs were modeled with different degrees of spike frequency adaptation based on previous experimental data (Quirk et al., 1995, 1997; Faber et al., 2001; Herry et al., 2008) and to account for the projection from LA to BA, the firing rate of BA fear inputs was assumed to be dependent on LA inputs due to similar firing patterns across training (Quirk et al., 1995; Herry et al., 2008). The IL inputs were modeled as Poisson-distributed spike trains with a duration of 300 msec and a mean frequency of 20 Hz (Milad and Quirk, 2002). In addition, Poisson-distributed random background inputs were delivered to all ITC and Ce neurons to achieve experimentally observed spontaneous firing rates.

Model runs were performed on a personal computer using the software package GENESIS with the Crank-Nicholson integration method, and a time step of 20 msec. A simulation of 5 sec of network activity took 15 min of CPU time.

Insights provided by the network model of ITC clusters

We developed the model to investigate how the electroresponsive properties of ITC cells shape their responsiveness to BLA/IL inputs, and how IL inputs might overcome the inter-ITC inhibition after extinction training and reduce the responsiveness of Ce. The model showed that ITC neurons could transform the transient CS-related signals arising in the BLA into a persistent pattern of activity. It also showed that over a wide range of stimulation strengths, brief IL activation can overwhelm inter-ITC inhibition and reduce the activity of fear output Ce neurons. Importantly, both intrinsic properties (i.e., bistability) and variations in the short-term synaptic dynamics of ITC neurons contributed to the effectiveness of IL stimulation. Similar to the LA model case discussed earlier, the ITC model provided several insights into the functioning of this cluster of cells and how they might modulate the expression of fear and extinction memories.

Can ITC neurons help in transforming transient LA fear inputs into sustained Ce output? The model showed that despite the presence of inhibitory connections between ITC cells, transient excitatory inputs from BLA or IL were transformed by ITC cells into 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 for a 2.5 fold increase in inhibitory synaptic weights. This finding suggests 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. It was recently shown that prelimbic (PL) neurons transform transient amygdala inputs into a sustained output that drives conditioned fear responses and gates the expression of extinction (Burgos-Robles et al., 2009). Our model suggests that ITC activity could add to the role of PL in sustaining the expression of conditioned fear. While PL seems to sustain fear by increasing the excitatory drive onto Ce via BA, the present study suggests that ITC_D neurons could contribute to this sustenance by increasing their inhibition on ITC_V neurons, resulting in disinhibition of Ce. During the high fear state, strongly adaptive LA inputs were transformed into a sustained output by ITC_D neurons, leading to persistent inhibition of ITC_V cells and consequent sustained firing in Ce. Also, ITC neurons can support the expression of extinction via persistent activity in ITC_V cells. In the extinction state, LA responses diminished and the LA–ITC_D connection depotentiated, while the BA–ITC connection potentiated (Amano et al., 2010). Strongly adaptive BA inputs were then transformed into sustained firing in ITC_V cells, resulting in lowered Ce responses (see fig 8 in Li et al., 2011).

Can IL overcome inter-ITC inhibition and reduce Ce responses? The model examined the impact of a brief 300 ms IL stimulation on the responsiveness of ITC cells to strongly adaptive CS-related BLA inputs, in the high fear state. Over a wide range of strengths, IL inputs consistently caused a marked increase in the firing rate of ITC cells, which then inhibited Ce, the fear output station. Also, IL-evoked firing caused a persistent inactivation of I_{SD} in ITC neurons and this extended IL's impact beyond the 300-msec stimulation window. The model also demonstrated that IL stimulation given shortly after tone onset was most

effective in reducing Ce firing, in agreement with experimental findings (Milad et al., 2004). This might be due to the fact that this timing most effectively combines the direct impact of IL in inhibiting early Ce spikes and its indirect (after IL is turned off) impact in inhibiting Ce firing subsequently, via the inactivation of the slowly de-inactivating current I_{SD}. The model also predicted that ITC neurons contacted by depressing synapses are more likely recruited by IL inputs than those contacted by facilitating or constant-type synapses.

