Published in final edited form as:

Brain Struct Funct. 2016 May ; 221(4): 2163–2182. doi:10.1007/s00429-015-1037-4.

Synaptic competition in the lateral amygdala and the stimulus specificity of conditioned fear: a biophysical modeling study

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Abstract

Competitive synaptic interactions between principal neurons (PNs) with differing intrinsic excitability were recently shown to determine which dorsal lateral amygdala (LAd) neurons are recruited into a fear memory trace. Here, we explored the contribution of these competitive interactions in determining the stimulus specificity of conditioned fear associations. To this end, we used a realistic biophysical computational model of LAd that included multi-compartment conductance-based models of 800 PNs and 200 interneurons. To reproduce the continuum of spike frequency adaptation displayed by PNs, the model included three subtypes of PNs with high, intermediate, and low spike frequency adaptation. In addition, the model network integrated spatially differentiated patterns of excitatory and inhibitory connections within LA, dopaminergic and noradrenergic inputs, extrinsic thalamic and cortical tone afferents to simulate conditioned stimuli as well as shock inputs for the unconditioned stimulus. Last, glutamatergic synapses in the model could undergo activity-dependent plasticity. Our results suggest that plasticity at both excitatory (PN–PN) and di-synaptic inhibitory (PN–ITN and, particularly, ITN–PN) connections are major determinants of the synaptic competition governing the assignment of PNs to the memory trace. The model also revealed that training-induced potentiation of PN–PN synapses promotes, whereas that of ITN–PN synapses opposes, stimulus generalization. Indeed, suppressing plasticity of PN–PN synapses increased, whereas preventing plasticity of interneuronal synapses decreased the CS specificity of PN recruitment. Overall, our results indicate that the plasticity configuration imprinted in the network by synaptic competition ensures memory specificity. Given that anxiety disorders are characterized by tendency to generalize learned fear to safe stimuli or situations, understanding how plasticity of intrinsic LAd synapses regulates the specificity of learned fear is an important challenge for future experimental studies.

Keywords

Hebbian learning; Amygdala; Competition; Specificity; Memory

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Conflict of interest The authors declare no competing financial interests.

Introduction

The ability to associate fear responses to new stimuli is typically studied using Pavlovian fear conditioning. In this paradigm, an innocuous conditioned stimulus (CS) acquires the ability to elicit fear responses after a few pairings with a noxious unconditioned stimulus (US). Fear conditioning induces synaptic plasticity in several brain regions, including the thalamus (Weinberger 2011), cortex (Letzkus et al. 2011), and amygdala (LeDoux 2000). Nevertheless, it is commonly believed that plasticity in the dorsal portion of the rodent lateral amygdala plays a critical role in the storage of CS–US associations. For instance, it was found that fear conditioning augments the CS responsiveness of LAd neurons (Quirk et al. 1995; Collins and Paré 2000; Repa et al. 2001; Goosens et al. 2003) with neurons in the most dorsal part of LAd (‘transient plastic’ cells) displaying increases that last for only a few CSs whereas more ventrally located LAd neurons showing persistent increases (‘long-term plastic’ cells), even resisting extinction training (Repa et al. 2001).

This contrasting pattern of CS responsiveness could be reproduced by a biophysical model of LAd (Kim et al. 2013a) that included compartmental models of 800 principal neurons (PNs) and 200 interneurons (ITNs), neuromodulatory inputs, and spatially heterogeneous intrinsic connections, all of which were constrained by prior experimental observations (Sah et al. 2003; Samson and Paré 2006; Pape and Paré 2010). To reproduce the continuum of spike frequency adaptation seen in PNs (Sah et al. 2003), the model featured three types of PNs, with high, intermediate, or low spike frequency adaptation, due to the differential expression of a Ca^{2+} -dependent K^{+} current (g_{KCa}) (see “Appendix”). Last, consistent with prior experimental and modeling studies, all model glutamatergic synapses could undergo short-term and long-term activity-dependent plasticity, except those delivering shock or background inputs (Pape and Paré 2010; Li et al. 2009).

The biophysical model was then used to investigate how particular LAd neurons are assigned to the fear memory trace (Kim et al. 2013b). Indeed, after fear conditioning, relatively few LAd neurons develop an increased CS responsiveness (Quirk et al. 1995; Repa et al. 2001) even though most receive the necessary inputs (Han et al. 2007). This led to the hypothesis that assignment of particular LAd neurons to the memory trace involves a competitive process (Han et al. 2007, 2009). Consistent with this notion, the model revealed that PNs with high intrinsic excitability were more likely to be integrated in the fear memory trace. Moreover, connectivity analyses in plastic and non-plastic cells revealed that subsets of PNs effectively band together by virtue of their excitatory interconnections to suppress plasticity in other PNs via the recruitment of inhibitory ITNs.

However, it remains unclear how synaptic plasticity between different subtypes of LAd neurons contributes to the competition and how it relates to the specificity of conditioned fear responses. In various training paradigms, conditioned responses are largest for the original CS and decrease gradually with increasingly dissimilar stimuli (Honig and Urciuoli 1981; Domjan 2006). Therefore, we tested the hypothesis that competition and stimulus specificity mechanisms are related and depend on intra-amygdalar plasticity in intrinsic LAd excitatory and inhibitory connections. In particular, we hypothesized that plasticity at synapses between principal cells contributes to reduce the specificity of conditioned fear

responses, whereas plasticity at the synapses contributed by inhibitory interneurons enhances their specificity. To test these hypotheses, we conducted two types of modeling experiments with multiple CSs of the same or different modalities and examined the impact of inactivating plasticity at different subsets of synapses.

Materials and methods

Overview and development of the LAd biophysical model

Past computational models of fear learning have typically used connectionist or firing rate formalisms and have provided valuable insights (Armony et al. 1997; Balkenius and Moren 2001; Vlachos et al. 2011; Krasne et al. 2011; Moustafa et al. 2013). However, these types of models cannot incorporate data about the cellular, synaptic, and neuromodulatory mechanisms involved in fear learning. For instance, firing rate (Ball et al. 2012) and Izhikevich-based spiking (Hummos et al. 2014) model cells could not match passive properties and current injection responses simultaneously. Moreover, studying phenomena such as effects of voltage clamping at different levels, of neuro-modulation, of calcium-based plasticity rules, and of blocking particular current channels is typically difficult in networks with such cells. In contrast, multi-compartmental biophysical models can incorporate such neurophysiological data (Kim et al. 2013a) and potentially lead to insights into the cellular mechanisms of fear learning. Thus, we have developed such a model, as described below.

It was estimated that there are 24,000 PNs in rat LAd (Tuunanen and Pitkanen 2000). To keep computation times practical while capturing the complexity of the intra-LAd network, we developed a scaled down (30:1) biophysical model of LAd that included 800 PNs. Because the proportion of ITNs to PNs is 20:80, the model also included 200 ITNs. PNs and ITNs were distributed randomly in a realistic tri-dimensional representation of the horn-shaped LAd (Fig. 1A). As detailed below, the LAd network also featured neuromodulatory inputs from brainstem dopaminergic and noradrenergic neurons (Johnson et al. 2011), as well as probabilistic gradients of inhibitory and excitatory connections (Fig. 2, Kim et al. 2013a) within LAd.

Because the model included so many neurons connected by nearly 40,000 synapses, individual low-level aspects of the model (e.g., intrinsic properties of the cells, specific connections between them, learning rates for excitatory and inhibitory synapses, selection of cells to place neuro-modulator receptors) could not be tuned directly to achieve particular impacts on high-level model behavior. Rather, for each low-level property, we searched the experimental literature for model constraints and adjusted model parameters until the modeled aspect reproduced prior experimental findings, independently of other low-level model properties, as described below.

Single cell models

The distribution and density of ionic conductances in biophysical model cells was adjusted to reproduce the electroresponsive properties of LAd cell types, as observed experimentally (reviewed in Sah et al. 2003). The PN model had three compartments representing a soma

(diameter 24.75 μm ; length 25 μm), where GABAergic synapses were placed, an apical dendrite (a-dend; diameter 2.5 μm ; length 119 μm) on which glutamatergic synapses were placed, and another dendrite (dend; diameter 5 μm ; length 400 μm). The differential distribution of glutamatergic and GABAergic synapses in the different compartments of principal cells is based on prior electron microscopic studies (reviewed in Pape and Paré 2010). Values of specific membrane resistance, membrane capacity and cytoplasmic (axial) resistivity used in Eq. (1) were, respectively, $R_m = 55 \text{ K}\Omega\text{-cm}^2$; C_m for soma/a-dend/dend were 1.4, 2.8 and 2.5 $\mu\text{F/cm}^2$, respectively; and R_a for soma/a-dend/dend were 147, 200 and 200 $\Omega\text{-cm}$, respectively. Leakage reversal potential (E_L) was set to -67 mV . The resulting V_{rest} was -69.5 mV , input resistance (R_{IN}) was $\sim 150 \text{ M}\Omega$, and time constant τ_m was 30 ms, all within the ranges reported in previous physiological studies (Washburn and Moises 1992; Faber et al. 2001). To reproduce the diversity of spike frequency adaptation seen in PNs (Faber et al. 2001; Faber and Sah 2003; Power et al. 2011), we modeled three types of regular spiking PNs, with high (type A; $n = 400$), intermediate (type B; $n = 240$), or low (type C, $n = 160$) spike frequency adaptation, due primarily to the differential expression of a Ca^{2+} -dependent K^+ current [see Eq. (1) and Table 3]. Details of all current types, equations and densities can be found in the “Appendix”.

