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Synaptic Mechanisms of Associative Memory in the Amygdala

Minireview

Stephen Maren*
Department of Psychology and
Neuroscience Program
University of Michigan
Ann Arbor, Michigan 48109

Do associative learning and synaptic long-term potentiation (LTP) depend on the same cellular mechanisms? Recent work in the amygdala reveals that LTP and Pavlovian fear conditioning induce similar changes in postsynaptic AMPA-type glutamate receptors and that occluding these changes by viral-mediated overexpression of a dominant-negative GluR1 construct attenuates both LTP and fear memory in rats. Novel forms of presynaptic plasticity in the lateral nucleus may also contribute to fear memory formation, bolstering the connection between synaptic plasticity mechanisms and associative learning and memory.

It is widely believed that encoding and storing memories in the brain requires changes in the number, structure, or function of synapses. Other possibilities include the wholesale addition of new neurons or changes in intrinsic neuronal excitability, but it would be hard to imagine a memory mechanism that did not involve synaptic plasticity. This axiomatic view that synaptic plasticity is critical for learning and memory is supported by data derived from many different memory systems, neural circuits, and molecular pathways mediating an array of different behaviors. Ideally, however, one would want a systematic analysis of this problem in a brain circuit known to contain synapses that are essential for the formation and storage of a localizable long-term memory that is easily indexed in behavior.

Fortunately, a model system that is amenable to this sort of analysis exists. Pavlovian fear conditioning is an associative memory system that rapidly encodes memories of aversive events in both man and animals. In the laboratory, fear conditioning is established by presenting a neutral stimulus (the conditional stimulus, or CS), such as a tone, together with a noxious stimulus (the unconditional stimulus, or US), such as an electric shock to the feet. After a single conditioning trial, the CS will elicit a learned fear response (the conditional response, or CR), and this fear memory will persist for months, years, even a lifetime. Importantly, the population of synapses that is essential for encoding and storing fear memories has been identified.

At Home with Fear

The hub of the fear memory circuit lies in the amygdala, a collection of functionally and anatomically heterogeneous neurons deep within the temporal lobe (for reviews see LeDoux, 2000; Maren, 2001). Within the amygdala there exist two core nuclear groups important in fear conditioning: the basolateral complex (which contains the lateral, basolateral, and basomedial nuclei) and the central nucleus (Figure 1). Early conceptual models for fear conditioning posited a serial circuit whereby sensory information (e.g., information about the CS and US) entered and was associated in the lateral nucleus. This associative signal was then conveyed to the central nucleus for the expression of fear behavior. There is now evidence, however, that the lateral, basolateral, and central nuclei perform certain associative functions in parallel (Everitt et al., 2003; Paré et al., 2004) and that the central nucleus alone can itself mediate associative learning under some conditions (Figure 1). For instance, rats with basolateral complex lesions acquire conditioned fear after overtraining (Maren, 1999b), and this is mediated by the central nucleus (J. Zimmerman, C.A. Rabinak, and S. Maren, 2005, Soc. Neurosci., abstract).

Nevertheless, it is clear that neurons in the lateral nucleus (LA) play a critical role in the acquisition of fear memories and that these neurons are essential for maintaining fear memory, at least as indexed by conditional freezing, for at least 1 year in rats (Gale et al., 2004). Consistent with this observation, neurons in the lateral nucleus of the amygdala increase their firing rates to CSs that have been paired with USs (for review see Maren and Quirk, 2004), and these changes in neuronal firing are due to the associative relationship of the CS and US (i.e., memory) and not the fear and arousal such CSs engender (i.e., performance; Goosens et al., 2003). The lateral nucleus is therefore a key target for examining the molecular basis for associative memory.

Mapping Memory Molecules

Emerging in parallel with the mapping of brain circuits for fear memory has been outstanding progress in understanding the molecular mechanisms of synaptic plasticity in brain structures important for memory, including the hippocampus and amygdala. The field has now advanced sufficiently to apply these molecular tools to ask the critical question: are the molecular mechanisms for the induction and expression of long-term synaptic plasticity, such as long-term potentiation (LTP), also required for Pavlovian fear conditioning?

