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Cellular Mechanisms in the Amygdala Involved in Memory of Fear Conditioning

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1. Introduction

Natural disaster can occur in all countries of the world. Now Japan suffers triple disasters: massive earthquake, vast tsunami and the world's worst nuclear crisis. The victims with fear in their minds deeply might fall into fear-related disorders in future.

Fear is a conserved emotion in response to danger and triggers some defensive mechanisms for adapting to threatening events for survival. Moreover, fear can lead to a number of anxiety disorders when aberrantly expressed. Defining the cellular and synaptic mechanisms underlying fear memory will enhance our understanding of biological mechanism to enemies, as well as our ability to develop treatments for individual afflicted with anxiety disorders, including posttraumatic stress disorder (PTSD).

The discovery of long-term potentiation (LTP), a phenomenon in which repetitive stimulation of afferent fibers results in a prolonged enhancement of synaptic strength provided the cellular mechanism to explain learning and memory formation. While LTP has been described at many synapses in different brain regions, it has been studied most intensively at glutamatergic synapses in the mammalian hippocampus. However, linking changes in synapse transmission of the hippocampus to specific behavioral changes has proved to be difficult largely because of the complex behaviors which the hippocampus is involved in.

We have to apply a simpler system to investigate such changes. Auditory fear conditioning induces LTP-like enhancement of synaptic transmission in cortical input to the principal neurons of the amygdala that both mimic and occlude LTP in acute slice induced with electrical stimulation, thus providing a simple linkage between changes of synaptic strength and behavior in auditory fear conditioning (Tsvetkov et al., 2002). The conservation of the anatomy and physiology of the amygdala between species allows studies in different animals to get potential implications for fear memory and associated disorders in human.

In the rat, one of the most important researches is the finding that auditory fear conditioning is linked to the persistent strength at glutamatergic synapse in both thalamic and cortical inputs to the principal cell of the amygdala (Rogan et al., 1997; McKernan & Shinnick-Gallagher, 1997).

It has been widely accepted that LTP in the amygdala is the basic factor in fear conditioning and better understanding mechanism for LTP will leads the mechanism for fear conditioning (Sah et al., 2008).

The aim of this chapter is to improve our understanding of cellular mechanism, in particular, synaptic mechanism underlying fear memory and permit rational development of better therapeutic treatments for PTSD and other anxiety disorders. We will focus on the lateral nucleus of amygdala (LA) because molecular and synaptic changes in this area have been shown to make essential contributions to the fear memory formation, storage, and expression of the learned fear (LeDoux, 1998).

To get a better understanding synaptic mechanism underlying the learned fear, we firstly will review auditory fear conditioning in detail and the basic anatomy and properties of synaptic transmission of the LA. Secondly we will summarize the molecular mechanisms that contribute to synaptic plasticity in the auditory fear conditioning.

Although neutral and aversive information enters via both thalamic and cortical inputs to the LA, the individual role of both inputs are still debated. Moreover there are a lot of studies about pre- and postsynaptic modifications in the induction and expression of LTP during auditory fear conditioning (Shin et al., 2010; Rumpel et al., 2005).

However, the specific roles of both modifications remain elusive.

Finally we review recent studies and discuss the two questions above.

2. The involvement of the amygdala in the learned fear

The most detailed behavioral studies from bilateral lesion of the primate temporal lobe suggest that the temporal lobe including amygdala is involved in processing emotion (Klüver & Bucy, 1937). In this study, monkeys with bilateral temporal lobe lesions tried to eat inedible objects, to copulate with same-sex partners or even with other species, and lost their fear of snakes. The key feature of this phenomenon, called psychic blindness by Klüver and Bucy (1937), was seemed to lose their emotional implications despite their fine visual perception. In experiment using functional magnetic resonance imaging, human amygdalae was preferentially activated by emotional stimuli such as fearful faces (Breiter et al. 1996). These reports suggested that the amygdala plays an important role on processing emotion in response to aversive stimulus.

In rodent experiment, anatomical tracing and lesion studies indicated the importance of the LA for encoding fear memory. In particular, bilateral infusion of N-methyl-D-aspartate (NMDA) receptor antagonist, D-2-amino-5-phosphonovaleric acid (D-AP5) into the rat LA decreased the amount of the learned fear (Miserendino et al., 1990). This result suggests two

possibilities: the first is that we can confirm that the amygdala is the very important region which fear memory could be stored. The second is that NMDA receptor-dependent plasticity change can occur in the amygdala during fear memory formation. In 1996, two teams indicated that auditory fear conditioning is associated with persistent synaptic enhancement in auditory inputs to the LA: Rogan et al (1997) have shown the enhancement of evoked field potential in the LA of the conditioned rat in using *in vivo* recording; McKernan and Shinnick-Gallagher (1997) have reported that the probability of synaptic transmitter release in thalamic input to the principal neurons was increased in the acute slice prepared from the conditioned rat. Moreover, fear conditioning induced LTP-like enhancement of synaptic transmission in auditory input to the LA the occlude NMDA receptor-dependent LTP in acute slice induced by electrical stimulation (Tsvetkov et al., 2002). In addition, gastrin-releasin peptide suppressed NMDA receptor-dependent LTP induction by the enhanced excitability of local inhibitory interneuron, result in the decreased fear memory (Shumyatsky et al., 2002). These reports demonstrat that neural activity by NMDA receptor activation in the LA is involved in the encoding of fear memory.

Recently, Cho et al (2012) have been shown that the kainite receptor-dependent LTP was occluded in the slice of the conditioned rat and infusion of kainite receptor blockade into the LA attenuated auditory fear conditioning, indicating that the kainite receptor in the LA is essential to auditory fear conditioning by investigating LTP induction and behavioural experiments.

