To appear in *Neuronal Mechanisms of Memory Formation* (ed. C. Hölscher)

Multiple Roles for Synaptic Plasticity in Pavlovian Fear Conditioning

Stephen Maren
Department of Psychology and Neuroscience Program
University of Michigan
525 E. University Ave.
Ann Arbor, MI 48109-1109

Proofs and correspondence:
Stephen Maren
Department of Psychology
University of Michigan
525 E. University Avenue
Ann Arbor, MI 48109-1109
Tel: (734) 936-6532
Fax: (734) 763-7480
E-mail: maren@umich.edu

Summary

Long-term potentiation (LTP) is a form of synaptic plasticity that has been proposed to mediate certain forms of learning and memory. In this chapter, it is argued that LTP in the hippocampus and amygdala plays a crucial role in the acquisition of a simple form of emotional learning and memory: Pavlovian fear conditioning. The distinct roles for hippocampal and amygdaloid LTP and the roles for short-term synaptic plasticity mechanisms in the acquisition of learned fear responses are discussed.

I would have you imagine, then, that there exists in the mind of man a block of wax, which is of

different sizes in different men; harder, moister, and having more or less purity in one than another,

and in some an intermediate quality. Let us say that this tablet is a gift of Memory, the mother of the

Muses, and that when we wish to remember anything which we have seen, or heard, or thought in our

own minds, we hold the wax to the perceptions and thoughts, and in that material receive the

impression of them as from the seal of a ring; but when the image is effaced, or cannot be taken, then

we forget and do not know.

Plato, ca. 400 B.C.

Throughout history, humans have been fascinated by the nature of memory, the

permanent storehouse of the mind's experience. The foregoing passage excerpted from

Plato's *Theaetetus* (ca. 400 B.C.) represents an early attempt to describe the process of

memory formation in the human brain. In this passage, Plato envisions that memories are

established in the brain when perceptions or thoughts render lasting impressions in the

mnemonic wax of the mind. He implies that memories, once impressed in this way, are

permanent, unless their trace in the mental 'wax' is erased. Despite the figurative nature

of Plato's 'wax tablet hypothesis', the notion of a pliable or plastic substrate for memory

storage continues to be a central tenet of nearly all current neurobiological models of

memory formation. And although our comparatively enlightened understanding of brain

function has taken the analysis of the neurobiological mechanisms of learning and

memory to a molecular level, the question remains: What is the nature of the 'waxy'

neural substrate for memory and how does it retain a mnemonic 'impression'?

Since the turn of the century, there has been a general consensus among psychologists,

neurobiologists, and even philosophers that memories are represented in the brain as

enduring changes in the brain's neuronal circuitry--the 'wiring' that interconnects

individual nerve cells. In the last twenty-five years, this view has been extensively

elaborated. Indeed, it is now generally believed that synapses, which are the sites for

chemical communication between interconnected neurons, are the pliable substrate for

memory traces to make their impression.

   The involvement of synapses in memory formation has received considerable

empirical support from the discovery of a long-lasting change in synaptic function in the

mammalian brain. In the early 1970s, a pair of papers was published that described a

long-lasting enhancement of synaptic transmission following high-frequency stimulation

of excitatory synapses in the brain (Bliss and Gardner-Medwin, 1973; Bliss and Lomo,

1973). This form of synaptic plasticity was discovered in the hippocampus, a seahorse-

shaped structure known to be crucial for memory in humans (e.g., Squire and Zola-

Morgan, 1991). Bliss, Lomo, and Gardner-Medwin termed the phenomenon long-lasting

or long-term potentiation (LTP), because it was considerably more enduring than any

previously described form of synaptic enhancement, which typically lasted only minutes.

Although it was not evident at the time, the discovery of LTP heralded a new era for the

study of the neural mechanisms of learning and memory.

   In the years after the discovery of hippocampal LTP, a massive research effort has

revealed that it exhibits many properties typical of memory and occurs in brain structures

known to be important for learning and memory (Bliss and Collingridge, 1993; Maren

and Baudry, 1995).   For these and other reasons, there has been great interest in the

possibility that LTP serves as a cellular mechanism for learning and memory. Indeed,

LTP has served as the primary experimental model for learning and memory in the

mammalian brain in the last decade. However, questions have recently been raised

concerning the utility of using LTP as an experimental model for the neurobiology of

learning and memory (Diamond and Rose, 1994; Shors and Matzel, 1997). For example,

the involvement of hippocampal LTP in rodent spatial learning, which has been the

preferred behavioral paradigm for examining the LTP-learning connection, has come into

question (e.g., Cain, 1997).   This confusion may be due, at least in part, to the

complicated and multiply determined nature of spatial learning tasks.

    To circumvent this problem, a number of laboratories are now exploring the role for

LTP in less complicated associative learning paradigms in which the experimenter has

exquisite control over the behavioral contingencies. One paradigm that is becoming

increasingly popular in this regard is Pavlovian fear conditioning, a robust and rapidly

acquired form of emotional learning and memory that is exhibited by mammals

throughout the animal kingdom (e.g., Fanselow, 1984).   The neurobiological analysis of

Pavlovian fear conditioning has revealed exciting insight into the involvement of LTP in

learning and memory (Davis et al., 1994; Maren, in press; Maren and Fanselow, 1996;

Rogan and LeDoux, 1996). This is particularly true insofar as the analysis of LTP has

moved beyond the hippocampus into the amygdala, an almond-shaped temporal lobe

structure that is crucial for fear conditioning (Davis, 1992; Fanselow, 1994; LeDoux,

1998; Maren, 1996). The aim of this chapter is to describe the considerable progress that

has been made in elucidating the roles for hippocampal and amygdaloid LTP in

Pavlovian fear conditioning. It will be argued that LTP in the hippocampus and

amygdala play complimentary, but distinct, roles in mediating different aspects of

Pavlovian fear conditioning.

Pavlovian fear conditioning in rodents : A model system for exploring the LTP-

learning connection

After the discovery of LTP in the hippocampus and the realization that it exhibits properties that are expected of a cellular learning mechanism, considerable interest emerged in the role hippocampal LTP might play in behavioral learning and memory. It is not surprising then that the earliest empirical approaches to this issue sought to determine whether forms of learning that were known to require the hippocampus, such as spatial learning in the Morris water maze, also required hippocampal LTP (Morris et al., 1986). As a result of this work, a substantial body of evidence has emerged that implicates hippocampal LTP in spatial learning (Elgersma and Silva, 1999; Morris et al., 1990). However, there are a number of recent studies that have cast doubt on an essential role for LTP in spatial learning (Bannerman et al., 1995; Cain, 1997; Saucier and Cain, 1995). For example, there are several recent reports that genetic mutants which exhibit impaired hippocampal LTP do not exhibit deficits in spatial learning and memory (Meiri et al., 1998; Zamanillo et al., 1999). These disparate results may be due to a number of factors, not the least of which is the fact that successful performance in spatial learning tasks involves acquiring several types of information, only some of which involves the hippocampal system.

As an alternative to spatial learning tasks, a number of laboratories have begun to examine the LTP-learning connection in well characterized and simple associative learning paradigms. The prototypical task of this sort is a Pavlovian (or classical) fear conditioning task, in which rats or mice learn that certain innocuous stimuli (conditional stimuli or CSs), such as tones, lights, or places, are predictive of aversive events (unconditional stimuli or USs), such as electric footshocks. When re-exposed to the CSs that were associated with the aversive event, animals exhibit learned or conditional fear responses (CRs), such as freezing (immobility except movement associated with

breathing), enhanced acoustic startle, increased heart rate and blood pressure, pupillary dilation, and defecation. This type of learning is not unique to rodents, but has been described in animals ranging from sea slugs to humans. The ubiquity of fear conditioning is not surprising. It allows animals to encode and remember experiences that are a threat to their survival, and as a result, to engage defensive systems when stimuli predictive of these aversive events are re-encountered (Fanselow, 1984).

Pavlovian fear conditioning is an outstanding model system for the study of the neurobiological substrates of learning and memory for a number of reasons. First, the psychological processes underlying Pavlovian fear conditioning have been well characterized. Indeed, it is from this behavioral task that much of the behavioral data forming the core of modern associative learning theory has emerged. Second, Pavlovian fear conditioning is rapidly acquired (with as little training as a single trial) and extremely long-lasting. This makes it an ideal task for studying time-dependent memory processes, such as consolidation. Moreover, because the fear conditioning procedure takes little time to administer, experimental variance can be greatly reduced by running large numbers of animals in parallel. Third, the neural circuitry underlying fear conditioning has been described in some detail. Importantly, the essential locus for CS-US association formation has been identified and many of the sensory pathways that transmit information to this learning center have been mapped (LeDoux, 1998).

