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Amygdala-dependent and amygdala-independent pathways for contextual fear conditioning

Ravikumar Ponnusamy, Ph.D., Andrew M Poulos, Ph.D., and Michael S. Fanselow, Ph.D

Psychology and the Brain Research Institute, University of California, Los Angeles, Los Angeles, California 90095

Abstract

The basolateral amygdala (BLA), consisting of the lateral and basal nuclei, is considered to be essential for fear learning. Using a temporary inactivation technique, we found that rats could acquire a context-specific long-term fear memory without the BLA but only if intensive overtraining was used. BLA-inactivated rats' learning curves were characterized by slow learning that eventually achieved the same asymptotic performance as rats with the BLA functional. BLA inactivation abolished expression of overtrained fear when rats were overtrained with a functional BLA. However, BLA-inactivation had no effect on the expression of fear in rats that learned while the BLA was inactivated. These data suggest that there are primary and alternate pathways capable of mediating fear. Normally, learning is dominated by the more efficient primary pathway, which prevents learning in the alternate pathway. However, alternate pathways compensate when the dominant pathway is compromised.

Keywords

Amygdala; context; fear conditioning; anxiety; freezing; muscimol; BLA; basolateral amygdala; hippocampus; neural plasticity; anxiety disorders; PTSD

Introduction

The currently dominant view of the neurobiology of learning and memory is that there are specialized sets of independent circuits dedicated to particular functions such as spatial learning, declarative memory and emotional learning (Gold, 2004; Poldrack and Rodriguez, 2004; Squire, 2004; White, 2004). However, this "multiple memory systems" view conflicts with anatomical evidence that the brain is a highly plastic complex recurrent network or dynamical system, with multiple pathways between any two structures (Young et al., 1995). The present series of experiments seek to reconcile these two very different positions applying them to Pavlovian contextual fear conditioning.

The most widely accepted view of fear is based on the assumption that there is an essential neuroanatomical circuit for fear learning and behavior that is centered on the amygdala (Fanselow and LeDoux, 1999; Davis, 2000; Pare et al., 2004; Maren, 2005). Applying this view to contextual fear conditioning (e.g., (Maren and Fanselow, 1995), contextual information is encoded by the hippocampus (HPC) and converges with aversive information at the basolateral

Corresponding author: Dr. Michael S. Fanselow Psychology and the Brain Research Institute, University of California, Los Angeles, Los Angeles, California 90095 mfanselow@gmail.com

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amygdaloid complex which contains lateral, basolateral, basomedial nuclei. Plasticity in the basolateral amygdaloid complex (BLA) supports the formation and storage of an association between environmental information and shock and passes this information to the CeA, whose efferents to the ventral periaqueductal gray (vPAG) trigger the expression of fear as indexed by conditional freezing (Fanselow, 1991; LeDoux et al., 1991; Davis, 1992; Kim and Davis, 1993; Fanselow and LeDoux, 1999; Pare et al., 2004). There is a tremendous amount of evidence from lesion, pharmacological, genetic and electrophysiological studies that support this role for the BLA in fear memory and its expression. For instance, reversible inactivation or lesions of the BLA prior to training block the acquisition of fear, while inactivation or lesions of the BLA prior to testing completely abolish the expression of fear (e.g., Miserendino et al., 1990; Helmstetter and Bellgowan, 1994; Maren et al., 1996a; Wilensky et al., 1999). Moreover, a recent study demonstrated that lesions of the BLA made either 1 day or 1.3 years following fear learning completely abolished the expression of fear, suggesting that the BLA is a site of formation and permanent storage of CS-US associative learning (Gale et al., 2004). All of these data have led to the widely held view that the BLA is an essential component of a specialized HPC→BLA→CeA→vPAG circuit that is necessary for contextual fear learning.