Overall neural activity induced by glutamatergic receptor activation in the LA can promote the encoding of fear memory, therefore, we review the relationship between excitability of the LA and fear memory in the following subchapters.

3. The animal model of fear memory: Auditory fear conditioning

Behavior related to fear could be relatively described in human; we can easily notice the fear from human face expression, however, it is very difficult to achieve when it concerns experimental animal. To assess the quantity of learned fear, a simpler behavioral method is required. Auditory fear conditioning provides an animal model that is commonly used to study associative learning, such as fear conditioning.

In the rat, fear conditioning learning consists to the presentation of an initially biologically insignificant conditioned neutral stimulus (CS; for example an auditory sound) that is paired with the presentation of an unconditioned aversive stimulus (US; for example an electric footshock). During conditioning, neutral stimulus to experimental animal is paired with an aversive stimulus. Following a single or a few such pairing, the neutral stimulus elicits a defensive response as if he is threatened by an aversive stimulus. Lesions of the amygdala disrupt both the acquisition and expression of fear conditioning. As subsequent presentation of the CS without the US elicits, in the conditioned animal, defensive behavioral responses (freezing responses), autonomic nervous system responses (change in blood pressure and heart rate) and neuroendocrine responses (release of hormones from the pituitary and adrenal glands). This simple form of learning is exceptionally robust and rapidly

acquired, as it can be achieved as quickly as after a couple of trials (LeDoux, 1998; Whilen-sky et al., 2000; Repa et al., 2001). Some of these defensive responses are genetically deter-mined: animals are innate species-specific responses to threats and express defensive res-ponses automatically despite appropriate stimuli. In fear conditioning, therefore, when the CS is used as an initially biological insignificant stimulus, such as sound, light or touch, experimental animals can show learned “fear” that had never occurred in response to the neutral CS.

In the laboratory, the experimental cage for fear conditioning, equipped with stainless-steel shocking grids and a sound making apparatus, is placed in a sound-attenuating enclosure. On the training day the animal is placed in the chamber for a couple of minutes (habitua-tion: Figure 1A) before the onset of the CS, auditory cue that is co-terminated with the US, footshock (fear conditioning: Figure 1B), and returns to its home cages after several CS-US pairings (paired group: Figure 1D). For unpaired control group (Figure 1E), animal receives tones and footshocks in an unpaired manner (tones and footshock are separated by random intervals of some minutes). During the test 24-72 hours after training, animals are placed in a novel cage in which the tone that had been presented during training is given after habitu-ation period. Freezing scores are calculated as the fraction (percentage) of the total CS dura-tion in which the animal remained immobile (Frozen) (Figure 1C).

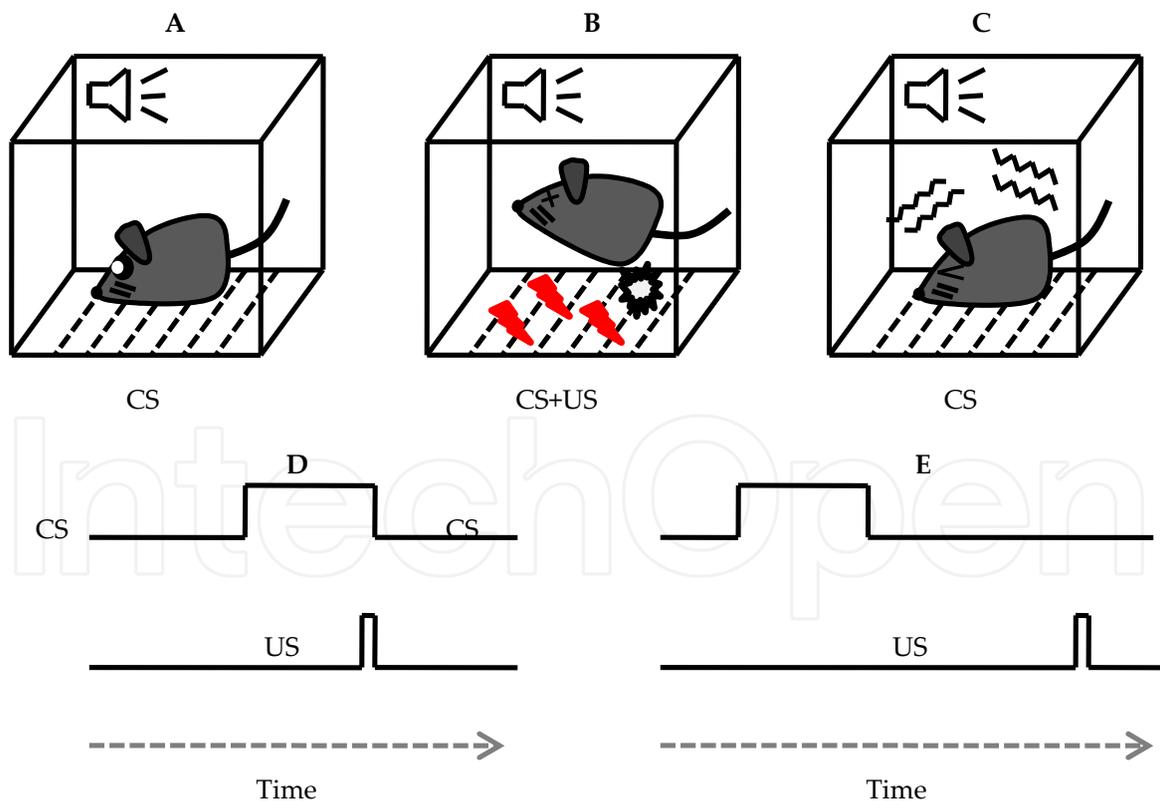


Figure 1. Auditory fear conditioning in rats.

