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Molecular Mechanisms Underlying Emotional Learning and Memory in the Lateral Amygdala

Review

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Fear conditioning is a valuable behavioral paradigm for studying the neural basis of emotional learning and memory. The lateral nucleus of the amygdala (LA) is a crucial site of neural changes that occur during fear conditioning. Pharmacological manipulations of the LA, strategically timed with respect to training and testing, have shed light on the molecular events that mediate the acquisition of fear associations and the formation and maintenance of long-term memories of those associations. Similar mechanisms have been found to underlie long-term potentiation (LTP) in LA, an artificial means of inducing synaptic plasticity and a physiological model of learning and memory. Thus, LTP-like changes in synaptic plasticity may underlie fear conditioning. Given that the neural circuit underlying fear conditioning has been implicated in emotional disorders in humans, the molecular mechanisms of fear conditioning are potential targets for psychotherapeutic drug development.

Memories formed during emotional experiences are stored for future use in similar situations. For example, if we are injured, we acquire information about the stimuli that were associated with the event so that we may later avoid harm. Aversive emotional memories of this type can be studied in the laboratory using classical fear conditioning, a behavioral task in which an initially neutral conditioned stimulus (CS), such as a light or tone, gains emotional properties after being paired with a noxious unconditioned stimulus (US), such as a footshock. Once the CS-US association is learned, the occurrence of the CS initiates behavioral, autonomic, and endocrine responses that help the organism cope with the aversive situation. The expression of these responses in the presence of the CS serves as a measure of the emotional memory created during the learning experience.

Fear conditioning is a powerful procedure for studying the neural basis of emotional memory and of memory in general, because the learning occurs rapidly (within a single trial under some circumstances) and the memory is extremely persistent (it can last a lifetime). Moreover, the fact that the learning involves a specific stimulus that is under the control of the investigator and that comes to elicit well-defined, easily measurable, and quantifiable responses greatly aids in the search for the neural system, as well as the cellular and molecular changes in this system, that underlie learning and memory.

A large body of evidence implicates the lateral nucleus of the amygdala (LA) as a key component of the neural system involved in the formation of memories during fear conditioning, especially when an auditory CS is used (LeDoux, 2000; Maren, 2001; Walker and Davis, 2002; Fanselow and LeDoux, 1999; Blair et al., 2001). For example, damage to the LA prevents fear conditioning from occurring, neurons in the LA are responsive to both the CS and US before conditioning, neural responses elicited by the CS greatly increase after conditioning, and pharmacological blockade of neural activity and its biochemical concomitants interferes with the formation of memories of the fear conditioning experience. The LA is not necessarily the only region in which neural activity changes during fear conditioning. However, it is the only region where changes in neural activity have been shown to be essential for fear conditioning.

The changes in neural activity that occur in LA during fear conditioning are believed to be mediated by enhanced transmission at synapses that process the CS, allowing the CS to come to elicit defensive responses (LeDoux, 2000; Blair et al., 2001; Schafe et al., 2001; Sah et al., 2003; Pare et al., 2004). This synaptic plasticity involves cellular and molecular events induced by the convergence of CS and US inputs onto LA cells.

Long-term potentiation (LTP), an artificial means of inducing synaptic plasticity and a physiological model of learning and memory (Lynch, 1986; Bliss and Collingridge, 1993; Malenka and Nicoll, 1999; Martin et al., 2000), is believed to be a valuable tool for studying the cellular and molecular mechanisms that underlie synaptic plasticity in the LA (for review, see Blair et al., 2001; Maren, 2001; Sah et al., 2003). LTP occurs at CS input synapses in LA (Chapman et al., 1990; Rogan and LeDoux, 1995; Huang and Kandel, 1998; Weisskopf et al., 1999), and fear conditioning induces associative LTP-like changes in the responses of LA neurons (McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997).

Below, we will summarize research on the cellular and molecular mechanisms engaged by fear conditioning. We will consider both the initial events that induce synaptic plasticity during CS-US pairing and the later events that convert the early plasticity into persistent modifications of synaptic transmission and thus that consolidate the memory.

In surveying this literature, we will focus on studies that have used drug infusions into the LA to manipulate fear learning and memory at various time points related to stages of memory formation. We will also consider the effects of drugs on synaptic plasticity produced by induction of LTP in LA. Genetic manipulations have also provided many important insights into the molecular basis of fear memory and amygdala LTP. We will mention such studies in passing when relevant, but we do not emphasize this approach, since it typically involves molecular modifications in widespread brain regions and thus does not usually specifically implicate mecha-

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nisms within the LA (for summaries of genetic studies related to fear conditioning and amygdala LTP, see Tsien, 2000; Kandel, 2001; Maren, 2001; Matynia et al., 2002; Frankland et al., 2003; Silva, 2003; Tonegawa et al., 2003).

Phases of Fear Memory Defined through Drug Manipulations

We concentrate on the molecular mechanisms underlying two specific phases in the life of a fear memory. These are called learning, or *acquisition*, and long-term memory (LTM) formation, or *consolidation*. Acquisition and consolidation are assessed by using behavioral tasks that involve a training component (this is what prompts acquisition) and a test component in which the effects of training are assessed after an appropriate delay (this is how you determine whether learning occurred and whether LTM was formed). Other important aspects of fear memory, such as extinction and reconsolidation, will not be discussed here (for reviews, see Nader et al., 2000; Dudai, 2002; Quirk and Gehlert, 2003; Sotres-Bayon et al., 2004).

To determine whether a particular molecule is involved in the acquisition or consolidation of memory, drugs that interfere with that molecule are delivered at certain time points in relation to training or testing. In evaluating whether a drug affects acquisition or consolidation, it is thus necessary to consider both the time the drug is administered with respect to training or testing and the time or times when memory is tested.

When Should Drugs Be Administered and Memory Be Tested in Order to Implicate Molecules in Acquisition of Conditioned Fear?

In fear conditioning studies of learning or acquisition, the aim is to understand the molecular events that are involved in the formation of an association between the CS and US. Thus, the drug treatment is typically administered prior to training. Memory is then tested by measuring fear responses elicited by the CS, typically 24 hr after training. If memory is impaired (that is, conditioned fear responses are reduced relative to a control group), the usual conclusion is that the drug disrupted learning. However, to conclude that the drug specifically blocked learning, as opposed to routine neural processes required for processing the auditory stimulus in the LA during acquisition, it is necessary to show that administration of the drug immediately prior to the testing of fear responses has no effect. This is called an *expression test* of fear memory.

Studies of acquisition thus typically include groups that receive the drug or vehicle before training, as well as other groups that receive the drug before testing, with testing typically occurring 24 hr after training. However, additional drug infusion and memory test points are useful in drawing the conclusion that pretraining infusions affect the acquisition of the CS-US association. Ideally, the drugs should be administered both pre- and immediately posttraining. This additional condition is not usually included.

Posttraining drug treatment is important, because the half-life of most drugs is such that, although administered prior to training, the drug will still be active in the minutes or even hours after training, especially in tasks such as fear conditioning that often involve one or at most a few training trails. This is relevant because in other aversive learning paradigms, immediate posttraining infusions of drugs into the LA have been shown to modulate the strength of memory formation (McGaugh, 2000). Thus, to conclude that a drug given before training affects only acquisition, it is necessary to show that delivery of the drug immediately after training has no effect on memory.