Contradicting this view that the BLA is essential for fear learning, (Maren, 1999, 2001) reported that learning deficits normally produced by BLA lesions can be mitigated with extensive overtraining (see also (Gale et al., 2004). However, post-training BLA lesions, regardless of the amount of training completely abolish the expression of conditional fear. Together these results suggest that if BLA function or the primary neural pathway is compromised an alternate neural pathway has the capacity to acquire conditional fear. However, since, post-training lesions abolished fear in rats it appears that plasticity in this alternate pathway does not normally play a role in fear learning. Rather, the alternate pathway plays a compensatory role only when the primary fear learning circuitry (i.e., BLA) fails.

It is not known why this compensatory circuitry is recruited when the BLA is damaged. There are a number of possibilities. The stress of the overtraining session may result in a generalized and nonspecific response that does not occur when the normal systems for dealing with fear are functioning properly. Perhaps freezing in the lesioned and overtrained rats reflects this sort of nonspecific stress response rather than context-specific fear (Fanselow, 1980). Alternatively, it is possible that the permanent lesion-induced denervation of the structures communicating with the amygdala generated some degree of rewiring or new patterns of network activity in the rest of the fear circuit during the week that intervened between lesion and training. A third possibility is that the animal's inability to predict shock on so many trials may have provided sufficient error-correction signals to establish plasticity in a slower learning neural circuit. To address these issues, the current experiments used temporary inactivation of the BLA that only compromised neural activity, not the neurons themselves, and did so only during training and/or testing in both the trained and a novel context. We also examined the context specificity of the overtrained fear.

Experimental Procedures

Subjects

Male Long-Evans rats initially weighing 250–280 gram were obtained from a commercial supplier (Harlan). After arrival, the rats were housed individually in standard stainless-steel cages on a 12/12 hr light/dark cycle and were provided free access to food and tap water. After being housed, the rats were handled daily (60–90 sec per rat) for 5 days to acclimate them to the experimenter. All procedures were approved by the UCLA Animal Care and Use Committee.

Surgery

Under aseptic conditions, animals were given atropine methyl nitrate (0.04 mg/kg, i.p.), anesthetized with sodium pentobarbital (65 mg/kg, i.p.), and mounted into a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The scalp was incised and retracted, and head position was adjusted to place bregma and lambda in the same horizontal plane.

Cannula implantation—Small burr holes were made to implant 26 gauge guide cannulas (Plastics One, Roanoke, VA) bilaterally into the amygdala (from bregma: anteroposterior, -3.1 mm; mediolateral, ± 5.2 mm; dorsoventral, -7.6 mm). Implanted cannulas were cemented to the skull using two anchoring screws to stabilize the dental acrylic. After surgery the cannulae were kept patent by inserting “dummy cannulae.” These dummies were replaced daily with clean ones. This adapts the rat to handling during the 12–13 day recovery period making it easy to insert the injectors in awake animals at the time of MUSC or ACSF infusion. For drug infusion, 33-g injectors were inserted so that they extend 1 mm below the guide cannula.

MUSC infusion

Muscimol free base (Sigma-Aldrich, St. Louis, MO), dissolved in artificial CSF (ACSF) (1mg/mL, pH 7.4) was micro-infused into the BLA (bilaterally) by back loading the drug up a 33 gauge infusion cannula into polyethylene (PE 20) tubing connected to 10 μ L Hamilton microsyringes (Hamilton Company, Reno, NV). The infusion cannula protruded 1 mm beyond the guide cannula. An infusion volume of 0.25 μ L/side was delivered using a Harvard # 22 syringe pump (Harvard Apparatus, South Natick, MA) at a rate of 0.1 μ L/min. The injector was replaced with a dummy cannula 1 min after completion of the injection.