Not surprisingly, there is an abundance of evidence implicating amygdaloid LTP in the acquisition of Pavlovian fear (for a review see Maren, 1999a). When infused into the basolateral complex of the amygdala (BLA; including the lateral, basolateral, and basomedial nuclei), NMDA receptor antagonists, which block some forms of synaptic potentiation, prevent the acquisition of fear memory. There has been some debate concerning the selectivity of these pharmacological effects for encoding (as opposed to retrieving or expressing) fear memory, but recent data support a selective role for amygdaloid NMDA receptors in fear learning (Goosens and Maren, 2004; Rodrigues et al., 2002). More recent pharmacological work has shown that disrupting protein kinases that are coupled to NMDA receptor activation prevents the acquisition, but not expression, of fear memory (Rodrigues et al., 2004). Moreover, protein syn-

*Correspondence: maren@umich.edu

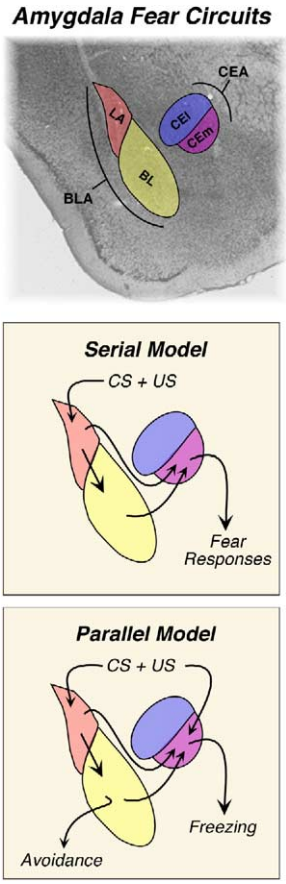

Figure 1. Amygdaloid Nuclei Involved in Pavlovian Fear Conditioning

(Top) Color overlays indicate critical nuclei. BLA, basolateral complex of the amygdala; LA, lateral nucleus; BL, basolateral nucleus; CEA, central nucleus of the amygdala; CEl, central nucleus (lateral division); CEm, central nucleus (medial division). (Middle) The standard model of fear conditioning posits that the BLA, particularly the LA, is the sensory interface of the amygdala receiving and associating CS and US information during fear conditioning. The associative memory encoded in LA neurons is then conveyed to CEm either via indirect projections through BL or through intercalated neurons interposed between CEA and BLA (for simplicity, the arrow interconnecting LA and CEm is meant to convey this indirect connection through the intercalated neurons). (Bottom) Emerging evidence suggests that the BL and CE may mediate independent associative functions in aversive conditioning tasks (Everitt et al., 2003). According to this view, processing of CS and US information in LA may drive Pavlovian fear responses such as freezing through the CE and direct instrumental responses, such as avoidance, through BL projections to the ventral striatum. The CEA may have sufficient sensory input to mediate fear conditioning in the absence of the BLA under some conditions.

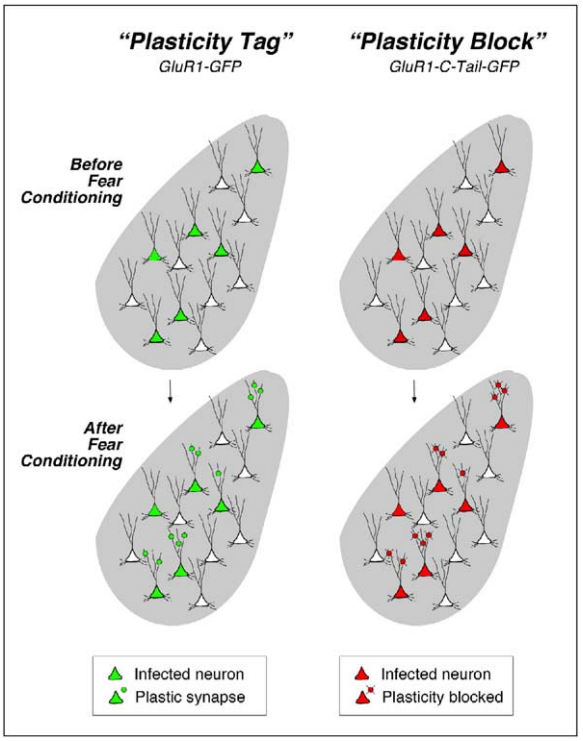

Figure 2. Viral-Mediated Gene Transfer Procedures

Herpes simplex amplicons were used to express modified AMPA-type glutamate receptor subunits. In one case, a "plasticity tag" was created by expressing GluR1 subunits conjugated with green fluorescent protein (GluR1-GFP); homomeric GluR1 receptors, which are driven into synapses during long-term potentiation and experience-dependent synaptic plasticity, exhibit a unique electrophysiological signature that can be used to identify plastic synapses after fear conditioning. Fear conditioning induced synapse-specific increases in GluR1-mediated conductances in infected neurons, suggesting that roughly 30% of LA neurons were modified by the conditioning experience. In the other case, a "plasticity block" was created by expressing only the GluR1 carboxyl tail conjugated to GFP (GluR1-C-Tail-GFP). These modified subunits act as a dominant-negative mutation by competing with native GluR1 subunits for synaptic delivery during long-term potentiation. Infection of LA neurons with viruses carrying the GluR1-C-Tail-GFP construct impaired the acquisition of short- and long-term fear memories. These procedures were used by Rumpel et al. (2005).