In addition to using both pretraining and posttraining infusions, acquisition studies should also test both short-term memory (STM) and LTM. STM is usually assessed by testing between 1 and 4 hr after training, whereas LTM is usually tested 24 hr after training. These times are based on the belief that LTM but not STM depends on protein synthesis, which is supported by findings showing that administration of protein synthesis inhibitors immediately after training disrupts LTM but disruption of protein synthesis later (after 4–5 hr) does not affect LTM, presumably because STM has been converted into LTM (for review, see Schafe et al., 2001). Testing of both STM and LTM is important to distinguish an effect on acquisition from an effect on STM. A drug that affects STM when given before training could have its effect by disrupting acquisition or by interfering with the maintenance of the association in STM. However, if the same drug has no effect on STM when given after training, it is possible to conclude that the drug disrupted the acquisition of the CS-US association rather than interfered with the maintenance of STM. In practice, most studies use STM as a test of acquisition. Below, we will therefore discuss drug effects on STM in the acquisition section. However, it is important to remember that acquisition and STM are likely to be mediated by unique molecular mechanisms that are potentially separable. The mechanisms that mediate STM itself have not been explicitly explored in fear conditioning.

When Should Drugs Be Administered and Memory Be Tested in Order to Implicate Molecules in the Consolidation of Conditioned Fear?

So far, we've emphasized posttraining drug infusions as a control in acquisition studies, but they are most typically used to study memory consolidation, the processes that occur after information is acquired and convert the STM into a persistent LTM (McGaugh, 2000). In memory consolidation studies, drugs are usually administered immediately after and at additional time points within the first several hours after training. Two conditions usually have to be satisfied to conclude that the drug has disrupted consolidation. First, administration of the drug immediately after training should leave memory intact for some period (usually 1–4 hr), but the memory should be impaired when tested several hours later and remain so when tested on subsequent days. In other words, the drug should have no effect on STM but prevent the conversion of STM to LTM. Although many consolidation studies only test LTM, conclusive proof that the drug affects the conversion of STM to LTM requires that STM be shown to be intact. As noted above, STM is itself a memory process with a molecular basis, and it is likely that the molecules that maintain STM are different from those that consolidate LTM, just as the molecules that maintain STM are likely to differ

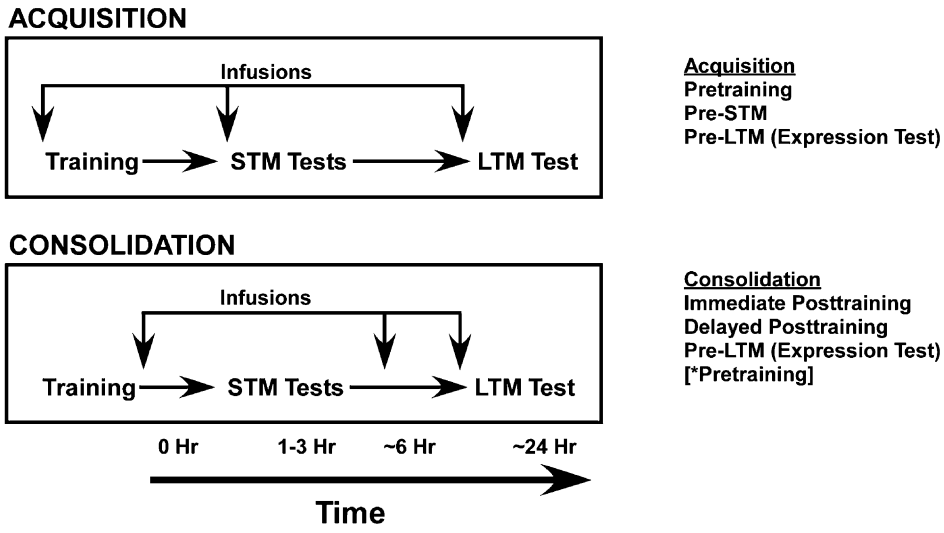

Figure 1. Drug Manipulation of Phases of Memory

*Pretraining infusions are sometimes used in consolidation studies. If both STM and LTM are affected, posttraining infusions should be used to determine whether the effects on LTM are due to a disruption of consolidation rather than acquisition. STM, short-term memory; LTM, long-term memory.

from those involved in acquisition—that is, in mediating the initial effects of CS-US pairing. The second condition that is required to show that the drug disrupts consolidation is that the same drug treatment that disrupts LTM when given shortly after training should have no effect when given several hours later, after STM has been converted into LTM. In other words, it takes several hours for memory traces to be consolidated, and once that happens the drug should leave the memory unaffected. It should also be noted that studies showing that posttraining drug infusions block consolidation are most often interpreted in terms of a failure of memory storage, but it is sometimes difficult to distinguish storage and retrieval deficits (see Hoz et al., 2004).

Above, we argued that it is important in acquisition studies to test both STM and LTM and to give drugs both pre- and posttraining. These are also important in the effort to understand consolidation. Drugs administered before training block processes that occur during training but do not necessarily affect LTM by preventing acquisition. That is, they can have effects that are triggered by stimuli occurring during training but express their effects on consolidation processes rather than on acquisition. For example, suppose that a drug given pretraining affects LTM but not STM (the classic consolidation effect) but that the same drug given immediately after training has no effect on either LTM or STM (not a typical consolidation effect). The conclusion in this case, determined by the use of pre- and posttraining drug delivery and STM and LTM tests, would be that the drug interfered with processes that occurred during training, but rather than disrupting acquisition the effect was on consolidation. We will see an example of this when we discuss the effects of voltage-gated calcium channel (VGCC) blockade on acquisition.

Controlling for State Dependency. In both studies of acquisition and consolidation, it can be useful to assess whether the drug treatment affects learning or memory directly or whether instead the drug effects are due to a condition called state dependency. This condition exists when the retrieval of memory during a test is only possible when the subject is in the same sensory context and physiological state as during training (Gordon and Klein, 1994). State dependency is mainly a problem in studies that employ pretraining drug infusions, since with posttraining studies the subject is typically trained and tested drug free. Thus, in studies using pretraining infusions, it is important to have a control condition in which subjects receive the drug during both training *and* testing. Relatively few studies of fear conditioning have employed this control (but see Maren et al., 1996; Helmstetter and Bellgowan, 1994; Muller et al., 1997; Goosens et al., 2000; Schafe and LeDoux, 2000). It is nonetheless an important measure for ensuring that the endogenous state of the brain and the environment during learning do not have to be reinstated in order to gain access to newly acquired information.

Summary of Drugs and Memory Phases. Drugs that block the development of both STM and LTM when given before training but not immediately after training are said to block acquisition, provided that they do not interfere with the expression of the memory when given right before the memory tests. In contrast, drugs that block the development of LTM when given immediately after training are said to block memory consolidation, provided that they do not also interfere with STM. Systematic administration of the drug at different time points after learning, as well as the use of several tests of memory over the course of 24 hr, allows fine discrimination of how the drug affects learning and memory.

Table 1. Effects of Drug Manipulations of the Lateral Amygdala on Short-Term Memory and Long-Term Memory of Fear Conditioning and on Long-Term Potentiation

	Pretraining Infusions		Preexpression Test Infusions	Posttraining Infusions (Immediate)		LTP[b]
	STM	**LTM**	**LTM**	**STM**	**LTM**	
NMDAR	✓	✓	✓[a]	?	?	✓
• NR2B	✓	✓	×	?	?	✓
• polyamines	?	✓	?	?	✓	?
mGluR5	✓	✓	×	×	×	✓
CaMKII	✓	✓	×	?	?	✓
L-VGCC	×	✓	?	?	?	✓
PKA	?	?	?	×	✓	✓
• AKAPs	×	✓	×	?	?	?
MAPK	×	✓	?	?	?	✓
RNA synthesis	?	✓	?	×	✓	✓
Protein synthesis	?	?	?	×	✓	✓
ROCK	×	✓	×	?	?	?

[a]Lack of pretesting effect in fear-potentiated startle studies.
[b]See text for induction protocol details.