Because our intra-amygdalar MUSC infusion parameters are similar to those used in previous fear-conditioning studies (Helmstetter and Bellgowan, 1994; Muller et al., 1997; Wilensky et al., 1999; Maren et al., 2001; Wilensky et al., 2006) the extent of MUSC diffusion in the amygdala should be comparable. Based on studies that examined 3H-MUSC spreading (Krupa et al., 1996; Arikan et al., 2002) in the cerebellum in which a 1 μ L volume infusion diffused a radius of 1.6–2.0 mm, it was estimated that 0.25 μ L of MUSC used in the present study would spread within a radius of 0.5–0.7 mm from the infusion needle tip. Hence, it is likely that infused MUSC would have diffused to the lateral, and basal nuclei of the amygdala and possibly to portions of central nucleus and adjacent neighboring structures.

Conditioning apparatus

Context-A—The context A environment consisted of aluminum (side walls) and Plexiglas (front, back, and top) chambers (28 $\times$ 21 $\times$ 22 cm; Lafayette Instruments, Lafayette, IN). The floor of each chamber had 18 stainless steel rods (4 mm diameter, 1.5 cm apart) connected to a shock scrambler and generator (which, along with internal ventilation fans, supplied background noise of 70 dB, A scale). The chambers were cleaned with 5% ammonium hydroxide solution and scented with 0.1% Benzaldehyde in 100 % Ethanol. These computer-controlled (Med-Associates, Lafayette, IN) chambers were in a well lit room separate from the observers.

Context B—The context B environment was in a separate room. These chambers (same size as above) had a white rear wall inserted and two white plastic side walls (24 $\times$ 21 cm) placed at 60° to the floor, forming a triangular enclosure. The floors consisted of 17 staggered rods (two rows, 1 cm vertically apart; in each row, each rod was 2.6 cm apart). Background noise (70 dB) was supplied by a white-noise generator, and the chambers were cleaned and scented with 1% acetic acid solution. This room was illuminated by a 30 W red light bulb.

Experimental Design

Experiment 1: The effect of BLA inactivation on learning and expression of over-trained fear

The goal of experiment 1 was to determine whether animals can learn and express fear while BLA was inactivated. To test this, the GABAA receptor agonist MUSC was used to inhibit amygdalar neurons before overtraining and/or testing. We used freezing as a measure of fear. Freezing during over training and during testing was scored from videotape by an observer blind to the treatment conditions. The design was a 2 (Training Drug Treatment) X 2 (Testing Drug Treatment) factorial. Half the rats received MUSC injected bilaterally into the BLA 20 minutes prior to overtraining. To equate MUSC experience in all animals, the other half received the same MUSC injection 20 min after training. It has been previously shown that post-training infusions of MUSC do not disrupt cue fear memory consolidation (Wilensky et al., 1999). Three days later, half of each condition received MUSC and other half received the ACSF 20 min prior to an 8 min context test in the training context.

Overtraining Procedure: After a 2 minute exposure to the chamber, rats were given 76 unsignaled shocks (1 mA, 2 sec) with a 64 sec ITI. Two minutes after the final shock, rats were removed from the chamber and returned to the home cage. Three days after training they were again infused with MUSC and tested in the original training context.

Experiment-2: BLA inactivation on the context specificity of over trained fear

Rats were given the same overtraining as experiment 1, except that all rats received pretraining MUSC. To test for context specificity these rats were tested twice (3 days and 6 days after training), once in the training chamber and once in a novel chamber (test order was counterbalanced between animals). Freezing was measured as described for experiment 1.

Experiment-3: Intra-BLA MUSC infusions at two different time points with normal training

Rats were trained with 5 shocks under MUSC and the specificity of context fear was tested in the same manner as Experiment 2. We choose 5 trials because we know that with these training parameters fear is asymptotic after 3 trials in intact rats (e.g., Young and Fanselow 1992, see also Figure 2A). Thus 5 trials would be considered post-asymptotic but not over-trained for intact rats. We used the same MUSC infusion parameters as Experiment 1 but training consisted of only 5 shocks. Three days after training, they were again infused with MUSC and tested in either the original training context (Context A) or a different context (Context B). A second test was given three days later in the other (original or different) context under MUSC (order counterbalanced). Therefore, this experiment determined if rats normally trained under MUSC could retain fear and if that fear was context specific. It is possible that during the long overtraining session (in experiments 1 & 2) MUSC lost its efficiency and that is why the overtraining rats learned. Therefore, we gave another set of rats ($n = 8$) 5 shocks time-matched with respect to MUSC infusion to the last 5 shocks of 76 shocks. The experiment had the same design as above described experiment, except that normal training was used 1 hour and 35 minutes after MUSC infusion.