LA also contains local GABAergic ITNs that exhibit various firing patterns, even among neurochemically homogeneous subgroups (Pape and Paré 2010; Spanpanato et al. 2011). However, the majority displays a fast-spiking pattern, which was reproduced in the model. The ITN model had two compartments, a soma (diameter 15 μm ; length 15 μm) and a dendrite (diameter 10 μm ; length 150 μm) which had glutamatergic synapses. The passive membrane properties were as follows: $R_m = 20 \text{ K}\Omega\text{-cm}^2$, $C_m = 1.0 \mu\text{F/cm}^2$, $R_a = 150 \Omega\text{-cm}$, and $E_L = -70 \text{ mV}$. The ITN model could reproduce the non-adapting repetitive firing behavior of fast-spiking cells, as observed experimentally. ITNs synapsed onto the soma of principal cells.

Network inputs and connectivity

Background synaptic inputs—LA projection neurons have low spontaneous firing rates in control conditions (Gaudreau and Paré 1996). To reproduce this, Poisson-distributed, random excitatory background inputs (3 Hz to PNs and 5 Hz to ITNs) were delivered to all model cells, resulting in average spontaneous firing rates of 0.8 Hz for PNs and 8.7 Hz for ITNs.

Tone and shock inputs—Auditory fear conditioning is thought to depend on the convergence of inputs relaying information about the CS (tone) and US (footshock) in LA (LeDoux 2000; Pape and Paré 2010; Quirk et al. 1995). In the model, the CS and US inputs were represented by glutamatergic synapses acting via AMPA and NMDA receptors. The frequency of thalamic and cortical tone inputs during habituation was set to 20 Hz. In light of evidence that fear conditioning leads to plasticity in CS afferent pathways, the frequency of thalamic and cortical tone inputs was increased to 40 Hz after the first and sixth conditioning trials, respectively (Bordi and LeDoux 1994; Quirk et al. 1995; Maren et al. 2001). The following distribution of inputs was used for the simulations: uniform total tone density throughout LAd with 70 % of the LAdd cells receiving thalamic and 35 % receiving

cortical tone projections, and the opposite for LAdv, i.e., 35 % of LAdv cells receiving thalamic and 70 % receiving cortical tone projections. The shock inputs were distributed uniformly to 70 % of LAd cells.

Intrinsic LAd network—By comparing the responses of LA cells to local applications of glutamate at various positions with respect to recorded neurons, general principles were inferred for connectivity among PNs, as well as between ITNs and PNs (Samson and Paré 2006). We used probabilistic gradients of excitatory and inhibitory connectivity so that the model could reproduce prior experimental observations about the spatially heterogeneous intrinsic connectivity that exists in different parts of LA. See Sect “Intrinsic connectivity in LAd” of the “Appendix” for an explanation of how this was implemented (Kim et al. 2013a).

Activity-dependent synaptic plasticity

Model synapses could undergo activity-dependent synaptic plasticity, consistent with the experimental literature (Pape and Paré 2010). All AMPA synapses in the model were endowed with long-term postsynaptic plasticity except for those delivering shock or background inputs. Also, all GABAergic synapses had long-term plasticity. Long-term plasticity was implemented using a learning rule that depends on the concentration of a postsynaptic Ca^{2+} pool at each modifiable synapse (Shouval et al. 2002a, 2002b). In keeping with the experimental literature (Pape and Paré 2010), at excitatory synapses, Ca^{2+} entered postsynaptic pools via NMDA receptors (and AMPA receptors for ITNs; Mahanty and Sah 1998) as well as voltage-gated Ca^{2+} channels (VGCCs). At inhibitory synapses (only), Ca^{2+} from postsynaptic intracellular stores and from VGCCs also contributed to the postsynaptic Ca^{2+} pools (Li et al. 2009); details in Sect. “Calcium dynamics and Hebbian learning” of “Appendix”.

For both types of synapses, synaptic weights decreased when the Ca^{2+} concentration was below a lower threshold and increased when it exceeded an upper threshold. Importantly, properties of plastic synapses in LAdd and LAdv were not adjusted differently to reproduce the contrasting distribution of transient and persistent plastic cells in different sectors of LAd, as reported previously (Repa et al. 2001). Rather, except for synapses delivering shock or background inputs, uniform properties were implemented at all synapses so that they could undergo activity-dependent plasticity, while maintaining overall network stability and avoid runaway behavior. Finally, all model glutamatergic and GABAergic synapses also exhibited short-term presynaptic plasticity, with short-term depression at PN–PN, ITN–PN and PN–ITN connections. Additional details and equations related to the implementation of these plasticity mechanisms can be found in the “Appendix”.

Neuromodulator effects

Neuromodulators have long been implicated in fear and anxiety, and are known to regulate Pavlovian fear learning and synaptic plasticity in LA (Bissiere et al. 2003; Tully and Bolshakov 2010). Conditioned aversive stimuli alter the activity of ventral tegmental area and locus coeruleus neurons, which in turn modulate fear and anxiety through their widespread forebrain projections, including to the amygdala. Therefore, the model

incorporated the effects of dopamine (DA) and norepinephrine (NE) on LAd cells, based on prior experimental reports (for DA: Durstewitz et al. 2000; Kroner et al. 2004; Loretan et al. 2004; Martina and Bergeron 2008; Muller et al. 2009; for NE: Hu et al. 2007; Sara 2009; Farb et al. 2010; Tully and Bolshakov 2010; Johnson et al. 2011; Kim et al. 2013a). Details of how DA and NE modify the intrinsic and synaptic conductances in the model are listed in Table 5 of the “Appendix”.

Training paradigm

The training paradigm we used (Fig. 1b) was based on prior in vivo tests (Quirk et al. 1995). It included three phases (habituation, conditioning, and within-session extinction), comprised of 8, 16, and 20 trials, respectively. As cited earlier in this section, the frequency of tone inputs during habituation was set to 20 Hz and was increased to 40 Hz after the first and sixth conditioning trials for thalamic and cortical tone inputs, respectively. During extinction, the frequency was maintained at 40 Hz. The first 4 trials of extinction were considered as a recall trial. Each trial consisted of a 0.5-s tone CS that followed a 3.5-s gap. During conditioning only, a shock was administered 0.1 s prior to the end of the tone, so that they co-terminated. Figure 2a shows examples of tone-evoked responses before (left) and after conditioning (right) with different colors used for PNs that displayed (red) or did not (blue) display increases in tone responsiveness. We also note that the network did not exhibit any form of instability or runaway behavior, due to the sparse connectivity among PNs (~3 %; synapse onto dendrites) and the strong ITN–PN connections.

It should be noted that we use the term habituation to match the nomenclature used in fear conditioning studies. In these studies, habituation trials are required to attenuate fear responses elicited by novel stimuli (a phenomenon termed neophobia). The last few habituation trials are then used to measure the baseline CS responsiveness of recorded cells. The model of course displayed no neophobic responses. In the model, we only used habituation trials to assess the baseline (control) responsiveness of model neurons to the various stimuli. Importantly, habituation trials in the model did not alter the weight of connections because they did not cause sufficient elevation of simulated intracellular Ca^{2+} levels. As a result, there is no need to counterbalance the order Ca^{2+} of CS presentations since whatever the order, the outcome will be the same: no change in responsiveness will occur.

Definition of plastic cells

We used a more stringent definition of plastic cells than in Kim et al. (2013b). This was implemented by replacing spikes/tone in the Repa et al. (2001) criterion by z score and selecting only cells that had $z > 5.5$ in any of the conditioning trial blocks. Here, z score is defined as $z = (x - \mu)/\sigma$, where x is spikes/tone in the first 300 ms of the trial, and μ and σ are average and SD, respectively, of spontaneous spikes/tone during habituation. The higher the z score, the more plastic the cell. Both definitions of plastic cells yielded similar results. However, with z scores, only cells with large differences in tone responsiveness between conditioning and habituation responses were selected as plastic cells.

Stimulus specificity studies

For stimulus specificity studies, to accommodate five different CSs, we reduced the tone density from 70 % in the control case of Kim et al. (2013b) to 50 % (Fig. 3a), with no change in shock inputs (70 % of PNs and ITNs). All five CSs each targeted 50 % of ITNs and PNs with varying degrees of overlap (Fig. 3a). Specifically, CS2, CS3, CS4, and CS5, respectively, targeted 75, 50, 25, and 0 % of the cells targeted by CS1. CS1 was co-presented with the US, and so is termed CS+. Because of the complete lack of overlap between CS1 and CS5, CS5 is also termed CS5/- or just CS-. Each CS randomly targeted PNs of the three types (A–C) to ensure that none of the CSs preferentially targeted highly excitable cells. For instance, the numbers of cell types (A–C) targeted by the CSs for one particular run were as follows (order: #s of A, B, C types): CS1—198, 114, 88; CS2: 189, 121, 90; CS3—188, 119, 93; CS4—197, 114, 89; CS5—202, 126, 72. Similar numbers were obtained for other runs. We followed the protocol in Lissek et al. (2008) and Repa et al. (2001) and conditioned the network to CS+ (paired CS and US) and to CS- (unpaired CS and US) (Ball et al. 2012). The other CSs were used only for testing.