What is the role of synaptic heterogeneity within ITC cells? Geracitano et al. (2007) have shown that the synapses within the ITC region, i.e., ITC-ITC synapses, exhibit short-term presynaptic plasticity that is distributed equally between facilitating, depressing and constant types. Their experiments also show that the ITC_V–Ce connections (fig. 8) have to be of the facilitating or constant types. Model experiments suggest that this specificity could be functionally relevant in the inhibitory control of Ce by IL inputs. The model, as expected, showed that ITC_V–Ce connections of the facilitating and constant types were more effective in inhibiting Ce output. However, for the inter-ITC connections, pure facilitating or constant synapses decrease the firing rates of both ITC_D and ITC_V neurons when IL inputs are active, resulting in elevated Ce responses. Hence, depressing inter-ITC synapses, together with inactivation of I_{SD}, would allow IL inputs to overcome the inter-ITC inhibition. These insights suggest that the specific distribution of heterogeneous short-term plasticity of the inter-ITC connections enables sufficiently high activity levels in ITC_V cells for an efficient control of fear-related Ce outputs when BA and IL neurons are active.

C. Modeling the other amygdalar nuclei, and modulation by cortical structures

The primary structures of the fear circuit, as presently understood, include the amygdala, the prefrontal cortex, and the hippocampus. These structures, in turn, are themselves composed of different sub-circuits, with different roles in auditory fear learning. The amygdala, as cited, consists of several nuclei LA, BA, Ce and the ITC clusters, all acting in concert to store auditory fear and extinction memories, and express them later via the fear output station Ce. Interestingly, these individual nuclei themselves are not homogeneous. For instance, the dorsal and ventral regions of LA participate in fear learning in different ways (Repa et al., 2001); BA has different nuclei, BL and BM, which have recently been shown to relay fear differently to Ce (Amano et al., 2011); the two different ITC cell clusters associated with the fear circuit again contribute in different ways to the expression of fear and extinction memories (Royer et al., 2000); and the output nucleus Ce has also been shown recently to have very distinct sub-circuits whose specific roles as far as influencing fear await further investigation (Coicci et al., 2010; Haubensak et al., 2010).

Amygdalar fear, in turn, is known to be modulated by mPFC (both PL and IL) and by the hippocampus, and so the expression of fear and extinction memories is also under control of these 'higher' level structures. At present, modulation of amygdalar fear by mPFC (see Burgos-Robles et al., 2009) is better understood in comparison to modulation by the hippocampus. As discussed earlier, studies related to contextual fear conditioning

(Fanselow, 2010), which involve the hippocampus, have been complicated by this lack of understanding. Hence computational models of the hippocampus and its linkages to the amygdala and mPFC in auditory fear may have to await progress in our understanding of anatomical linkages between these regions and experimental data on their interactions during the different phases of fear learning.

Opportunities and challenges. There is consensus on the critical involvement of amygdala in the fear circuit. However, it is not presently clear how the various amygdalar nuclei with different internal sub-circuits act in concert to store fear and extinction memories in a distributed manner, and what roles they play in expressing these memories (Paré et al., 2004). Neurobiological information continues to accumulate at an increasing rate, including at intracellular, cellular, circuits and behavioral levels, providing opportunities for integrating such information via 'system' level models such as the ones discussed here. Such models can then be used to address several interesting challenges related to the fear circuit: What is the distribution of tone (thalamic and cortical) and shock in the lateral amygdala? What are the different types of learning mechanisms in the amygdala (pre and post synaptic, short and long term), and what synapses do they impact? Neuromodulatory systems have been shown to play an important role in the fear circuit, but how are the receptors distributed and how do they influence intrinsic excitability and synaptic efficacy? What is the level of robustness and redundancy in storage and expression of fear and extinction memories within each structure and at the circuit level? What are the pathways for modulation of amygdalar fear by the prefrontal cortex and hippocampus (and possibly other cortical structures)?

These are important challenges that have to be addressed in order to gain an understanding of the functioning of the mammalian fear circuit. As demonstrated in the discussion above, biologically realistic models can potentially supplement experimental modalities such as patch clamp recordings, single unit recordings, pharmacological manipulations and transgenic approaches, and assist with reverse engineering the functioning of this critical circuit. They also provide tremendous opportunities for research in interdisciplinary settings with participation of neuroscientists, electrophysiologists and computational experts. For instance, similar to the insights obtained for LA and ITC clusters, such interdisciplinary research would also aid in elucidating the roles of the various sub-circuits within the other nuclei, BA and Ce. After development of the individual models of these nuclei, they can be integrated into an overall model of the amygdalar fear circuit. The level of robustness and redundancy that the circuit components and the circuit as a whole possesses (e.g., in BA as reported by Amano et al., 2011) can then be addressed effectively by such models. As cited, the role of context in fear learning is not well understood at the present time. Thus, improved neurobiological understanding of information processing within the hippocampus and of the anatomical connectivity (hippocampus to mPFC and to the amydgala) will have to precede modeling efforts related to contextual auditory fear circuits.