Freezing

Our measure of fear was the freezing response which is known to be purely a conditional response in situations using electric shocks (Fanselow, 1980,1986). Freezing behavior was defined as the absence of any visible movement (including the vibrissae), except that required for respiration. It was scored by a blind observer according to instantaneous time sampling procedure in which each animal was observed every eight sec. For each minute (defined as 8 samples or 64 sec), the number of observations scored as freezing were summed and converted to a percentage (number of freezing observations/total number of observations $\times$ 100). This

probability estimate for freezing conforms to the assumptions of parametric statistical analyses. During over training in experiments 1 and 2 freezing was measured after the 1st, 5th, 10th, 25th, 50th, 75th and 76th shocks. During the test, freezing was measured for the entire 8 minutes test.

Histology

At the completion of behavioral testing (for all experiments), the rats were overdosed with sodium pentobarbital and perfused intracardially with 0.9% saline followed by 4 % buffered formalin. The brains were removed and stored in 10% formalin for at least 2 weeks before slicing. These brains were cryoprotected in 30 % sucrose –PBS mixture for 48 hours and were sectioned on a cryostat. Coronal sections (50 μ m) were taken through the extent of the cannula tract, mounted on gelatinized slides, and stained with cresyl violet. Stained sections were examined with a light microscope (Zeiss, Oberkochen, Germany). Cannula tip positions were verified with a rat brain atlas (Paxinos and Watson, 1998).

Statistical analyses

All experiments were analyzed by appropriate ANOVA's (Experiment-1) or paired t-test (Experiment 2 &3). When interactions were reliable, follow-up contrasts were made using the Bonferroni correction to hold $p < 0.05$ for the number of contrasts made.

Results

Histology for all experiments

Figure-1 shows a photomicrograph of a coronal section stained with cresyl violet from a rat with cannula tip placement in the BLA and schematic diagram of each cannula placement. Animals with misplaced cannula tips were excluded from the data analysis. Histological verifications were done by an observer blind to the experimental conditions.

Effects of Muscimol infusion into BLA during over training

To control for any non-specific or long-term effects of MUSC, rats trained in the absence of drug received an infusion of MUSC 20 minutes after training. Rats received a single contextual fear conditioning session containing 76 shocks. Half the animals ($n = 15$) received a muscimol (MUSC) infusion prior to training. To control for any enduring effects of MUSC, the remaining rats ($n = 15$) received the same MUSC infusion but after training. To assess learning during this session we sampled post-shock freezing at several time points during the session (Figure-2A). Postshock freezing is a reliable measure of context conditioning (Fanselow, 1980). While the non-infused animals displayed high levels of freezing following the 3rd or 4th trials, infusions substantially reduced the freezing for the first 10 trials. Thereafter infused rats began to display significant levels of freezing. Freezing was marginally higher in the controls for the first 40 trials, but the rats infused with MUSC caught up by the 50th trial. These impressions were supported by a two-factor ANOVA that indicated a reliable between-group effect of MUSC ($F(1,216) = 7.83$; $p < 0.01$) and a reliable repeated measure factor for trials ($F(6,216) = 25.93$; $p = 0.0001$). There was also a reliable interaction between these factors $F(6,216) = 6.90$; $p < 0.0001$) that supports the idea that the two groups learned at different rates.

