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Research report

NR2B subunit of the NMDA receptor in the basolateral amygdala is necessary for the acquisition of conditioned defeat in Syrian hamsters

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ABSTRACT

Reversible inactivation of the basolateral amygdala (BLA) disrupts the acquisition and expression of conditioned defeat (CD), an ethological model of conditioned fear, suggesting that the BLA may be a critical component of the neural circuit mediating behavioral plasticity associated with the experience of social defeat. We have also shown that this effect is *N*-methyl-D-aspartic acid (NMDA) receptor-dependent, because infusion of D,L-2-amino-5-phosphovalerate (APV) into the BLA also impairs the acquisition of CD. APV is a non-selective NMDA antagonist, however, thus it disrupts the entire heteromeric receptor complex, making it difficult to distinguish the relative contributions of either the NR2A or NR2B receptor subtypes on the acquisition of CD. There is ample evidence, however, that the NR2B subunit of the NMDA receptor in the amygdala is critical for mediating long-term potentiation and plasticity related to fear learning. The purpose of the present experiment was to determine whether infusion of ifenprodil, a selective antagonist of the NR2B subunit, into the BLA would block the acquisition (but not expression) of CD. In Experiment 1, infusion of ifenprodil immediately before defeat training significantly decreased submissive behaviors and restored territorial aggression when hamsters were later paired with a non-aggressive intruder (NAI). Conversely, infusion of ifenprodil immediately before CD testing failed to inhibit the expression of submissive behaviors in previously defeated hamsters. These results support the hypothesis that the BLA is a critical site for the plasticity underlying social defeat-induced changes in behavior.

1. Introduction

Over the past several years our lab has utilized an ethologically based model of conditioned fear called conditioned defeat (CD), wherein Syrian hamsters (*Mesocricetus auratus*) are first defeated by a larger, more aggressive opponent (the resident aggressor, or RA) in the resident aggressor's cage. Upon subsequent exposure to a smaller, non-aggressive intruder (NAI), the defeated hamster will display an array of submissive and defensive behaviors instead of their normal territorial aggression [14]. Strikingly, this learned response is elicited even when the defeated animal is tested in its own home cage, and it persists for at least one month post-defeat in the majority of animals [5]. We maintain that developing an understanding of how the brain changes in response to social defeat in hamsters will offer important insights into the mechanisms of experience-induced behavioral plasticity and perhaps into the mechanisms whereby social stress increases social avoidance and other depressive-like symptoms.

Previous studies in our lab have suggested that the basolateral amygdala (BLA) is a critical component of the neural circuit mediating the acquisition and expression of CD and that neural activity underlying the defeat experience is *N*-methyl-D-aspartic acid (NMDA) receptor-dependent. Thus, we have demonstrated that reversible inactivation of the BLA using muscimol significantly impairs both the acquisition and expression of CD [6], results that are consistent with studies using more traditional models of fear learning such as Pavlovian fear conditioning and fear-potentiated startle (FPS) [8,3,17]. Next, we have shown that infusion of APV, an NMDA receptor antagonist, into the BLA also blocks the acquisition and expression of CD [7]. This is also consistent with a wide body of evidence suggesting that the acquisition of fear learning is mediated via NMDA receptors [16,1,2,21].

NMDA receptors contain of two families of receptor subunits, NR1 and NR2 [15], and within the amygdala two subtypes of the NR2 subunit have been identified, NR2A and NR2B [12]. Because APV is a non-selective NMDA antagonist, it blocks the entire receptor complex, making it impossible to distinguish the relative contributions of each subunit to constitutive neural transmission in the BLA versus a role in the actual synaptic plasticity surround-