Neural circuitry of fear conditioning

It has been recognized for decades that the amygdala is involved in emotional processes, including aversively motivated learning (see Fendt and Fanselow, 1999 for an excellent review). The major amygdaloid nuclei and projections have been well

characterized (see (Swanson and Petrovich, 1998 for a review of amygdaloid anatomy).

Recent anatomical and behavioral evidence indicates that there are at least two distinct

subsystems within the amygdala that are important for Pavlovian fear conditioning. The

first subsystem of the amygdala consists of the lateral, basolateral and basomedial nuclei.

These nuclei, which are collectively referred to as the basolateral amygdaloid complex

(BLA), are the primary sensory interface of the amygdala. Thus, the BLA receives

synaptic input from many primary sensory structures, and lesions in these structures yield

deficits in Pavlovian fear conditioning. For example, projections from the auditory

thalamus and auditory cortex to the BLA are essential for conditioning to auditory CSs

(Campeau and Davis, 1995a; Romanski and LeDoux, 1992), projections from the

hippocampal formation to the BLA appear to underlie conditioning to contextual CSs

(Kim and Fanselow, 1992; Maren and Fanselow, 1997; Phillips and LeDoux, 1992), and

projections from the perirhinal cortex transmit visual CS information to the BLA

(Campeau and Davis, 1995a; Rosen et al., 1992). Information about the aversive

footshock US might reach the BLA via parallel thalamic and cortical pathways (Shi and

Davis, 1999). Consistent with this anatomy, single neurons in the BLA respond to

auditory, visual and somatic (shock) stimuli (Romanski et al., 1993), which indicates that

the amygdala is a locus of convergence for information about CSs and USs. Thus, the

BLA is anatomically situated to integrate information from a variety of sensory domains.

The information processed by the BLA is either relayed back to afferent structures or

sent to the second major subsystem of the amygdala, the central nucleus of the amygdala

(CEA). The CEA projects to many brainstem targets and is the amygdala's interface with

the fear-response systems. For example, the CEA projects to nuclei in the hypothalamus,

midbrain and medulla that control a variety of defensive responses, including freezing and

acoustic startle. Electrical stimulation of the CEA produces responses that are similar to those elicited by stimuli paired with shock, and lesions of the CEA also produce profound deficits in both the acquisition and expression of conditional fear (Hitchcock and Davis, 1986; Iwata et al., 1987). Moreover, lesions placed in structures that are efferent to the CEA, such as the lateral hypothalamus or periaqueductal grey, produce selective deficits in either cardiovascular or somatic conditional fear responses, respectively (LeDoux et al., 1988). This suggests that the CEA is the final common pathway for the generation of learned fear responses. Thus, the amygdala contains two distinct subsystems that represent areas of either sensory convergence (BLA) or response divergence (CEA).

Much evidence indicates that the BLA is the crucial neural locus for the formation and storage of fear memories. Selective lesions of the BLA abolish both acquisition and expression of conditional fear in several behavioral paradigms (Campeau and Davis, 1995b; Helmstetter, 1992; Maren, 1998b; Maren et al., 1996a; Sananes and Davis, 1992). In addition, BLA lesions yield deficits in conditional fear when they are made up to one month after training (Cousens and Otto, 1998; Lee et al., 1996; Maren et al., 1996a) or after extensive overtraining (Maren, 1998b). Moreover, manipulations that temporarily disable amygdaloid neurons prevent both the acquisition and expression of fear conditioning (Helmstetter and Bellgowan, 1994; Muller et al., 1997). Fear-conditioning deficits are associative in nature, because rats with BLA lesions can perform the freezing response under some conditions (Maren, 1998a; Maren, in press).

The view that the BLA is a locus of plasticity during aversive learning is further supported by electrophysiological studies of neuronal activity in the amygdala during auditory fear conditioning. For example, neurons in the amygdala exhibit short-latency, CS-elicited firing during aversive learning (Maren et al., 1991; Quirk et al., 1995).

Associative neuronal firing in the BLA precedes the development of both behavioral CRs and associative firing in other brain structures, including the auditory cortex (Quirk et al., 1997). Moreover, an intact amygdala is required for the acquisition of at least some forms of neuronal plasticity in the auditory cortex (Armony et al., 1998). Because of its essential role in forming and storing fear memories, the BLA serves as an ideal anatomical substrate for analyzing the relationship of synaptic plasticity mechanisms, such as LTP, to behavior.

NMDA receptor antagonists and Pavlovian fear conditioning

An important breakthrough in understanding the role LTP plays in behavior came with the discovery that some forms of LTP induction require activation of the NMDA subclass of glutamate receptors. Hence, a typical strategy for investigating the role for LTP in learning involves administering NMDA receptor antagonists to animals prior to a learning episode. Methods for delivering NMDA receptor antagonists range from systemic administration of drugs that permeate the blood-brain barrier to discrete intracranial infusion of antagonists into small brain areas. Insofar as some forms of LTP require NMDA receptor activation, the assumption is that learning tasks that are sensitive to NMDA receptor antagonists may also require NMDA receptor-dependent LTP. Of course, NMDA receptors mediate other cellular functions in addition to LTP (Leung and Desborough, 1988; Sah et al., 1989). So, the fact that a learning task is sensitive to NMDA receptor antagonists is a necessary, but not sufficient, condition for involvement of NMDA receptor-dependent LTP in the task.

Studies that have examined the contribution of NMDA receptors to fear conditioning have used both intracerebroventricular (ICV) infusion of NMDA receptor antagonists into the lateral ventricles and local drug infusions into discrete brain areas. Collectively,

the data reveal an important role for NMDA receptors in the acquisition, and, in some

cases, the expression of learned fear. For example, Fanselow and colleagues have

performed an extensive examination of the effects of ICV administration of the NMDA

receptor antagonist, APV, on the acquisition and expression of conditional freezing to

contextual stimuli in rats (Fanselow et al., 1994; Kim et al., 1991; Kim et al., 1992).

This work indicates that pretraining infusions of APV eliminate the acquisition of

conditional freezing to the contextual cues associated with footshock without affecting

either footshock sensitivity or the performance of the freezing response (Kim et al.,

1991). The disruption of fear conditioning by APV was dose-dependent and

stereospecific (i.e., only D-APV blocked conditioning). Moreover, the conditional

freezing impairments were not state-dependent, insofar as rats both trained and tested

after APV infusion exhibited equally robust deficits in conditional freezing. The

impairment in fear acquisition was complete insofar as there was no evidence of savings

(learning that is not reflected in performance) following reacquisition training in a drug-

free state (Kim et al., 1992). Importantly, APV was only effective in preventing

acquisition of conditional freezing if it was present during or before training--immediate

posttraining administration of APV did not impair the acquisition of freezing.

    In subsequent studies, Fanselow and colleagues examined whether the memory

impairments produced by ICV APV were time-limited and/or modality-specific. It was

found that rats treated with APV did in fact exhibit conditional freezing immediately

following footshock, so-called immediate postshock freezing (Kim et al., 1992). This

indicates that APV did not effect the encoding of a short-term memory for the context-

shock association. However, APV did interfere with the establishment of a long-term

memory for this association. In an effort to determine the extent of fear conditioning

deficits after APV infusion, Fanselow and colleagues next examined whether fear

conditioning to an auditory CS would be impacted by ICV APV infusion. In these

experiments, footshock was always signaled by a tone CS during training. For fear

testing, conditional freezing to the contextual CS was assessed in the training chamber,

and conditional freezing to the tone CS was assessed in a novel context. These

experiments revealed that APV infusion before training only disrupted the acquisition of

contextual fear conditioning; auditory fear conditioning was only minimally affected by

APV infusion (Fanselow et al., 1994). Unfortunately, ICV methodology does not allow

one to determine the locus upon which the APV was exerting its effects. However, the

selective effect of APV on contextual fear conditioning suggests that APV was affecting

the hippocampus, because hippocampal lesions typically yield deficits in contextual, but

not auditory, fear conditioning (Kim and Fanselow, 1992; Phillips and LeDoux, 1992) but

see (Maren et al., 1997). Therefore, these studies indicate that NMDA receptor activation

is required for contextual fear conditioning, and they implicate NMDA receptor-

dependent LTP in the hippocampus in this form of learning.