Effects of MUSC infusions during the test of long-term fear memory

Three days after training both groups of rats were treated with MUSC or the ACSF vehicle and given a test for long-term memory of context fear. MUSC nearly abolished freezing in rats trained with a functional BLA, but had no effect on rats trained with the BLA inactive (Figure 2B).

A 2×2 factorial (Training Drug Condition X Testing Drug Condition) ANOVA revealed a significant main effect of Test Drug ($F(1, 26) = 11.46$; $p < 0.005$) but not Training Drug Treatment ($F(1, 26) = 1.91$). Most importantly, there was a reliable interaction between training and testing drug ($F(1, 26) = 15.64$; $p = 0.001$). The interaction occurred because inactivation of the BLA during testing only reduced the freezing in rats trained with a functional amygdala. That is the animals trained with the BLA intact and tested with the BLA inactivated froze reliably less than the other 3 groups, which did not differ. Thus, when animals were trained with a functional BLA, MUSC blocked the expression of freezing. The lack of difference between the two MUSC-trained groups ($p > 0.05$) indicates that state-dependent learning does not cause the pattern of effects, that is, MUSC is not acting as a retrieval cue for learning under MUSC.

The rats trained under MUSC did show somewhat less fear than the Post-ACSF group, however this difference was not statistically significant.

Context specificity of overtrained fear

In this experiment, we determined the context-specificity of BLA-independent fear memory established with overtraining. Eight naïve rats were trained with 76 shocks under MUSC (Figure -2A shows fear acquisition for the rats of both experiments). After a three-day rest period, the rats were tested while the BLA was again inactivated in both the original training context and a different context 72 hours apart with the order of test-context counterbalanced between the animals. The data were analyzed using a Paired t test (two tailed). As shown in figure-3A, MUSC treated overtrained rats froze more in the trained context than the novel context (Paired $t(7) = 2.96$, $p < 0.02$) indicating that the BLA-independent fear acquired through overtraining was context specific.

Rats with inactivated BLA need extensive over training

In the previous experiments, rats that were infused with MUSC before overtraining showed a moderate percentage of freezing even in the earlier parts of the training session. Specifically, during initial shocks (5–10 shocks) of over training, rats treated with MUSC froze 30–50 % of the time (Figure - 2B). This experiment determined if the overtraining is actually necessary to form a long-term fear memory when the BLA is inactivated.

The experiment had the same design as experiment as previous experiment, except that normal training was used. Naïve rats ($n = 7$) were infused with MUSC and 20 minutes later trained with 5 shocks. We selected 5 shocks because previous work with this procedure has found that fear is asymptotic in 3 trials (Young and Fanselow, 1992). The data in Figure 2A are consistent with this view as the post-MUSC animals show stable freezing levels after 5 shocks. Thus 5 trials is post-asymptotic in intact animals but would not be considered over training. Three days after training they were again infused with MUSC and tested in either the original training context or a novel context. A second test was given three days later in the other (original or different) context under MUSC (counterbalanced). Rats trained with 5 shocks showed very little freezing and the small amount of freezing observed did not differ in the trained and untrained context (Paired $t(6) = 0.812$, $p = 0.45$), see figure-3B). Thus overtraining is indeed necessary for fear conditioning when the BLA is inactivated. Note that in vehicle-infused rats, 5 or fewer training trials is sufficient to produce freezing levels ranging from 50–80% when rats are tested 1–15 days after training (Helmstetter and Bellgowan, 1994; Maren et al., 1996b; Muller et al., 1997).

Note that the 5 shocks administered in this experiment occurred at the same time with respect to the MUSC infusion, as the first 5 shocks of the overtraining. It is possible that during the long overtraining session MUSC lost its efficiency and that is why the overtraining rats learned.