✓ **effect**
× **no effect**
? **unknown**

STM, short-term memory; LTM, long-term memory; LTP, long-term potentiation.

Ideally, to implicate a drug and its underlying mechanisms in learning and memory, the drug should be administered at each of these time points: before training, immediately after training, at several points within the first few hours, and immediately before testing one or more days after training (Figure 1). Further, regardless of when the drug is administered and whether the focus is on acquisition or consolidation, memory should always be systematically tested at several points along the way: tests within the first 3 hr are needed to assess STM, additional tests after 3–4 hr are needed to assess the rate of conversion of STM to LTM, and tests at 24 hr and longer time points are needed to assess LTM.

Below, as we survey the research on the molecular basis of fear conditioning, it will become obvious that relatively few studies to date have performed the full range of tests. In some cases in which drugs have only been given pretraining, we have to interpret whether the effect is on acquisition or consolidation based on current understanding in the field. Also, some drug effects are not easily interpreted, even if all appropriate tests are done—for example, when a drug has the same impairment when given before and immediately after training, its effects could be due to a disruption of either acquisition or consolidation. In spite of these caveats, the pattern of results obtained for a number of drugs is beginning to provide an understanding of the molecular basis of acquisition and consolidation, even if some of the pieces of the puzzle remain missing (Table 1).

Molecular Mechanisms Underlying the Acquisition of Fear Conditioning

Acquisition of fear conditioning is believed to involve the convergence of neural inputs from the CS and US pathways onto LA cells during training (LeDoux, 2000; Blair et al., 2001; Maren, 2001). Specifically, it has been proposed that the CS inputs lead to the release of glutamate, which binds to glutamate receptors, including AMPA receptors (AMPARs), NMDA receptors (NMDARs), and metabotropic glutamate receptors (mGluRs) on LA cells. The US then depolarizes these cells while glutamate is bound to NMDARs, allowing Ca^{2+} to enter through NMDARs. The elevation of intracellular Ca^{2+} through NMDARs activates protein kinase second messenger cascades that are also essential for memory formation. In addition, Ca^{2+} entry through VGCCs during training is required. Each of these molecular steps that are engaged during acquisition will be summarized below.

The Role of NMDARs in the LA in the Acquisition of Fear Conditioning

It has long been believed that NMDARs play a key role in the synaptic plasticity that underlies learning (Lynch, 1986; Collingridge and Bliss, 1995). Unlike other iono-

tropic glutamate receptor channels, the NMDAR is gated by Mg^{2+} at resting membrane potentials and only opens when glutamate is bound to the receptor during depolarization (Mayer et al., 1984; Nowak et al., 1984). Current flow through the channel is thus maximal when glutamate is bound to the receptor and the neuron is depolarized. Because of these two requirements, NMDARs are viewed as quintessential coincidence detectors (see Tsien, 2000; Riedel et al., 2003) and are believed to be capable of activating the biochemical cascades behind experience-dependent synaptic modification. Ca^{2+} entry through the NMDAR is believed to activate numerous Ca^{2+}-dependent second messenger cascades that contribute to the maintenance of neural plasticity (Dingledine et al., 1999). Indeed, a wealth of evidence has shown that NMDARs play a central role in synaptic plasticity, as well as in learning and memory, in a variety of brain systems (for review, see Nicoll and Malenka, 1999; Riedel et al., 2003; Martin et al., 2000).

Below, we discuss pharmacological studies that have implicated NMDARs in the LA in the acquisition of fear conditioning. We separately consider studies that have used two different antagonists, since these have provided different pictures of the role of NMDARs in fear learning. For reasons described above, we do not discuss studies that have used genetic manipulations to implicate NMDARs in fear conditioning, although these have been numerous (Kiyama et al., 1998; Tang et al., 1999; Huerta et al., 2000; Moriya et al., 2000; Shimizu et al., 2000; Tang et al., 2001; Cui et al., 2004).

Blockade of the NMDAR Complex with the Broad Spectrum Antagonist APV. A number of studies have infused the NMDAR antagonist D,L-2-amino-5-phosphovalerate (APV) into the LA and adjacent regions (such as the basal nucleus [B]) prior to and immediately after fear conditioning. These studies have consistently found that APV prevents the acquisition (Miserendino et al., 1990; Campeau et al., 1992; Fanselow and Kim, 1994; Lee and Kim, 1998; Bauer et al., 2002) of fear conditioning. Generalizing from the fact that immediate posttraining infusions of APV have no effect on the consolidation of contextual fear conditioning (Maren et al., 1996), we propose that pretraining APV blocks acquisition and STM (Walker and Davis, 2000; Bauer et al., 2002) but has no effect on consolidation and retention of auditory fear conditioning. However, some but not all studies have also found that NMDAR blockade with APV prior to testing prevents the expression of previously conditioned responses (Maren et al., 1996; Lee and Kim, 1998; Lee et al., 2001). This obviously complicates the interpretation of the acquisition results, since it means that APV disrupts either stimulus processing or response control.

The studies described above fall into one of two groups that differ in terms of how conditioned fear is assessed. Some measured behavioral (e.g., freezing) or physiological responses (e.g., changes in heart rate, blood pressure, respiration, or other autonomic nervous system responses) that were directly elicited by the CS. Others assessed the effects of conditioning indirectly by measuring the degree to which startle reflexes are modulated by fear conditioning. Interestingly, all of the studies that found an effect of APV on acquisition but not expression used the fear-potentiated startle paradigm (Miserendino et al., 1990; Gewirtz and Davis, 1997; Walker and Davis, 2000; but see Fendt, 2001), while all of the studies that found an effect on expression measured fear conditioning directly (Maren et al., 1996; Lee and Kim, 1998; Lee et al., 2001). Why APV has an effect on expression in some paradigms but not others is not known. However, the pattern of results obtained with APV makes it difficult to conclude with confidence that NMDAR antagonism selectively affects synaptic plasticity underlying fear conditioning without disrupting routine synaptic function.

Selective Blockade of the NR2B Subunit of the NMDAR. APV competitively inhibits all NMDARs, regardless of their subunit composition (McBain and Mayer, 1994). An alternative way to assess the function of NMDARs in fear conditioning is to target specific subunits of the NMDAR. The NMDAR is comprised of the NR1 subunit, which is essential for receptor function, and a combination of NR2 subunits, which contribute to receptor gating, Mg^{2+} sensitivity, conductance, and pharmacology (Monyer et al., 1992). Because the NR2A and NR2B subunits are more strongly blocked by extracellular Mg^{2+} than other NR2 subtypes, they are more sensitive to voltage and may be better equipped to detect presynaptic glutamate release coupled with postsynaptic depolarization (Tsien, 2000). While NR2A and NR2B share approximately 70% sequence homology (Monyer et al., 1992), they contain different functional properties. For example, the NR1-NR2B complex exhibits longer EPSPs than the NR1-NR2A complex (Monyer et al., 1994), and this allows a longer time window for coincidence detection in the former. NR2B works together with scaffolding proteins that enable dendritic clustering and synaptic targeting (Shiraishi et al., 2003). In addition, the NR2B subunit has a modulatory site for polyamines (Gallagher et al., 1996), regulatory molecules that regulate the functioning of the NMDAR (Williams, 1997; Johnson, 1996). NMDAR polyamine binding site inhibition and activation in the LA directly disrupt and facilitate fear memories, respectively (Rubin et al., 2004). Although NR2B levels are especially high in early development (Sheng et al., 1994; Portera-Cailliau et al., 1996), NR2B is still abundantly expressed in adult brains, particularly in areas that must remain plastic throughout life, including the hippocampus (Charton et al., 1999), cortex (Charton et al., 1999), and amygdala (S.M. Rodrigues et al., 2000, Soc. Neurosci., abstract).

