N-METHYL-D-ASPARTATE RECEPTORS IN THE AMYGDALA ARE NECESSARY FOR THE ACQUISITION AND EXPRESSION OF CONDITIONED DEFEAT

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Abstract—Here, we describe a biologically relevant model called conditioned defeat that is used to examine behavioral responses to social defeat in Syrian hamsters. In this model experimental animals that are normally aggressive experience social defeat and consequently display high levels of submissive/defensive behavior even in response to non-threatening conspecifics. N-methyl-D-aspartate (NMDA) receptors within the amygdala play an important role in conditioned fear; therefore, the purpose of this study was to examine whether NMDA receptors within the amygdala are necessary for the acquisition and expression of conditioned defeat. Specifically, the present study examined whether bilateral infusions of the NMDA receptor antagonist DL-2-amino-5-phosphonopentanoic acid (AP5; 0.625, 1.25, 2.5, 5.0, 10.0 μg) into the amygdala would block the acquisition of conditioned defeat. Subsequently, we examined whether bilateral infusions of AP5 (0.625, 1.25, 2.5, 5.0, 10.0 μg) into the amygdala prior to testing would block the expression of conditioned defeat. Infusions of AP5 into the amygdala immediately before the initial social defeat significantly reduced submissive/defensive behavior when hamsters were tested the following day with a non-aggressive intruder. Similarly, infusions of AP5 into the amygdala immediately before exposure to a non-aggressive intruder significantly attenuated the display of submissive/defensive behavior. These data demonstrate that NMDA receptors are necessary for both the acquisition and expression of conditioned defeat. We believe that conditioned defeat is a unique and valuable animal model with which to investigate the neurobiology of fear-responsive behavior. Conditioned defeat is not simply a transient change in behavior, but rather a prolonged response to social defeat. For instance, following four 5 min exposures to social defeat, male hamsters become submissive to non-aggressive intruders for a period that can last from at least 16 days to over 1 month without further experience of defeat (Hueman et al., 2003). Interestingly, there is a sex difference in the response to social defeat in this species; females show a greatly attenuated response to defeat compared with males, and this difference may be due to actions of the steroid hormone, estrogen (Hueman et al., in preparation). Recently, we have begun to uncover some of the mechanisms underlying the striking change in social behavior observed in males in this model. The amygdala plays an important role in regulating both the acquisition and expression of conditioned defeat. Specifically, infusion of the GABA<sub>A</sub> agonist, muscimol, into the central nucleus of the amygdala, either before the initial experience of defeat or immediately before testing, greatly reduces the duration of submissive behavior displayed by defeated hamsters (Jasnow et al., 2000). These data are consistent with previous studies implicating the amygdala in fear and anxiety (Muller et al., 1997; Wilensky et al., 2000; Hitchcock and Davis, 1986; Hitchcock et al., 1989) In addition, corticotropin-releasing hormone (CRH) is also involved in regulating conditioned defeat. Central infusion of a non-specific CRH antagonist into the lateral ventricle immediately before testing also reduces the duration of submissive behavior displayed by defeated hamsters (Jasnow et al., 2000).

Key words: fear, anxiety, social stress, emotion, agonistic behavior, submission.

Social stress, primarily in the form of conflict between individuals, is one of the most pervasive forms of stress experienced by many animal species. Exposure to social stress often produces pronounced changes in physiology and behavior. Over the last several years, our laboratory has been examining the mechanisms underlying an abrupt and prolonged change in social behavior that is observed following single and repeated exposure to social defeat. We have been using an ecologically relevant model that has been previously established in Syrian hamsters, called conditioned defeat (Potegal et al., 1993). Male Syrian hamsters are normally aggressive animals that readily defend home territories from intruding conspecifics. However, if these animals are placed in a situation in which they experience a mild social defeat from a larger, more aggressive animal, they subsequently become highly submissive and are virtually unable to reverse their subordinate social status. Following this experience, defeated hamsters do not defend their home territory, even against smaller, non-aggressive animals that would have previously attacked and defeated. Instead of attacking intruding animals, defeated hamsters vigorously avoid social interaction and submit to intruders without provocation. We believe that conditioned defeat is a unique and valuable animal model with which to investigate the neurobiology of fear-responsive behavior.
One of the major targets of CRH-modulation of conditioned defeat appears to be the bed nucleus of the stria terminalis (BNST). Infusion of a CRH antagonist directly into the BNST, but not the central nucleus of the amygdala, immediately before testing significantly reduces the duration of submissive behavior. Moreover, the CRH acting on the BNST may be originating in neurons within the central nucleus of the amygdala (Jasnow et al., unpublished observations). These data are in accord with previous literature suggesting that CRH, acting within the BNST, modulates behavioral as well as physiological responses to stressful stimuli (Lee and Davis, 1997; Erb and Stewart, 1999; Erb et al., 2001).