Therefore, we gave another set of rats ($n=8$) 5 shocks time-matched to the last 5 shocks of 76 shocks. The experiment had the same design as the previous experiment, except that the 5 shocks occurred 1 hour and 35 minutes after MUSC infusion. Three days after training they were again infused with MUSC and tested in either the original training context or a novel context. A second test was given three days later in the other (original or different) context under MUSC (counterbalanced). Consistent with the previous experiments rats trained with 5 shocks showed very little freezing and the small amount of freezing observed did not differ in the trained and untrained context (Paired $t(7) = 1.01$, $p = 0.34$, see figure-3C). Thus MUSC prevents learning of fear when the BLA is inactivated with normal training and overtraining is necessary to overcome MUSC effects.

Discussion

The data indicate that normally, neuronal activity in the amygdala supports fear learning probably through the mechanisms of NMDA-dependent synaptic plasticity that have been demonstrated in this region (Chapman et al., 1990;Clugnet and LeDoux, 1990;Miserendino et al., 1990;Fanselow and Kim, 1994;Maren and Fanselow, 1995;Rodrigues et al., 2004). Activity in this region is also necessary for performance of fear responses *when the BLA has undergone plasticity* suggesting that fear memory is also stored in the BLA (Figure 2B and 3A, see (Helmstetter, 1992) for an example with minimal training). However, the present results demonstrate that when activity in the BLA is prevented by MUSC fear can be learned, albeit slowly (Figure 2A), and importantly can be expressed without the BLA (Figures 2B & 3A). This suggests that plasticity in another region can support conditional fear learning.

It is important to note that overtraining alone is not sufficient to support learning in this alternate pathway because MUSC virtually abolished expression of freezing in the rats overtrained with an unmanipulated BLA (Figure 2B). The alternate pathway only becomes critical for behavioral expression when the BLA is inactivated while the animal is being overtrained. It is well known that following damage to one brain region other regions may take on increased function. For example, when the medial geniculate is deprived of auditory input because of damage to the inferior colliculus, it becomes innervated by visual projections from the retina (von Melchner et al., 2000;Newton et al., 2004). Unlike permanent lesion studies (e.g.,(Maren, 1999), we chose a procedure that specifically precluded the possibility that loss of function in the BLA caused a rewiring in other neural circuits. We exposed all rats to the same MUSC infusions and simply changed whether the BLA was inactivated during or after training. Experiencing both overtraining and BLA inactivation separately is not sufficient to produce BLA-independent fear; rather such learning depends on BLA inactivation during training. Our procedure makes clear that the alternate circuitry engages specifically because the BLA is not learning fear.

These experiments do not tell us what regions compensate to support contextual fear conditioning when the BLA is inactivated. Compensation could be mediated by increased plasticity in a region that normally contributes to expression of context fear or it may be through recruitment of additional regions. One possibility is the CeA as learning related activity in this structure has been shown to be necessary for auditory fear conditioning (Wilensky et al., 2006). It is unclear at present whether CeA plasticity related to auditory fear conditioning depends on processes within the BLA or is mediated independently by direct CS and US inputs to CeA (LeDoux et al., 1988;Bernard and Besson, 1990;Turner and Herkenham, 1991;Jasmin et al., 1997;McDonald, 1998). The CeA would be an especially attractive candidate if CeA plasticity proceeds independently from BLA plasticity as has been suggested by some investigators (Cardinal et al., 2002;Balleine and Killcross, 2006).

It should be noted however, that Koo et al., (2004) showed that electrical but not chemical lesions of CeA reduced context fear, suggesting that fibers of passage through CeA and not CeA neurons themselves support context fear. Furthermore, Maren, (1998) reported that rats with combined lesions of the BLA and CeA acquired significant levels of fear when they were overtrained with 50 shocks spread over 2 sessions. Additionally, Kim and Davis, (1993) reported that rats given extensive Light-Shock training both before and after large lesions of the amygdala that included both BLA and CeA could express light-cued fear measured with potentiated startle. Thus it is unlikely that CeA plasticity could completely account for the findings reported here.