   In addition to the ICV experiments, a number of studies have examined the effects of

local administration of APV into discrete brain areas.   In the first study of its kind, Davis

and colleagues demonstrated that infusion of APV into the basolateral amygdala prevents

the acquisition of conditional fear to a visual CS in a fear-potentiated startle paradigm

(Miserendino et al., 1990). This effect was dose-dependent and was not due to an APV-

induced shift in footshock sensitivity. Importantly, APV infusion into the amygdala

before testing did not affect the expression or performance of an already-learned CR.

Furthermore, APV infusion into the cerebellar interpositus nucleus, a brain structure that

is not required for fear conditioning, did not affect acquisition of fear-potentiated startle.

Subsequent work has demonstrated that intra-amygdala APV also blocks the acquisition, but not expression of fear-potentiated startle to acoustic CSs (Campeau et al., 1992). The deleterious effect of APV on fear-potentiated startle acquisition has also been demonstrated in second-order conditioning, in which a CS (CS1) that has previously been paired with shock serves as the reinforcer for a novel CS (CS2) in a second phase of training. Under these conditions, conditional fear accrues to CS2 despite the fact that CS2 itself is never paired with the US. APV infusion into the amygdala prior to second-order conditioning does not affect expression of conditional fear to CS1, but does affect the acquisition of the second-order CR to CS2 (Gewirtz and Davis, 1997). This suggests that APV impairs fear conditioning by attenuating an associative mechanism, rather than affecting CS or US processing *per se*.

In addition to blocking the original acquisition of fear-potentiated startle, intra-amygdala APV infusions also prevent the extinction of this conditional fear response (Falls et al., 1992). Lee and Kim (1998) have also demonstrated that intra-amygdala APV blocks the extinction of a conditional freezing response (Lee and Kim, 1998). Because extinction appears to represent the acquisition of a new inhibitory CS-'no US' association (as opposed to the erasure of the original CS-US association), these data extend the role for NMDA receptors in the amygdala to inhibitory forms of learning. It would be of interest to examine whether APV effects the acquisition of other inhibitory associations, such as those acquired during conditioned inhibition training.

In addition to the fear-potentiated startle paradigm, the effects of intra-amygdala APV have also been examined in the conditional-freezing paradigm (Maren et al., 1996b). In these experiments, we found that intra-amygdala APV administered before training produced a robust impairment in the acquisition of conditional freezing that was not do a

change in footshock sensitivity or motor activity. Intra-amygdala APV only blocked conditioning when it was present in the BLA during training; immediate posttraining infusion of APV did not affect conditional freezing (Maren et al., 1996b). The effects of intra-amygdala APV did not appear to be time-limited, insofar as immediate postshock freezing was impaired by APV infusion. However, another study has not found impairments in immediate postshock freezing after APV infusion (Lee and Kim, 1998). As with ICV infusions, immediate posttraining administration of NMDA receptor antagonists did not affect the acquisition of conditional freezing. However, unlike the results obtained from the fear-potentiated startle paradigm, we found that the effects of intra-amygdala APV were not specific to acquisition; the expression or performance of previously acquired fear CRs was also impaired by intra-amygdala APV (Maren et al., 1996b). This pattern of results has recently been replicated (Lee and Kim, 1998) and may be due to the influence of NMDA receptor antagonists on evoked-potentials in the amygdala (Li et al., 1995; Maren and Fanselow, 1995). Thus, it appears that NMDA receptor activation is generally involved in the acquisition of fear CRs, but selectively involved in the expression of the conditional freezing (see Lee and Kim (1998) for a discussion of this issue). Nonetheless, the evidence implicates NMDA receptor-dependent processes in the amygdala, and possibly amygdaloid LTP, in the acquisition of fear conditioning.

Work in the Morris water maze has revealed that the deficits in learning incurred after administration of NMDA receptor antagonists are often ameliorated by prior familiarization with the task requirements in a drug-free state (Bannerman et al., 1995; Saucier and Cain, 1995). To determine whether this was true for fear conditioning paradigms, Lee and Kim (1998) examined whether the acquisition of a fear CR prior to

APV administration affected the subsequent acquisition of a novel CS-US association

under the influence of APV. In contrast to the results obtained in the Morris water maze,

rats that received aversive 'pretraining' still exhibited robust deficits in the acquisition of

conditional freezing when subsequently trained on a new CS-US association after intra-

amygdala APV infusion. These results strongly suggest that intra-amygdala APV

attenuates the acquisition of conditional fear associations, rather than having a

nonassociative effect on task performance.

   The amygdala is not the only brain area to have been targeted in APV infusion

experiments. The effects of intra-hippocampal administration of APV on the acquisition

of fear conditioning have also been examined. Fanselow and colleagues found that the

acquisition of contextual fear conditioning was impaired by pretraining infusions of APV

into the dorsal hippocampus (Young et al., 1994). Unlike the effects of intra-amygdala

APV infusion, intra-hippocampal APV infusion produces a selective effect on contextual

fear conditioning (Stiedl, 1998).   Of great importance, these studies indicate that NMDA

receptors in the hippocampus and amygdala play different roles in fear conditioning.

   It is well documented that NMDA receptor activation is only the first step in a

biochemical cascade that ultimately leads to synaptic modification. Activation of

intracellular protein kinases, which are stimulated by NMDA receptor activation, is

essential for the induction of LTP. Examinations of the role for protein kinases in fear

conditioning have just begun, but there is already evidence that various kinases are

required for establishing long-term fear memories. For instance, Kandel and colleagues

have shown that post-training ICV administration of protein kinase A (PKA) inhibitors

impairs memory consolidation for contextual fear conditioning (Bourtchouladze, 1998).

Likewise, LeDoux and colleagues have found that posttraining ICV administration of

PKA and mitogen-activated protein kinase (MAPK) inhibitors disrupts the memory for

contextual and auditory fear conditioning (Schafe et al., 1999). Furthermore, Davis and

colleagues have reported that intra-amygdala administration of either PKA or CamKII

inhibitors attenuates the acquisition of fear-potentiated startle (Ding et al., 1998).

Although these studies are in an early stage, they seem to indicate that many of the

kinases that have already been implicated in LTP have a role in Pavlovian fear

conditioning.

Less work has been performed on the influence of AMPA receptor ligands on the

acquisition of conditional fear. Davis and colleagues have shown that intra-amygdala

infusion of AMPA receptor antagonists impair both the acquisition and expression of

fear-potentiated startle (Kim et al., 1993; Walker and Davis, 1997). Additionally,

LeDoux and colleagues have shown that AMPA receptor agonists infused into the

amygdala prior to training enhance the acquisition of conditional freezing (Rogan et al.,

1997a).

Collectively, these data reveal an important role for both NMDA and AMPA receptors

in the amygdala in the acquisition and expression of Pavlovian fear conditioning. Insofar

as these receptors are essential for the induction and expression of LTP, these data also

suggest that both hippocampal and amygdaloid LTP are required for the acquisition of

learned fear responses. Importantly, however, these results reveal that NMDA receptors

in the amygdala and hippocampus play different roles in the acquisition of fear

conditioning. Specifically, hippocampal NMDA receptors are only required for the

acquisition of long-term conditional fear memories to contextual CSs, whereas

amygdaloid NMDA receptors are required for the acquisition and, in some cases, the

expression of short- and long-term fear memories to contextual, visual, and auditory CSs.

The different involvement of amygdaloid and hippocampal NMDA receptors in the acquisition of fear conditioning suggests that LTP in these structures subserves different roles in this form of learning.

Correlations between LTP and Pavlovian fear conditioning

The foregoing studies indicate that both hippocampal and amygdaloid NMDA receptors are involved in the acquisition of Pavlovian fear conditioning in rats. By extension, these results implicate NMDA receptor-dependent LTP in these brain areas in the acquisition of conditional fear.   With respect to the involvement of hippocampal LTP in contextual fear conditioning, there are no studies that have attempted to directly measure hippocampal synaptic transmission during the acquisition of contextual fear conditioning. However, a number of studies have used a correlational approach to examine hippocampal LTP, particularly perforant path-dentate granule cell (PP-GC) LTP, in relation to contextual fear conditioning.