Although we are just beginning to determine the neurobiological mechanisms regulating conditioned defeat, a great deal is known about the mechanisms underlying conditioned fear. Specifically, the amygdala is necessary for the acquisition and expression of conditioned fear responses. For example, pre- or post-training lesions of the central, lateral or basolateral amygdala decrease conditioned freezing to shock or to a context paired with a shock (LeDoux et al., 1990; Rozendaal et al., 1991a,b; Helmstetter, 1992; Phillips and LeDoux, 1992). Post-training lesions of the central or basolateral amygdala block the expression of fear-potentiated startle (Hitchcock and Davis, 1986; Sananes and Davis, 1992; Kim and Davis, 1993; Campeau and Davis, 1995; Lee et al., 1996), and pre-training chemical or electrolytic lesions of the basolateral or central amygdala block both the acquisition and expression of fear-potentiated startle (Sananes and Davis, 1992; Kim and Davis, 1993). Finally, post-training lesions or functional inactivation of amygdala also block contextual fear and consolidation of inhibitory avoidance learning (Parent and McGaugh, 1994; Wilensky et al., 2000; Muller et al., 1997; Liang et al., 1982). Together, these data indicate that the amygdala is essential for fear learning. In fact, data suggest that the basolateral complex of the amygdala is an important site of the synaptic plasticity underlying fear conditioning (Clugnet and LeDoux, 1990; Rogan and LeDoux, 1995; Rogan et al., 1997). Moreover, the synaptic plasticity that is essential for fear learning appears to be dependent upon N-methyl-D-aspartate (NMDA)-type glutamate receptor activation. A number of studies have demonstrated that infusion of the NMDA receptor antagonist DL-2-amino-5-phosphonopentanoic acid (AP5) into the amygdala blocks the acquisition of both fear-potentiated startle and conditioned freezing (Miserendino et al., 1990; Campeau et al., 1992; Fanselow and Kim, 1994; Gewirtz and Davis, 1997; Lee and Kim, 1998).

In addition, a number of studies have shown that infusion of AP5 into the amygdala also blocks the expression of conditioned freezing (Maren et al., 1996; Lee and Kim, 1998), and fear-potentiated startle (Fendt, 2001; Lee et al., 2001). These data suggest that NMDA receptors are essential for the acquisition of fear learning and might also be important for the expression of fear, as well.

Conditioned defeat is an ideal model with which to study the role of the amygdala in learning because of its similarities with fear conditioning. Without traditional mod-els of conditioned fear, identifying the mechanisms underlying fear learning would not have been possible. However, these mechanisms most likely did not evolve to respond to electric shock, but rather to respond to stimuli in the environment such as predators, food poisons and potentially threatening conspecifics. Given that NMDA receptors within the amygdala are essential in several types of conditioned fear models, the present study tested the hypothesis that NMDA receptors within the amygdala are necessary for the acquisition and expression of conditioned defeat in Syrian hamsters (Mesocricetus auratus).

EXPERIMENTAL PROCEDURES

Animals and housing conditions

Adult male Syrian hamsters (M. auratus), weighing 120–130 g at the beginning of the experiment, were obtained from Charles River Laboratories (Wilmington, MA, USA). Hamsters were housed individually for 2 weeks prior to testing in a temperature-controlled (20 °C ± 2 °C) colony room on a 14/10 h light/dark cycle with lights off at 11:00 h. Additional hamsters weighing 150–180 g were used as resident aggressors for the initial social defeat, and hamsters weighing 100–110 g at the beginning of the experiment were used as non-aggressive intruders during behavioral testing. All animals were housed in Plexiglas cages (20×40×20 cm) with wire mesh tops, and food and water were available ad libitum. Resident aggressors were housed individually, whereas non-aggressive intruder animals were group-housed (5–6 animals/cage).