Another possibility are the bed nuclei of the stria terminalis, which seems to play a role in context fear (Sullivan et al., 2004) and some other forms of anxiety (Walker and Davis, 1997; Walker et al., 2003). This region receives information from the hippocampal formation which is important for contextual fear (Maren and Fanselow, 1995; Dong and Swanson, 2004, 2006). It also projects to the vPAG, which is necessary for generating the freezing response (De Oca et al., 1998; Dong and Swanson, 2004, 2006). Electrolytic lesions of CeA may reduce context conditioning because they disrupt fibers from the BLA to the Bed nuclei that pass through CeA (Koo et al., 2004). Therefore, ongoing research in our lab is investigating the contribution of plasticity in this region to the phenomenon reported here.

These data suggest that the standard model of fear conditioning based on the necessity of a simple linear circuit (Maren and Fanselow, 1996; LeDoux, 2000; Walker and Davis, 2000) is an oversimplification. In its place we suggest a more dynamic model based on four key hypotheses:

1. There are primary and alternate pathways capable of mediating fear behavior.
2. The alternate pathways are less efficient than the primary pathway.
3. The more efficient primary pathway dominates the learning, and simultaneously prevents significant learning in the alternate pathway(s).
4. The alternate pathways compensate when the dominant pathway is compromised.

The first hypothesis is supported by the demonstration of both BLA-dependent and BLA-independent fear (Figure 2A & 2B). Support for the second hypothesis is provided by a comparison of the learning curves in Figure 2A that shows learning curves with similar asymptotes but very different growth rates. The dominance of the primary pathway (hypothesis 3) is demonstrated by the fact that BLA inactivation abolishes fear in rats overtrained while the BLA is active (Figure 2B). And the last hypothesis is derived from the robust long-term memory observed in rats overtrained while the BLA was inactivated (Figures 2B & 3A).

This model integrates several surprising findings in the fear conditioning literature and reaches beyond the structures and specific preparation used here. For example, (Maren et al., 1997) reported that while post-training hippocampal lesions abolished context fear, pretraining lesions did not prevent learning. Similar to our results in Figure 2A, (Wiltgen et al., 2006) reported that rats with pretraining hippocampal lesions were less efficient in learning context fear but could reach the same asymptotic level of overtraining under optimal training parameters. Anglada-Figueroa and Quirk (2005), demonstrated a similar pattern within the amygdala. Using auditory fear conditioning, (Anglada-Figueroa and Quirk, 2005) they reported that small lesions of the basal nuclei of the amygdala that spared the lateral and central nuclei, produced pronounced deficits if made after but not before conditioning. In both of these cases (Anglada-Figueroa & Quirk, 2005; Maren et al., 1997) structures that supported expression of fear when the animal was trained intact, were not necessary for acquisition of behavior in the damaged brain.

While we have focused on fear conditioning here, the model may be applicable to other sorts of learning. For example, Richmond et al., (1997) showed that post-training lesions of the HPC abolished a well-trained negative patterning discrimination. However, these animals reacquired this discrimination with continued post-lesion training. This suggests that the HPC is not absolutely necessary for learning configural discriminations such as negative patterning as initially proposed by Sutherland and Rudy, (1989) but it normally does play an essential role in such learning. When the HPC is damaged other regions seem to compensate. Rather than saying a particular circuit is necessary for learning and memory it may be more appropriate to ask what is normally used in the intact animal. The idea that learning and memory is restricted to specialized sets of independent circuits necessary for particular functions may underestimate the rich potential the brain's recurrent and dynamic networks can provide. The model we have presented here may better reflect this dynamic flexibility.