Limitations. Although computational modeling is increasingly used as a tool for studying complex neuronal brain circuits, some general limitations should be acknowledged. Computational models are typically designed to answer specific questions, and so consider only the relevant structures and associated functions. The size of reported model networks incorporating biological realistic cells is typically much smaller than the actual biological networks at the present time (e.g., Durstewitz et al., 2000; Morgan et al., 2007). This is compensated for presently by careful modeling including preservation of key network features and by extensive parametric studies (Morgan et al., 2007). Increasing computing speeds and specialized architectures should enable the development of larger networks directly in the near future. Lack of biological data necessitates using values for parameters such as ionic conductances from multiple brain areas, or tuning them (e.g., synaptic weights) to match behavioral output. Improved characterization of the physiology including identification of neuronal types, connectivity (including spatial constraints and axonal distributions), parameter values, and learning mechanisms will help improve the fidelity of such models. The diversity of interneurons and the networks they form in the elements of the fear circuit continue to intrigue researchers, although we are beginning to understand the functions that they might subserve, e.g., synchronization of neuronal assemblies (Bissiere et al., 2011). Also, active afferents from areas omitted in the model are typically considered as 'background' input, and better characterization of such afferents under in vivo conditions will be needed. Finally, computational research can be most effective only if the neuroscientist and electrophysiologist play an active role in the model development process, particularly in 'constraining' the model, and the computational expert is committed to an in-depth understanding of neurobiology. Notwithstanding such limitations, researchers continue to take advantage of the fact that information related to neurophysiology is accumulating at an increasing pace, and are developing very large scale biologically realistic networks (e.g., Miller, 2011; Morgan et al., 2007) to reverse engineer the functions implemented by brain circuits.

5. Summary and potential applications

There has been a surge in interest related to the role of intra-amygdaloid structures in Pavlovian fear learning. Research papers have risen from an average of 25/year in the 1980s to 200/year in the 2000s (Paré et al., 2004). Although this has resulted in an improved understanding of the underlying mechanisms, it has also highlighted the complexity of the circuit, including possible distributed storage of fear and extinction memories in the various nuclei/structures, distinct mechanism of LTP/LTD at different synapses, and recruitment of alternative pathways providing redundancy that is probably an important trait of such a critical circuit. This complexity renders the understanding of the amygdalar involvement in fear learning a bigger challenge than previously envisaged. Furthermore, modulation of the amygdala by the prefrontal cortex, hippocampus and other related regions is only beginning to be understood. Computational modeling has the potential to play an important role in our efforts to unravel this complex fear circuit.

Insights into the functioning of the sub-circuits using computational models would also be useful for studying disruptions associated with the fear circuit, leading to PTSD and anxiety disorders. For instance, studies have shown that humans with PTSD exhibit a delay in acquisition of extinction as compared to controls (Rothbaum and Davis, 2003). 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 with PTSD and provide insights into the pathology of the illness. The model could also shed light on how therapeutic approaches such as cognitive restructuring provide a new emotional significance to a negative cognition and reduce physiological arousal (Debiec et al., 2006).