The CS, only a single sensory modality or cue such as audible sound, light, smell or touch, can be unimodal. It is well established that unimodal (cued) fear conditioning is dependent

on the amygdala but not the hippocampus by the results of behavioral studies utilizing pharmacological inactivation of the amygdala (Marea et al., 2001; Fanselow & LeDoux, 1999).

However, animals also exhibit fear response in the absence of CS when returned to the chamber in which the tone and footshock were paired or unpaired. This is called multimodal (contextual) fear conditioning and requires both the hippocampus and the amygdala (Dityatev & Bolshakov, 2005).

In a typical auditory fear conditioning procedure, rats are habituated to the chamber with the US (auditory sound) (A). During fear conditioning (B), the electric footshock (US) is paired with an auditory sound several times. The sound is presented alone in the test session to estimate the effects of conditioning (C). To assess the amount of learned fear, most researchers measure the time of “freezing” behavior elicited by the auditory sound alone. During fear conditioning, an auditory sound is co-terminated with a footshock (D: paired group). An unpaired group in which the auditory sound and electric footshock in a nonoverlapping manner (E).

4. The neural circuit of auditory fear memory

Recent studies indicate that some cortices are involved in fear memory formation during auditory fear conditioning; the disinhibition of pyramidal neurons of the auditory cortex is required for auditory fear conditioning (Letzkus et al., 2011); auditory fear memory is stored in the secondary auditory cortex in cue-specific manner (Sacco & Sacchetti, 2010).

The preceding section gives us a general scheme and better understanding for the neural circuit between the amygdala complex and other brain regions and /or within the amygdala complex in the acquisition of fear memory. Here, this section will summarize the neuronal network about the flow of CS (auditory sound) and US (electric footshock).

Anatomical tracing studies combined with single unit recording in experimental animals suggest that the LA is a site of convergence of somatosensory input carrying the information relative to the footshock US and afferent inputs carrying the CS information (whatever the sensory modality) (Pitkanen et al., 1997). Therefore, it has been suggested that the LA is the site, where the association of learned information about CS and US apparently occurs during fear conditioning (Fanselow & LeDoux, 1999).

During auditory fear conditioning, the sensory information that mediates the CS (an audible cue), reaches the LA by the two pathways, both of which are essential to the learned fear (Romanski & LeDoux, 1992). One input, consisting of the direct thalamo-amygdala projections, originates in the medial division of the thalamic medial geniculate nucleus (MGm) and in the posterior intralaminar nucleus (PIN) of the thalamus (Dityatev & Bolshakov, 2005; Figure 2). The second input, the indirect cortico-amygdala projections, extends from the ventral division of the thalamic medial geniculate nucleus (MGv) to the auditory cortex (TE) and includes further projections that relay the auditory information from the cortex to the LA (Maren, 2001). The LA is neither highly laminar structure nor homogenous: the LA is

composed of at least three subnuclei: the dorsolateral (LAd), ventrolateral (LAvl), and medial division (LAm) (Pitkanen et al., 1997). Because auditory-evoked responses recorded in the different parts of the amygdala were the shortest in the LAd, it has been suggested that this part of the amygdala constitutes the entrance site to the fear conditioning (Bordi & LeDoux, 1992). After the information is processed in the LA, the final signals are sent to periaqueductal gary and brain stem via the central nucleus of the amygdala (CE), resulting in freezing, autonomic responses and release of stress hormones as the index of fear expression.