All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee, and all methods were in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals. Every effort was made to minimize the number of subjects used as well as to minimize any suffering by the animals.

Surgical procedures

At the beginning of each experiment, adult male, Syrian hamsters were anesthetized deeply with sodium pentobarbital (90 mg/kg) and were placed in a stereotaxic instrument. Hamsters were then implanted bilaterally with two 4 mm, 26-gauge guide cannulae aimed at the basolateral amygdala. The guide cannulae did not extend down into the structure toward which they are aimed. The final depth is reached only when a smaller (33 gauge) injection needle is inserted into the guide cannula. The advantage of this procedure is that there is very little tissue damage caused by the smaller injection needle. Final stereotaxic coordinates were 0.4 mm posterior and ±3.9 mm lateral to bregma and 6.3 mm below dura. The skull was level between lambda and bregma prior to implantation of the guide cannulae. Dummy stylets were placed in the guide cannulae in order to maintain patency. Hamsters were allowed 1 week of recovery before being used in behavioral experiments. Following surgery, all animals were handled every day and their dummy stylets removed and replaced to minimize handling and restraint stress on testing days.

Social defeat training and testing

All hamsters were matched by weight and randomly assigned to experimental or control groups. On the day of defeat, animals were transported from the colony room to the behavioral room for the procedure. The defeat procedure consisted of a single resident/intruder pairing in which animals were placed into the cage of a resident aggressor for 15 min. All behavioral procedures occurred during the first 2 h of the dark phase of the daily light/dark cycle to control for circadian rhythmicity of physiology and behav-
ior. During the 15-min defeat session, experimental animals were routinely attacked by resident aggressors and displayed submissive and defensive behaviors toward the resident aggressors. Behavioral testing began 1 day after the acquisition procedure. A similar resident/intruder model was used for testing, but in this case a non-aggressive intruder was placed into the home cage of the defeated animal for 5 min. We have previously shown that 5-min testing sessions are sufficient to observe an adequate sample of behaviors and to ensure that the behaviors remain consistent within tests (Potegal et al., 1993). All training and testing sessions were recorded on VHS tape, transferred to CD-ROM, and scored by an observer blind to experimental condition using Noldus Observer (version 4; Noldus Information Technology, Wageningen, Netherlands). The following classes of behaviors were recorded as total duration in seconds: 1) non-social: locomotor/exploratory, self-groom, nesting, feeding, pouching; 2) social: attend, approach, investigate, sniff, touching nose; 3) submissive/defensive: upright and side defense, tail lift, flee, tooth chatter, full submissive posture, risk assessment, attempted escape from cage; 4) aggressive: upright and side offense, chase, attack, bite. In the conditioned defeat model, a statistically significant reduction in the duration of submissive/defensive behaviors and/or the display of territorial aggression signifies a reduction in, or blockade of, conditioned defeat.

Drug infusions and site verification

Infusions into the amygdala were administered to hamsters restrained gently by hand over 1 min with a Hamilton syringe mounted on a syringe pump (Harvard apparatus PHD 2000, South Natick, MA, USA) connected to a 33-gauge needle via polyethylene tubing. The needle was kept in place for another minute before being removed and the dummy stylet replaced. All hamsters were administered infusions of the NMDA antagonist AP5 (Sigma) or vehicle control (300 nl saline). At the conclusion of each experiment, hamsters were given a lethal dose of sodium pentobarbital and infused with 300 nl of Methylene Blue dye in order to verify location of needle placement. Brains were removed and frozen immediately on dry ice and then stored at -80 °C. Brains were blocked and sectioned on a cryostat at 30 μm and stained with neutral red. Sections were coverslipped with DPX mountant and examined under light microscopy for verification of injection. Only animals with injection sites within 0.5 mm of the basolateral amygdala were included in the statistical analysis.