Application to drug discovery research. An important future application of such models would be in identifying new pharmacological targets for therapeutic interventions (Li et al., 2008). For instance, the LA computational model predicted that three types of NMDA-glutamatergic synapses and one type of GABAergic synapse could be involved in storing fear and extinction memories. These predictions seem to be consistent with two recent experimental findings. First, a partial NMDA agonist D-cycloserine has been shown to facilitate extinction of fear conditioning in rats (Akirav et al., 2009; Walker et al., 2001). D-Cycloserine was also effective in treating social anxiety disorder and acrophobia in combination with psychotherapy (Ressler et al., 2004). The mechanism of action of D-Cycloserine and other drugs acting on the glutamatergic system can now be modeled both at receptor and cellular levels in the specific LA neurons indicated by the model. Second, NMDA receptors in the amygdala activate an intracellular signaling cascade leading to new protein synthesis. 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 (Quirk, 2002). The return to baseline level of gephyrin is associated with an increase in the surface expression of $GABA_A$ receptors, corresponding to increasing inhibitory neurotransmission in the amygdala (Harris et al., 1998). Drugs that impact these mechanisms would have a potential role 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 (Chhatwal et al., 2005). 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. With increasing availability of neurobiological information and easy-to-use software tools, such *in silico* models of brain circuits have the potential to become common place in drug discovery research.

Author details

Satish S. Nair
Department of Electrical and Computer Engineering
University of Missouri - Columbia, Columbia, MO, USA

Acknowledgement

The author is grateful to his former PhD student Guoshi Li, and to Gregory Quirk and Denis Paré for their contributions, in many ways, to the collaborative research that this review significantly draws upon. This work was supported by NIMH grant MH087755 to SSN.

6. References

Akirav I, Segev A, Montanis H, Maroun M (2009) D-Cycloserine into the BLA reverses the impairing effects of exposure to stress on the extinction of contextual fear, but not conditioned taste aversion. Learn Mem 16: 682-686.

Amano T, Unal CT, Paré D (2010) Synaptic correlates of fear extinction in the amygdala. Nat Neurosci 13: 489–494.

Amano T, Duvaci S, Popa D, and Paré D (2011) The fear circuit revisited: Contributions of basal amygdala nuclei to conditioned fear. J Neurosci 31(43): 15481-15489.

Anagnostaras SG, Maren S, Fanselow MS (1999) Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. J Neurosci 19:1106 –1114.

Aradi I, Érdi P (2006) Computational neuropharmacology: dynamical approaches in drug discovery. Trends in Pharm Sci 27(5): 240-243.

Armony JL, Servan-Schreiber D, Cohen JD, LeDoux JE (1995) An anatomically constrained neural network model of fear conditioning. Behav Neurosci 109:246–257.

Balkenius C, Morén J (2001) Emotional learning: a computational model of the amygdala. Cybern Syst 32: 611–636, 2001.

Bissiere S, Zelikowsky M, Ponnusamy R, Jacobs NS, Blair HT, Fanselow MS (2011) Electrical synapses control hippocampal contributions to fear learning and memory. Science 331:87–91.

Blair HT, Schafe GE, Bauer EP, Rodrigues SM, and LeDoux JE (2001) Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. Learn Mem 8: 229–242.

Blanchard DC, Blanchard RJ (1972) Innate and conditioned fear reactions to threat in rats with amygdaloid lesions. J Comp Phys Psychol 81:281–290.

Blum S, Hebert AE, Dash PK (2006) A role for the prefrontal cortex in recall of recent and remote memories. Neuroreport 17:341–344.

Boeing 777, In *Wikipedia*. Retrieved March 9, 2012, from http://en.wikipedia.org/wiki/777-200ER#777-200ER.

Bouton ME, King DA (1983) Contextual control of the extinction of conditioned fear: tests for the associative value of the context. J Exp Psychol Anim Behav Process 9:248-265.

Bouton ME. Context and behavioral processes in extinction. Learn Mem 11: 485-494, 2004.

Bower JM, Beeman D (2003) The book of GENESIS: *Exploring realistic neural models with the GEneral NEural SImulation System*, Internet Edition.

Buchel C, Morris J, Dolan RJ, and Friston KJ (1998) Brain systems mediating aversive conditioning: an event-related fMRI study. Neuron 20: 947–957.

Burgos-Robles A, Vidal-Gonzalez I, Quirk GJ (2009) Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure. J Neurosci 29: 8474–8482.

Byrne JH, Roberts JL (2004) *From Molecules to Networks – An Introduction to Cellular and Molecular Neuroscience*. Elsevier Academic Press.

Carnevale NT, Hines ML (2006) *The NEURON Book*. Cambridge, UK: Cambridge University Press.

Chhatwal JP, Myers KM, Ressler KJ, Davis M, et al. (2005) Regulation of gephyrin and $GABA_A$ receptor binding within the amygdala after fear acquisition and extinction. J Neurosci 25:502-506.