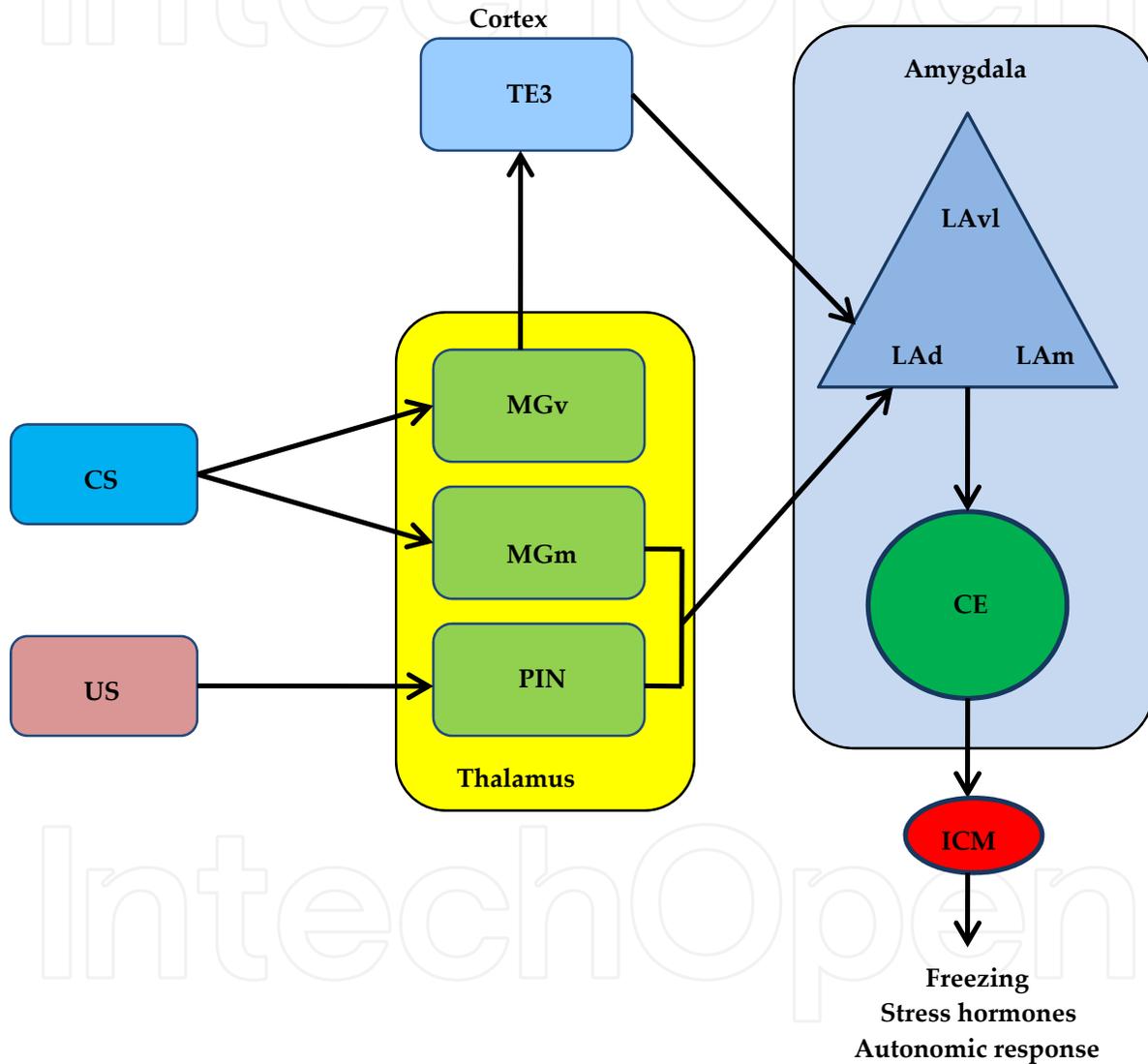


Figure 2. Auditory fear conditioning circuit

The CS (auditory sound) enters the LA directly via MGm and indirectly via MGm and TE.

The US (electric footshock) enters the LA via PIN. Convergence of CS and US enter in the LA, especially in the LAd, leading to synaptic plasticity. These signal processed in the LA projects to CE. The LA connects with CE directly and indirectly by way of other nucleus, such as ICM. Finally CE promotes some defensive responses, freezing, increased blood

pressure and heart rate as the expression of conditioned fear by trigger of hypothalamic and brainstem areas. Abbreviation: LAd, LAVl, LAM: the dorsolateral, ventrolateral and medial division of the LA. MGv, MGm: the ventral and medial division of the thalamic medial geniculate nucleus. PIN: the posterior intralaminar nucleus of the thalamus. ICM: intercalated cell masses. TE: the auditory cortex, CE: the central nucleus of the amygdala

5. The electrophysiological properties of the LA

We are just beginning to explore the properties of basic synaptic physiology in the LA for synaptic mechanism underlying fear conditioning because the LA is the site where the CS and the US information convey and encoding fear memory is formed (LeDoux et al., 1990). LeDoux et al (1990) has reported that bilateral lesion of the LA impaired fear conditioning.

It is necessary to characterize the properties of neuron typing, excitatory and inhibitory synapse and neural network involving distinct types of neurons. I will describe the rules governing and modulation of synaptic plasticity in the next subchapter 6.

The whole-cell patch-clamp technique has widely been used to explore the synaptic character in the amygdala because the LA is not highly laminar, making field potential observations difficult.

5.1. Diversity of cell types dependent on electrophysiological classification

On the basis of neuronal morphology and chemical analysis, the LA contains two main cell types: (1) pyramidal-like shape projection neurons (principal neurons), which use glutamate as neurotransmitter, form the majority of the constituent neurons, and (2) non-pyramidal neurons, observed in far fewer numbers, function primarily as local inhibitory circuit by releasing γ -aminobutyric acid (GABA) (Shin et al., 2006; Faber et al., 2001).

In slice preparation, the cells are readily identifiable as principal neurons with the pyramidal shape of their soma and the ability to show spike frequency accommodation in response to prolonged current injections by using patch-clamp method (Figure 3A; Shin et al., 2006; Tsvetkov et al., 2002; Mahanty & Sah. 1998). In contrast, interneurons are identified as non-accommodation pattern, which show spiking at regular intervals for the duration of the depolarization and increasing spike frequency with increasing depolarization (Figure 3B; Shin et al., 2006; Mahanty & Sah. 1998).

The dendrites of interneurons of the LA lack spines and pyramidal neurons exhibit dendritic arbors without spatial polarization (Faber et al., 2001). By fine investigation of *in vitro* two-photon microscopy, the size of dendritic spines contacted by thalamic, with the ability large Ca^{2+} transients during action potential backpropagation, was larger than that by cortical input to the principal cell of the LA. This thalamic spine could induce Hebbian plasticity by activation of R-type voltage-dependent Ca^{2+} channel in input-specific manner (Humeau et al., 2005).

Recent report has demonstrated that fear conditioning increases the rate of spin elimination of layer-V pyramidal neurons of the mouse frontal association cortex connected to the LA using *in vivo* transcranial two-photon microscopy, indicating that fear conditioning can promote modification of fear conditioning-related neural circuit. (Lai et al, 2012).