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ing the defeat experience. Several studies, however, now suggest that the NR2B subunit plays a more critical role in the plasticity mediating acquisition of conditioned fear. For example, Rodrigues et al. [16] have shown that infusion of ifenprodil, a selective antagonist of the NR2B receptor subtype, into the basolateral complex of the amygdala causes a significant impairment in the acquisition of auditory and contextual fear conditioning. More recently, Walker and Davis [21] have shown that blockade of the NR2B subunit via infusion of CP101,606 into the amygdala inhibits the acquisition (but not expression) of FPS. Together, these findings provide strong evidence that the NR2B subunit in the amygdala plays a critical role in fear memory formation. The previously discussed fear conditioning studies, however, use models that are comparatively simple in terms of the causative stimuli as well as the motor outputs involved. CD, on the other hand, is a complex, ethologically relevant social behavior with relatively undefined antecedents and a complex and variable outcome.

In the present study, we tested the hypothesis that NMDA receptors in the BLA play a critical role in the synaptic plasticity related to the defeat experience by selectively blocking the NR2B subtype of the NMDA receptor. We infused ifenprodil into the BLA either before initial defeat training (acquisition) or before testing with the NAI (expression). If the NR2B subunit is a critical for the formation of fear memories related to social defeat, the infusion of ifenprodil should impair the acquisition, but not expression, of CD.

2. Materials and methods

2.1. Animals and housing conditions

Male Syrian hamsters, weighing 120–130 g at the beginning of the experiment, were obtained from Charles River Laboratories (New York, NY) and group housed for one week prior to surgery. Hamsters serving as RA's were individually housed and weighed between 150 and 180 g, while non-aggressive intruders (NAI) were group housed and weighed between 100–110 g at the start of the experiment. All animals were housed in a temperature and humidity-controlled room on a 14:10 h light/dark cycle with lights off at 11:00 h and kept in clear polycarbonate cages (20 cm × 40 cm × 20 cm) with wire tops and food and water available ad libitum. All testing occurred in the first three hours of the dark phase of the daily light/dark cycle to minimize circadian variation of the behaviors. All procedures were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with National Institutes of Health and United States Department of Agriculture guidelines.

2.2. Surgical procedures

Hamsters were anesthetized with sodium pentobarbital (90 mg/kg, i.p.) and placed into a Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA). Guide cannula (26 gauge stainless steel; Plastics One, Roanoke, VA) were bilaterally implanted and aimed at the BLA (0.4 mm posterior and 3.9 mm lateral to bregma, and 2.1 mm below dura). The guide cannula was secured to the skull using cyanoacrylate ester gel, wound clips and dental acrylic. A removable obturator needle was kept in place to maintain patency of the cannula. Following surgery, all animals were handled daily and their obturators removed and replaced into the cannula to habituate each animal to the infusion procedure. During the injection procedure, a 33-gauge needle with a 4.2 mm projection from the base of the cannula guide was lowered into the BLA to give a final dorsal-ventral depth of 6.3 mm. This was done in order to minimize tissue damage in and around the amygdala.

2.3. Social defeat and behavioral testing

Hamsters were matched by weight and randomly assigned to experimental or control groups. Animals were transported from the colony room to the behavioral testing room on the day of defeat training. Training sessions consisted of one 15-min exposure to the RA in the aggressor's home cage, upon which the subject was reliably attacked by the RA within 60 s. This training duration is based on previous studies from our lab showing that a single 15 min defeat results in reliable levels of conditioned defeat during testing with the NAI. The testing session occurred 24 h later. Subjects were again transported to the same testing room and were exposed for 5 min to a NAI in the experimental animals' own home cage. All training and testing sessions were recorded on VHS tape, transferred to CD-ROM and scored by observers blind to the experimental condition using Noldus Observer (version 3; Noldus Information Technology, Wageningen, Netherlands). The total duration of four classes of behaviors were scored during the test session: (a) social behavior (stretch, approach, sniff, nose touching and flank marking), (b) non-social behavior (locomotion, exploration, grooming, nesting, feeding and sleeping), (c) submissive/defensive behaviors (flight, avoidance, tail up, upright, side defense, full submissive posture, stretch attend, head flag, attempted escape from cage, and (d) aggressive behaviors (upright and side offense, chase and attack, including bite).