It is possible that subunit-specific manipulations might help clarify the role of NMDARs in amygdaloid plasticity. Tang et al. (1999), using genetic manipulations, found that NR2B overexpression in the forebrain enhanced several forms of memory, including cued and contextual fear STM and LTM. To investigate the contribution of the NR2B subunit specifically in the LA during fear conditioning, Rodrigues et al. (2001) assessed the effects of ifenprodil, a selective NR2B antagonist (Chenard and Menniti, 1999). Infusion of ifenprodil into the LA prior to training significantly disrupted both STM and LTM of fear conditioning (at 1 and 24 hr, respectively). However, when ifenprodil was infused immediately before testing of STM or LTM, there was no effect (Rodrigues et al., 2001).

In conclusion, blockade of the NR2B subunit of the NMDAR with ifenprodil provides a more specific and selective means of manipulating NMDARs in the LA. The

results from studies using ifenprodil confirm the role of NMDARs in fear acquisition that was suggested by studies using APV and, at the same time, avoid the confounding effects on fear expression that can occur with APV.

VGCCs

Membrane depolarization not only allows Ca^{2+} to enter cells through NMDARs but also opens VGCCs. Although there are several types of VGCCs, the L-type VGCC (L-VGCC) is particularly relevant to the fear conditioning circuitry (Weisskopf et al., 1999; Bauer et al., 2002; Karst et al., 2002; Shinnick-Gallagher et al., 2003). L-VGCCs are opened by strong depolarizing stimuli, especially those that produce postsynaptic spiking and back-propagating action potentials (BPAPs) (Yuste and Tank, 1996; Magee and Johnston, 1997; Stuart et al., 1997; Johnston et al., 1999).

In behavioral studies, intraamygdala infusion of an L-VGCC antagonist prior to training but not expression testing blocks fear conditioning (Bauer et al., 2002; Shinnick-Gallagher et al., 2003). Interestingly, unlike the effects seen with NMDAR antagonists, fear memory deficits produced with pretraining L-VGCC blockade are not evident until hours after training—that is, STM is intact in the hours immediately after training, while LTM (24 hr later) is impaired (Bauer et al., 2002). Because L-VGCCs are believed to be opened by the strong depolarizing effects of the US during CS-US pairing, the effects of L-VGCC blockade are interpreted as a deficit induced during training. That is, Ca^{2+} entry through L-VGCCs during training (CS-US pairing) is thought to set in motion processes that are not needed for acquisition (since STM is intact) but that are needed for the consolidation of LTM. To show this definitively, though, it would be necessary to show that immediate posttraining infusions have no effect on STM or LTM.

Metabotropic Glutamate Receptors

Metabotropic glutamate receptors (mGluRs) are unique amongst glutamate receptors in that they are G protein-coupled receptors (see Nakanishi, 1992; Hollmann and Heinemann, 1994). The group I mGluRs (mGluR1 and mGluR5) are especially important for synaptic plasticity via their activation of second messenger cascades and the generation of a Ca^{2+} signal from intracellular stores (see Hermans and Challiss, 2001). These stores are loaded by Ca^{2+} that enters through L-VGCCs and NMDARs (Rae et al., 2000).

mGluRs may be particularly important in short-term synaptic plasticity and memory via their close mutual relationship with the NMDAR. mGluR5, in particular, is a principal partner of NMDAR activity in synaptic plasticity. These two classes of glutamate receptors are physically tethered together at the synapse via scaffolding proteins, and here they can potentiate each other's activity (see Alagarsamy et al., 2001). Consistent with a role of mGluR5 in STM, pretraining intraamygdala infusion of the mGluR5 antagonist 2-methyl-6-(phenylethynyl) pyridine hydrochloride (MPEP) disrupts the acquisition and STM formation of fear conditioning, while immediate posttraining injections have no effect on STM (Rodrigues et al., 2002) or the consolidation of STM into LTM (Fendt and Schmid, 2002; Rodrigues et al., 2002). mGluR5 in the LA, like NMDARs, thus contributes importantly to the acquisition and STM of fear conditioning.

One mechanism whereby mGluR5 might promote STM formation is by stimulation of protein kinase C (PKC). PKC is known to target two phosphorylation sites on the C-terminal domain of NR2B (Liao et al., 2001), which can modulate NMDAR conductance (see Anwyl, 1999; de Blasi et al., 2001; Liao et al., 2001). In mGluR5 KO mice, for example, LTP of NMDAR currents in CA1 is absent but can be rescued by activators of PKC (Jia et al., 1998). Further, an mGluR5 agonist (CHPG) has been reported to induce a slowly developing, long-lasting potentiation of NMDAR currents via PKC (Doherty et al., 1997). Intraamygdala infusion of inhibitor 1-(5′-isoquinolinesulfonyl)-2-methylpiperazine (H7), a broad spectrum kinase inhibitor that affects PKC as well as PKA and PKG (Quick et al., 1992), impairs fear conditioning (Goosens et al., 2000). While such studies do not specifically implicate PKC, mice with a specific deletion of the β isoform of PKC have impaired fear conditioning when tested 24 hr after training (Weeber et al., 2000). Additional pharmacological studies are nevertheless necessary to elucidate the precise role of mGluR-mediated activation of PKC in the LA in fear conditioning.

Ca^{2+}/Calmodulin-Dependent Protein Kinase II

One important downstream effector of Ca^{2+} influx through the NMDAR is Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). CaMKII is also activated by L-VGCCs (Yasuda et al., 2003) and induces frequent long openings of their channels (Dzhura et al., 2000). The α isoform of CaMKII (αCaMKII), in particular, has been implicated as a major contributor to synaptic strengthening (Thiagarajan et al., 2002; Fink et al., 2003) and associative learning (see Fukunaga and Miyamoto, 2000; Lisman et al., 2002). A wealth of literature has shown this kinase to be necessary for hippocampal-dependent learning (see Lisman et al., 2002; Silva, 2003). Upon activation, αCaMKII undergoes rapid translocation to the postsynaptic density (Strack et al., 1997; Shen and Meyer, 1999; Shen et al., 2000). αCaMKII is often called a "memory molecule," because autophosphorylation on a specific threonine residue (Thr^{286}) allows the enzyme to remain activated after intracellular Ca^{2+} concentrations fall to basal levels (see Soderling et al., 2001; Lisman et al., 2002). In fact, this persistent activation occurs after contextual fear conditioning (Atkins et al., 1998) and acute stress (Blank et al., 2002) in the hippocampus. The autophosphorylation-dependent targeting of αCaMKII is specifically linked to the NR2B subunit over other NMDAR subunits (Strack and Colbran, 1998), and stimulus-dependent translocation of CaMKII to NR2B locks the kinase in an active state (Bayer et al., 2001).

Studies of fear conditioning using molecular-genetic methods in mice have also implicated αCaMKII (Chen et al., 1994; Mayford et al., 1996; Frankland et al., 2001; Ohno et al., 2001; Bejar et al., 2002; Elgersma et al., 2002; Miller et al., 2002; Wang et al., 2003). Particularly interesting are studies that have limited the αCaMKII alteration to forebrain areas and showed reversible deficits in cued and contextual fear LTM (Mayford et al., 1996; Wang et al., 2003). While the LA was included in this manipulation, so were other regions, such as the striatum.

Recent studies have implicated CaMKII in the initial phases of fear memory formation. Fear conditioning, for

example, results in an increase of active αCaMKII in LA synapses 15 min after fear conditioning (Rodrigues et al., 2004). Further, studies using pretraining infusions of 1-[NO-bis-1,5-isoquinolinesulfonyl]-N-methyl-l-tyrosyl-4-phenylpiperazine (KN-62), a blocker of CaMKII activity, targeted to the LA found a disruption of STM and LTM of both cued and contextual fear conditioning (Rodrigues et al., 2004). As we'll discuss below, posttraining manipulations of CaMKII also have an effect on STM (and thus also on LTM). Thus, CaMKII may therefore be involved in STM rather than in CS-US pairing during acquisition.