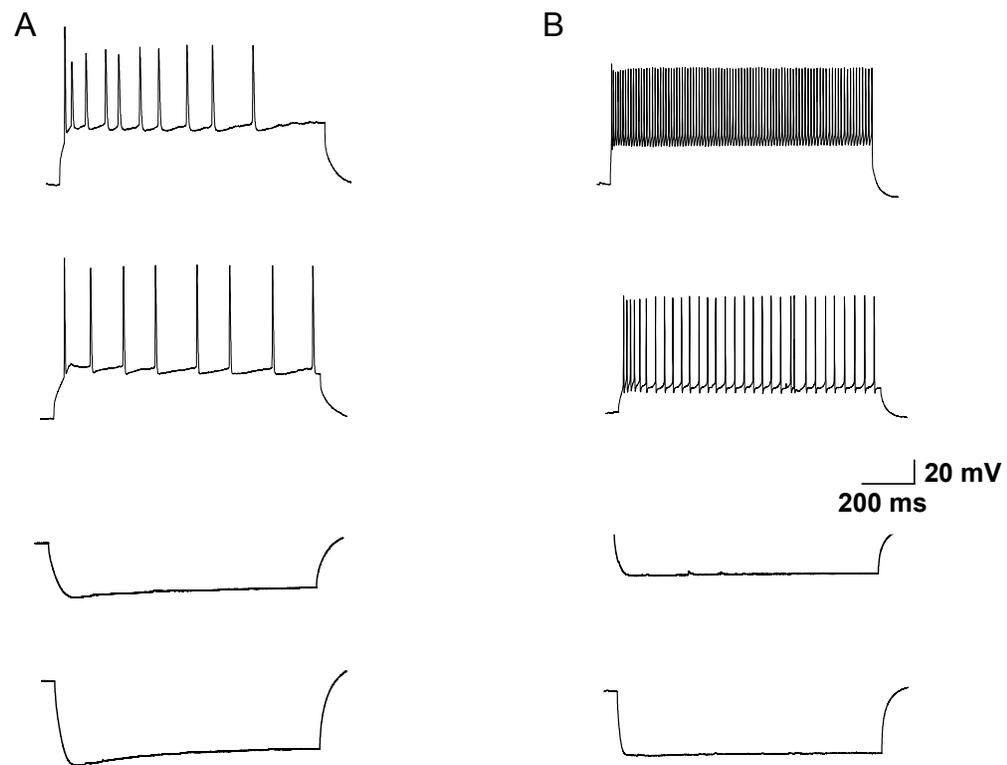


Figure 3. Membrane physiological characters of two representative cells of the LA. Typical firing patterns of the two neurons recorded under current-clamp mode in response to increasing current injection steps. The accommodation neuron (A) showed fewer action potentials per depolarization. By contrast, the nonaccommodation neuron (B) showed spiking at regular intervals for the duration of the depolarization and their spiking frequency was increased with the effect of depolarization.

5.2. Diversity in synaptic transmission: electrophysiological properties of LA neurons

5.2.1. Excitatory transmission

The early *in vitro* whole studies about excitatory and inhibitory synaptic transmission in the amygdala had done (Rainnie et al., 1991a, b). The afferent projections from thalamic nucleus or auditory cortex to the LA form excitatory synapses on both principal neurons and inhibitory interneurons (Rainnie et al., 1991a, b). The studies by using intracellular recording from

rat acute slice indicated that postsynaptic potentials elicited stimulation of the LA is composed of excitatory postsynaptic potentials (EPSPs) followed by either a fast inhibitory postsynaptic potential (f-IPSP) only, or by a fast- and subsequent slow-IPSP (s-IPSP). The EPSPs at the resting membrane potential consisted of dual fast and slow, components. The f-EPSPs increased in amplitude with membrane hyperpolarization and was insensitive to the NMDA receptor antagonist, D-AP5, but was blocked non-NMDA receptor antagonist, 6-cyano-7-nitro-quinoxaline-2, 3-dione (CNQX). In contrast, the slow-EPSPs decreased with membrane hyperpolarization, were blocked APV but were insensitive to the CNQX receptor antagonist, indicating that slow-EPSPs are mediated by NMDA receptor activation (Rainnie et al., 1991a).

The stimulation of the cortical (auditory cortex) and thalamic (thalamus) afferent inputs that convey auditory information related to the CS during auditory fear conditioning induces synaptic response in principal neuron in the LA. In the presence of GABA_A receptor antagonist, picrotoxin (PTX), such stimulation evokes a monosynaptic EPSP or excitatory postsynaptic currents (EPSC) with a short and constant latency in recorded cell in current- or voltage clamp mode at resting membrane potential (almost -70 mV) in brain slice (Shin et al., 2010, 2006; Kodirov et al., 2006; Shumyatsky et al., 2005, 2002; Tsvetkov et al., 2004, 2002; Weisskopf & LeDoux, 1999b). EPSC recorded by stimulation at either thalamic or cortical input was abolished by α - amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor antagonist, CNQX, thus suggesting that EPSCs recorded in thalamic or cortical inputs are mediated by AMPA receptor (Tsvetkov et al., 2002; Weisskopf & LeDoux, 1999b).

Moreover, fine electrophysiological studies indicated that the glutamatergic input from both cortical and thalamic to principal cell of the LA do not differ in either their basal probability or quantal amplitude (Shin et al., 2006). Plus, both inputs do not overlap (Tsvetkov et al., 2004), indicating that both cortical and thalamic input to the LA are independent from each other. It has been reported that the vesicular Zn²⁺ released from cortical, but not thalamic afferents, to the principal cell of the LA can induce NMDA receptor-dependent LTP (Kodirov et al., 2006).

Some studies have been demonstrated that NMDA receptor-mediated components were identified in both thalamic and cortical afferent inputs to the principal neurons in the LA (Shin et al., 2006; Shumyatsky et al., 2005; Tsvetkov et al., 2004, 2002; Weisskopf & LeDoux, 1999b), and the NMDA receptors in both inputs play the important role on the induction of LTP (Shin et al., 2006; Tsvetkov et al., 2004, 2002).

NMDA receptor are also functionally expressed at both cortico- and thalamo- LA synapses becomes evident at membrane depolarization. NMDA receptors in the LA are represented by a complex of NR1, NR2A and NR2B subunits. Pharmacological studies using the selective NR2A antagonist ifenprodil and the NR2A antagonist NVP-AAM077 have indicated that subunit composition of synaptic NMDA receptors in cortical input is not different from that in thalamic pathway (Shin et al., 2006). However, there are distinct subsets mediated by NMDA receptor in thalamic input versus cortical input to the LA on the basis of voltage and Mg²⁺ sensitivity (Weisskopf & LeDoux, 1999b).