Ciocchi S, Herry C, Grenier F, Wolff SBE, Letzkus JJ, Vlachos I, Ehrlich I, Sprengel R, Deisseroth K, Stadler MB, et al. (2010) Encoding of conditioned fear in central amygdala inhibitory circuits. Nature 468:277–282.

Corcoran KA, Desmond TJ, Frey KA, Maren S (2005) Hippocampal inactivation disrupts the acquisition and contextual encoding of fear extinction. J Neurosci 25: 8978-8987.

Corcoran KA, Quirk GJ (2007) Activity in prelimbic cortex is necessary for the expression of learned, but not innate, fears. J Neurosci 27:840–844.

Corcoran KA, Quirk GJ (2007) Recalling safety: Cooperative functions of the ventromedial prefrontal cortex and the hippocampus in extinction. CNS Spect 12:200-206.

Debiec J, Doyere V, Nader K, Ledoux JE (2006) Directly reactivated, but not indirectly reactivated, memories undergo reconsolidation in the amygdale. Proc Natl Acad Sci USA 103:3428 -3433.

Durstewitz D, Seamans JK, Sejnowski TJ (2000) Dopamine-mediated stabilization of delay-period activity in a network model of prefrontal cortex. J Neurophysiol 83:1733-1750.

Faber ESL, Callister RJ, Sah P (2001) Morphological and electrophysiological properties of principal neurons in the rat lateral amygdala in vitro. J Neurophysiol 85:714-723.

Fanselow MS (2010) From contextual fear to a dynamic view of memory systems. Trends Cogn Sci 14:7–15.

Gaudreau H, Paré D (1996) Projection neurons of the lateral amygdaloid nucleus are virtually silent throughout the sleep-walking cycle. J Neurophysiol 75:1301-1305.

Geracitano R, Kaufmann WA, Szabo G, Ferraguti F, Capogna M (2007) Synaptic heterogeneity between mouse paracapsular intercalated neurons of the amygdala. J Physiol 585: 117–134.

Grossberg S, Schmajuk NA (1987) Neural dynamics of attentionally modulated Pavlovian conditioning: conditioned reinforcement, inhibition, and opponent processing. Psychobiology 15: 195–240.

Harris JA, Westbrook RF (1998) Evidence that GABA transmission mediates context-specific extinction of learned fear. Psychopharmacology 140:105-115.

Haubensak W, Kunwar PS, Cai H, Ciocchi S, Wall NR, Ponnusamy R, Biag J, Dong HW, Deisseroth K, Callaway EM, et al. (2010) Genetic dissection of an amygdala microcircuit that gates conditioned fear. Nature 468: 270–276.

Herry C, Garcia R (2002) Prefrontal cortex long-term potentiation, but not long-term depression, is associated with the maintenance of extinction of learned fear in mice. J Neurosci 22:577–583.

Herry C, Ciocchi S, Senn V, Demmou L, Muller C, Luthi A (2008) Switching on and off fear by distinct neuronal circuits. Nature 454: 600–606.

Hobin JA, Goosens KA, Maren S (2003) Context-dependent neuronal activity in the lateral amygdala represents fear memories after extinction. J Neurosci 23: 8410-8416.

Hoover WB and Vertes RP (2007) Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct 212: 149–179.

Koch C, Segev I (2001) *Methods in Neuronal Modeling- From Ions to Networks*. The MIT Press.

Krasne FB, Fanselow MS, Zelikowsky M. Design of a neurally plausible model of fear learning (2011) Frontiers in Behavioral Neuroscience 5:41.

Leblois A, Boraud T, Meissner W, Bergman H, Hansel D (2006) Competition between feedback loops underlies pathological dynamics in the basal ganglia J Neurosci 26(13):3567-3583.

LeDoux JE (1994) Emotion, memory and the brain. Sci Am. 50-57.

LeDoux JE (1996). *The Emotional Brain: The Mysterious Underpinnings of Emotional Life*. Simon & Schuster: New York.

LeDoux JE (2000) Emotion circuits in the brain. Annu Rev Neurosci 23: 155–184.

Li G, Amano T, Paré D, Nair SS (2011) Impact of infralimbic inputs on intercalated amygdala neurons: a biophysical modeling study, Learn Mem 18:226-240.