In one series of experiments, we attempted to determine whether a behavioral manipulation that was known to enhance acquisition rate in several learning paradigms (e.g., Berry and Swain, 1989) would have facilitatory effects on the induction of hippocampal LTP in anesthetized rats. The behavioral manipulation we chose for this purpose was acute water deprivation, for which access to water was restricted to 1-hour per day for four days. In line with the previously reported facilitatory effects of water deprivation on learning, we found that water deprivation reliably enhanced the magnitude of PP-GC LTP induced by high-frequency stimulation (Maren et al., 1994c). This effect was not due to a change in the properties of baseline synaptic transmission at PP-GC synapses. However, water deprivation did increase the proportion of theta-frequency

activity in the electroencephalogram, which might have favored greater levels of LTP

induction (Huerta and Lisman, 1995).

   With this LTP enhancing manipulation in hand, we next sought to determine whether

water deprivation would affect the acquisition of contextual fear conditioning, a

hippocampus-dependent task that others had suggested might be mediated by

hippocampal LTP (e.g., Kim et al., 1991). We found that water deprivation prior to fear

conditioning significantly enhanced the acquisition of contextual fear conditioning

(Maren et al., 1994c). The enhancement in conditional freezing to the contextual CSs

was only observed with sub-asymptotic levels of training; therefore water deprivation

increased the rate, but not asymptote, of contextual fear conditioning. This effect was not

due to enhanced footshock sensitivity. Interestingly, the enhancement of fear

conditioning by water deprivation was specific to contextual fear conditioning; water

deprivation did not enhance auditory fear conditioning (Maren et al., 1994b). We have

also recently reported that this enhancement of contextual fear conditioning is specific to

water deprivation. That is, a comparable period of food deprivation does not affect fear

conditioning (Maren and Fanselow, 1998). Collectively, these results reveal that water

deprivation has a specific effect on the acquisition rate of contextual fear conditioning, a

hippocampus-dependent task, and also augments hippocampal LTP induction.

   These experiments suggest a role for hippocampal LTP in contextual fear

conditioning. To further explore this relationship, we examined whether individual

differences in aversive learning correlated with hippocampal LTP induction, insofar as

we had previously demonstrated a relationship between hippocampal LTP and individual

differences in nonassociative fear responses (Maren et al., 1993; Maren et al., 1994d;

Mitchell et al., 1993). To this end, we examined whether well known sex differences in

aversive learning correlate with hippocampal LTP. As a first step, we simply assessed

the magnitude of PP-GC LTP in adult male and female rats. Somewhat to our surprise,

we found a robust sex difference in the magnitude of PP-GC LTP induced *in vivo*: males

exhibited reliably more LTP than females (Maren, 1995; Maren et al., 1994a). Given the

sex difference in hippocampal LTP, we hypothesized that male and female rats should

exhibit a sex difference in contextual fear conditioning. To test this hypothesis, we

simply examined Pavlovian fear conditioning in male and female rats. Consistent with

the sex difference in hippocampal LTP, male rats exhibited greater levels of contextual

freezing than female rats (Maren et al., 1994a). There was no sex difference in fear

conditioning to an auditory CS, consistent with the specific role for hippocampal LTP in

contextual fear conditioning. The basis for the sex difference in hippocampal LTP and

fear conditioning is not known, although a role for circulating testosterone in adult male

rats has been ruled out (Anagnostaras et al., 1998).   Preliminary results from our

laboratory indicate that the ovarian steroid, estrogen, in adult female rats contributes to

these sex differences.

   The parallel between deprivation-induced enhancements in hippocampal LTP

induction and contextual fear conditioning, and the correlation between sex differences in

both hippocampal LTP and contextual fear conditioning provides further evidence for a

role for hippocampal LTP in the mediation of contextual fear conditioning. And while

these correlations do not necessarily implicate causation with regard to the role for LTP

in contextual fear conditioning, they are nevertheless consistent with such a role. Indeed,

such correlations are necessary for hypotheses that invoke hippocampal LTP as a

mechanism for contextual fear conditioning (Fanselow, 1997; Maren, 1997).

In addition to hippocampal LTP, the role for amygdaloid LTP in Pavlovian fear

conditioning has received considerable attention (Maren, in press). Amygdaloid LTP,

which has been studied in thalamic (Clugnet and LeDoux, 1990; Rogan and LeDoux,

1995) and hippocampal (Maren and Fanselow, 1995) projections to the amygdala,

exhibits several properties that are similar to those exhibited by hippocampal LTP

(Maren, 1996). Some forms of amygdaloid LTP are NMDA receptor-dependent (Huang

and Kandel, 1998; Maren and Fanselow, 1995), although, as in the hippocampus (Grover

and Teyler, 1990), there also are NMDA receptor-independent forms of LTP in the

amygdala (Chapman and Bellavance, 1992). Amygdaloid LTP also appears to require

the activation of protein kinases, such as PKA (Huang and Kandel, 1998), that are also

involved in hippocampal LTP. Thus, while differences between the two mechanisms

may exist, we will assume for the purpose of this discussion that they are largely similar.

The first non-pharmacological evidence to link amygdaloid LTP to fear conditioning

has emerged from studies in the LeDoux laboratory examining auditory evoked-

potentials in the amygdala following either LTP induction or fear conditioning. Rogan

and LeDoux (1995) have shown that induction of LTP at thalamoamygdaloid synapses *in

vivo* potentiates auditory evoked-potentials in the amygdala that are transmitted through

the thalamus. This suggests that experimental induction of LTP at thalamoamygdaloid

synapses has functional consequences for the processing of acoustic stimuli that use these

synapses. In a related study, LeDoux and colleagues have recently demonstrated that

auditory evoked-potentials in the thalamoamygdaloid pathway are also augmented during

the acquisition of auditory fear conditioning (Rogan et al., 1997b). The increase in the

amplitude of auditory evoked-potentials in the amygdala was only observed in rats

received paired CS and US presentations. Moreover, the change in the evoked-potentials

was uncoupled from freezing behavior, insofar as rats receiving unpaired training exhibited similar levels of freezing behavior during training, but did not exhibit an increase in the amplitude of auditory evoked-potentials. The similar increases in auditory evoked-potentials in the amygdala following both tetanic LTP induction and fear conditioning suggests that LTP-like increases in thalamoamygdaloid synaptic transmission contribute to the acquisition of auditory fear conditioning.

In a related study, McKernan and Shinnick-Gallagher (1997) have shown that fear-potentiated startle training enhances the amplitude of synaptic currents in amygdaloid neurons *in vitro*. In these experiments, rats were first trained in the fear-potentiated startle task, and then sacrificed for *in vitro* electrophysiological experiments. Intracellular recordings were obtained from lateral amygdaloid neurons, and currents in these cells were evoked by electrical stimulation of axons presumed to originate from the auditory thalamus. Rats receiving paired CS-US trials, but not those receiving unpaired trials, exhibited a marked increase in stimulus-evoked currents in amygdaloid neurons, and this effect appeared to be limited to synaptic currents derived from the AMPA subclass of glutamate receptors. Fear conditioning also reduced paired-pulse facilitation, in which the evoked response to the second stimulus of a pair is larger than that to the first stimulus. This indicates that fear-potentiated startle training had increased neurotransmitter release in the thalamoamygdaloid pathway. Synaptic transmission in the endopyriform nucleus, which is not believed to play a role in fear conditioning, was not altered by the conditioning procedures. Insofar as tetanus-induced amygdaloid LTP is associated with increased evoked responses and enhanced neurotransmitter release (Huang and Kandel, 1998; Maren and Fanselow, 1995), it would appear that fear-potentiated startle training induced a form of 'behavioral' LTP. These results provide

strong support for a role for amygdaloid LTP in the acquisition of fear conditioning. Of course, it would be of interest to determine whether these conditioning-related changes in amygdaloid synaptic transmission are blocked by NMDA receptor antagonist administration during training.

Gene targeting, LTP, and fear conditioning

   A more direct test of the role for hippocampal LTP in fear conditioning has been provided by several recent studies that have taken advantage of genetically modified mice, which have been engineered with genetic manipulations that either disable or eliminate key proteins in the intracellular biochemical cascades that mediate LTP. Although this technique is clearly a powerful and exciting approach to studying the neural substrates of learning and memory, it must be kept in mind that these studies have several caveats. For instance, the irreversible nature of the mutations, the opportunity for developmental compensation, and the inability to localize the mutations within discrete neural subregions need to be considered when interpreting the results from these studies.