In contrast to pyramidal neuron, glutamatergic inputs to interneurons of the LA may not contain NMDA receptor (Mahanty & Sah, 1998). Synaptic response mediated by AMPA receptors in interneurons show inward rectification and sensitivity to external polyamines, indicating that GluR2 subunit-lacking AMPA receptor is permeable to Ca^{2+} .

5.2.2. Inhibitory transmission

As described above, Rainnie et al (1991a) showed that the EPSPs recorded in the LA are, followed by both fast- and slow-IPSPs. Both IPSPs were reduced the presence of APV, and were abolished by CNQX, indicating that both IPSPs were mediated by multi-synapse pathways. The CNQX-resistant fast-IPSPs were abolished by bicuculline methiodide, GABA_A receptor antagonist, suggesting direct inhibition by local GABA_A ergic circuit. The slow-IPSP, which reversal potential is deeper (-95 mV), was depressed by 2-hydroxy-saclofen, GABA_B receptor antagonist (Rainnie et al., 1991b).

The afferent projections from thalamic nucleus or auditory cortex to the LA form excitatory synapses on both principal neurons and inhibitory interneurons (Rainnie et al., 1991a, b). The inhibitory interneurons send inhibitory inputs to principal neurons and other interneurons and their feedback and feed-forward GABA_A ergic inputs to principal neurons determine how the information conveying to principal neurons are processed (Wang et al., 2001).

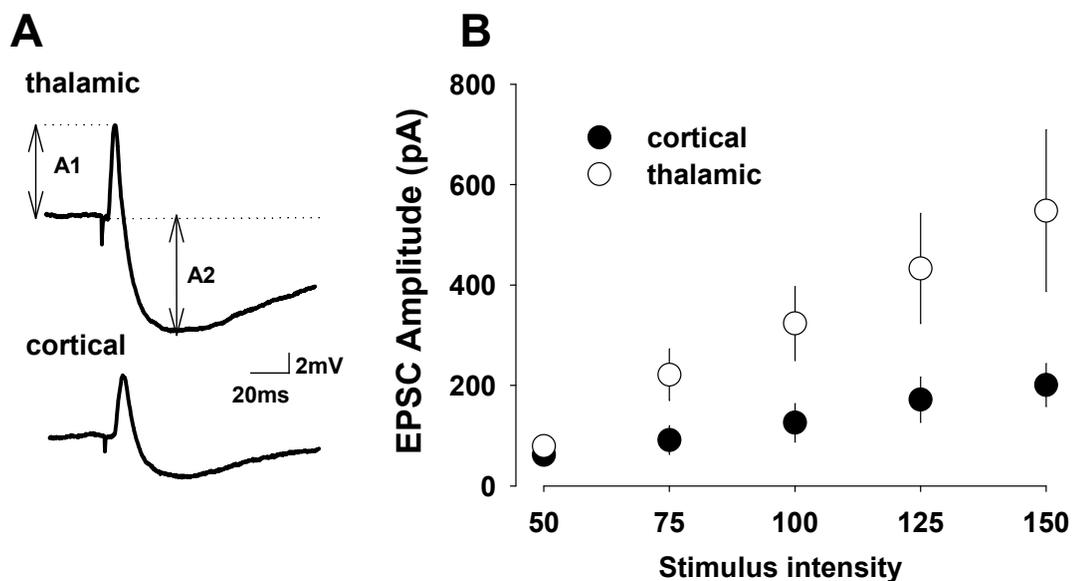


Figure 4. Feedforward GABA receptor-mediated inhibition of principal neuron is stronger in thalamic input. (A) Examples of the EPSP/IPSP sequences recorded at a membrane potential of -55 mV in convergent cortical and thalamic pathways. (B) Input-output curves for the AMPA receptor-mediated EPSC recorded in interneurons at holding potential of -70 mV in the presence of GABA_A receptor antagonist, PTX at convergent cortical and thalamic pathways. Reproduced with permission from Shin et al (Shin et al., 2006).

In Figure 4A, the EPSP/IPSP sequences elicited by both inputs to the same neuron display both monosynaptic glutamatergic EPSP (A1) and disynaptic GABA_A ergic IPSP (A2). The

ratios of each EPSP and IPSP amplitude (A2/A1) were enhanced in thalamic input when compared with cortical input. Strengthening of AMPA receptor-mediated inputs to interneurons leads to increased inhibition in principal cells in the LA (Mahanty & Sah, 1998). This suggests the possibility that input-specific differences in excitatory inputs to interneuron result in different inhibitory drives in individual pathway. In Figure 4B, the glutamatergic synaptic efficacies at convergent inputs (both thalamic and cortical input) to same interneuron were compared by analyzing two input-output curves of EPSCs. A leftward shift in the input-output curves obtained in thalamic input, as compared with those in cortical pathway was observed (Figure 4B). These results indicate the stronger inhibitory drive in thalamic pathway, as compared to cortical input.

6. Synaptic mechanism for fear memory

The hypothesis that NMDA-dependent LTP is involved in the cellular mechanism underlying fear conditioning arose initially from the finding that blockade of NMDA receptor within the LA decreased the amount of learned fear (Miserendino et al., 1990). Behavioral study combined with electrophysiological investigation showed that learned fear produced a persistent enhanced synaptic strength by both cortico- and thalamo-amygdala pathways (Tsvetkov et al., 2002; McKernan & Shinnick-Gallagher, 1997; Rogan et al., 1997). These synaptic modifications, observed along behavioural responses of learned fear, are mechanically similar to LTP induced artificially by electrical stimulation in acute slices of the LA. The NMDA receptor-dependent LTP induced by a pairing protocol (low-frequency presynaptic stimulation with postsynaptic depolarization) in the cortico-amygdala pathway was occluded in the acute slice of the conditioned rat (Tsvetkov et al., 2002). This result indicated that the NMDA receptors in the cortico-amygdala synapse are critical for fear conditioning. LTP induced by pairing protocol in the both the cortico- and thalamo-amygdala synapses required NMDA receptor activation (Shin et al., 2006). Other studies found that the LTP induced by a pairing protocol depended on both postsynaptic NMDA receptor and L-type voltage-dependent calcium channels (Tsvetkov et al., 2002; Weisskopf & LeDoux, 1999b; Huang & Kandel, 1998).