thesis inhibitors in the BLA prevent the consolidation of fear memory. There is also considerable electrophysiological data consistent with the induction of synaptic potentiation in the amygdala during fear conditioning (McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997). Together, these data build a strong case for the involvement of synaptic plasticity in the amygdala in fear memory. What has been lacking, however, is the ability to selectively visualize and manipulate molecules involved in synaptic plasticity within the population of neurons in the amygdala that are known to mediate memory storage—until now.

Trafficking Fear in the Amygdala

In an elegant study, Malinow and colleagues harnessed viral-mediated gene delivery to hijack the protein synthetic machinery of LA neurons to either label plastic synapses or prevent synaptic plasticity during fear conditioning (Rumpel et al., 2005). This study used modified AMPA-type glutamate receptors (AMPARs) to measure both learning-induced synaptic potentiation in single LA neurons after fear conditioning and to examine the consequences of blocking synaptic plasticity in a subset of LA neurons on fear learning and memory (Figure 2). This approach takes advantage of the fact that the induction of LTP drives GluR1-containing AMPARs into synapses and, further, that preventing AMPAR delivery reduces the magnitude of LTP. To introduce these modified AMPARs into LA neurons, they in-

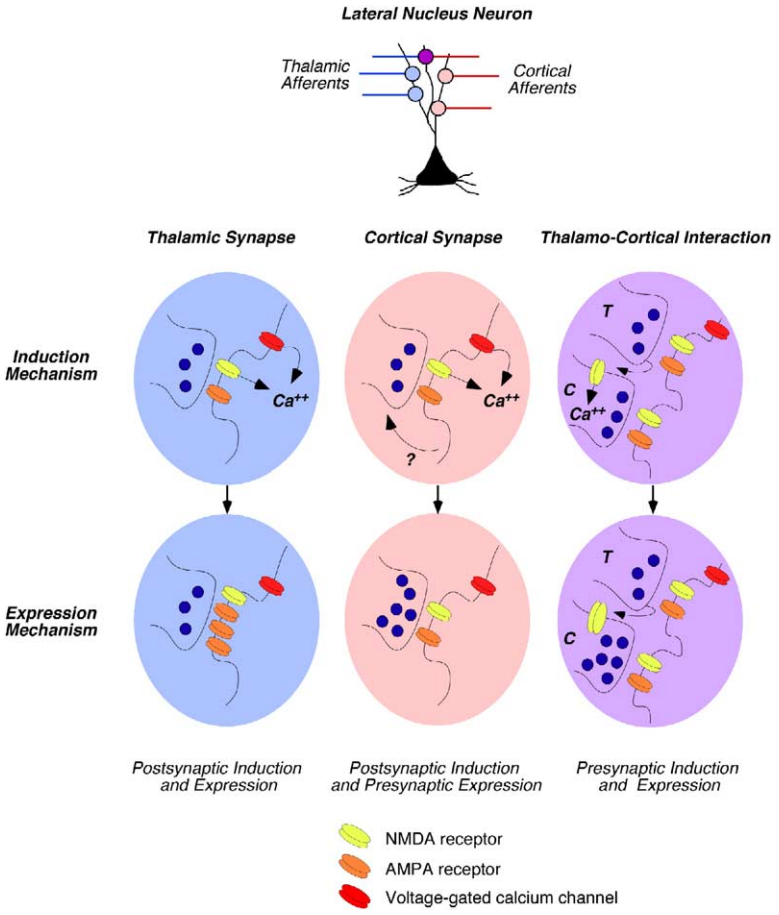

Figure 3. Synaptic Plasticity Mechanisms in the Lateral Amygdala

The induction and expression of long-term potentiation (LTP) at thalamo-amygdala synapses (left) relies primarily on postsynaptic mechanisms (although presynaptic increases in neurotransmitter release may also occur after LTP induction). In contrast, plasticity at cortico-amygdala synapses may be induced either postsynaptically (middle) or presynaptically (right), but is mediated primarily by increases in presynaptic neurotransmitter release. Presynaptic LTP induction is mediated by a novel synaptic mechanism in which activation of presynaptic NMDA receptors on cortical terminals by thalamic afferents induces an associative and heterosynaptic LTP at the cortico-amygdala synapse.

fected LA neurons with nonreplicating herpes simplex viral amplicons expressing GFP-tagged GluR1 constructs.