   The first study to examine the relationship between hippocampal LTP and Pavlovian fear conditioning involved the use of mice that lacked the   isoform of protein kinase C (PKC), an enzyme that has been implicated in both LTP and learning and memory. Mice that lack PKC exhibited mild deficits in contextual, but not auditory, fear conditioning (Abeliovich et al., 1993b). In contrast, they exhibited normal immediate postshock freezing, suggesting that their short-term memory for contextual fear was intact. The normal level of freezing to the auditory CS indicates that the deficits in contextual freezing were not due to an inability to perform the freezing response. The selective deficit in contextual conditioning is interesting insofar as the mice also exhibited

impairments in the induction of hippocampal LTP in area CA1 *in vitro* (Abeliovich et al.,

1993a). There were no deficits in either baseline synaptic transmission or paired-pulse

facilitation and post-tetanic potentiation (PTP), which are measures of presynaptic

neurotransmitter release. Interestingly, the deficits in hippocampal LTP induction could

be overcome with low-frequency priming stimulation. The fact that some capacity to

exhibit LTP was evident in these mice might explain the incomplete nature of the

contextual freezing deficits.   Unfortunately, amygdaloid LTP was not examined in these

animals, although the pattern of behavioral results suggests that it would be normal.

Together, these data suggest that hippocampal LTP is involved in establishing memories

for long-term contextual fear.

A more recent study has examined the influence of a targeted mutation of the cAMP-

responsive element-binding (CREB) protein, which is a transcription factor thought to

play an important role in establishing long-term memories, on both fear conditioning and

hippocampal LTP. Mice with a disruption of the   and   isoforms of CREB were found

to exhibit robust impairments in both contextual and auditory fear conditioning

(Bourtchuladze et al., 1994). These impairments were time-dependent insofar as freezing

to both contexts and tones was found to be intact when measured within 30 or 60 minutes

of training, respectively. However, conditional freezing was nearly absent at long (24

hour) retention intervals. Thus, the sparing of short-tem fear memories reveals that the

CREB mutants were capable of normal freezing under some conditions. Moreover, the

time-dependent loss of conditional freezing over long retention intervals indicates that

CREB is essential for consolidating long-term fear memories. Paralleling the time-

dependency of fear conditioning, mice that lack CREB also exhibited impairments in

hippocampal LTP in area CA1 *in vitro* (Bourtchuladze et al., 1994). While CREB

mutants exhibited normal paired-pulse facilitation and PTP, they showed a marked deficit in LTP. This was manifest as a more rapid decay of the potentiation over a 2-hour period. Interestingly, the time period over which LTP decayed appeared to parallel the time period over which fear memories are lost in CREB mutants. A similar pattern of results has been obtained in mice that express an inhibitory form of the regulatory subunit for PKA, an enzyme known to play a role on LTP (Abel et al., 1997). In this case mice also exhibited long-term, but not short-term, impairments in both contextual and auditory fear conditioning. Although neither group of investigators has measured amygdaloid LTP in these mice, the behavioral data would suggest that it should be impaired in CREB mutants. In agreement with the role for CREB in fear conditioning , it has recently been reported that both contextual and auditory fear conditioning rapidly induce CREB in the hippocampus and amygdala (Impey et al., 1998).

In yet another series of studies, Kandel and colleagues have examined mice that overexpress a mutant form of Ca^{2+}-calmodulin-dependent protein kinase II (CamKII) in the amygdala, hippocampus and striatum (Mayford et al., 1996). In the hippocampus, CamKII is activated by Ca^{2+}-influx through the NMDA-receptor. It interacts with a number of substrates within neurons, including CREB.   Not surprisingly, CamKII has been demonstrated to have a role in LTP induction and mice that overexpress the transgene for this protein do not exhibit LTP in the hippocampus following theta-frequency stimulation, for example (Mayford et al., 1996). Interestingly, these transgenic mice (Mayford et al., 1996), as well as mice that lack CamKII (Chen et al., 1994), also exhibit impairments in Pavlovian fear conditioning to both contextual and auditory cues. The global impairment in conditional fear to both contextual and auditory cues suggests the existence of amygdala dysfunction in these genetically modified mice. And although

the nature of this dysfunction is not yet known, it is tempting to speculate that it will take the form of impaired amygdaloid LTP.

A more direct demonstration of a specific role for amygdaloid LTP in fear conditioning has come from studies of mice that lack Ras-GRF, a neuron-specific guanine nucleotide releasing factor that is activated by both Ca^{2+} and G-protein-coupled messengers.   Electrophysiological recordings from brain slices obtained from mice lacking Ras-GRF has revealed a pronounced deficit in LTP in the basolateral nucleus of the amygdala (Brambilla et al., 1997). Interestingly, these mice also exhibit impairments in consolidating long-term memories for Pavlovian fear conditioning to both contextual and acoustic stimuli. These deficits in LTP and learning were selective for the amygdala and Pavlovian fear conditioning insofar as both hippocampal LTP and spatial learning in Ras-GRF knockouts were normal (Brambilla et al., 1997). Interestingly, Ras-GRF modulates CREB activity through the MAPK pathway. A role for MAPK in fear conditioning has recently been demonstrated (Atkins et al., 1998). Together, these results provide strong support for the view that synaptic LTP in the amygdala is required for the establishment and maintenance of emotional memories.

_____

Insert Figure 1 about here

_____

Differential roles for hippocampal and amygdaloid LTP in Pavlovian fear conditioning

Several lines of study converge upon a role for both hippocampal and amygdaloid LTP in the acquisition of Pavlovian fear conditioning. As we have seen, the LTP-

learning connection for Pavlovian fear conditioning is supported by behavioral, pharmacological, electrophysiological, and molecular experiments. Fear conditioning is 1) prevented by NMDA receptor antagonists, 2) associated with an induction of behavioral LTP, 3) correlated with tetanic LTP, 4) positively modulated by behavioral manipulations that facilitate tetanic LTP and 5) impaired by manipulations that disrupt or eliminate crucial elements of the biochemical cascade underlying LTP.   It is also apparent from the studies discussed to this point that LTP in the amygdala and hippocampus play different, yet complementary, roles in fear conditioning. This is an important outcome that deserves further attention. It illustrates that even within a simple learning paradigm, such as Pavlovian fear conditioning, synaptic plasticity mechanisms such as LTP play multiple and distinct roles in the learning process.

The roles for amygdaloid and hippocampal LTP in Pavlovian fear conditioning diverge along at least three dimensions: time, sensory modality, and associative function. With respect to the temporal dimension, there is good evidence that LTP is only required for establishing long-term fear memories (i.e., memories that are at least two hours old). Short-term fear memories (i.e., memories that last only minutes), such as those evidenced by immediate postshock freezing, do not appear to require LTP. Rather, it is more likely that short-term synaptic plasticity mechanisms, such as PTP, mediate these memories. The requirement for LTP in the establishment of long-term memories might be explained by the important role for protein synthesis in Pavlovian fear conditioning. Indeed, it has recently been demonstrated that infusion of protein synthesis inhibitors into the amygdala prior to fear conditioning prevents the establishment of long-term, but not short-term fear memories to both contextual and auditory cues (Bailey et al., 1999). However, the role for LTP in conditional fear that is expressed over very long retention interval (> 30 days)

has not been explored. Recent studies demonstrate a time-limited role for the

hippocampus in the expression of contextual fear conditioning (Anagnostaras et al., 1999;

Kim and Fanselow, 1992; Maren et al., 1997) and a time-independent role for the

amygdala in the expression of contextual and auditory fear conditioning (Kim and Davis,

1993; Lee et al., 1996; Maren et al., 1996a). This suggests that amygdaloid LTP might

have a much more temporally enduring role in the consolidation and maintenance of fear

memories than hippocampal LTP.