The NMDA receptor-mediated response in both cortical and thalamic input to principal cells is dependent on the NR1, NR2A and NR2B subunits. Despite the fact that NMDA receptor response in both inputs were largely mediated by NR2A-containing receptors (almost 75%), the blockade of NR2B subunit abolished pairing induced LTP in the LA (Shin et al., 2006). In addition, injection of NR2B subunit antagonist into the LA reduced the acquisition of fear conditioning without affecting expression of fear memories or basal synaptic transmission (Rodrigues et al., 2001). However, the activation of NMDA receptors in the LA following high frequency thalamic input stimulation is not necessary for the induction LTP (Weisskopf & LeDoux, 1999a).

By contrast, it has been shown that Ca^{2+} influx into interneurons resulting from the activation of AMPA receptor lacking GluR2 subunit may be implicated in the plasticity induction in the thalamic input (external capsule) to the amygdala interneuron (Mahanty & Sah, 1998).

In vivo electrophysiological recording of principal cells in the LA display unusually low firing pattern even during emotional arousal (Pare & Collins, 2000), providing that local GABAergic interneuron circuit's tight inhibitory control on the excitability of principal neurons in the LA. Therefore, it is thought that LTP in the LA is significantly diminished when pairing protocol is delivered in the absence of GABA_A receptor antagonist. However, by using a pairing protocol in the absence of PTX, (GABA_A receptor antagonist), a cortical input stimulation was able to induce LTP in the principal cell of the LA, probably because the inhibitory circuits activated by the cortical inputs are weaker, as compared with thalamic input (Shin et al, 2006; Figure 5; see 5.2.2 Inhibitory transmission). Other electrophysiological studies have shown that norepinephrine and dopamine gate the LTP induction in the thalamo-amygdala synapse by suppressing excitability of feedforward GABAergic circuit in thalamic input to the LA (Tully et al., 2007; Bissiere et al., 2003). Moreover, Hu et al (2007) have reported that emotion can enhance memory via norepinephrine regulation AMPA receptor trafficking into glutamatergic synapse. In addition, the highly expressed gastrin-releasin peptide in the LA suppressed both the fear conditioning responses and the magnitude of LTP recorded in cortical input. This effect being probably due to an enhancement of the excitability of feedforward GABAergic circuit related to the cortical input (Shin et al., 2006; Shumyatsky et al., 2002).

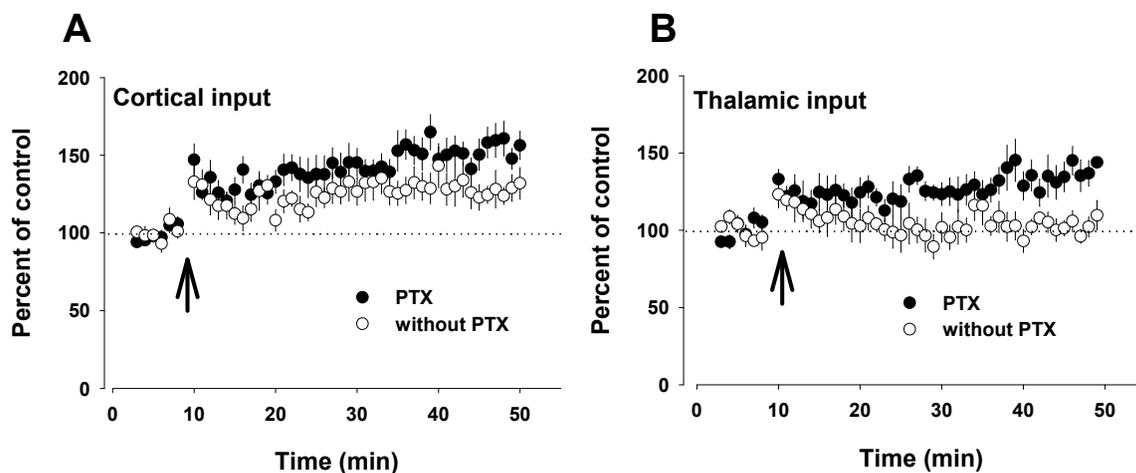


Figure 5. Pairing protocol induced LTP at the cortico-amygdala and thalamo-amygdala synapses. The graphs represent electrophysiological recordings of the LTP observed in cortical (A) and thalamic (B) inputs in the presence of PTX and without PTX. Reproduced with permission from Shin et al (Shin et al., 2006).

Most researchers have focused on postsynaptic modifications that occur in the principal cell of the LA during fear conditioning. However, some evidences also provided that presynaptic molecular alterations are important for encoding fear (Cho et al., 2012; Shin et al., 2010; Humeau et al., 2003; Tsvetkov et al., 2002; Pan et al, 2008; McKernan & Shinnick-Gallagher, 1997). Although the persistent increased synaptic strength in both thalamic and cortical inputs to the LA contributed to fear conditioning, the specific roles of both inputs are still debated. *In vivo* study revealed that the increased potential of the LA by stimulation of the auditory thalamus was observed, indicating that projections from the auditory thalamus to the LA are critical for auditory fear conditioning (Clugnet & LeDoux, 1990). According to this, it has been shown that the