Experiment 1: acquisition of conditioned defeat

The purpose of Experiment 1 was to determine whether infusion of AP5 into the basolateral amygdala would block the acquisition of conditioned defeat. Animals (n=46) were matched by weight and randomly assigned to one of two conditions. Hamsters received infusions of either AP5 (0.625, 1.25, 2.5, 5.0 or 10.0 μg in 300 nl saline) or vehicle immediately before being placed in the cage of a dominant resident aggressor for 15 min. On the following day animals were tested for 5 min in their own home cage in the presence of a non-aggressive intruder, as described above.

Experiment 2: expression of conditioned defeat

The purpose of Experiment 2 was to determine if infusion of AP5 into the basolateral amygdala would block the expression of conditioned defeat. Animals (n=40) were matched by weight and randomly assigned to one of two conditions. All hamsters were placed into the cage of a resident aggressor for 15 min for conditioned defeat training. On the following day, animals received infusions of either AP5 (0.625, 1.25, 2.5 or 5.0 μg in 300 nl saline) or vehicle immediately before being tested in their own home cage for 5 min with a non-aggressive intruder.

Experiment 3: post-defeat infusions of AP5

The purpose of Experiment 3 was to determine if the decrement in submissive behavior that we observed in Experiment 1 could be explained by residual drug effects of AP5 still present 24 h later during testing. Hamsters (n=22) were matched by weight and randomly assigned to one of four groups. Animals received infusions of AP5 (5.0 μg in 300 nl saline) or vehicle either immediately before being defeated in the home cage of an aggressive opponent or 4 h after being defeated. If AP5 given before defeat is diminishing submissive behavior due to hangover effects, then the decrement in submissive behavior should be even greater when the drug is given closer to the time of testing with the non-aggressive intruder.

Statistical analyses

For all experiments, the total duration (seconds) of each behavior displayed (Submissive/Defensive, Aggressive, Social, Non-social) was determined. The mean total duration of each behavior was analyzed using a one-way between-subjects analysis of variance (ANOVA) with dose as the between-subjects factor. Significant differences for all analyses were ascribed at P<0.05. Statistically significant differences were analyzed further using a Tukey-Kramer multiple comparison post-hoc test to compare all pairwise differences among group means.

RESULTS

Effects of infusion of AP5 into the amygdala on the acquisition of conditioned defeat

Histological analysis revealed that needle placements were mainly within the basolateral amygdala. Two animals had unilateral needle placements within the central amygdala on one side with a basolateral placement on the other side. Two additional animals received bilateral infusions into the central nucleus of the amygdala. Although there were not enough animals to run a statistical analysis there did not appear to be any differences in behavior between these animals and animals having bilateral needle placements within the basolateral amygdala (Fig. 1). This may be due to diffusion of AP5 from the injection site into the basolateral amygdala. Infusion sites for two animals were unable to be verified as a result of blocked cannulae at the time of dye infusion. One animal received a unilateral basolateral amygdala infusion and the other infusion was placed ventral to the central amygdala on the other side. Thus, a total of 43 animals were used in the statistical analysis: vehicle (n=15); 0.625 μg (n=9); 1.25 μg (n=6); 2.5 μg (n=5); 5.0 μg (n=5); 10.0 μg (n=3). Infusion of AP5 immediately before defeat significantly reduced the display of submissive/defensive behavior (F(5,42)=7.05; P<0.05; Fig. 2). Post hoc analysis revealed that infusion of AP5 into the amygdala reduced submissive/defensive behavior at all doses compared with animals receiving vehicle control (P<0.05). No differences in submissive/defensive behavior were observed among doses of AP5. Infusion of AP5 also increased aggressive behavior (F(5,42)=3.88; P<0.05). Post hoc analysis revealed that animals receiving 5.0 μg of AP5 displayed significantly more aggressive behavior compared with animals re-
ceiving 0.625 μg of AP5 and animals receiving vehicle control (P<0.05; Fig. 2). In addition, there was a statistically significant difference observed in social behavior (F(5,42)=3.20; P<0.05). Post hoc analysis revealed that animals receiving 5.0 μg of AP5 displayed more social behavior than did animals receiving vehicle control. Finally, there were no significant differences observed in nonsocial behavior (F(5,42)=1.25; P>0.05; Fig. 2).