CaMKII may participate in STM via its ability to undergo autophosphorylation and also to phosphorylate AMPA receptors (AMPARs), increase their conductance, and drive them into synapses (Barria et al., 1997a, 1997b; Benke et al., 1998; Hayashi et al., 2000; Krapivinsky et al., 2004). Upregulation of AMPAR conductance and/or number is in fact one mechanism believed to underlie the temporary persistence of memory (Malinow, 2003). Recent findings showing that AMPA receptors are trafficked to LA synapses following fear conditioning support this notion (S. Rumpel et al., 2004, Soc. Neurosci., abstract; S. Rumpel et al., submitted). Thus, activation of αCaMKII during training may regulate AMPARs at LA synapses and thereby contribute to the formation and maintenance of STM.

Summary of Acquisition Results. Evidence from fear learning experiments suggests that NMDARs, especially the NR2B subunit, as well as mGluR5 and αCaMKII are involved in the acquisition and/or initial formation of STM of a fear conditioning experience. Pre- but not posttraining administration of NMDAR and mGluR5 antagonists block STM and LTM and are thus implicated in fear acquisition. Pre- and posttraining blockade of CaMKII, on the other hand, disrupts LTM formation, very likely by disrupting STM. Thus, during training, NMDARs, mGluR5, and αCaMKII trigger and/or maintain STM in the LA. In contrast, pretraining blockade of L-VGCC blockade has no effect on STM but blocks LTM. Although not yet tested, we predict that L-VGCC blockade immediately after training would have no effect on fear conditioning. This finding would suggest that in spite of the fact that pretraining L-VGCC blockade disrupts processes that occur during training, these channels are not necessary for acquisition; they are instead necessary for memory consolidation.

Consolidation of Fear Conditioning

As described above, during CS-US pairing, Ca^{2+} enters LA cells through NMDARs and L-VGCCs. The combined Ca^{2+} signal provided by these two sources then triggers a variety of additional intracellular steps that consolidate the short-lasting STM into a persistent LTM by initiating macromolecular synthesis. In this section, we begin by considering the protein kinase second messenger pathways that are downstream of Ca^{2+}. We then discuss how these second messenger systems initiate macromolecular changes in the cell that promote LTM formation.

Second Messenger Pathways Underlying LTM Formation

The rise in intracellular Ca^{2+} during training (CS-US pairing) leads, either directly or indirectly, to the activation of protein kinase second messenger pathways that are necessary for LTM. The role of two of these in the LA has been studied fairly extensively in relation to fear conditioning: the cyclic AMP-dependent protein kinase (PKA) and the mitogen-activated protein kinase (MAPK).

Posttraining administration of PKA inhibitors into the cerebral ventricles (Bourtchouladze et al., 1998; Schafe et al., 1999) or into the LA (Schafe and LeDoux, 2000) shortly after training leaves STM intact but prevents the formation of LTM. Also, PKA binding to the A-kinase anchoring protein (AKAP) in the LA is necessary for LTM but not STM (Moita et al., 2002). In addition, transgenic mice that overexpress R(AB), an inhibitory isoform of PKA, show deficits in LTM but not STM formation of fear conditioning (Abel et al., 1997).

PKA signals, as well as those of other upstream kinases, including PKC, CaMKII, and Akt, are known to converge on the MAPK signaling pathway (Adams and Sweatt, 2002; Chen et al., 1998; Wang et al., 2004; Lin et al., 2001). MAPK, and specifically the extracellular-regulated kinase (ERK), has been widely implicated in long-term synaptic plasticity in both vertebrates and invertebrates (English and Sweatt, 1997; Martin et al., 1997; Thomas and Huganir, 2004). More recently, ERK/MAPK has been implicated in memory formation (Berman et al., 1998; Atkins et al., 1998; Blum et al., 1999) including fear memory formation (Atkins et al., 1998; Brambilla et al., 1997; Schafe et al., 2000). In the LA, ERK/MAPK undergoes a transient activation by phosphorylation that peaks within an hour after conditioning (Schafe et al., 2000). Further, pretraining intraamygdala infusion of a MAPK inhibitor spares STM at 1 and 3 hr after training, but LTM is impaired at 24 hr (Schafe et al., 2000).

Although the preceding studies involved pretraining infusions, the assumption is that posttraining consolidation mechanisms (e.g., translocation of ERK to the nucleus) are affected. However, it should also be noted that pretraining infusion of ERK inhibitors, like pretraining L-VGCC blockade, may impair consolidation by affecting processes that are engaged during training. ERK/MAPK, for example, is known to target and phosphorylate the dendritic A-type potassium (K^+) channel Kv4.2 (Adams et al., 2000), which is a critical regulator of dendritic BPAPs in neurons (Yuan et al., 2002). The binding of phosphorylated ERK to Kv4.2 has been shown to decrease the A-type dendritic K^+ current in CA1 dendrites, leading to enhanced dendritic excitability (Yuan et al., 2002). Thus, under normal circumstances, ERK/MAPK acts to promote dendritic BPAPs and synaptic plasticity in part, by dampening K^+ currents. Administration of ERK inhibitors, however, blocks this effect (Yuan et al., 2002). Given that we know that BPAPs are an important means of opening L-VGCCs (Magee and Johnston, 1997; Stuart et al., 1997; Johnston et al., 1999), it remains possible that pretraining administration of an ERK inhibitor may have blunted the generation of BPAPs in LA neurons during fear learning, preventing the opening of L-VGCCs and the initiation of downstream consolidation mechanisms that they promote. Therefore, studies employing immediate posttraining infusions of ERK inhibitors will be necessary to directly test the hypothesis that ERK plays a role in fear memory consolidation

by signaling to the nucleus to initiate transcriptional changes.

Above, we argued that CaMKII is involved in STM. It is also possible that CaMKII plays a role in consolidation. This is suggested by studies that have targeted genetic manipulations of αCaMKII activity to specific areas in the forebrain, including the LA, *after* fear conditioning, and found that LTM was disrupted (Mayford et al., 1996; Wang et al., 2003). In light of these findings, systematic time course studies of CaMKII blockade in the LA immediately after fear conditioning would be especially useful. If such studies show that immediate posttraining infusions disrupt LTM formation, then CaMKII would be clearly be implicated in consolidation. On the other hand, if such studies show that pre- but not posttraining inhibition of CaMKII disrupts LTM formation, CaMKII would be implicated in acquisition but not consolidation. At this point, though, the weight of evidence is strongest for a role of CaMKII in STM. It is certainly likely, however, that CaMKII plays at least some role in both STM and LTM.

Transcription Factors and Genes

In their active state, PKA and MAPK are thought to translocate to the cell nucleus, where they stimulate gene transcription factors, including the cAMP response element binding protein (CREB; Alberini et al., 1995; Silva et al., 1998; Milner et al., 1998). When activated by phosphorylation on its Ser^{133}, CREB binds to DNA apparatuses and aids in the expression of the genes and ultimately the proteins that are needed for temporary cellular changes to be turned into persistent modifications and thus LTM (see Yin and Tully, 1996; Kandel, 1997; Milner et al., 1998; Silva et al., 1998; Alberini, 1999; Lamprecht, 1999). CREB has been implicated in fear conditioning in several studies of rodents, including genetic manipulations in mice (Bourtchuladze et al., 1994; Kida et al., 2002). Viral-mediated CREB overexpression in the LA and adjacent areas results in an enhancement of long-term fear memories in both fear conditioning (Wallace et al., 2004) and fear-potentiated startle (Josselyn et al., 2001) paradigms. Likewise, an increase of phosphorylated CREB occurs in these regions of the amygdala after fear conditioning (Stanciu et al., 2001).