thalamic, but not cortical input to the LA, is essential for fear conditioning by using genetic methods (Humeau et al., 2007; Rumpel et al., 2005). In addition, rapid plasticity change occurred in the auditory thalamus rather than the auditory cortex during fear conditioning (Quirk et al., 1997). In contrast, some lesion studies have shown that either pathway alone is sufficient for auditory fear conditioning; the lesion of one of them being ineffective to disrupt learning of conditioned auditory fear (Romanski & LeDoux, 1992). Also, post-training lesion of cortical, but not thalamic input to the LA, abolished the learned fear, indicating that cortical input may be a dominant role in fear conditioning in intact brain (Campeau & Davis, 1997). Finally, combination of *in vivo* and *in vitro* studies has revealed that synaptic plasticity in cortical input was essential for fear conditioning (Cho et al., 2012; Tsvetkov et al., 2002). It has been reported that presynaptic GABA_B receptor in the LA prevented the generalization of the learned fear via the regulation of LTP in cortical, but not thalamic input to the LA, by using genetic deletion technique (Shaban et al., 2006). Further, presynaptic GABA_B receptor was also reported to suppress LTP in cortical input to the principal cell of the LA via the control of glutamate release in acute slice experiment (Pan et al., 2009).

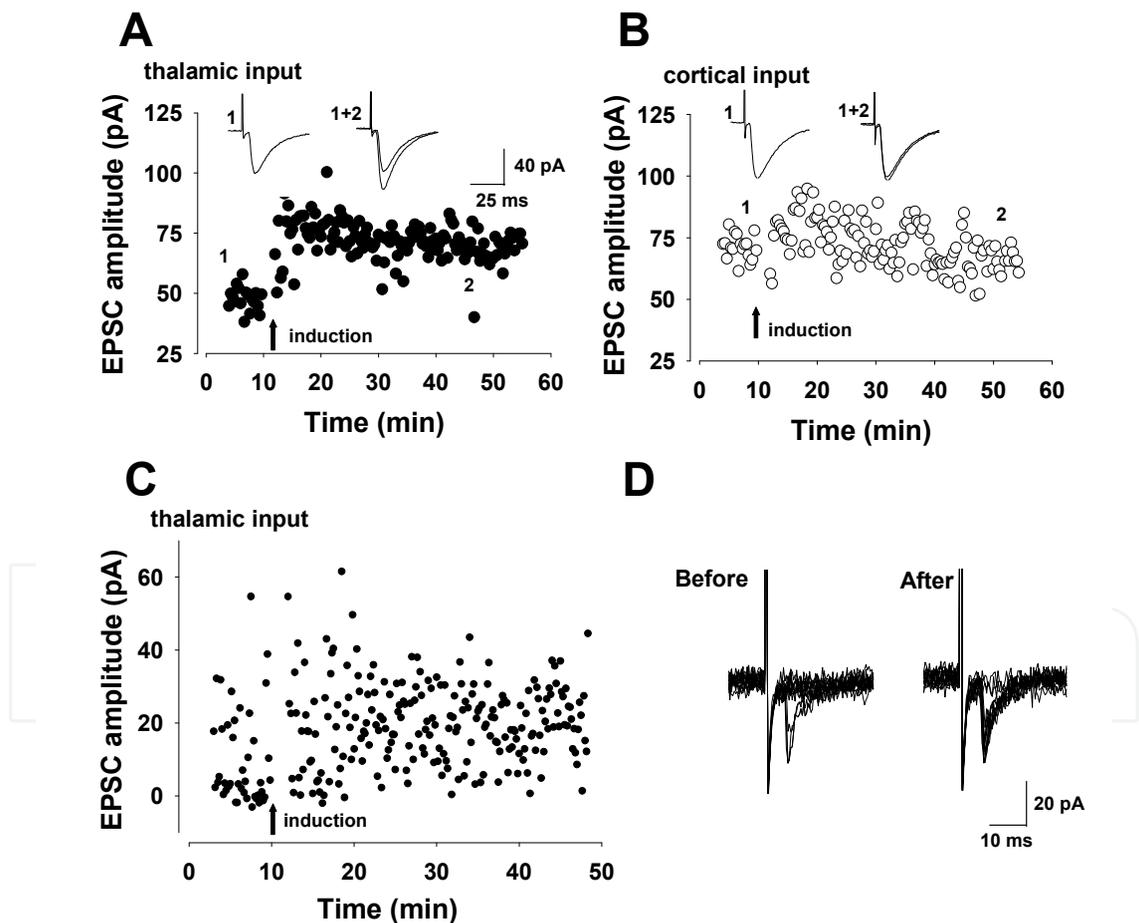


Figure 6. Properties of presynaptic form of LTP in thalamic input to the LA. LTP in thalamic input to the LA was induced by the presynaptic stimulation without postsynaptic depolarization (A). The same protocol did not induce LTP in cortical input (B). The course of LTP in thalamic input experiment by using unitary EPSCs recording (C). (D) Superimposed individual EPSCs before and after LTP experiment (C). Reproduced with permission from Shin et al (Shin et al., 2006).

Recently it has been found that LTP in thalamic, but not cortical input to the pyramidal neuron of the LA, expressed presynaptically, was resulted from low-frequency presynaptic stimulation in the absence of postsynaptic depolarization (Shin et al., 2010; Figure 6). In Figure 6C, D, same protocol above produced the increased probability of neurotransmitter release of glutamate in thalamic input to the LA in unitary recording, indicating that an expression of this LTP, observed in thalamic input alone, was presynaptic. Overall these studies revealed the possibility that each of the thalamic and cortical inputs may have distinct function in fear conditioning but their specific roles remain to be demonstrated.

7. Conclusion

Basic synaptic mechanisms in the amygdala that support auditory fear processes are now commonly known. However, in the light of recent findings some important questions remain to be answered. First: between the thalamic and cortical inputs, which is the afferent that control auditory fear conditioning? Second: Is it the pre- or postsynaptic modification of the induction and expression of LTP that is important during fear conditioning? I hope that young people or student will throw themselves into the research of fear conditioning.

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