In their first experiment, Malinow and colleagues infected LA neurons with wild-type GluR1 subunits to bias these neurons to express homomeric AMPARs (Rumpel et al., 2005). Synapses containing these tagged receptors were then identified using electrophysiological procedures in brain slices, taking advantage of the fact that homomeric AMPARs exhibit greater rectification than native AMPARs. After viral infection, rats were submitted to auditory fear conditioning and then sacrificed for in vitro electrophysiological analysis of synaptic currents in excitatory projections from the auditory thalamus to LA. They found that over one-third of LA neurons exhibited greater inward rectification in animals that received fear conditioning (indicating they had incorporated homomeric AMPARs). Moreover, there was significantly more rectification in animals that received paired conditioning trials (in which the CS and US occur together), compared to unpaired controls (in which the CS and US are not presented together to discourage the formation of a CS-US association). Interestingly, synaptic delivery of AMPARs was specific to a subset of synapses on infected neurons, consistent with the synaptic-specificity of LTP.

Does preventing the delivery of AMPARs to LA synapses compromise fear conditioning? To answer this question, Malinow and colleagues infected LA neurons with a truncated GluR1 subunit tagged to GFP (the GluR1 protein was limited to the carboxyl cytoplasmic tail). As in the hippocampus, they showed that LA neurons infected with this "plasticity block" construct exhibited normal electrophysiological characteristics, but could not sustain pairing-induced LTP in the LA. Importantly, animals infected with this construct prior to fear conditioning exhibited impairments in both short- (3 hr) and long-term (24 hr) retention of fear memory, despite exhibiting normal freezing behavior (a standard and easily-measured fear response) on the conditioning day. By varying levels of infection, they found that infection in only about 25% of the neurons was required to produce a deficit in fear memory. This suggests that associative memory may be sparsely coded in the LA network and that disruption of only a small portion of the network is sufficient to yield behavioral impairments (also see Tsvetkov et al., 2002).

Together, these results strongly support the hypothesis that LTP, expressed through an increase in synaptic AMPARs, occurs in LA neurons during fear conditioning. Moreover, amygdaloid LTP requiring the synaptic delivery of AMPARs appears to be necessary for establishing long-term memories of the conditioning experience (at least as it is manifest in conditional fear responses, such as freezing behavior). Nonetheless, there was some evidence that the plasticity block con-

struct impaired normal synaptic transmission in the LA. This may have impaired conditioning by limiting sensory transmission rather than plasticity per se, for example. An experiment examining the influence of infecting LA neurons with the plasticity block construct on the expression of conditioned freezing would address this issue.

Synaptic Model of Fear Memory: Taking Sides

The involvement of GluR1-mediated synaptic plasticity in the lateral amygdala during fear conditioning provides important new evidence for long-term potentiation in sensory afferents to the LA in the acquisition of fear memory (Rumpel et al., 2005). This agrees with considerable data indicating that Pavlovian fear conditioning relies on molecular mechanisms that support long-term synaptic plasticity in the brain (Rodrigues et al., 2004). A central theme in this model is that the locus of the induction and expression of conditioning-related synaptic plasticity is postsynaptic to sensory afferents from the thalamus and neocortex. However, it is now clear that LTP is expressed, in part, by presynaptic modifications in LA (Huang and Kandel, 1998), that associative LTP in the LA can be sustained by presynaptic NMDA receptor activation and increases in presynaptic release probability (Humeau et al., 2003), and that presynaptic plasticity in LA may contribute to fear memory (Apergis-Schoute et al., 2005; McKernan and Shinnick-Gallagher, 1997; Tsvetkov et al., 2002).

Importantly, the nature of synaptic plasticity within the LA is different at thalamic and cortical afferents (Figure 3). The bulk of the evidence suggests that LTP in thalamic afferents to the LA is induced postsynaptically (involving NMDA receptors and L-type voltage-dependent calcium channels) and expressed by postsynaptic modifications (Humeau et al., 2005). In contrast, cortical afferents express synaptic plasticity primarily through presynaptic modifications in neurotransmitter release, and induction may involve both pre- and postsynaptic mechanisms (Huang and Kandel, 1998; Humeau et al., 2003). Interestingly, presynaptic NMDA receptors can mediate an associative form of plasticity in LA that is independent of postsynaptic depolarization (Humeau et al., 2003) but required concurrent activity in thalamic afferents. Interactions between thalamic and cortical inputs therefore have an important influence on the nature and causes of LA plasticity, but the precise functional role for the different forms of plasticity at thalamic and cortical synapses on LA neurons requires further investigation.