With respect to the sensory dimension, there is strong evidence that hippocampal LTP

plays a unique role in fear conditioning to contextual stimuli, whereas amygdaloid LTP

has a more general role in fear conditioning to both contextual and discrete CSs, such as

tones and lights (see Figure 1). This is supported by the fact that manipulations that

selectively interfere with hippocampal LTP disrupt only contextual fear conditioning,

whereas manipulations that interfere with amygdaloid LTP disrupt both contextual and

auditory fear conditioning. The different roles for hippocampal and amygdaloid LTP in

conditioning to contextual and discrete CSs is imposed by the anatomy of the fear

conditioning circuit. That is, the hippocampus appears to be part of a sensory pathway

that conveys contextual CSs to the amygdala for association with footshock. In contrast,

as described above, the amygdala appears to be the essential locus for encoding and

storing CS-US associations. The different functional roles for the hippocampus and

amygdala in fear conditioning provides the foundation for understanding the unique roles

LTP in these structures plays in different aspects of Pavlovian fear conditioning.

The role for the hippocampus in processing contextual CSs has been established in a

number of studies. From this work, it appears that the hippocampus plays a unique role

in forming contextual representations, that is, the process by which the configure or the

conjunction of the contextual cues present during fear conditioning is encoded (Fanselow, 1986; Rudy and Sutherland, 1995). Good and colleagues have suggested that the hippocampus plays an important role in acquiring incidental information about the conditioning context (Good et al., 1998), which might occur during exploration of the conditioning chamber, for example (Maren et al., 1997). In either case, it does not appear that the hippocampus is important for establishing context-US associations (Cho, 1999; Frankland et al., 1998; Maren et al., 1997; Young et al., 1994) -- this is the domain of the amygdala (e.g, (Maren et al., 1996a). The involvement of the hippocampus in forming contextual representations suggests a unique role for hippocampal LTP in this process (Figure 1). There is at least one line of evidence that is consistent with this proposal. It is well established that manipulations that affect CS processing, such as increasing the salience of CSs, affect the rate at which conditional behavior is acquired, but do not affect the level of asymptotic CR performance (Rescorla, 1988).   Insofar as hippocampal LTP has a role in representing contextual CSs, manipulations that modulate hippocampal LTP should affect acquisition rate, but not asymptotic CR performance. This selective rate effect might be obscured by LTP impairing manipulations, unless care is taken to train animals to a behavioral asymptote. To avoid this problem, we have examined the effect on contextual fear conditioning of a manipulation that facilitates hippocampal LTP.   As predicted, we found that enhancing hippocampal LTP enhanced the rate, but not asymptote, of contextual fear conditioning (Maren et al., 1994c). This reveals that hippocampal LTP has a special role in contextual CS processing during Pavlovian fear conditioning. More specifically, we have suggested that hippocampal LTP represents the conjunction of contextual stimuli experienced at the time of conditioning, and that contextual CS salience is modulated by the strength and/or the inclusiveness of the

contextual representation (Maren et al., 1994c). Thus, hippocampal LTP is presumed to regulate phenomena such as the facilitatory effects of context preexposure on the acquisition of contextual fear conditioning (Fanselow, 1990; Rudy, 1996).

In contrast to the hippocampus, the amygdala plays a crucial role in associating CSs and USs during fear conditioning. Thus, the amygdala is also involved in representing stimulus conjunctions, but in this case they are the conjunctions between the CS and the US (Figure 1). Therefore, the role for amygdaloid LTP in fear conditioning is quite different from that of hippocampal LTP, insofar as its induction is driven by the US and is expected to occur in CS pathways to the amygdala. The involvement of amygdaloid LTP in CS-US association is demonstrated by the fact that manipulations that impair amygdaloid LTP also disrupt the acquisition of both contextual and auditory fear conditioning. Insofar as amygdaloid LTP is hypothesized to reflect the levels of associative strength that a CS has acquired, it should be highly correlated with CR performance. Unfortunately, studies have not been performed that systematically examine the relationship of amygdaloid LTP to either the rate or asymptote of fear conditioning. One might expect, however, that manipulations that facilitate amygdaloid LTP would enhance both the acquisition rate and asymptotic performance of conditional fear. Further work is required to examine this hypothesis. Nevertheless, the available evidence strongly support the view that amygdaloid LTP is the crucial plasticity underlying formation and storage of CS-US associations acquired during fear conditioning.

Conclusions

In conclusion, there is now an abundance of data reinforcing the view that there are multiple roles for synaptic plasticity in the acquisition of a simple and adaptive form of associative learning: Pavlovian fear conditioning. It has been established that acquisition of long-term fear memories to the contexts in which shock occurs requires LTP in the hippocampus, whereas the acquisition of conditional fear to both contexts and other discrete cues requires LTP in the amygdala. Hippocampal LTP is proposed to play a special role in the acquisition of contextual CS representations, whereas amygdaloid LTP is proposed to underlie the acquisition of CS-US associations during fear conditioning. Short-lasting forms of synaptic plasticity, such as PTP, may play an important role in encoding short-term fear memories, such as those expressed immediately following an aversive event. Considering all of the available evidence, it is concluded that LTP and related forms of synaptic plasticity are viable mechanisms for memory formation and storage during emotional learning and memory.

References

Abel T, Nguyen PV, Barad M, Deuel TA, Kandel ER, Bourtchouladze R (1997) Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. Cell 88:615-626.

Abeliovich A, Chen C, Goda Y, Silva AJ, Stevens CF, Tonegawa S (1993a) Modified hippocampal long-term potentiation in PKC gamma-mutant mice. Cell 75:1253-1262.

Abeliovich A, Paylor R, Chen C, Kim JJ, Wehner JM, Tonegawa S (1993b) PKC gamma mutant mice exhibit mild deficits in spatial and contextual learning. Cell 75:1263-1271.

Anagnostaras SG, Maren S, DeCola JP, Lane NI, Gale GD, Schlinger BA, Fanselow MS (1998) Testicular hormones do not regulate sexually dimorphic Pavlovian fear conditioning or perforant-path long-term potentiation in adult male rats. Behav Brain Res 92:1-9.

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

Armony JL, Quirk GJ, LeDoux JE (1998) Differential effects of amygdala lesions on early and late plastic components of auditory cortex spike trains during fear conditioning. J Neurosci 18:2592-2601.

Atkins CM, Selcher JC, Petraitis JJ, Trzaskos JM, Sweatt JD (1998) The MAPK cascade is required for mammalian associative learning. Nat Neurosci 1:602-609.

Bailey DJ, Kim JJ, Sun W, Thompson RF, Helmstetter FJ (1999) Acquisition of fear conditioning in rats requires the synthesis of mRNA in the amygdala [In Process Citation]. Behav Neurosci 113:276-282.

Bannerman DM, Good MA, Butcher SP, Ramsay M, Morris RG (1995) Distinct components of spatial learning revealed by prior training and NMDA receptor blockade. Nature (Lond) 378:182-186.

Berry SD, Swain RA (1989) Water deprivation optimizes hippocampal activity and facilitates nictitating membrane conditioning. Behav Neurosci 103:71-76.

Bliss TV, Collingridge GL (1993) A synaptic model of memory: Long-term potentiation in the hippocampus. Nature (Lond) 361:31-39.

Bliss TV, Gardner-Medwin AR (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol (Lond) 232:357-374.

Bliss TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol (Lond) 232:331-356.

Bourtchouladze R, Abel, T., Berman, N., Gordon, R., Lapidus, K., Kandel, E. R. (1998) Different training procedures recruit either one or two critical periods for contextual memory consolidation, each of which requires protein synthesis and PKA. Learn Mem 5:365-374.

Bourtchuladze R, Frenguelli B, Blendy J, Cioffi D, Schutz G, Silva AJ (1994) Deficient long-term memory in mice with a targeted mutation of the cAMP- responsive element-binding protein. Cell 79:59-68.

Brambilla R, Gnesutta N, Minichiello L, White G, Roylance AJ, Herron CE, Ramsey M, Wolfer DP, Cestari V, Rossi-Arnaud C, Grant SG, Chapman PF, Lipp HP, Sturani E, Klein R (1997) A role for the Ras signalling pathway in synaptic transmission and long-term memory. Nature 390:281-286.

Cain DP (1997) LTP, NMDA, genes and learning. Curr Opin Neurobiol 7:235-242.

Campeau S, Davis M (1995a) Involvement of subcortical and cortical afferents to the lateral nucleus of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. J Neurosci 15:2312-2327.

Campeau S, Davis M (1995b) Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. J Neurosci 15:2301-2311.

Campeau S, Miserendino MJ, Davis M (1992) Intra-amygdala infusion of the N-methyl-D-aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. Behav Neurosci 106:569-574.