The numbers of trials for the various phases of the training protocol were unchanged. However, during the pre-conditioning test (when measuring baseline responses), we turned off plasticity and presented the stimuli as follows: CS1, CS2, CS3, CS4, CS5, CS1, CS2, CS3, etc., 10 trials each, with a 3.5-s inter-tone interval, and no shock. The presentations during the other phases were as follows. During habituation: CS1, CS5, CS1, CS5, etc., 8 trials, with plasticity turned on. During conditioning: identical to habituation with plasticity turned on, except that the shock was paired to CS1, but specifically unpaired with CS5 for 16 trials. The post- and pre-conditioning tests were identical.

Model runs and analyses

The mathematical details related to the biophysical model described earlier in this section can be found in the “Appendix”. All model runs were performed using parallel NEURON (Carnevale and Hines 2006) running on a Beowulf supercluster with a time step of 50 μ s. The model used in this study is available on the ModelDB public database (<http://senselab.med.yale.edu/ModelDB/>) as part of our previous publication (Kim et al. 2013b). All reported values in results are averages $\pm$ SEM, and tone responses are computed as spikes per tone within 300 ms of tone onset. We used MATLAB R2013b for the calculation involving Students *t* test and Chi-square tests, and SAS 9.4 for calculation of ANOVA and MANOVA with post hoc Bonferroni corrections.

Results

Contribution of different synapse types to the assignment of LAd cells to the memory trace

As reviewed in the introduction, prior experimental and modeling results indicate that both intrinsic excitability and competitive synaptic interactions contribute to the assignment of LAd neurons to fear memory traces. However, the contribution of plasticity at synapses between different subtypes of LAd neurons in these competitive interactions is unknown. To address this question, we conducted two types of simulations. In the first, plasticity at

particular subsets of intra-LAd synapses was allowed during conditioning but the weights of the synapses were returned to control levels during the recall test. In the second, we simply clamped the weight of the same synapses at habituation level during conditioning and the recall test. Note that in both cases, the connections were not altered; only activity-dependent changes in synaptic efficacy were prevented. Also, plasticity of tone inputs was not interfered with and could therefore support potentiation of tone responses as a result of conditioning.

Both sets of manipulations yielded qualitatively similar results during the recall test. Thus, for brevity, we only report the results of the second set of simulations. As detailed below, interfering with plasticity at subsets of intrinsic LAd synapses affected the CS responsiveness of plastic PNs in significantly different ways (MANOVA, $F = 19.33$, $p < 0.0001$; see Fig. 2b1, b2).

To check the effect of inactivation of plasticity at different synapses, we performed two-way ANOVAs for the inactivation at four different synapses and ten different blocks of trials and found them to be significant (overall $F = 118.58$, $DF = 39$, $p < 0.0001$). A significant effect of inactivation of either synapse types (control, PN–PN, PN–ITN, ITN–PN) was found ($F = 479.40$, $DF = 3$, $p < 0.0001$). Also, a significant effect of blocks of trials (1 block of Hab, 4 blocks of Cond and 5 blocks of Ext) ($F = 317.09$, $DF = 9$, $p < 0.0001$), and a significant effect of interaction between synapse types and trial blocks were found ($F = 12.32$, $DF = 27$, $p < 0.0001$).

Inactivation of plasticity at synapses between PNs—Compared to the control case (Fig. 2b1, filled circles), fear conditioning in the absence of PN–PN plasticity (gray diamonds) produced a large decrease in CS responsiveness during conditioning (last trial block, unpaired t test, $p < 0.0001$), which was still present during the recall test (first trial block, unpaired t test, $p < 0.0001$). The p values for unpaired t test throughout the protocol were as follows: habituation: $p < 0.0001$; each of the 4 trial blocks of conditioning: $p < 0.0001$; each of the 5 trial blocks of extinction: $p < 0.0001$. Importantly, this effect was paralleled by a significant decrease in the number of plastic PNs from 211 to 151 (Chi-square test, $p < 0.0001$; Table 1). In particular, inactivation of PN–PN plasticity resulted in 72 of the originally plastic cells becoming non-plastic (losers; 0 of type A, 72 of types B and C) and 12 of the former non-plastic cells becoming plastic (winners; 0 of type A, 12 of types B and C). Also, inactivation of PN–PN plasticity resulted in a major decrease in the spontaneous firing rate of the plastic cells ($71 \pm 3\%$ reduction, paired t test, $p < 0.0001$), suggesting a substantial drop in excitability in the nucleus.

Inactivation of PN–ITN plasticity—Compared to the control case (Fig. 2b2, filled circles), fear conditioning in the absence of PN–ITN plasticity (gray diamonds) produced a minor and non-significant decrease in CS responsiveness during conditioning (last trial block, unpaired t test, $p = 0.6769$), which changed to a minor increase during the recall test (first trial block, unpaired t test, $p = 0.6968$). The p values for unpaired t test throughout the protocol were as follows: habituation: $p < 0.0001$; conditioning: 1st trial block: $p = 0.0037$, 2nd trial block: $p < 0.0001$, 3rd trial block: $p = 0.0017$, 4th trial block: $p = 0.6769$; extinction: 1st trial block: $p = 0.6968$, 2nd trial block: $p = 0.4169$, 3rd trial block: $p =$

0.1074, 4th trial block: $p = 0.4493$, 5th trial block: $p = 0.1754$. Moreover, the proportion of plastic cells was not affected significantly by this manipulation (211–197; Chi-square test, $p = 0.2506$), and their spontaneous firing rate decreased slightly (22 ± 2 % reduction, paired t test, $p < 0.0001$).

Inactivation of ITN–PN plasticity—Inactivating plasticity at the synapses formed by ITNs with PNs had a major impact (Fig. 2b2, diamonds). This included a large potentiation of CS responses (conditioning, last trial block, unpaired t test, $p < 0.0001$; recall, first trial block, unpaired t test, $p < 0.0001$) and an increase in the number of plastic cells (211–236; Chi-square test, $p = 0.0526$). The p values for unpaired t test throughout the protocol were as follows: habituation: $p < 0.0001$; conditioning: 1st trial block: $p = 0.0613$, 2nd trial block: $p = 0.3702$, 3rd trial block: $p < 0.0001$, 4th trial block: $p < 0.0001$; each of the 5 trial blocks of extinction: 1st $p < 0.0001$. In particular, 14 of the originally plastic cells lost their plasticity (losers: 0 of type A, 14 of types B and C) and 39 of the former non-plastic cells became plastic (winners: 0 of type A, 39 of types B and C). This manipulation also resulted in a significant decrease in the spontaneous discharge rate of plastic cells (25 ± 2 % decrease, paired t test, $p < 0.0001$).

To check the robustness of these results, we carried out model runs with five different random seeds for synaptic delays and background noise. This was performed by running the model on different numbers of parallel nodes of the supercluster computer which resulted in different seeds being used. The tone responses for the various cases, as well as the changes in the numbers of plastic cells were nearly identical to the above. Overall, the above simulations indicate that plasticity at intrinsic LAd synapses plays a critical role in shaping the competitive assignment of PNs to the fear memory trace.

Plasticity in synapses of representative cells

Next, to gain insights into the role of plasticity at intrinsic LAd synapses, we considered the changes in synaptic weights of 40 randomly selected plastic and non-plastic PNs of types B and C, all of which received tone inputs and were of the persistent type (Fig. 2c). These two groups of cells each provided samples of 856 PN–PN, 816 ITN–PN, 224 PN–ITN and 42 tone–PN connection weights. We performed two-way ANOVAs for the two types of cells and four different synapses and found them to be significant (overall $F = 470.97$, $DF = 7$, $p < 0.0001$). A significant effect of types of cells (plastic vs. non-plastic) was found ($F = 235.82$, $DF = 1$, $p < 0.0001$). Also, a significant effect of types of synapses (tone–PN, PN–PN, ITN–PN and PN–ITN) ($F = 967.5$, $DF = 3$, $p < 0.0001$), and of interaction between them (types of cells $\times$ types of synapses) ($F = 52.83$, $DF = 3$, $p < 0.0001$) was found. As described below, conditioning produced significant alterations in the weights of all synapse types and it was differentially expressed in plastic vs. non-plastic cells (unpaired t tests: tone–PN, $p < 0.0001$; PN–PN, $p < 0.0001$; ITN–PN, $p < 0.0001$; PN–ITN, $p < 0.0001$).

During the recall test (first trial block), the weight of tone–PN synapses (Eq. 7) increased by 156 ± 4 % in plastic cells (paired t test, $p < 0.0001$) and by 49 ± 13 % in non-plastic cells (paired t test, $p < 0.0001$). Also, the weight of PN–PN synapses (Eq. 7) between plastic cells

increased by $66 \pm 5\%$ (paired t test, $p < 0.0001$), while synapses from plastic to non-plastic PNs did not change significantly ($2 \pm 4\%$; paired t test, $p = 0.54$).

Plasticity at ITN–PN connections was considered by averaging weight changes at all connections from ITNs to each PN. Interestingly, ITN–PN plasticity was found to be higher for plastic than non-plastic PNs: the weight of ITN–PN connections to plastic cells increased $75 \pm 2\%$ compared to $38 \pm 1\%$ for non-plastic cells (unpaired t tests, $p < 0.0001$). Finally, the weight of PN–ITN synapses increased by $126 \pm 9\%$ for presynaptic plastic cells compared to $58 \pm 6\%$ for non-plastic cells (unpaired t tests, $p < 0.0001$).