Perhaps, the most intriguing hypothesis in this model is the third, which suggests that learning in the primary pathway down-regulates learning in other potential circuits. Some speculative insight about this apparent regulation between pathways is suggested by that fact that the learning curves for BLA-dependent and BLA-independent fear had different growth rates to the same asymptote (Figure 2A). In Pavlovian conditioning, when two stimuli predict the same outcome, the stimulus with the more rapid learning rate comes to dominate the learning at the expense of the other stimulus often in a winner take all fashion (e.g (Mackintosh, 1976). This overshadowing effect has been incorporated into most formal models of Pavlovian conditioning (e.g.,(Rescorla and Wagner, 1972;Mackintosh, 1975;Pearce and Hall, 1980;Miller and Matzel, 1988;Gallistel, 1992;Schmajuk and Larrauri, 2006). It is possible that the same negative feedback circuitry responsible for this competition between stimuli for the control of behavior is also responsible for this competition between neural circuits for the control of behavior (Fanselow, 1998). This competition between potential learning circuits may have considerable adaptive significance. If a potent reinforcer like shock caused synaptic strengthening in all pathways that could mediate fear behavior, the magnitude of the fear response might rapidly exceed the magnitude of the threat. If these circuits were strengthened in a manner limited by competitive learning, the level of fear would be graded to the magnitude of threat. In this regard, levels of conditional fear tend to grade with factors such as shock intensity (Young and Fanselow, 1992). It is entirely possible that anxiety disorders result when this regulation fails.

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Figure 1. Histology-Location of needle tips for Experiments 1, 2 and 3

A.) A photomicrograph of coronal sections through the amygdala stained with cresyl violet showing the guide cannula track and infusion cannula tip from a rat used in the experiment. Cannula tips were targeted at the BLA (lateral and basal nuclei). (L = lateral, BL = basal nucleus, Ce = central nucleus)

B.) This diagram represents histology from 53 animals whose behavioral data are depicted in Figure 3; Figure 4; and Figure 5 (MUSC -black squares; ACSF -grey circles). Because of the extensive overlap between the infusion needle tips of these animals, not all tip locations are resolvable on this diagram. Coronal brain sections images were adapted from Paxinos and Watson (1998).

Figure 2. Experiment-1 Effect of intra-amygdala infusions of muscimol on extensive fear conditioning

A) Mean ($\pm$ SEM) percentage of freezing during an over-training procedure. Since experiments 1 and 2 had identical training procedures this curve contains the rats of both experiments. The learning curves of the two experiments were almost identical. On the training day rats received 0.25 μ l muscimol (1 μ g/ μ l) infused into the BLA before (Pre-) or after (Post-) overtraining with 76 shocks. Posttraining infusion serves as a control for any permanent effect of muscimol (MUSC) or infusion. MUSC infusion significantly retarded the freezing until 10 shocks (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ when compared to PostMUSC group)

B) Mean ($\pm$ SEM) percentage of freezing during the 8 min shock-free test of experiment 1. Three days after training the rats were infused with either MUSC or the artificial cerebrospinal fluid vehicle (ACSF) prior to test (* * $p < 0.01$ when compared to all other groups, ns = not significant).

Figure 3. Experiment -2 and 3 Effect of intra-BLA infusions of muscimol on memory expression in original and different contexts

A) Mean ($\pm$ SEM) percentage of freezing during the shock free 8 min context test of experiment 2. Rats were overtrained under muscimol (Day-1 fear acquisition is shown in figure 2). Three days after training they were again infused with muscimol and tested in either original training context or a different context. A second test was given three days later in the other (original or different) context under muscimol. * $p < 0.02$ when compared to the freezing in different context

B) & C) Mean ($\pm$ SEM) percentage of freezing during the shock-free 8 min context test of experiment 3 (begin group and end group) ($p > 0.05$ when compared to the freezing in different context). The design remains the same as Experiment-2 except that rats were not over trained. These rats received normal training (5 shocks) time-matched to the first 5 shocks of 76 shocks (begin group). Another set of rats received normal training (5 shocks) time-matched to the last 5 shocks of 76 shocks (end group). These rats were given normal training 1 hour and 35 minutes after MUSC infusion.