Outstanding Questions

The last few years have brought considerable progress in understanding the nature of synaptic plasticity in the amygdala and its relationship to Pavlovian fear conditioning. At the same time, associative processes in the amygdala appear to extend beyond the boundaries of the lateral nucleus and might be mediated by bidirectional synaptic plasticity in internuclear connections within the amygdala (Fu and Shinnick-Gallagher, 2005; Heinbockel and Pape, 2000; Mahanty and Sah, 1998; Samson and Paré, 2005). The cellular induction and expression mechanisms for the various forms of synaptic plasticity in the amygdala have important implications for understanding the encoding, storage, and retrieval of fear memories. It is also essential to understand how learning to suppress fear, for example, during the extinction of fear, interacts with these cellular mechanisms. Indeed, there is considerable evidence suggesting that NMDA receptor-dependent plasticity in the amygdala may be involved in the extinction of fear (Lin et al., 2003; Walker et al., 2002). It will be important to determine whether the same molecular endpoints (e.g., GluR1 delivery) are involved in extinction memory and, if so, how competing excitatory and inhibitory memories are instantiated into the network using a common synaptic plasticity mechanism.

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Selected Reading

Apergis-Schoute, A.M., Debiec, J., Doyere, V., LeDoux, J.E., and Schafe, G.E. (2005). J. Neurosci. *25*, 5730–5739.

Everitt, B.J., Cardinal, R.N., Parkinson, J.A., and Robbins, T.W. (2003). Ann. N Y Acad. Sci. *985*, 233–250.

Fu, Y., and Shinnick-Gallagher, P. (2005). J. Neurophysiol. *93*, 3012–3015.

Gale, G.D., Anagnostaras, S.G., Godsil, B.P., Mitchell, S., Nozawa, T., Sage, J.R., Wiltgen, B., and Fanselow, M.S. (2004). J. Neurosci. *24*, 3810–3815.

Goosens, K.A., and Maren, S. (2004). Eur. J. Neurosci. *20*, 537–548.

Goosens, K.A., Hobin, J.A., and Maren, S. (2003). Neuron *40*, 1013–1022.

Heinbockel, T., and Pape, H.C. (2000). J. Neurosci. *20*, RC68.

Huang, Y.Y., and Kandel, E.R. (1998). Neuron *21*, 169–178.

Humeau, Y., Shaban, H., Bissière, S., and Lüthi, A. (2003). Nature *426*, 841–845.

Humeau, Y., Herry, C., Kemp, N., Shaban, H., Fourcaudot, E., Bissière, S., and Lüthi, A. (2005). Neuron *45*, 119–131.

LeDoux, J.E. (2000). Annu. Rev. Neurosci. *23*, 155–184.

Lin, C.H., Yeh, S.H., Lu, H.Y., and Gean, P.W. (2003). J. Neurosci. *23*, 8310–8317.

Mahanty, N.K., and Sah, P. (1998). Nature *394*, 683–687.

Maren, S. (1999a). Trends Neurosci. *22*, 561–567.

Maren, S. (1999b). J. Neurosci. *19*, 8696–8703.

Maren, S. (2001). Annu. Rev. Neurosci. *24*, 897–931.

Maren, S., and Quirk, G.J. (2004). Nat. Rev. Neurosci. *5*, 844–852.

McKernan, M.G., and Shinnick-Gallagher, P. (1997). Nature *390*, 607–611.

Paré, D., Quirk, G.J., and Ledoux, J.E. (2004). J. Neurophysiol. *92*, 1–9.

Rodrigues, S.M., Schafe, G.E., and LeDoux, J.E. (2002). J. Neurosci. *22*, U1–U2.

Rodrigues, S.M., Schafe, G.E., and LeDoux, J.E. (2004). Neuron *44*, 75–91.

Rogan, M.T., Staubli, U.V., and LeDoux, J.E. (1997). Nature *390*, 604–607.

Rumpel, S., LeDoux, J., Zador, A., and Malinow, R. (2005). Science *308*, 83–88.

Samson, R.D., and Paré, D. (2005). J. Neurosci. *25*, 1847–1855.

Tsvetkov, E., Carlezon, W.A., Benes, F.M., Kandel, E.R., and Bolshakov, V.Y. (2002). Neuron *34*, 289–300.

Walker, D.L., Ressler, K.J., Lu, K.T., and Davis, M. (2002). J. Neurosci. *22*, 2343–2351.