Sustained CREB activation relies on activation of the MAPK pathway but also on L-VGCCs (Dolmetsch et al., 2001), both of which, as noted, are activated during training and are especially involved in LTM of fear conditioning (Schafe et al., 2000; Bauer et al., 2002). Moreover, group I mGluRs (mGluR5 and mGluR1) possess the ability to upregulate CREB phosphorylation via the intracellular Ca^{2+} release that is induced by activation of L-VGCCs (Mao and Wang, 2002). Ca^{2+} entry from both NMDARs and L-VGCCs leads to the translocation of calmodulin to the nucleus and the activation of nuclear CaMKIV (see Platenik et al., 2000). Further, studies of transgenic mice show that CaMKIV, a CaMK that phosphorylates CREB (see Soderling, 1999), is also important for fear memory (Wei et al., 2002).

Among the genes that are stimulated by the activation of CREB is the gene for brain-derived neurotrophic factor (BDNF) (West et al., 2001). BDNF signaling via the tyrosine kinase TrkB receptor enhances open probability of NR2B-containing NMDARs (Levine and Kolb, 2000) and is involved in the acquisition fear conditioning in the LA (Rattiner et al., 2004). CREB also interacts with nuclear factor κB, which is activated in the amygdala after fear conditioning and is involved in LTM but not STM of fear conditioning (Yeh et al., 2002, 2004).

Fear conditioning induces the expression of immediate-early (Beck and Fibiger, 1995; Rosen et al., 1998; Malkani and Rosen, 2000; Hall et al., 2001; Scicli et al., 2004; but see Campeau et al., 1997) and downstream genes (Stork et al., 2001; Ressler et al., 2002) in the LA. These genes can modulate transcription factors and cytoskeletal proteins and influence signal transduction, structural reorganization, and synaptic tagging, all of which may underlie experience-dependent plasticity (Lanahan and Worley, 1998; Stork et al., 2001).

In spite of this progress, much remains unknown about the transcription factors and genes underlying fear conditioning. This is likely to be an important future area of research.

Macromolecular Synthesis

Cytoplasmic protein kinases activated by Ca^{2+} translocate to the cell nucleus and activate gene transcription factors such as CREB. A major consequence of this is the induction of new macromolecular synthesis (new RNA and protein). Indeed, in a variety of neural systems in diverse organisms, LTM depends on macromolecular synthesis, whereas STM does not (for review, see Flexner et al., 1965; Davis and Squire, 1984; Goelet et al., 1986; Kandel et al., 1986; McGaugh, 2000; Kandel, 2001; Schafe et al., 2001; Dudai, 2002; Dubnau et al., 2003). Specifically, blockade of macromolecular synthesis prior to training or immediately after leaves STM intact for several hours but prevents the consolidation of STM to LTM. That the effects can be achieved by posttraining infusions alone, as well as the fact that with either pre- or posttraining infusions STM is intact and only LTM suffers, suggests that macromolecular blockade affects consolidation of STM into LTM rather than acquisition.

In fear conditioning, inhibition of protein synthesis in the LA immediately after training disrupts LTM (Schafe and LeDoux, 2000; Maren et al., 2003) but not STM (Schafe and LeDoux, 2000). In addition, antagonism of mRNA synthesis before training also impairs LTM formation of fear conditioning (Bailey et al., 1999), suggesting that upstream transcription, in addition to translation, is necessary for the production of new proteins involved in the conversion of STM to LTM. Indeed, posttraining blockade of mRNA synthesis disrupts LTM but not STM of fear conditioning (S. Duvarci et al., 2003, Soc. Neurosci., abstract).

Cellular Consequences of Macromolecular Synthesis

Macromolecular synthesis is believed to lead to changes in cell (especially synaptic) structure that stabilizes memory (Bailey and Kandel, 1993; Woolf, 1998; Rampon and Tsien, 2000; Sweatt, 2004), presumably by altering the actin cytoskeleton underlying synaptic organization (van Rossum and Hanisch, 1999; Matus, 2000; Kasai et al., 2003). While there is little evidence that changes in synaptic structure occur following fear conditioning, recent studies have shown that interference with molecular pathways known to be involved in structural plasticity during early development, such as the Rho-GAP pathway, disrupts memory formation (Lamprecht et al., 2002). While structural changes are most often thought of as necessary for LTM stabilization, the fact that forma-

tion of complexes involved in the structural remodeling of dendrites and spines occurs soon (within minutes) after fear conditioning (Lamprecht et al., 2002) is consistent with the idea that changes in synaptic structure are rapidly induced following activation of neural pathways (see Lamprecht and LeDoux, 2004). Structural changes may therefore participate in short-term plasticity as well as LTM.

Summary of Consolidation Results. In summary, Ca^{2+} influx through NMDARs and L-VGCCs during CS-US pairing recruits a number of protein kinases, including PKA and MAPK, which, in turn, activate downstream nuclear substrates, including CREB. These nuclear substrates, in turn, promote consolidation of fear memories by initiating the production of new RNA and proteins that, among other consequences, may lead to the restructuring of synapses and thereby contribute to memory persistence.

Molecular Parallels between LTP in the LA and Fear Conditioning

Synaptic plasticity has been studied extensively using LTP, a cellular model of memory involving artificial activation of pathways (Malenka and Nicoll, 1999; Lynch, 1986; Bliss and Collingridge, 1993; Martin et al., 2000). While studies of LTP offer important technical advantages in the effort to identify mechanisms that are involved in synaptic plasticity, because of the artificial nature of this approach it is important to relate results obtained from LTP studies to findings from actual behavioral learning. In fact, ideally, to relate LTP to learning of a specific behavior, LTP should be studied in the exact synaptic circuits suspected of being involved in the learning, rather than simply in a brain region believed to participate in the behavior. It is generally accepted that LTP that is induced in the auditory CS input pathways to the LA and recorded from LA cells is one of the few examples that satisfy this circuit requirement (Rogan et al., 1997; Stevens, 1998; Barnes, 1995; Eichenbaum, 1997; Martin et al., 2000). Below, we review evidence that shows parallels between fear conditioning and LTP in auditory CS input pathways to LA.

Ideally, we would like to discuss findings relevant to the induction of LTP (similar to learning or acquisition) and maintenance of LTP (similar to consolidation). However, this would require the same kinds of manipulations described above, where drugs are administered prior to LTP induction and after induction at various times, including immediately postinduction and prior to testing the expression of LTP at short and longer intervals after induction. The common practice in LTP studies of the amygdala and other regions, including the hippocampus, is to administer drugs prior to induction. Thus, little is known about the effects of postinduction drug delivery. This compromises our ability to draw conclusions about the induction versus maintenance phases. An alternative approach to this question has been to use different LTP induction protocols. A single train of high-frequency stimulation (HFS), for example, produces a short-lived enhancement of synaptic transmission called early LTP (E-LTP), while multiple trains of HFS produce a longer-lasting form of LTP called late LTP (L-LTP). Pharmacologically, E-LTP is like STM (it does not require new RNA or protein synthesis), and L-LTP is like LTM (it requires new RNA and protein synthesis) (Huang and Kandel, 1994; Nguyen and Kandel, 1997; Huang et al., 2000).