Summarizing this section, the above analyses indicate that all types of model intrinsic LAd synapses undergo activity-dependent plasticity during fear conditioning. In the model, plasticity at both excitatory and inhibitory synapses plays a role in the competitive assignment of PNs to the fear memory trace.

Role of intra-amygdalar plasticity in maintaining stimulus specificity

To analyze how the competitive synaptic mechanisms documented above influence the CS specificity of conditioned fear associations, we conducted two types of experiments with multiple CSs of the same or different modalities. Below, we will use the terms “Intra-modal” and “Inter-modal” to refer to these two types of experiments, respectively. The only difference between experiments assessing intra- vs. inter-modal specificity resided in the following. For intra-modal specificity experiments, when there was overlap between the PNs targeted by different CSs, the two CSs influenced the common PNs via the same synapses. This approach simulates instances where the CSs are auditory stimuli with frequency spectra that overlap to different degrees. In contrast, for inter-modal specificity experiments, different synapses to the common PNs were activated by the two CSs, reproducing a situation where the CSs are tones and light stimuli, for instance. It should be noted that LA receives somatosensory, auditory, and visual information through posterior thalamic nuclei and associative cortical areas, as recently reviewed (Pape and Paré 2010).

In both types of simulations, we used 5 different stimuli (CS1–5) but only one of them (CS1+) was paired to the footshock during conditioning; CS2–4 were only used for testing. CS5– was presented during conditioning but never paired to the footshock. The sensory inputs activated by the different CSs targeted sub-populations of PNs with a progressively decreasing amount of overlap from CS2 to CS5– relative to CS1+ (Fig. 3a, gray shading). Namely, the inputs activated by CS1+ and CS2 recruited subpopulations of PNs with 75 % overlap, whereas the cells targeted by CS1+ and CS5– did not overlap at all (Fig. 3a).

For panels B and C in Fig. 3, since we were not interested in comparing between the ‘Post-conditioning control’ and ‘No PN–ITN and ITN–PN plasticity’ case, we had a total of 10 cases ($5 \text{ CSs} \times 2 \text{ plasticity}$) for comparison. We first computed paired t tests for these 10 cases and found the following: For Fig. 3b1, all of the comparison cases were found to be significant (paired t tests, $p < 0.05$) except the following two: red vs. gray for CS4 and for CS5. For Fig. 3b2, all of the comparison cases were found to be significant (paired t tests, $p < 0.05$) except the following five: blue vs. gray for CS1, red vs. gray for CS2, red vs. gray for CS4 and red vs. gray and blue vs. gray for CS5. For Fig. 3c1, the red vs. gray case was

found to be not significant, while the blue vs. gray case was significant (paired t tests, $p < 0.05$) for all the five CSs types. For Fig. 3c2, all of the cases were found to be significant (paired t tests, $p < 0.05$) except the following four: blue vs. gray for CS1, red vs. gray for CS2, red vs. blue for CS3 and blue vs. gray for CS5.

Intra-modal specificity—After training the network with CS1+ (paired to the US) and CS5– (not paired to the US), we first identified PNs that did ($n = 218$) or did not ($n = 582$) develop significant increases in responsiveness to CS1 (plastic and non-plastic, respectively). Then, we tested their responses to the 5 CSs for the three separate cases shown in Fig. 3b1, b2 (plastic and non-plastic cells combined MANOVA, $F = 237.34$, $p < 0.0001$; plastic cells, MANOVA, $F = 51.81$, $p < 0.0001$; non-plastic cells, MANOVA, $F = 64.11$, $p < 0.0001$). Given the gradual decrease in overlap between the cells targeted by CS1 to CS5, we expected a progressive reduction in the post-conditioning responsiveness of plastic cells from CS1 to CS5 (Lissek et al. 2010). As expected, the responses elicited by CS1–3 in plastic cells (Fig. 3b1) were significantly higher after conditioning (red vs. gray triangles; Bonferroni-corrected post hoc t tests, $p < 0.034$), whereas responses to CS4–5 did not change significantly (red vs. gray triangles; Bonferroni-corrected post hoc t tests, $p > 0.9$). By contrast, the conditioning-induced changes in responsiveness exhibited by non-plastic cells were small and inconsistent (red vs. gray triangles; Fig. 3b2, Bonferroni-corrected post hoc t tests, $p > 0.8$).

In keeping with the plasticity inactivation experiments, blocking plasticity at interneuronal synapses (Fig. 3b1, blue triangles) produced a significant increase in the responsiveness of plastic cells to CS1–5 (blue vs. gray triangles; Bonferroni-corrected post hoc t tests, $p < 0.0006$). In non-plastic cells, blocking plasticity at interneuronal synapses similarly produced a significant potentiation of tone responses elicited by CS3–4 (Fig. 3b2; blue vs. gray triangles; Bonferroni-corrected post hoc t tests, $p < 0.0001$).

Inter-modal specificity—In these simulations, because the various CSs affected the overlapping PNs via different synapses, we anticipated greater CS specificity than in intra-modal simulations. To test this prediction, we tested responses evoked by the five CSs (CS*1–5) for the three separate cases shown in Fig. 3c1, c2, and observed significant differences (plastic and non-plastic cells combined MANOVA, $F = 131.33$, $p < 0.0001$; plastic cells, MANOVA, $F = 27.93$, $p < 0.0001$; non-plastic cells, MANOVA, $F = 32.87$, $p < 0.0001$). As expected, responsiveness of plastic cells to CS*1–5 did not change significantly (Fig. 3c1, red vs. gray triangles, Bonferroni-corrected post hoc t tests, $p = 0.9$). Nevertheless, blocking plasticity at interneuronal synapses produced a small yet significant potentiation of the responses (Fig. 3c1, blue vs. gray triangles, Bonferroni-corrected post hoc t tests, $p < 0.0001$) elicited by all CSs, as seen in the intra-modal simulations. Training-induced changes in the CS responsiveness of non-plastic cells also reached significance. In these cells, blocking plasticity at interneuronal synapses produced significant potentiation of responses to CS*2–4 (Fig. 3c2, blue vs. gray triangles, paired t tests, $p < 0.0001$), but were not significant for CS*1 and CS*5 (paired t test, $p = 0.41$ and 0.58 , respectively). The mechanisms underlying the differential changes in responsiveness to the various CSs in Fig. 3b and c await investigation.

Next, to better isolate the impact of plasticity at intrinsic LA synapses on stimulus generalization, we considered the responses of PNs with non-overlapping CSs inputs (Fig. 3a, yellow squares; see details in figure legend). Thus, these PNs receive no direct CS1 tone inputs, only indirect CS1 inputs via other LA neurons receiving such synapses. As a result, any modification in the CS responsiveness of the non-overlapping cells has to be dependent on changes in the weight of intrinsic LAd synapses.

Figure 4 illustrates the responsiveness of the non-overlapping PNs for the intra- (Fig. 4a) and inter-modal (Fig. 4b) cases in three conditions: before (gray circles) or after conditioning in control conditions (red triangles) and after blocking interneuronal synaptic plasticity (blue triangles). For both the intra- and inter-modal cases, we restricted our attention to non-overlapping PNs with the 25 % highest responsiveness to the particular CS* in the blocked inhibitory plasticity case. Note that responsiveness to CS5 is not provided here because all PNs receive inputs about either CS1 or CS5. In other words, there are no non-overlapping cells for this combination of CSs. Also, because training with CS1+ is expected to cause a potentiation of PN–PN synapses in a subset of cells; Fig. 4 contrasts the responses of the same non-overlapping cells to CS1 (panel 1 in Fig. 4a) and to CS*1 (panel 1 in Fig. 4b) vs. CS2–4 (panel 2 in Fig. 4a) and CS*2–4 (panel 2 in Fig. 4b).

Although training with CS1+ marginally increased the responses elicited in the non-overlapping cells by CS1 for the intra-modal case (Fig. 4a1; red triangles; Bonferroni-corrected post hoc t tests, $p < 0.049$), the potentiation of responses was not significant for the inter-modal case (Fig. 4b1; Bonferroni-corrected post hoc t tests, $p > 0.95$). However, blocking interneuronal plasticity caused a response potentiation (Fig. 4a1, b1; dark blue triangles) that reached significance for all subsets of non-overlapping cells (Bonferroni-corrected post hoc t tests, $p = 0.02$), for both the intra- (Fig. 4a1) and inter-modal (Fig. 4b1) cases.

In Fig. 4a1 and a2 (note different y-axes), the increased responsiveness to CS1–4 observed after blocking interneuronal plasticity could reflect the influence of two non-exclusive mechanisms: (1) a potentiation of CS1 inputs to PNs afferent to the non-overlapping PNs and/or (2) a potentiation of synapses to the non-overlapping PNs contributed by PNs that receive CS1 inputs. To shed light on the relative importance of these two factors, we compared the effectiveness of inter-modal CS inputs (CS*1–4) in the same non-overlapping cells (Fig. 4b1, b2). Note that in these inter-modal cases, potentiation of responses produced by blocking interneuronal plasticity has to depend on potentiation of PN–PN connections since CS*1–4 were never paired with the US. Therefore, these results together indicate that plasticity of interneuronal synapses promotes CS specificity whereas plasticity at PN–PN synapses promotes generalization (Ball et al. 2012).