**Effects of infusion of AP5 into the amygdala on the expression of conditioned defeat**

Histological analysis revealed that infusions were localized mainly within the basolateral amygdala. Some animals had needle placements within the central amygdala, but there were no differences in behavior between these animals and animals having needle placements within the basolateral amygdala (Fig. 1). A total of seven animals were removed from the statistical analysis. Infusion sites for five animals were unable to be verified due to blocked cannulae at the time of dye infusion. One additional animal had a misplaced infusion. This animal received only a unilateral basolateral amygdala infusion; the infusion on the other side was located in the ventral endopiriform cortex, lateral to the basolateral amygdala. Interestingly, this animal received AP5 and displayed similar behavior to other animals receiving bilateral AP5 infusions into the basolateral amygdala. One animal received misplaced infusions bilaterally that were at least 0.5 mm away from the amygdala. Importantly, this animal received an infusion of 2.5 μg of AP5 but displayed submissive behavior similar to animals receiving vehicle. Specifically, this animal displayed 100 s of submissive/defensive behavior, which is 88.76 s beyond the mean of animals receiving 2.5 μg of AP5, but only 12.97 s below the mean of animals receiving vehicle control. Two animals received infusions into the central nucleus of the amygdala. Although there were not enough animals to run a statistical analysis, there did not appear to be any differences in behavior between these animals and animals receiving infusions into the basolateral amygdala. This may be due to diffusion of the drug from the injection site into the basolateral amygdala, or due to the fact that AP5 inhibits synaptic transmission and, when infused into the central amygdale, can block the expression of conditioned defeat. Therefore, a total of 33 animals used in the statistical analysis: vehicle (n=12); 0.625 μg (n=4); 1.25 μg (n=4); 2.5 μg (n=6); 5.0 μg (n=7). Infusion of AP5 into the amygdala immediately before animals were tested with a non-aggressive intruder significantly decreased the display of sub-
missive/defensive behavior compared with animals receiving vehicle control ($F_{(4,32)} = 11.69; P < 0.05$). Post hoc analysis revealed that animals receiving all doses of AP5 displayed significantly less submissive/defensive behavior compared with animals receiving vehicle control ($P < 0.05$; Fig. 3). There were no differences in the display of submissive/defensive behavior among animals receiving any dose of AP5. Infusion of AP5 into the amygdala also increased social behavior compared with animals receiving vehicle control ($F_{(4,32)} = 5.73; P < 0.05$; Fig. 3). Animals receiving 0.625 μg, and 5.0 μg of AP5 displayed significantly more social behavior compared with animals receiving vehicle control ($P < 0.05$). No differences were observed in aggressive behavior ($F_{(4,32)} = 1.44; P > 0.05$) or nonsocial behavior ($F_{(4,32)} = 1.87; P > 0.05$; Fig. 3).

Fig. 2. Total duration (mean±S.E.M.) of submissive/defensive (a), aggressive (b), social (c) and nonsocial (d) behavior displayed by defeated hamsters during a 5-min test with a non-aggressive intruder. Animals received a bilateral infusion of 0.0, 0.625 μg, 1.25 μg, 2.5 μg, 5.0 μg or 10 μg of AP5 into the basolateral amygdala immediately before being defeated for 15 min. (*) Denotes significantly different ($P < 0.05$), than vehicle control. (△) Denotes significantly different ($P < 0.05$) than 0.625 μg.
Effects of post-training infusions of AP5 on the expression of conditioned defeat

All hamsters included in the statistical analysis had bilateral needle placements within the basolateral amygdala. ANOVA revealed a significant effect of treatment ($F(3,18) = 3.59; P<0.05$) on submissive behavior. Post hoc analysis indicated that hamsters receiving pre-training infusions of AP5 exhibited significantly less submissive/defensive behavior than did animals receiving pre-training infusions of vehicle. Hamsters receiving AP5 or vehicle 4 h after the initial defeat exhibited intermediate levels of submissive/defensive behavior that were not significantly different from any other group (Fig. 4). Levels of aggression ($F(3,18) = 0.75, P>0.05$), social ($F(3,18) = 1.02, P>0.05$) and non-social ($F(3,18) = 0.75, P>0.05$) behavior were not significantly different among groups (data not shown).
DISCUSSION