Induction of LTP in LA

In an effort to understand the plasticity that might underlie the acquisition of fear conditioning, LTP has been studied both in vivo and in vitro at synapses formed by auditory CS input projections to the LA from the thalamus and cortex (for review, see Rogan and LeDoux, 1996; Maren, 1999; Blair et al., 2001; Schafe et al., 2001; Goosens and Maren, 2002; Chapman et al., 2003). Two different induction protocols have been used. In one, LTP is induced by pairing presynaptic stimulation of auditory CS pathways to the LA with injection of depolarizing current into the postsynaptic cell in the LA (*pairing protocol*). The presynaptic stimulation is like a CS (a weak stimulus), and the depolarizing current is like a US (a strong stimulus) delivered to the same cell that receives the CS input. In the other form of LTP induction, HFS is given to one of the CS input pathways to the LA (*tetanus protocol*). The presynaptic stimulation serves as both the CS input and the depolarizing stimulus. Thus, both forms of LTP combine presynaptic stimulation of CS pathways with postsynaptic depolarization.

Weisskopf et al. (1999) showed that pairing-induced LTP is blocked by L-VGCC inhibitors and the Ca^{2+} chelator BAPTA but not by blockade of NMDARs with APV. Although L-VGCCs may be opened widely throughout the neurons by BPAPs (Yuste and Tank, 1996; Magee and Johnston, 1997; Stuart et al., 1997; Johnston et al., 1999), the fact that L-VGCC-dependent LTP in LA is synapse specific (Weisskopf et al., 1999) suggests that cell-wide effects are not responsible for the LTP. Specificity could come from the fact that BPAPs preferentially invade distal dendrites where excitatory postsynaptic potentials (EPSPs) have just occurred (Magee and Johnston, 1997). Alternatively, it is also possible that L-VGCCs might be opened by local dendritic spikes rather than somatic action potentials (see Blackstone and Sheng, 1999).

In contrast to the lack of an effect of APV on pairing-induced LTP, Huang and Kandel (1998) found that APV partially blocked LTP induced with a 100 Hz tetanus, while Lee et al. (2002) found that LTP induced by a 100 Hz tetanus could be disrupted by either APV or L-VGCC blockade. These latter studies suggest that tetanus-induced LTP may, under certain conditions, depend on Ca^{2+} entry through both NMDARs and L-VGCCs.

Because L-VGCCs are opened by strong depolarizing stimuli, especially those that produce postsynaptic spiking and BPAPs (Yuste and Tank, 1996; Magee and Johnston, 1997; Stuart et al., 1997; Johnston et al., 1999), the possibility arises that induction of LTP with a tetanus that depolarizes postsynaptic cells but without eliciting spikes might produce a pure NMDAR-dependent form of LTP in LA. To test this, Bauer et al. (2002) delivered a 30 Hz tetanus to the thalamic input pathway. This stimulus elicited few if any spikes but induced LTP. This LTP was unaffected by L-VGCC blockade but was disrupted by NMDAR blockade with either the broad antagonist APV or the NR2B-specific antagonist ifenprodil. This LTP was also disrupted by the mGluR5 antagonist MPEP (Rodrigues et al., 2002) and the CaMKII

antagonist KN-62 (Rodrigues et al., 2004). Unlike APV, which has been shown to produce a partial disruption of synaptic transmission (Rainnie et al., 1991; Li et al., 1995; Danober and Pape, 1998; Mahanty and Sah, 1999; Weisskopf and LeDoux, 1999; Bauer et al., 2002), each of the other treatments that blocked LTP had no effect on routine synaptic transmission. Thus, while NMDAR blockade with APV interferes with routine baseline activity in the LA (just as it interferes with the expression of fear responses), NR2B-specific blockade does not (just as it fails to affect the expression of fear) (Rodrigues et al., 2001; Bauer et al., 2002).

Maintenance of LTP in LA

The maintenance or consolidation of LTP, as noted above, has been studied using the L-LTP paradigm. Although only one such study has been conducted in the LA, it provides evidence for similarities between consolidation of LTM of fear conditioning and LTP persistence (Huang et al., 2000). For example, just as MAPK, PKA, and new RNA and protein synthesis are required for LTM but not for STM of fear conditioning (see Schafe et al., 2001), MAPK, PKA, and RNA and protein synthesis are also required for L-LTP but not E-LTP in LA (Huang et al., 2000). In hippocampus, NMDARs and CaMKII are required for E-LTP (Huang and Kandel, 1994), just as they are for fear acquisition and STM formation in the LA (Rodrigues et al., 2001, 2004; Walker and Davis, 2000; Bauer et al., 2002). Additional experiments will be necessary to determine whether NMDAR and CaMKII activity also contribute to E-LTP in the LA.

As noted already, very few LTP studies have examined the effects of postinduction drug delivery. One exception is a recent study showing that administration of ERK inhibitors both pre- and postinduction impairs LTP in area CA1 of hippocampus, suggesting that ERK plays a role in the consolidation of LTP *after* the tetanus is delivered (Rosenblum et al., 2002). Similar experiments should also be performed in the LA to tease apart the contribution of second messenger and other signaling pathways to events at the time of LTP induction from those that occur after LTP has been induced. Only then will it be possible to distinguish the molecular basis of the induction and maintenance phases of LTP.

Summary of LTP Induction and Maintenance in LA

In sum, auditory input pathways to the LA exhibit LTP that can depend on either NMDARs or L-VGCCs, depending on the stimulation conditions during induction. When induction involves postsynaptic spiking, L-VGCCs are involved, whereas in the absence of spiking NMDARs are required. Under natural conditions, both NMDARs and L-VGCCs are likely to participate in plasticity, since postsynaptic cells are likely to spike in the presence of strong stimuli like footshocks (H.T. Blair and J.E.L., unpublished data). Consistent with this view, as noted above, fear conditioning depends on both NMDARs and L-VGCCs to activate second messenger pathways sufficiently to induce macromolecular synthesis. Also, as with fear conditioning, the NMDAR-mediated form of LTP in the LA also requires mGluRs and CaMKII. Short-lasting plasticity or early LTP (similar to STM) does not require macromolecular synthesis, but longer-lasting plasticity or late LTP (similar to LTM) does. The second messenger pathways downstream to calcium involved in LTP maintenance appear to include at least MAPK and PKA. However, in drawing parallels between phases of LTP and fear conditioning, it should be noted that studies of LTP have not used the kind of drug delivery schedule we described for fear conditioning. This complicates the task of precisely identifying whether a given molecule is involved in induction versus maintenance of LTP.

Inhibitory Plasticity in LA

Before leaving this discussion of synaptic plasticity in the LA, it is important to mention the contribution of inhibitory synaptic transmission. Although most LTP studies in the LA have focused on excitatory neurons, considerable evidence also suggests that GABAergic inhibitory interneurons and their receptors in the LA play an important role in amygdaloid plasticity. For example, recent studies have shown two forms of inhibitory plasticity in the LA. One involves LTP of excitatory inputs to inhibitory interneurons (Mahanty and Sah, 1998; Bauer and LeDoux, 2004), mediated by Ca^{2+} entry through special AMPARs located on the interneurons (Mahanty and Sah, 1998). Another involves LTP of the inhibitory synapses of interneurons (Bauer and LeDoux, 2004). In addition, LTD has been found in the LA (Heinbockel and Pape, 2000; Rammes et al., 2001; Kaschel et al., 2004) and in the adjacent intercalated cell group (Royer and Pare, 2002, 2003). GABAergic terminals innervate all CaMKII postsynaptic domains (McDonald et al., 2002). Interestingly, NR2B subunits seem to contribute to the function of LA interneurons (including transmission), as well as to excitatory processes (Szinyei et al., 2000, 2003). CS presentation leads to a reduction of extracellular GABA in the LA and adjacent regions (Stork et al., 2002), and expression of GABA-related genes (GAD65 but not GAD67) is differentially regulated after fear conditioning (Pape and Stork, 2003).