Discussion

In the present study, we used a realistic biophysical model of LAd to shed light on the competitive mechanisms that determine the assignment of PNs to the fear memory trace. Prior experimental and modeling results indicated that two factors underlie the competition: intrinsic excitability and synaptic mechanisms. However, little was known about the latter

factor. Using a realistic computational model of the LAd network, we found that fear conditioning induces plasticity at intrinsic LAd synapses and that this plasticity is differentially expressed depending on whether the target cells acquired increased CS responses or not. Interfering with plasticity at the synapses between PNs produced a major decrease in CS responsiveness and a reduction in the number of plastic PNs whereas blocking plasticity at interneuronal synapses had opposite effects. Importantly, our analyses also revealed that conditioning-induced plasticity of intrinsic LAd synapses also promotes the CS specificity of conditioned fear. Indeed, blocking plasticity at interneuronal synapses or PN–PN connections, respectively, decreased and enhanced the CS specificity of conditioning-induced changes in responsiveness.

Intrinsic and synaptic factors regulate the assignment of PNs to the fear memory trace

It was proposed that intrinsically more excitable PNs are more likely to be integrated into the fear memory trace (Han et al. 2007, 2009). Consistent with this proposal, PNs with a higher intrinsic excitability because they express activated CREB (Viosca et al. 2009; Zhou et al. 2009; Benito and Barco 2010) were more likely to be integrated in the memory trace (Han et al. 2007, 2009). However, it was observed that CREB overexpression or downregulation in LA did not alter the proportion of PNs recruited into the memory trace, indicating that a competitive synaptic process is also involved (Han et al. 2007).

The biophysical model used here could replicate these observations (Kim et al. 2013b). First, model PNs with a higher intrinsically excitability (types B and C) were more likely to develop potentiated CS responses as a result of fear conditioning. Second, modeling CREB overexpression or downregulation by converting randomly selected subgroups of less excitable PNs (type A) into more excitable ones (types B and C) or vice versa did not alter the number of plastic PNs (Kim et al. 2013b), as seen experimentally (Han et al. 2007). Third, an analysis of intrinsic connectivity revealed that a major substrate of this competition is the differential distribution of excitatory connections between PNs and the amount of di-synaptic inhibition they generate in other PNs. These factors conspire to enhance the likelihood that some PNs will fire more strongly to the CS and US at the expense of others. Last, neuromodulators played an important role in shaping the tone responsiveness of LA neurons during fear conditioning, with preferentially larger numbers of DA and NE receptors on plastic vs. non-plastic model cells (Kim et al. 2013a). Neuromodulation thus has a direct impact on plasticity and competition, and the specifics of these relationships remain to be unraveled.

Building on these earlier findings, the present study examined the role of plasticity at different types of intra-LAd synapses in this competitive process. To examine this question, we clamped the weight of different types of intra-LAd synapses at habituation level and compared the number of plastic PNs to that seen in control conditions. Blocking plasticity at PN–PN synapses produced a major decrease in CS responsiveness and a reduction in the number of plastic PNs. In contrast, blocking plasticity at ITN to PN synapses produced a large potentiation of CS responses and an increase in the number of plastic PNs.

Importantly, plasticity of interneuronal and PN to PN connections was differentially expressed depending on whether the target PN was a plastic cell or not, with the former

invariably expressing the largest changes in activity-dependent plasticity. Together, these observations indicate that activity-dependent potentiation of interneuronal synapses and PN–PN connections plays a major role in shaping the competitive assignment of PNs to the fear memory trace.

Plasticity of intrinsic LAd synapses contributes to the specificity of conditioned fear

In operant and classical conditioning paradigms, it was observed that conditioned responses are largest for the original CS and decrease gradually with increasingly dissimilar stimuli (Honig and Urcuioli 1981; Domjan 2006; Schechtman et al. 2010; Resnik et al. 2011). In our simulations, we examined this question for two types of stimulus conditions: with multiple CSs of the same or different modalities. The former reproduces the approach commonly observed in experimental studies where two stimuli of the same modalities (e.g., pure tones of different frequencies or white noise; Duvarci et al. 2009; Herry et al. 2008) are used as CSs. The latter reproduces another common experimental approach where two CSs of different modalities are used (tones, or light stimuli; Headley and Weinberger 2014). Consistent with prior observations, our LAd model exhibited an interesting behavior whereby stimuli that were progressively less similar to the original CS recruited a gradually decreasing proportion of PNs, a phenomenon hereafter termed gradient of generalization. Also, the generalization gradient was wider for stimuli of the same than different sensory modalities. Yet, in both cases, suppressing plasticity of interneuronal synapses produced a marked reduction in the CS specificity of PN recruitment. Analysis of the responsiveness of PNs that did not receive direct inputs about the original CS revealed that in the absence of plasticity at interneuronal synapses, potentiation of PN to PN synapses contributed to widen the stimulus generalization gradient. Together these modeling experiments suggest that the competitive synaptic mechanisms that govern the assignment of PNs to the memory trace also determine CS specificity. In particular, it appears that the configuration of synaptic plasticity imprinted in the LAd network during conditioning not only limits the number of PNs that participate in memory storage, but also ensures specificity of the memory trace.

Are there precedents in the experimental literature for interneuronal synaptic plasticity?

Compared to the vast amount of experimental data on PNs (reviewed in Pape and Paré 2010), relatively few experimental studies have examined properties of activity-dependent plasticity in ITNs (reviewed in Spanpanato et al. 2011). Nevertheless, the available evidence indicates that they too exhibit synaptic plasticity. On the input side, it was reported that both thalamic and cortical inputs onto ITNs of LA can undergo activity-dependent long-term potentiation (LTP; Mahanty and Sah 1998; Szinyei et al. 2000; Bauer and LeDoux 2004; Polepalli et al. 2010). In particular, a form of AMPA-dependent LTP was reported as some ITNs express Ca^{2+} -permeable AMPA receptors because they lack the GluR2 receptor subunit (Mahanty and Sah 1998; Szinyei et al. 2000; Polepalli et al. 2010). On the output side, it was also shown that ITN synapses onto PNs can undergo activity-dependent plasticity via a Ca^{2+} -dependent mechanism (Bauer and LeDoux 2004). This was demonstrated by electrically stimulating ITNs within LA in the presence of glutamate receptor antagonists. The resulting potentiation was expressed postsynaptically, but the underlying mechanisms are currently unknown (Bauer and LeDoux 2004; Sigurdsson et al. 2007). Thus, while activity-dependent plasticity at interneuronal synapses is under-studied,

there are precedents in the literature for the competitive mechanisms disclosed in our modeling experiments.

Conclusions

Overall, our results indicate that several interacting factors govern the competitive assignment of PNs to fear memory traces. A first factor is their intrinsic excitability: more excitable PNs are at a competitive advantage compared to less excitable ones. A second factor is the differential distribution of excitatory connections between PNs and the amount of di-synaptic inhibition they generate in other PNs. Combined with differences in intrinsic excitability, this differential connectivity enhances the likelihood that some PNs (“winners”) will fire more strongly in response to the CS and US at the expense of others (“losers”). A third factor is the potentiation of intrinsic LAd synapses. Potentiation of interneuronal synapses further reduces the likelihood that the loser PNs will increase their CS responsiveness as a result of conditioning. Indeed, potentiation of PN–PN connections, because it is stronger between winner PNs, promotes a banding effect where plastic PNs gang up to suppress plasticity in loser PNs via ITNs.

Importantly, these dynamic interactions also determine the CS specificity of PN recruitment. Given that stimulus generalization is a hallmark of anxiety disorders, understanding mechanisms of plasticity at intrinsic LAd synapses emerges as a key challenge for future experimental studies. Identifying drugs that can alter the efficacy of these synapses or their activity-dependent potentiation constitute promising strategies to treat anxiety disorders.

Acknowledgments

This research was supported in part by grants from the National Institute of Mental Health (MH083710 to DP and MH087755 to SSN).

Appendix

Here, we list additional information related to methods, including the mathematical equations, implementation of the effects of neuromodulators, and the iterative procedures. All model runs were performed using parallel NEURON (Carnevale and Hines 2006) running on a Beowulf supercluster with a time step of 50 μ s. Simulation output was analyzed using MATLAB.

Mathematical equations for voltage-dependent ionic currents

The equation for each compartment (soma or dendrite) followed the Hodgkin–Huxley formulations (Byrne and Roberts 2004) in Eq. (1),

$$C_m dV_s/dt = -g_L(V_s - E_L) - g_c(V_s - V_d) - \sum I_{cur,s}^{int} - \sum I_{cur,s}^{syn} + I_{inj} \quad (1)$$

where V_s/V_d are the somatic/dendritic membrane potential (mV), $I_{cur,s}^{int}$ and $I_{cur,s}^{syn}$ are the intrinsic and synaptic currents in the soma, I_{inj} is the electrode current applied to the soma, C_m is the membrane capacitance, g_L is the conductance of leak channel, $g_c = 1/R_a$ is the

coupling conductance between the soma and the dendrite (similar term added for other dendrites connected to the soma), and E_L is the leak reversal potential. Eq. (1) represents a current balance, with the sum of all currents being equal to the injected current. The term on the left represents the capacitance current. The intrinsic current $I_{\text{cur},s}^{\text{syn}}$ was modeled as $I_{\text{cur},s}^{\text{int}} = g_{\text{cur}} m^p h^q (V_s - E_{\text{cur}})$, where g_{cur} is its maximal conductance, m its activation variable (with exponent p), h its inactivation variable (with exponent q), and E_{cur} its reversal potential (a similar equation is used for the synaptic current $I_{\text{cur},s}^{\text{syn}}$ but without m and h). The kinetic equation for each of the gating variables x (m or h) takes the form

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

where x_{∞} is the steady state gating voltage- and/or Ca^{2+} -dependent gating variable and τ_x is the voltage- and/or Ca^{2+} -dependent time constant. The equation for the dendrite follows the same format with 's' and 'd' switching positions in Eq. (1). Details related to the model, including types of channels and parameter values are provided in Tables 2 and 3.