These data demonstrate that infusion of the NMDA receptor antagonist, AP5, into the amygdala reduces the acquisition and expression of conditioned defeat. Animals receiving bilateral infusions of AP5 into the amygdala immediately before the initial social defeat displayed significantly less submissive/defensive behavior when tested 24 h later than did animals receiving infusions of vehicle. There was no significant change in submissive/defensive behavior if the infusion of AP5 or vehicle was administered 4 h after the initial defeat. In addition, infusion of AP5 into the amygdala immediately before testing with a non-aggressive intruder also reduced the expression of submissive/defensive behavior. Social behavior was significantly increased following the infusion of AP5 when animals were tested with a non-aggressive intruder. Although there was a significant increase in Experiment 1 in aggressive behavior in animals receiving one of the doses of AP5, this effect was not replicated in Experiment 3. The change in behavior in Experiment 1 was very small (approximately 2 s; see Fig. 2), and this did not constitute a return to typical levels of territorial aggression. Importantly, there were no differences in nonsocial behavior, indicating that there were no nonspecific effects of AP5 on general activity. These data suggest that NMDA receptors play an important role in the acquisition and expression of conditioned defeat. In addition, the results from the present study suggest that there are similarities in the mechanisms regulating responses to social defeat and the mechanisms regulating other conditioned fear responses. Finally, these data indicate that NMDA receptors within the amygdala are not only necessary for the acquisition and expression of fear conditioning to a simple cue but also under more complex conditions in which animals must learn about their social environment.

One alternative explanation for the finding that pre-training infusions of AP5 significantly reduce submissive/defensive behavior when the animals are tested 24 h later is that AP5 caused hang-over or residual drug effects. To examine this possibility, we injected vehicle or a high dose of AP5 4 h after defeat training. If AP5 was decreasing conditioned defeat via residual drug effects, these animals should show decreases in submissive/defensive behavior at least as great as those observed when the drug was given 4 h earlier (before training). Instead, these hamsters exhibited intermediate levels of submissive/defensive behavior. It is thus possible that there were some minor effects of the drug given the preceding day; however the change in conditioned defeat was not as great as that seen with pre-training injections. In addition, we do not feel that hang-over effects account for the data because hamsters given vehicle 4 h after defeat also exhibited intermediate levels of submissive/defensive behavior. Finally, it seems

![Submissive/Defensive Behavior Graph](image_url)

Fig. 4. Total duration (mean±S.E.M.) of submissive/defensive behavior displayed by defeated hamsters during a 5-min test with a non-aggressive intruder. Animals received a bilateral infusion of 0.0 μg or 5.0 μg of AP5 into the basolateral amygdala immediately before or 4 h after being defeated for 15 min. (*) Denotes significantly different (P<0.05) than vehicle control pre-training.
likely that hang-over effects of a drug would reduce all agonistic behavior, whereas we observe a specific reduction of submissive/defensive behaviors and an increase in social behavior.

An additional alternative explanation for the present findings is that infusions of AP5 altered the experience of pain. Lesions of the amygdala produce analgesia in acute pain tests and following footshock (Hebert et al., 1999; Helmstetter andBellgowan, 1994). Therefore, when hamsters were attacked during the defeat period, it is possible that AP5 altered the experienced of being attacked or bitten. This possibility is unlikely, however, because previous studies have demonstrated that AP5 infused into the amygdala does not block pain sensitivity to footshock (Miserendino et al., 1990; Maren et al., 1996). In addition, physiological and behavioral responses to defeat often occur without actual physical contact between hamsters, suggesting that there is an important psychological component in the response to defeat (Huhman et al., 1992; Bronson and Eleftheriou, 1965).