Chapman PF, Bellavance LL (1992) Induction of long-term potentiation in the basolateral amygdala does not depend on NMDA receptor activation. Synapse 11:310-318.

Chen C, Rainnie DG, Greene RW, Tonegawa S (1994) Abnormal fear response and aggressive behavior in mutant mice deficient for alpha-calcium-calmodulin kinase II [see comments]. Science 266:291-294.

Cho YH, Friedman, E., Silva, A. J. (1999) Ibotenate lesions of the hippocampus impair spatial learning but not contextual fear conditioning in mice. Behav Brain Res 98:77-87.

Clugnet MC, LeDoux JE (1990) Synaptic plasticity in fear conditioning circuits: induction of LTP in the lateral nucleus of the amygdala by stimulation of the medial geniculate body. J Neurosci 10:2818-2824.

Cousens G, Otto T (1998) Both pre- and posttraining excitotoxic lesions of the basolateral amygdala abolish the expression of olfactory and contextual fear conditioning. Behav Neurosci 112:1092-1103.

Davis M (1992) The role of the amygdala in fear and anxiety. Annu Rev Neurosci 15:353-375.

Davis M, Rainnie D, Cassell M (1994) Neurotransmission in the rat amygdala related to fear and anxiety. Trends Neurosci 17:208-214.

Diamond DM, Rose GM (1994) Does associative LTP underlie classical conditioning? Psychobiol 22:263-269.

Ding C, Lee YL, Davis M (1998) Role of PKA and CaM kinases in fear conditioning assessed with fear-potentiated startle using local infusion of Rp-8-Br-cAMP. Soc Neurosci Abstr 24:926.

Elgersma Y, Silva AJ (1999) Molecular mechanisms of synaptic plasticity and memory. Curr Opin Neurobiol 9:209-213.

Falls WA, Miserendino MJ, Davis M (1992) Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. J Neurosci 12:854-863.

Fanselow MS (1984) What is conditioned fear? Trends in Neursosciences 7:460-462.

Fanselow MS (1986) Associative vs topographical accounts of the immediate shock-freezing deficit in rats: Implications for the response selection rules governing species-specific defensive reactions. Learn Motiv 17:16-39.

Fanselow MS (1990) Factors governing one-trial contextual conditioning. Anim Learn Behav 18:264-270.

Fanselow MS (1994) Neural organization of the defensive behavior system responsible for fear. Psychon Bull Rev 1:429-438.

Fanselow MS (1997) Without LTP the learning circuit is broken. Behavioral and Brain Sciences 20:616.

Fanselow MS, Kim JJ, Yipp J, De Oca B (1994) Differential effects of the N-methyl-D-aspartate antagonist DL-2-amino-5-phosphonovalerate on acquisition of fear of auditory and contextual cues. Behav Neurosci 108:235-240.

Fendt M, Fanselow MS (1999) The neuroanatomical and neurochemical basis of conditioned fear. Neurosci Biobehav Rev 23:743-760.

Frankland PW, Cestari V, Filipkowski RK, McDonald RJ, Silva AJ (1998) The dorsal hippocampus is essential for context discrimination but not for contextual conditioning. Behav Neurosci 112:863-874.

Gewirtz JC, Davis M (1997) Second-order fear conditioning prevented by blocking NMDA receptors in amygdala. Nature (Lond) 388:471-474.

Good M, de Hoz L, Morris RG (1998) Contingent versus incidental context processing during conditioning: dissociation after excitotoxic hippocampal plus dentate gyrus lesions. Hippocampus 8:147-159.

Grover LM, Teyler TJ (1990) Two components of long-term potentiation induced by different patterns of afferent activation. Nature (Lond) 347:477-479.

Helmstetter FJ (1992) The amygdala is essential for the expression of conditional hypoalgesia. Behav Neurosci 106:518-528.

Helmstetter FJ, Bellgowan PS (1994) Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. Behav Neurosci 108:1005-1009.

Hitchcock J, Davis M (1986) Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm. Behav Neurosci 100:11-22.

Huang YY, Kandel ER (1998) Postsynaptic induction and PKA-dependent expression of LTP in the lateral amygdala. Neuron 21:169-178.

Huerta PT, Lisman JE (1995) Bidirectional synaptic plasticity induced by a single burst during cholinergic theta oscillation in CA1 in vitro. Neuron 15:1053-1063.

Impey S, Smith DM, Obrietan K, Donahue R, Wade C, Storm DR (1998) Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. Nature Neuroscience 1:595-601.

Iwata J, Chida K, LeDoux JE (1987) Cardiovascular responses elicited by stimulation of neurons in the central amygdaloid nucleus in awake but not anesthetized rats resemble conditioned emotional responses. Brain Res 418:183-188.

Kim JJ, DeCola JP, Landeira-Fernandez J, Fanselow MS (1991) N-methyl-D-aspartate receptor antagonist APV blocks acquisition but not expression of fear conditioning. Behav Neurosci 105:126-133.

Kim JJ, Fanselow MS (1992) Modality-specific retrograde amnesia of fear. Science 256:675-677.

Kim JJ, Fanselow MS, DeCola JP, Landeira Fernandez J (1992) Selective impairment of long-term but not short-term conditional fear by the N-methyl-D-aspartate antagonist APV. Behav Neurosci 106:591-596.

Kim M, Campeau S, Falls WA, Davis M (1993) Infusion of the non-NMDA receptor antagonist CNQX into the amygdala blocks the expression of fear-potentiated startle. Behav Neural Biol 59:5-8.

Kim M, Davis M (1993) Lack of a temporal gradient of retrograde amnesia in rats with amygdala lesions assessed with the fear-potentiated startle paradigm. Behav Neurosci 107:1088-1092.

LeDoux J (1998) Fear and the brain: where have we been, and where are we going? Biol Psychiatry 44:1229-1238.

LeDoux JE, Iwata J, Cicchetti P, Reis DJ (1988) Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. J Neurosci 8:2517-2529.

Lee H, Kim JJ (1998) Amygdalar NMDA receptors are critical for new fear learning in previously fear-conditioned rats. J Neurosci 18:8444-8454.

Lee Y, Walker D, Davis M (1996) Lack of a temporal gradient of retrograde amnesia
    following NMDA-induced lesions of the basolateral amygdala assessed with the
    fear-potentiated startle paradigm. Behav Neurosci 110:836-839.

Leung LW, Desborough KA (1988) APV, an N-methyl-D-aspartate receptor antagonist,
    blocks the hippocampal theta rhythm in behaving rats. Brain Res 463:148-152.

Li XF, Phillips R, LeDoux JE (1995) NMDA and non-NMDA receptors contribute to
    synaptic transmission between the medial geniculate body and the lateral nucleus of
    the amygdala. Exp Brain Res 105:87-100.

Maren S (1995) Sexually dimorphic perforant path long-term potentiation (LTP) in
    urethane-anesthetized rats. Neurosci Lett 196:177-180.

Maren S (1996) Synaptic transmission and plasticity in the amygdala. An emerging
    physiology of fear conditioning circuits. Mol Neurobiol 13:1-22.

Maren S (1997) Arousing the LTP and learning debate. Behavioral and Brain Sciences
    20:622-623.

Maren S (1998a) Neurotoxic basolateral amygdala lesions impair learning but not
    performance of contextual fear conditioning in rats. Soc Neurosci Abstr 24:1682.

Maren S (1998b) Overtraining does not mitigate contextual fear conditioning deficits
    produced by neurotoxic lesions of the basolateral amygdala. J Neurosci 18:3088-
    3097.

Maren S (in press) Neurotoxic basolateral amygdala lesions impair learning and memory
    but not performance of conditional fear in rats. J Neurosci, in press.

Maren S (in press) Long-term potentiation in the amygdala: A mechanisms for
    emotional learning and memory. Trends Neurosci, in press.

Maren S, Aharonov G, Fanselow MS (1996a) Retrograde abolition of conditional fear
    after excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal
    gradient. Behav Neurosci 110:718-726.

Maren S, Aharonov G, Fanselow MS (1997) Neurotoxic lesions of the dorsal
    hippocampus and Pavlovian fear conditioning in rats. Behav Brain Res 88:261-274.

Maren S, Aharonov G, Stote DL, Fanselow MS (1996b) N-methyl-D-aspartate receptors
    in the basolateral amygdala are required for both acquisition and expression of
    conditional fear in rats. Behav Neurosci 110:1365-1374.