Mathematical equations for synaptic currents

Excitatory transmission was mediated by AMPA/NMDA receptors, and inhibitory transmission by GABA_A receptors. The corresponding synaptic currents were modeled by dual exponential functions (Durstewitz et al. 2000), as shown in Eqs. (3)–(5),

$$I_{\text{AMPA}} = w(t) \times G_{\text{AMPA}} \times (V - E_{\text{AMPA}}) \quad (3)$$

$$I_{\text{NMDA}} = w \times G_{\text{NMDA}} \times (V - E_{\text{NMDA}}) \quad (4)$$

$$I_{\text{GABA}} = w(t) \times G_{\text{GABA}} \times (V - E_{\text{GABA}}) \quad (5)$$

where V is the membrane potential (mV) of the compartment (dendrite or soma) where the synapse is located and w is the adjustable synaptic weight for the synapse (w was variable for AMPA and GABA synapses, but fixed for NMDA synapses), and G_X is the conductance of the particular synapse (see Sect. “Calcium dynamics and Hebbian learning” for expressions for G_X). The synaptic reversal potentials were $E_{\text{AMPA}} = E_{\text{NMDA}} = 0$ mV and $E_{\text{GABA}} = -75$ mV (Durstewitz et al. 2000).

Calcium dynamics and Hebbian learning

Intracellular calcium concentration, $[\text{Ca}^{2+}]_{\text{pool}}$, was regulated by a simple first-order differential equation shown in Eq. (6) (Warman et al. 1994),

$$\frac{d[\text{Ca}^{2+}]_{\text{pool}}}{dt} = -f \frac{I_{\text{Ca}}^X}{zFV} + \frac{[\text{Ca}^{2+}]_{\text{rest}} - [\text{Ca}^{2+}]_{\text{pool}}}{\tau_{\text{Ca}}} \quad (6)$$

where I_{Ca}^X is the relevant current (NMDA, AMPA, or GABA) contributing to the pool (refer to Eqs. 8–10); f is the fraction of the Ca^{2+} component of the relevant current ($f = 0.024$); volume $V = (4/3 \times \pi \times r_{pool}^3)$ with $r_{pool} = 0.9086$ mm, $z = 2$ is the valence of the Ca^{2+} ion; F is the Faraday constant; and τ_{Ca} is the time constant associated with Ca^{2+} removal. The resting Ca^{2+} concentration was $[Ca^{2+}]_{rest} = 50$ nmol/l (Durstewitz et al. 2000).

The biophysical Hebbian rule was implemented by adjusting the synaptic weight $w(t)$ in synaptic conductances (Eqs. 3, 5) using Eq. (7),

$$\Delta w_j = \eta \left([Ca^{2+}]_j \right) \Delta t \left(\lambda_1 \Omega \left([Ca^{2+}]_j \right) - \lambda_2 w_j \right) \quad (7)$$

where η is the Ca^{2+} -dependent learning rate and Ω is a Ca^{2+} -dependent function with two thresholds (θ_d and θ_p ; $\theta_d < \theta_p$) (for details, see Li et al. 2009); λ_1 and λ_2 represent scaling and decay factors, respectively; the local calcium level at synapse j is denoted by $[Ca^{2+}]_j$ and t is the simulation time step. With this learning rule, the synaptic weight decreases when $\theta_d < [Ca^{2+}]_j < \theta_p$, and increases when $[Ca^{2+}]_j > \theta_p$, with modulation by the decay term $\lambda_2 w_j$.

Concentration of calcium pools

The concentration of the calcium pool at synapse j followed the dynamics in Eq. (6), with $f_j = 0.024$ (Warman et al. 1994), $\tau_j = 50$ ms (Shouval et al. 2002b), V is the volume of a spine head with a diameter of 2 μ m (Kitajima and Hara 1997). All the synaptic weights were constrained by upper (W_{max}) and lower (W_{min}) limits (Li et al. 2009). Maximum (f_{max}) and minimum (f_{min}) folds were specified for each modifiable synapse so that $W_{max} = f_{max} \times w(0)$ and $W_{min} = f_{min} \times w(0)$.

Excitatory synapses onto principal cells

For tone-PN, and PN–PN connections, the calcium influx I_{Ca}^N which determines learning was estimated as in Li et al. (2009), using Eq. (8), $I_{Ca}^N = P_0 G_{NMDA} (V - E_{Ca})$, where

$$G_{NMDA} = g_{NMDA,max} \times STP_{NMDA} \times s(V) \times r_{NMDA}$$

$$r_{NMDA} = \alpha T_{max,NMDA} \times ON_{NMDA} \times (1 - r_{NMDA}) - \beta_{NMDA} \times r_{NMDA} \quad (8)$$

where $P_0 = 0.015$, the reversal potential of calcium $E_{Ca} = 120$ mV; the maximal conductance for NMDA current $g_{NMDA,max} = 0.5$ nS; STP_{NMDA} is the short-term plasticity factor (see Sect. “Short-term presynaptic plasticity”); the voltage-dependent variable $s(V)$ which implements the Mg^{2+} block was defined as: $s(V) = [1 + 0.33 \exp(-0.06 V)]^{-1}$ (Zador et al. 1990); r_{NMDA} is the fraction of bound receptors; $\alpha T_{max,NMDA} = 0.2659$ /ms and $\beta_{NMDA} = 0.0008$ /ms are specific synaptic current parameters; and $ON_{NMDA} = 1$ if the NMDA receptor is open, else 0.

Excitatory synapses onto interneurons

For tone-interneuron, and principal cell–interneuron connections, the calcium influx (used for learning) at the excitatory synapses on interneurons occurs through both NMDA and AMPA receptors Eqs. (8) and (9) (details in Li et al. 2009) with $P_0 = 0.001$ in Eq. (9).

$$I_{Ca}^A = P_0 w G_{AMPA} (V - E_{Ca}) / w(0), \text{ where}$$

$$G_{AMPA} = g_{AMPA, \max} \times STP_{AMPA} \times r_{AMPA}$$

$$r_{AMPA} = \alpha T_{AMPA} \times ON_{AMPA} \times (1 - r_{AMPA}) - \beta_{AMPA} \times r_{AMPA} \quad (9)$$

where the parameters are as defined in Eq. (8), with $P_0 = 0.001$; the maximal conductance for AMPA current $g_{AMPA, \max} = 1$ nS; r_{AMPA} is the fraction of bound receptors; $\alpha T_{\max AMPA} = 3.8142/\text{ms}$ and $\beta_{AMPA} = 0.1429/\text{ms}$, and $w(0)$ is the initial weight of the synapse. 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. 2002a; Li et al. 2009).

Inhibitory synapses onto principal cells

Several different mechanisms have been reported for potentiation at GABAergic synapses in other brain regions (e.g., Gaiarsa et al. 2002). A rise in postsynaptic intracellular Ca^{2+} concentration seems to be required in most mechanisms 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 that is dependent on stimulation of GABA receptors (Gaiarsa et al. 2002). Thus, both presynaptic activity (GABA receptor stimulation 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 G_{ABA} conductance (Li et al. 2009). Hence, we modeled this simplified process by considering Ca^{2+} release from the internal stores into a separate Ca^{2+} pool, using an equation similar to that for the AMPA/NMDA case cited above, as shown in Eq. (10), $I_{Ca}^G = P_0 G_{GABA} (V - E_{Ca})$, where

$$G_{GABA} = g_{GABA, \max} \times STP_{GABA} \times r_{GABA}$$

$$r_{GABA} = \alpha T_{\max GABA} \times ON_{GABA} \times (1 - r_{GABA}) - \beta_{GABA} \times r_{GABA} \quad (10)$$

with the parameters again as defined in Eq. (8), with $P_0 = 0.01$; the maximal conductance for GABA current $g_{\text{GABA,max}} = 0.6 \text{ nS}$; r_{GABA} is the fraction of bound receptors; $\alpha T_{\text{maxGABA}} = 7.2609/\text{ms}$ and $\beta_{\text{GABA}} = 0.2667/\text{ms}$.

The current 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. I_{Ca}^G , together with portion of postsynaptic voltage-dependent calcium current (I_{Ca}), contributed towards plasticity. The total calcium influx into the pool for learning was $I_{\text{Ca}}^G + 0.01 I_{\text{Ca}}$ for such synapses (Li et al. 2009).