The present results are consistent with previous reports demonstrating that infusion of AP5 into the amygdala blocks the acquisition of conditioned fear responses (Miserendino et al., 1990; Campeau et al., 1992; Gewirtz and Davis, 1997; Maren et al., 1996; Fanselow and Kim, 1994; Lee and Kim, 1998). Taken together, these data suggest that activation of NMDA receptors within the amygdala is a necessary component of fear learning in both highly controlled, but more artificial, models as well as in less controlled, but perhaps more biologically relevant, models such as conditioned defeat. The data presented here are also consistent with studies demonstrating that infusion of AP5 into the amygdala blocks the expression of conditioned freezing (Fanselow and Kim, 1994; Maren et al., 1996; Lee and Kim, 1998) as well as fear-potentiated startle (Fendt, 2001; Lee et al., 2001). A number of previous studies, however, have demonstrated that infusions of AP5 into the amygdala fail to block the expression of fear-potentiated startle (Miserendino et al., 1990; Campeau et al., 1992; Gewirtz and Davis, 1997), which is consistent with the hypothesis that NMDA receptors within the amygdala are principally involved in the synaptic plasticity associated with fear learning. LeDoux (2000) has suggested that NMDA receptors make significant contributions to synaptic transmission in addition to synaptic plasticity within pathways that provide input to the amygdala. A number of electrophysiological studies have demonstrated the ability of AP5 to block synaptic transmission within these pathways (Li et al., 1995, 1996; Maren and Fanselow, 1995; Weisskopf et al., 1999), and the recent behavioral data (Lee et al., 2001; Fendt, 2001; Lee and Kim, 1998) and the present results tend to support this hypothesis. At the present time it is not precisely clear why infusion of AP5 blocks the expression of fear conditioning in some instances and not others.

We have hypothesized that the mechanisms underlying conditioned defeat are similar to the mechanisms underlying other forms of conditioned fear. Data from our laboratory support this hypothesis; however, we also recognize that there are important differences between conditioned defeat and conditioned fear. In conditioned fear studies, conditions are arranged with a highly predictable temporal relationship between the onset of the conditioned and unconditioned stimuli. Once the conditioned stimulus is turned off, the subject can be sure that no aversive event will occur. In social defeat there is not a clear conditioned stimulus and unconditioned stimulus. In addition, the subordinate animal is at risk for attack, but the exact time when the attack may occur, if it does at all, is uncertain. Furthermore, we examine how previously defeated animals interact with novel stimulus animals over a range of behaviors used as indices for conditioned defeat. It is entirely possible that motor output for these responses are controlled by NMDA-dependent mechanisms that are different than those that control other conditioned fear responses. One final and important difference between the present study and previous reports is a difference in species. The present data were collected using Syrian hamsters, whereas the majority of previous reports have used either Sprague–Dawley or Wistar rats or laboratory mice.

There is evidence that the NR2B subunit of the NMDA receptor uniquely contributes to plasticity within the amygdala. A recent study demonstrated that both systemic and intra-amygdala infusion of the selective NR2B subunit antagonist, ifenprodil, dose-dependently decreased the acquisition, but not the expression, of freezing to a tone as well as to a context (Rodrigues et al., 2001). In addition, other studies have demonstrated that infusion of the metabotropic glutamate receptor (mGluR5) specific antagonist, 2-methyl-6-(phenylethynyl)-pyridine, into the lateral amygdala blocks the acquisition, but not expression, of fear-potentiated startle as well as auditory and contextual freezing (Fendt et al., 2000; Rodrigues et al., 2002). These studies suggest that both the NR2B subunit of the NMDA receptor and the mGluR5 receptor might uniquely contribute to the plasticity within the amygdala associated with the acquisition of fear conditioning without affecting the expression of fear conditioning. We are currently examining the hypothesis that more specific glutamate receptor antagonists might block the acquisition but not the expression of conditioned defeat.

Acknowledgements—The authors acknowledge Jeris Israel for her technical assistance as well as David Marshall and Patricia Hicks for their expert animal care. The authors also thank Dr. Charles F. Gillespie and Dr. Michael Davis for helpful discussions and comments during the preparation of this manuscript. This research was supported by USPHS grants MH 62044 to K.L.H., MH 12907 to A.M.J., and is based upon work supported in part by the STC program of the National Science Foundation under agreement No. IBN-9876754.

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(Accepted 3 October 2003)