2.4. Drug infusions and site verification

Ifenprodil (Sigma, 200 ng/200 μl and 400 ng/200 μl) dissolved in a 10% solution of dimethyl sulfoxide (DMSO) or vehicle control (200 μl 10% DMSO in saline) was infused bilaterally into the BLA over a 1 min period using a Hamilton syringe connected to a 33-gauge needle via polyethylene tubing. The needle was kept in place for an additional 1-min before being removed to ensure complete diffusion of the drug after which the dummy was replaced. Training or testing began 20 min after infusion. At the conclusion of the experiment, hamsters were given a lethal dose of sodium pentobarbital and infused with 200 nl of India ink in order to verify the location of the cannula placement. The brains were removed and flash frozen in dry ice and placed in a −80 °C freezer. The brains were then blocked and sectioned on a cryostat at 30 μm and stained with neutral red. Sections were cover slipped with DPX mountant and examined under light microscopy for placement of injection. Only animals with injection sites within 0.5 mm of the BLA were included in the statistical analysis.

2.5. Experiment 1a: acquisition of conditioned defeat

The goal of Experiment 1 was to determine whether blockade of the NMDA receptor subunit NR2B in the BLA would inhibit the *acquisition* of CD. Animals ($n = 40$) were matched by weight and randomly assigned to one of three conditions. Hamsters received ifenprodil (200 or 400 ng in 200 μl vehicle) or vehicle alone 20 min before being placed in the home cage of the RA for 15 min. One the following day, animals were tested drug-free in their own home cage against a NAI for 5 min.

2.6. Experiment 1b: no defeat control

Considering the high levels of aggression observed in ifenprodil-treated animals in Experiment 1, we performed an additional study wherein ifenprodil or vehicle was infused in undefeated animals to ensure that the heightened levels of aggression were not a by-product of the ifenprodil infusion, itself. Animals ($n = 20$) with guide cannula aimed at the BLA were infused with ifenprodil (400 ng/200 μl vehicle) or vehicle alone (200 μl) 20 min prior to being placed in the cage of a resident aggressor for 15 min without the aggressor being present. On the following day, animals were tested for 5 min in their own home cage in the presence of a NAI, as described above.

2.7. Experiment 2a: expression of conditioned defeat

The purpose of Experiment 2 was to determine whether blockade of the NMDA receptor subtype NR2B in the BLA would inhibit the *expression* of CD. Animals ($n = 32$) were matched by weight and defeated by a RA for 15 min. On the following day, animals were randomly assigned to one of two conditions and infused with either ifenprodil (400 ng in 200 μl vehicle) or vehicle alone 20 min prior to being tested in their own home cage for 5 min with a NAI. In this experiment, we used only the most effective dose of ifenprodil based on the results from Experiment 1.

2.8. Experiment 3: state-dependency controls

The reduction in submissive behaviors observed in Experiment 1 may have been the result of a difference in the drug state of subjects receiving only pre-training or pre-testing infusions of ifenprodil. In order to assess this possibility, we infused ifenprodil (400 ng/200 μl) or vehicle 20 min before defeat training and then again 20 min before testing with the NAI ($n = 10$) to ensure that CD was not restored when animals were trained and tested in the same drug state.

2.9. Statistical analysis

For all experiments, data was analyzed using a one-way between-subjects analysis of variance (ANOVA) with dose as the between-subjects factor. In cases where variance between groups were not homogenous, data was analyzed using Mann–Whitney *U* test when comparing only two groups and Kruskal–Wallis test when comparing three groups. Significant differences for all analyses were ascribed at $p < 0.05$. Statistically significant differences were analyzed using a LSD post hoc test to compare all pairwise differences. Exact probabilities and test values have been omitted for simplification and clarity of the presentation of these results.

3. Results

3.1. Histology

Fig. 1a and b shows the injections sites for animals in Experiments 1 and 2, respectively. Only bilateral placements of the