Intrinsic connectivity in LAd

By comparing the responses of LAd cells to local applications of glutamate at various positions with respect to recorded neurons, Samson and Paré (2006) inferred general principles of connectivity among principal cells, as well as between local-circuit and principal neurons. In particular, Samson and Paré (2006) determined that excitatory connections between principal cells prevalently run ventrally and medially with significant rostrocaudal divergence. In contrast, inhibitory connections prevalently run mediolaterally in the horizontal plane and have no preferential directionality in the coronal plane. Samson and Paré (2006) also recognized that principal LAd neurons located along the external capsule (in the “shell” region of LA) form different connections than those found more medially (in the “core” region of LA; shell thickness of $100 \mu\text{m}$). In the shell region, inhibitory neurons only affect nearby principal neurons, whereas excitatory connections between principal cells are spatially less limited. While not providing precise connectivity data, this information could be used to infer critical estimates about directionality and ratio of excitation to inhibition. The directionality information from the Samson and Paré (2006) study, described in the two paragraphs that follow, were implemented in the model, using a third of the connectivity numbers. Such an implementation for one model run showed that a model principal cell had, on average, 21.4 mono- and 40.6 di-synaptic excitatory inputs, and 20.4 mono-synaptic inhibitory inputs.

Coronal plane

Within a $100\text{-}\mu\text{m}$ coronal slice, principal shell neurons excite shell cells located $300\text{--}400 \mu\text{m}$ more ventrally with 10 % probability (mono-synaptic connectivity). Core to shell connections occur with a much lower probability (2 %). In addition, principal shell neurons are inhibited by more dorsally located interneurons (23 % connectivity for cells within $300 \mu\text{m}$). In the core region, excitatory connections between principal cells have a greater extent in the lateromedial direction ($50\text{--}800 \mu\text{m}$, 2–6 %, connectivity) than in the mediolateral direction ($50\text{--}200 \mu\text{m}$, 5 % connectivity), whereas inhibitory connections have similar strengths in all directions (interneurons formed inhibitory inputs with 10–24 % of principal cells at a distance of $50\text{--}600 \mu\text{m}$).

Horizontal plane

Within a $100\text{-}\mu\text{m}$ horizontal slice, connections were set in the following manner. Connection probability increased with distance for lateromedial connections, and the opposite for

mediolateral connections. As to inhibitory connections, they prevalently run in the mediolateral direction with 8–20 % connectivity in the range 50–600 μm and 5–20 % connectivity in the lateromedial direction within a distance of 50–600 μm . Principal cells project to all interneurons within a spherical radius of 100 μm . Figures related to these connectivity configurations can be found in Kim et al. (2013a; Fig. 2).

Short-term presynaptic plasticity

Short-term plasticity was implemented as follows (Varela et al. 1997; Hummos et al. 2014): For facilitation, the factor F was calculated using Eq. (11).

$$\tau_F \times \frac{dF}{dt} = 1 - F; F(0) = 1, \text{ and it is constrained to be } \geq 1 \quad (11)$$

After each stimulus, F was multiplied by a constant f (> 1) representing the amount of facilitation per presynaptic action potential, and updated as $F \rightarrow F \times f$. Between stimuli, F recovered exponentially back toward 1. A similar scheme was used to calculate the factor D for depression,

$$\tau_{Di} \times \frac{dD_i}{dt} = 1 - D_i; D_i(0) = 1, \text{ and it is constrained to be } \leq 1 \quad (12)$$

where i varied from 1 to the number of depression factors, permitting use of different time constants. After each stimulus, D_i was multiplied by a constant d_i (< 1) representing the amount of depression per presynaptic action potential, and updated as $D_i \rightarrow D_i \times d_i$. Between stimuli, D_i recovered exponentially back toward 1. We modeled depression using two factors d_1 and d_2 with d_1 being fast and d_2 being slow subtypes. The parameters for the short-term plasticity models, the initial weights and other learning parameters for the synapses are listed in Table 4.

Modeling neuromodulator effects

Blockade of DA and NE has been shown to impair the acquisition of fear memory in LA. These have been modeled by adjusting both intrinsic and synaptic parameters based on experimental reports (see Kim et al. 2013a for details) as shown in Table 5.

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Fig. 1.

Model properties and training paradigm. **a** Scheme of the LAd network model with 800 PNs (red and green dots, 400 each, represent PNs in LAd and LAdv, respectively) and 200 ITNs (black dots). Model PNs were populated randomly in the horn-shaped tri-dimensional structure with dimensions of 800 μm in the rostral–caudal, 800 μm in the ventral–dorsal, and 400 μm in the medial–lateral axes. **b** Fear conditioning protocol used in our modeling studies. The first trial block of extinction was used for the recall test

Fig. 2.

Impact of training on the responsiveness of PNs in the absence or presence of plasticity at intrinsic LAd synapses. **a** Raster showing CS-evoked responses (each *dot* represents a spike) before (*left*) and after (*right*) conditioning in plastic (*red*) and non-plastic (*blue*) cells. *x*-axis represents time. *y*-axis indicates cell number (each line is a different cell). Cells 1–25 are transient plastic (displaying increases that last for only a few CSs), 26–50 are long-term plastic (displaying increases that resist extinction training; Repa et al. 2001), and 51–100 are non-plastic. Both plastic and non-plastic cells start with similar CS-evoked responses before conditioning. However, after conditioning, plasticity causes much higher CS-evoked responses in plastic cells. **b** Average ($\pm$ SEM) tone responses in *z* scores (*y*-axis; blocks of 4 trials) during the various phases of the training protocol (*x*-axis). Various cases are considered in **b1** and **b2** (MANOVA, $F = 19.33$, $p < 0.0001$). **b1** Control (*solid circles*); or with inactivation of plasticity at PN to PN synapses (*empty diamonds*). A large decrease in CS responsiveness was observed during both conditioning (last trial block, paired *t* test, $p < 0.0001$) and the recall test (first trial block, unpaired *t* test, $p < 0.0001$). **b2** Control (*solid circles*); or with inactivation of plasticity at PN to ITN synapses (*empty triangles*), which produced a minor and non-significant decrease in CS responsiveness during conditioning (last trial block, unpaired *t* test $p = 0.6769$), but a minor increase during the recall test (first trial block, unpaired *t* test, $p = 0.6968$); or with inactivation of plasticity at ITN to PN synapses (*empty diamonds*), which produced a large potentiation of CS responses (conditioning, last trial block, unpaired *t* test, $p < 0.0001$; recall, first trial block, unpaired *t*

test, $p < 0.0001$). Tone responses are computed as spikes per tone within 300 ms of tone onset. **c** Changes (average $\pm$ SEM) in the weights (y -axis) of various types of synapses (x -axis) for representative groups of 40 PNs of persistent plastic (*dark gray*) and non-plastic (*pale gray*) types. Conditioning produced significant alterations in the weight of all synapse types and it was differentially expressed in plastic vs. non-plastic cells (t tests: tone-PN, $p < 0.0001$; PN-PN, $p < 0.0001$; ITN-PN, $p < 0.0001$; PN-ITN, $p < 0.0001$)

Fig. 3.

Role of plasticity at intrinsic amygdala synapses in maintaining stimulus specificity. **a** For specificity studies, 50 % of PNs (*x*-axis) received CS tone and US shock inputs. Five different tones, CS1–5, were used, and the cells they targeted are indicated by gray shading. For instance, CS1/+ targeted cells principal cells 1–400. CS2–5 exhibited a graded decline in similarity compared to CS1. CS2–4 were used for testing purposes only. CS5/– was also presented with unpaired US during conditioning, and targeted precisely the 50 % of pyramidal cells not targeted by CS1/+. Tone and shock inputs were distributed randomly and

independently to 70 % of the ITNs. *Yellow squares* cells tested in a later experiment, described in Fig. 4. **b, c** Impact of plasticity at intrinsic LAd synapses on stimulus generalization for the intra- (**b** plastic and non-plastic cells combined MANOVA, $F=237.34$, $p<0.0001$; plastic cells, MANOVA, $F=51.81$, $p<0.0001$; non-plastic cells, MANOVA, $F=64.11$, $p<0.0001$) and inter-modal (**c** plastic and non-plastic cells combined MANOVA, $F=131.33$, $p<0.0001$; plastic cells, MANOVA, $F=27.93$, $p<0.0001$; non-plastic cells, MANOVA, $F=32.87$, $p<0.0001$) cases. See text for t test results. Average ($\pm$ SEM) tone responses of plastic (**b1, c1**) and non-plastic (**b2, c2**) pyramidal cells to the different CS before (*circles*) and after (*triangles*) conditioning (*red triangles*, control plasticity; *blue triangles* no plasticity at interneuronal synapses). Tone responses were computed as spikes per tone within 300 ms of tone onset, which was then converted to z scores. CS for intra-modal and CS* for inter-modal

Fig. 4. Plasticity at intrinsic LAd synapses modulates stimulus generalization. **(a, b)** Average ($\pm$ SEM) tone responses of non-overlapping type B and C cells (*yellow squares* in Fig. 3a) for the intra- **(a)** and inter-modal **(b)** cases. **a1, b1** responses to CS1 and CS*1, respectively. **a2, b2** responses of the same cells to the CS indicated on the *x*-axis. In all four panels, $n = 25\%$ of 140, 98, and 53 from *left to right* (see text). All graphs show the responsiveness of the non-overlapping PNs before (*gray circles*) or after conditioning (*red triangles*), as well as with interneuronal plasticity suppressed (*blue triangles*). CS for intra-modal and CS* for inter-modal

Table 1

Plastic cell numbers and types when different intra-amygdala plasticity components are inactivated