Li G, Nair SS, Quirk GJ (2009) A biologically realistic network model of acquisition and extinction of conditioned fear associations in lateral amygdala neurons, Journal of Neurophysiology 101: 1629-1646.

Li G, Nair J, Quirk GJ, Nair SS (2008) Computational modeling – a tool for new psychiatric medication development, Psychiatric Annals, 28(4):296-304.

Malkani S and Rosen JB (200) Specific induction of early growth response gene1 in the lateral nucleus of the amygdala following contextual fear conditioning in rats. Neurosci 97: 693–702.

Maren S (2001) Neurobiology of Pavlovian fear conditioning. Annu Rev Neurosci 24: 897–931.

Maren S, Quirk GJ (2004) Neuronal signaling of fear memory. Nat Rev Neurosci 5:844-842.

Mauk MD (2000) The potential effectiveness of simulations verses phenomenological models. Nat Neurosci 23:649-711.

Milad MR, Quirk GJ (2002) Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420:70 –74.

Milad MR, Wright CL, Orr SP, Pitman RK, Quirk GJ, Rauch SL (2007) Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. Biolo Psychiatry 62:446-454.

Miller G (2011) Newsmaker interview: Henry Markram - Blue Brain founder responds to critics, clarifies his goals, Science 334 (6057): 748-749

Morgan RJ, Santhakumar V, Soltesz I (2007) Modeling the dentate gyrus, Prog in Brain Res 163:639-58.

Myers KM, Davis M (2007) Mechanisms of fear extinction. Mol Psychiatry 12:120-150.

Paré D, Quirk GJ, Ledoux JE (2004) New vistas on amygdala networks in conditioned fear. J Neurophysiol 92:1-9.

Pape HC, Paré D (2010) Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiol Rev 90: 419–463.

Quirk GJ, Russo GK, Barron JL, Lebron K (2000) The role of ventromedial prefrontal cortex in the recovery of extinguished fear. J Neurosci 20:6225– 6231.

Quirk GJ (2002) Memory for extinction of conditioned fear is long-lasting and persists following spontaneous recovery. Learn Mem 9:402-407.

Quirk GJ, Armony JL, LeDoux JE (1997) Fear conditioning enhances different temporal components of tone-evoked spike trains in auditory cortex and lateral amygdala. Neuron 19: 613–624.

Quirk GJ, Repa C, LeDoux JE (1995) Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. Neuron 15: 1029–1039.

Quirk GJ, Garcia R, Gonzalez-Lima F (2006) Prefrontal mechanisms in extinction of conditioned fear. Biol Psychiatry 60:337-343.

Quirk GJ, Mueller D (2008) Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology 33:56–72.

Repa JC, Muller J, Apergis J, Desrochers TM, Zhou Y, LeDoux JE (2001) Two different lateral amygdala cell populations contribute to the initiation and storage of memory. Nat Neurosci 4: 724–731.

Ressler KJ , Rothbaum BO, Anderson P, Zimand E, Tannenbaum L, Hodges L, Davis M (2004) D-Cycloserine, a putative cognitive enhancer, accelerates extinction of fear in humans. Arch General Psychiatry 61:1136-44.

Rothbaum BO, Davis M (2003) Applying learning principles to the treatment of posttrauma reactions. Ann N Y Acad Sci 1008:112-121.

Royer S, Martina M, Paré D (2000) Polarized synaptic interactions between intercalated neurons of the amygdala. J Neurophysiol 83: 3509–3518.

Tunnanen and Pitkanen (2000) Do seizures cause neuronal damage in rat amygdala kindling?, Epilepsy Research, 39(2):171-176.

Vlachos I, Herry C, Luthi A, Aertsen A, Kumar A (2011) Context-dependent encoding of fear and extinction memories in a large-scale network model of the basal amygdala. PLoS Comput Biol 7(3): e1001104.

Vidal-Gonzalez I, Vidal-Gonzalez B, Rauch SL, Quirk GJ (2006) Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. Learn Mem 13:728 –733.

Walker DL, Ressler KJ, Lu K-T, Davis M (2002) Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. J Neurosci 22:2343–2351.

Wehner JM, Radcliffe RA (2004) Current Protocols in Neuroscience 8.5C.1-8.5C.14, John Wiley & Sons, Inc.

IntechOpen