GABA interacts with a number of additional molecules in the LA. For example, LA interneurons express a high degree of the cannabinoid receptor CB1 (Katona et al., 2001; McDonald and Mascagni, 2001), which is involved in both GABAergic and glutamatergic synaptic transmission in the LA (Azad et al., 2003; Pistis et al., 2004). In addition, serotonergic receptors on GABAergic interneurons play a key role in the inhibitory tone of the LA (Rainnie, 1999; Koyama et al., 1999; Stutzmann and LeDoux, 1999; Koyama et al., 2002; Morales et al., 2004). Likewise, the gastrin-releasing peptide receptor on LA interneurons is involved in LA LTP and LTM of fear conditioning (Shumyatsky et al., 2002). The function of such inhibition might be to maintain synaptic homeostasis in the LA and thereby prevent runaway excitation during the presence of strong depolarizing inputs (Royer and Pare, 2003). Alternatively, it may serve to increase the signal-to-noise ratio of relevant synaptic inputs (Bauer and LeDoux, 2004).

How Might Short-Term Synaptic and Behavioral Changes Be Transformed into Long-Lasting Synaptic Modification and Permanent Memories?

We have proposed a model, based on the parallels between drug effects on fear conditioning and LTP in the LA, to account for how fear conditioning and resulting memories are created and maintained (Blair et al., 2001). Here, we will summarize the model and elaborate on it.

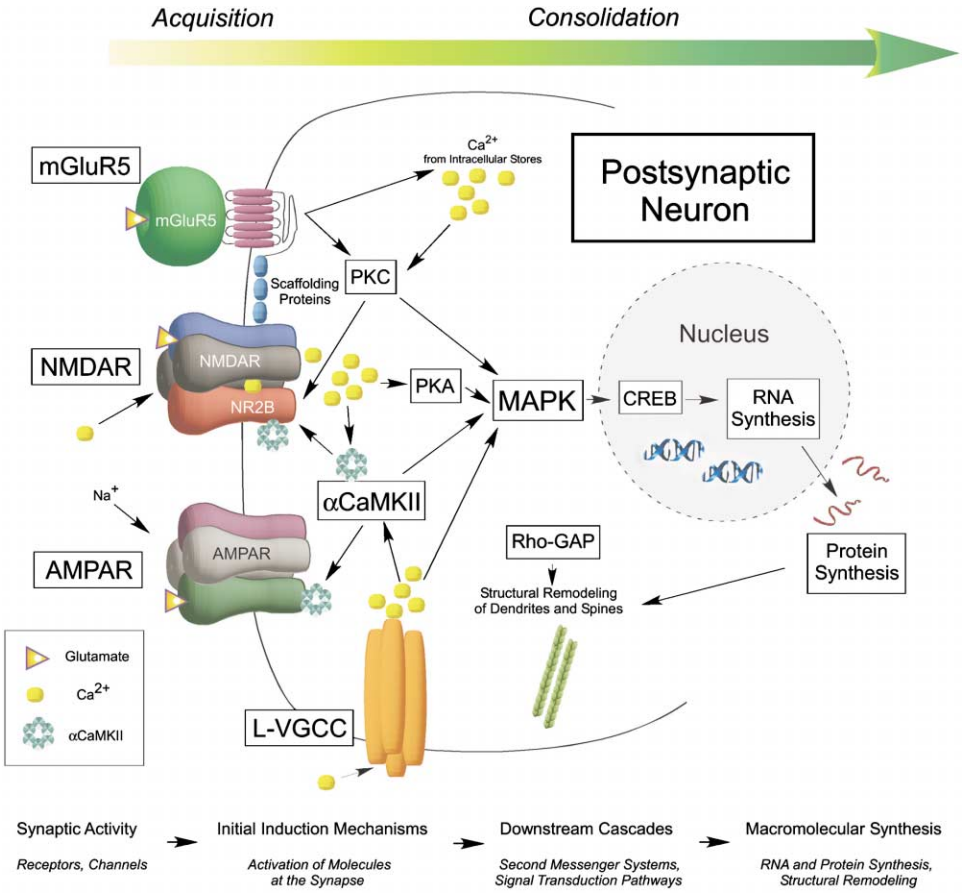

Figure 2. Molecular Mechanisms Underlying the Acquisition and Consolidation of Emotional Learning and Memory in the Lateral Amygdala

CS presentation leads to the release of glutamate in the LA from the presynaptic terminals of auditory input fibers (Farb and LeDoux, 1997; Li et al., 1995, 1996). The glutamate binds to AMPARs and NMDARs (Farb and LeDoux, 1997). Binding to AMPARs is sufficient to elicit single action potentials from postsynaptic cells during the first few milliseconds of stimulation. The cells quickly habituate to this level of presynaptic release, and additional spikes are sporadic (Clugnet et al., 1990; Li et al., 1995). We hypothesize that the terminals continue to release glutamate and that glutamate remains bound to postsynaptic receptors while the CS remains on. The arrival of the US, typically several seconds after the onset of the US, causes cells throughout the LA to be strongly depolarized (Blair et al., 2001). The Mg^{2+} block is only removed from those NMDARs where glutamate is bound—that is, at the CS input synapses. Ca^{2+} entry through glutamate bound NMDARs on depolarized neurons then leads to synapse-specific LTP (Weisskopf et al., 1999). However, the level of Ca^{2+} that enters through NMDARs is only sufficient to produce STM. In order for CS-US pairing to create a LTM, especially when a single or only a few pairings occur, a relatively large calcium signal is needed, and this is supplied by Ca^{2+} entry through L-VGCCs during the strong depolarizing influence of the US (Bauer et al., 2002). This secondary calcium signal does not participate in the acquisition process (which is mediated by NMDARs) or on STM (which is initiated and/or maintained by NMDARs, mGluR5, and αCaMKII) and only participates in the consolidation of LTM by activating downstream kinases (including at least PKA, MAPK, and PKC) that in turn activate gene transcription factors (e.g., CREB) leading to RNA and protein synthesis. New proteins in the form of AMPA receptors may be inserted into existing synapses (Malinow and Malenka, 2002), and recent studies suggest that this may occur in LA (S. Rumpel et al., 2004, Soc. Neurosci., abstract; S. Rumpel et al., submitted).

Given that half-life of the various molecules that are implicated in LTM is shorter than the memory itself, the question arises as to the basis of memory persistence (Dudai, 2002). Two solutions are typically proposed. One involves a mechanism that would allow molecules to remain persistently active, specifically autophosphorylation of CaMKII (Lisman et al., 2002). Autophosphorylation of CaMKII in the LA following fear conditioning has recently been shown (Rodrigues et al., 2004) but is unlikely by itself to account for the persistence of memory. The other mechanism that has been proposed to account for memory persistence involves changes in synaptic structure that would outlive molecular turnover (Bailey and Kandel, 1993). While convincing evidence that fear conditioning induces structural plasticity in the LA is still lacking, recent studies have shown that fear conditioning depends on molecules known to mediate structural plasticity during development, specifically

Rho GTPases and downstream kinases such as ROCK (Lamprecht et al., 2002; Lamprecht and LeDoux, 2004).

In summary, in this review we have emphasized some key synaptic events and downstream cellular cascades that are responsible for the initial triggering of synaptic plasticity during acquisition of fear conditioning and ultimately the persistent modifications that underlie LTM of fear conditioning (Figure 2). These mechanisms by no means exhaust the molecular events underlying acquisition and consolidation of memory in the LA, and future work will surely implicate many additional molecules. Because of the importance of fear conditioning as a means of studying brain circuits involved in emotional disorders (see Gorman et al., 2000; LeDoux, 2000; Pitman et al., 2001; Drevets, 2003), the discovery of molecules underlying fear conditioning provides important targets in the treatment of these disorders.

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