Model case (cells: A-400; B-240; C-160)	Cells receiving both tone and shock ($n = 449$)		Cells receiving only tone or only shock or neither ($n = 351$)		Total number of plastic cells (out of 800)
	Plastic A/B/C cells	Non-plastic A/B/C cells	Plastic A/B/C cells	Non-plastic A/B/C cells	
Control case	3/94/66	221/43/22	4/26/18	172/77/54	211
No PN-PN plasticity	3/70/54	221/67/34	2/11/11	174/92/61	151
No ITN-PN plasticity	3/103/74	221/34/14	6/28/22	170/75/50	236
No PN-ITN plasticity	1/90/63	223/47/25	0/26/17	176/77/55	197
No ITN-PN and PN-ITN plasticity	5/106/71	219/31/17	0/35/26	176/68/46	243

Type A, B, and C principal neurons (PNs) correspond to regular spiking PNs with high (A), intermediate (B) and low (C) frequency adaptations. A cell is plastic if $z > 5.5$ in any of the conditioning trial blocks. See text for t test results

Table 2

Gating variables for ion channels used in the single cell models

Current type	Gating variable	α	β	x_{∞}	τ_x (ms)
I_{Na}	$p = 3$	$\frac{-0.4(V+30)}{\exp[-(V+30)/7.2] - 1}$	$\frac{0.124(V+30)}{\exp[(V+30)/7.2] - 1}$	$\alpha/(\alpha + \beta)$	$\frac{0.6156}{\alpha + \beta}$
	$q = 1$	$\frac{0.03(V+45)}{\exp[-(V+45)/1.5] - 1}$	$\frac{0.01(V+45)}{\exp[(V+45)/1.5] - 1}$	$\frac{1}{\exp\left[\frac{V+50}{4}\right] + 1}$	$\frac{0.6156}{\alpha + \beta}$
I_{DR}	$p = 1$	$\exp[-0.1144(V - 13)]$	$\exp[-0.08(V - 13)]$	$\frac{1}{1 + \alpha}$	$\frac{30.78\beta}{1 + \alpha}$
I_{H1}	$p = 1$	$\exp[0.08316(V + 75)]$	$\exp[0.033264(V + 75)]$	$\frac{1}{\exp\left[\frac{V+81}{8}\right] + 1}$	$\frac{\beta}{0.0473(1 + \alpha)}$
I_{M1}	$p = 2$	$\frac{0.016}{\exp[-(V+52.7)/23]}$	$\frac{0.016}{\exp[(V - 52.7)/18.8]}$	$\alpha/(\alpha + \beta)$	$1/(\alpha + \beta)$
I_{Ca}	$p = 2$	$\frac{-15.69(V - 81.5)}{\exp[-(V - 81.5)/10] - 1}$	$\frac{0.29\exp[-V/10.86]}{\exp[-(V - 81.5)/10] - 1}$	$\alpha/(\alpha + \beta)$	$1/(\alpha + \beta)$
	$q = 1$	-	-	$\frac{0.001}{0.001 + [Ca]_i}$	-
I_A					
Soma	$p = 1$	$\exp\left[0.0381(V - 11) \left[-1.5 - \frac{1}{\exp[(V+40)/5+1]}\right]\right]$	$\exp\left[0.021(V - 11) \left[-1.5 - \frac{1}{\exp[(V+40)/5+1]}\right]\right]$	$\frac{1}{1 + \alpha}$	$\frac{6.4826\beta}{1 + \alpha}$
	$q = 1$	$\exp[0.1144(V + 56)]$	-	$\frac{1}{1 + \alpha}$	$\frac{0.26(V + 50)}{1 + \alpha}$
Dend	$p = 1$	$\exp\left[-0.0687(V + 1) + \frac{0.0382(V + 1)}{\exp[(V + 40)/5 + 1]}\right]$	$\exp\left[-0.026793(V + 1) + \frac{0.014898(V + 1)}{\exp[(V + 40)/5 + 1]}\right]$	$\frac{1}{1 + \alpha}$	$\frac{3.2413\beta}{1 + \alpha}$

Current type	Gating variable	α	β	x_{∞}	τ_x (ms)
I_{SAHP}	$p = I$	$\frac{0.0048}{\exp(-5\log_{10}([Ca]_{i2}) - 17.5)}$	$\frac{0.012}{\exp(2\log_{10}([Ca]_{i2}) + 20)}$	$\alpha/(\alpha + \beta)$	1000–2000
	$q = I$	$\exp[0.1144(V + 56)]$	$\exp[0.1144(V + 56)]$	$\frac{1}{1 + \alpha}$	0.26($V + 50$)

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Table 3

Maximal conductance densities of ion channels

Conductance (ms/cm ²)	<i>I</i> _{Na}	<i>I</i> _{DR}	<i>I</i> _M	<i>I</i> _H	<i>I</i> _{Ca}	<i>I</i> _A	<i>I</i> _{SAHP}	τ_{Ca}
Principal cell-type A								
Soma	54	3	0.55	–	0.2	1.43	–	–
Dend	27	3	0.55	0.0286	0.2	0.32	7	1000
Principal cell-type B								
Soma	54	3	0.39	–	0.2	1.43	–	–
Dend	27	3	0.39	0.0286	0.2	0.32	0.45	1000
Principal cell-type C								
Soma	54	3	0.4	–	0.2	1.43	–	–
Dend	27	3	0.4	0.0286	0.2	0.32	0.36	1000
Interneuron								
Soma	35	8	–	–	–	–	–	–
Dend	10	3	–	–	–	–	–	–

Table 4

Model synaptic strengths and learning parameters

Long-term postsynaptic plasticity									
Connection	Initial weight	$f_{\max}$ ($f_{\min} = 0.8$ for all)	Learning factor		Ca ²⁺ threshold				
			Scaling	Decay	Low	High			
Tone to pyr (thalamic)	5.5	3.5	80	0.04	0.40	0.53			
Tone to pyr (cortical)	6	3.5	10	0.04	0.40	0.53			
Tone to inter (thalamic)	4.5	4	5	0.01	0.45	0.5			
Tone to inter (cortical)	4	4	20	0.01	0.45	0.5			
PyrD to pyrD	1	4	80	0.03	0.3	0.55			
PyrD to pyrV	1	4	10	0.03	0.3	0.55			
PyrV to pyrD	1	4	80	0.03	0.3	0.55			
PyrV to pyrV	1	4	10	0.03	0.3	0.55			
InterD to pyrD	4.5	4	4	0.01	0.47	0.52			
InterD to pyrV	4.5	4	2	0.01	0.47	0.52			
InterV to pyrD	4.5	4	4	0.01	0.47	0.52			
InterV to pyrV	4.5	4	2	0.01	0.47	0.52			
PyrD to interD	1.5	3	3	0.01	0.4	0.45			
PyrD to interV	1.5	2	2	0.01	0.4	0.45			
PyrV to interD	1.5	3	3	0.01	0.4	0.45			
PyrV to interV	1.5	3	2	0.01	0.4	0.45			

Short-term presynaptic plasticity					
Connection	Short-term dynamics	Parameters			
		Lower limit for D	d_1/d_2	τ_{D1}/τ_{D2} (ms)	
Inter-pyr	Depression	0.6	0.9/0.95	40/70	
Pyr-pyr	Depression	0.5	0.9/0.95	40/70	
Pyr-inter	Depression	0.7	0.9/0.95	40/70	

Shock synapses do not potentiate (weight = 10 for synapses onto both principal cells and interneurons, in thalamic and cortical pathways)

Table 5

Variations in maximal conductances to model neuromodulator effects

NM	Receptor	Channel	Low level of NM (during trials 2–10 of conditioning)	High level of NM (during trials 11–16 of conditioning and trials 1–4 of extinction)	Highest level of NM (during shock)
Dopamine	D ₁ Rs (low affinity)	I_{Kdr}	–	Decrease g_{Kdr} by 10 %	Decrease g_{Kdr} by 20 %
		AP threshold	–	Change activation of Na ⁺ channel by –0.5 mV	Change activation of Na ⁺ channel by –1.5 mV
	D ₂ Rs (high affinity)	NMDA (pyr–pyr)	–	Decrease g_{NMDA} by 5 %	Decrease g_{NMDA} by 20 %
		GABA (interneuron–pyr)	–	Increase g_{GABA} by 40 %	Increase g_{GABA} by 60 %
Norepinephrine	NE- α (high affinity)	Input resistance	Decrease g_{leak} by 5 %	Decrease g_{leak} by 10 %	Decrease g_{leak} by 20 %
		GABA (interneuron–pyr)	Decrease g_{GABA} by 20 %	Decrease g_{GABA} by 20 %	Decrease g_{GABA} by 30 %
		NMDA (thalamic input to interneuron)	Increase g_{NMDA} by 5 %	Increase g_{NMDA} by 10 %	Increase g_{NMDA} by 30 %
	NE- β (low affinity)	NMDA (cortical input to principal cells)	Decrease g_{NMDA} by 10 %	Decrease g_{NMDA} by 30 %	Decrease g_{NMDA} by 30 %
		NMDA (thalamic input to principal cells)	Decrease g_{NMDA} by 5 %	Decrease g_{NMDA} by 10 %	Decrease g_{NMDA} by 20 %
		I_{sAHP}	–	Reduce g_K , $sAHP$ by 20 %	Reduce g_K , $sAHP$ by 30 %
		NMDA (cortical input to principal cells)	–	Increase g_{NMDA} by 20 %	Increase g_{NMDA} by 50 %
		NMDA (cortical input to interneurons)	–	Decrease g_{NMDA} by 20 %	Decrease g_{NMDA} by 30 %