Maren S, Baudry M (1995) Properties and mechanisms of long-term synaptic plasticity in
    the mammalian brain: relationships to learning and memory. Neurobiol Learn Mem
    63:1-18.

Maren S, De Oca B, Fanselow MS (1994a) Sex differences in hippocampal long-term
    potentiation (LTP) and Pavlovian fear conditioning in rats: positive correlation
    between LTP and contextual learning. Brain Res 661:25-34.

Maren S, DeCola JP, Fanselow MS (1994b) Water deprivation enhances fear conditioning to contextual, but not discrete, conditional stimuli in rats. Behav Neurosci 108:645-649.

Maren S, DeCola JP, Swain RA, Fanselow MS, Thompson RF (1994c) Parallel augmentation of hippocampal long-term potentiation, theta rhythm, and contextual fear conditioning in water-deprived rats. Behav Neurosci 108:44-56.

Maren S, Fanselow MS (1995) Synaptic plasticity in the basolateral amygdala induced by hippocampal formation stimulation in vivo. J Neurosci 15:7548-7564.

Maren S, Fanselow MS (1996) The amygdala and fear conditioning: Has the nut been cracked? Neuron 16:237-240.

Maren S, Fanselow MS (1997) Electrolytic lesions of the fimbria/fornix, dorsal hippocampus, or entorhinal cortex produce anterograde deficits in contextual fear conditioning in rats. Neurobiol Learn Mem 67:142-149.

Maren S, Fanselow MS (1998) Appetitive motivational states differ in their ability to augment aversive fear conditioning in rats (Rattus norvegicus). J Exp Psychol: Anim Behav Process 24:369-373.

Maren S, Patel K, Thompson RF, Mitchell D (1993) Individual differences in emergence neophobia predict magnitude of perforant-path long-term potentiation (LTP) and plasma corticosterone levels in rats. Psychobiol 21:2-10.

Maren S, Poremba A, Gabriel M (1991) Basolateral amygdaloid multi-unit neuronal correlates of discriminative avoidance learning in rabbits. Brain Res 549:311-316.

Maren S, Tocco G, Chavanne F, Baudry M, Thompson RF, Mitchell D (1994d) Emergence neophobia correlates with hippocampal and cortical glutamate receptor binding in rats. Behav Neural Biol 62:68-72.

Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER (1996) Control of memory formation through regulated expression of a CaMKII transgene. Science 274:1678-1683.

Meiri N, Sun MK, Segal Z, Alkon DL (1998) Memory and long-term potentiation (LTP) dissociated: normal spatial memory despite CA1 LTP elimination with Kv1.4 antisense. Proc Natl Acad Sci (USA) 95:15037-15042.

Miserendino MJ, Sananes CB, Melia KR, Davis M (1990) Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. Nature (Lond) 345:716-718.

Mitchell D, Maren S, Hwang R (1993) The effects of hippocampal lesions on two neotic choice tasks. Psychobiol 21:193-202.

Morris RG, Anderson E, Lynch GS, Baudry M (1986) Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature (Lond) 319:774-776.

Morris RG, Davis S, Butcher SP (1990) Hippocampal synaptic plasticity and NMDA receptors: a role in information storage? Phil Trans Royal Soc Lond Ser B 329:187-204.

Muller J, Corodimas KP, Fridel Z, LeDoux JE (1997) Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. Behav Neurosci 111:683-691.

Phillips RG, LeDoux JE (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106:274-285.

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

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

Rescorla RA (1988) Behavioral studies of Pavlovian conditioning. Annu Rev Neurosci 11:329-352.

Rogan MT, LeDoux JE (1995) LTP is accompanied by commensurate enhancement of auditory-evoked responses in a fear conditioning circuit. Neuron 15:127-136.

Rogan MT, LeDoux JE (1996) Emotion: systems, cells, synaptic plasticity. Cell 85:469-475.

Rogan MT, Staubli UV, LeDoux JE (1997a) AMPA receptor facilitation accelerates fear learning without altering the level of conditioned fear acquired. J Neurosci 17:5928-5935.

Rogan MT, Staubli UV, LeDoux JE (1997b) Fear conditioning induces associative long-term potentiation in the amygdala. Nature (Lond) 390:604-607.

Romanski LM, Clugnet MC, Bordi F, LeDoux JE (1993) Somatosensory and auditory convergence in the lateral nucleus of the amygdala. Behav Neurosci 107:444-450.

Romanski LM, LeDoux JE (1992) Equipotentiality of thalamo-amygdala and thalamo-cortico-amygdala circuits in auditory fear conditioning. J Neurosci 12:4501-4509.

Rosen JB, Hitchcock JM, Miserendino MJ, Falls WA, Campeau S, Davis M (1992) Lesions of the perirhinal cortex but not of the frontal, medial prefrontal, visual, or insular cortex block fear-potentiated startle using a visual conditioned stimulus. J Neurosci 12:4624-4633.

Rudy JW (1996) Postconditioning isolation disrupts contextual conditioning: an experimental analysis. Behav Neurosci 110:238-246.

Rudy JW, Sutherland RJ (1995) Configural association theory and the hippocampal formation: an appraisal and reconfiguration. Hippocampus 5:375-389.

Sah P, Hestrin S, Nicoll RA (1989) Tonic activation of NMDA receptors by ambient glutamate enhances excitability of neurons. Science 246:815-818.

Sananes CB, Davis M (1992) N-methyl-D-aspartate lesions of the lateral and basolateral nuclei of the amygdala block fear-potentiated startle and shock sensitization of startle. Behav Neurosci 106:72-80.

Saucier D, Cain DP (1995) Spatial learning without NMDA receptor-dependent long-term potentiation [see comments]. Nature 378:186-189.

Schafe GE, Nadel NV, Sullivan GM, Harris A, LeDoux JE (1999) Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. Learn Mem 6:97-110.

Shi C, Davis M (1999) Pain pathways involved in fear conditioning measured with fear-potentiated startle: lesion studies. J Neurosci 19:420-430.

Shors TJ, Matzel LD (1997) Long-term potentiation: what's learning got to do with it? Behav Brain Sci 20:597-614; discussion 614-555.

Squire LR, Zola-Morgan S (1991) The medial temporal lobe memory system. Science 253:1380-1386.

Stiedl O, Birkenfeld, K., Palve, M., Heine, C., Spiess, J. (1998) APV impairs context- but not tone-dependent fear in mice without discrimination between background and foreground conditioning. Soc Neurosci Abstr 24:1902.

Swanson LW, Petrovich GD (1998) What is the amygdala? Trends Neurosci 21:323-331.

Walker DL, Davis M (1997) Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. J Neurosci 17:9375-9383.

Young SL, Bohenek DL, Fanselow MS (1994) NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: immunization against amnesia by context preexposure. Behav Neurosci 108:19-29.

Zamanillo D, Sprengel R, Hvalby O, Jensen V, Burnashev N, Rozov A, Kaiser KM, Koster HJ, Borchardt T, Worley P, Lubke J, Frotscher M, Kelly PH, Sommer B, Andersen P, Seeburg PH, Sakmann B (1999) Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning. Science 284:1805-1811.

Index

Figure legends

Figure 1. Schematic diagram illustrating the role for hippocampal and amygdaloid long-term potentiation (LTP) in different associative processes in Pavlovian fear conditioning. Loci of synaptic plasticity are delineated in the figure by the shaded boxes. Hippocampal LTP is posited to play a role in the formation of contextual representations, which are configural or conjunctive representations formed from individual elemental stimuli (A, B, C, D, E). Amygdaloid LTP, on the other hand, is involved in associating conditional and unconditional stimuli (CSs and USs), such as tones and footshocks. The formation of CS-US associations in the amygdala results in the performance of conditional fear responses (CRs), such as freezing or potentiated acoustic startle. As described in the text, amygdaloid LTP is posited to play a role in establishing long-term fear memories for CSs in all modalities, where as hippocampal LTP is involved in establishing long-term memories of the contextual CS.

Figure 1

Contextual elements   Discrete CSs

A   B   C   D   E   Tone or Light

Contextual
configure

A   B   C   D   E

A   B   C   D   E

Hippocampal LTP

Fear CRs

CS - US   Freezing
or
Startle

Amygdaloid LTP

Footshock or Noise

Discrete USs