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Chronic stress impairs recall of extinction of conditioned fear

Angela D. Miracle, Michael F. Brace, Kellie D. Huyck, Samantha A. Singler, Cara L. Wellman *

Department of Psychological and Brain Sciences and Program in Neuroscience, Indiana University, Bloomington, IN 47405, USA

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Abstract

Chronic restraint stress produces retraction of apical dendrites of pyramidal neurons in medial prefrontal cortex. To begin to examine the functional significance of this dendritic reorganization, we assessed the effects of chronic restraint stress on a prefrontally mediated behavior, extinction of conditioned fear. After bar press training to obtain a baseline of activity against which to measure freezing, rats were either unstressed or stressed via placement in a plastic restrainer (3 h/day for 1 week). After an additional day of bar press training, rats underwent fear conditioning and extinction. Rats received five habituation trials to a 30-s tone (4.5 kHz, 80 db) followed by seven pairings of tone and footshock (500 ms, 0.5 mA). One hour later, rats received tone-alone extinction trials to criterion. The next day, rats received 15 additional extinction trials. Percent freezing was assessed during all phases of training. Stress did not significantly affect unconditioned responding to tone, acquisition of conditioned fear, or initial extinction, but significantly increased freezing on extinction day 2. Thus, consistent with the regressive dendritic changes seen in medial prefrontal cortex, one week of restraint stress specifically impaired recall of extinction, a pattern of deficits typical of animals with impaired medial prefrontal function.

Keywords: Medial prefrontal cortex; Fear conditioning; Extinction; Rat

1. Introduction

Medial prefrontal cortex (mPFC) is a target for glucocorticoids involved in the stress response (Meaney & Aitken, 1985), and exposure to stressors results in a variety of neurochemical changes in mPFC, including increases in glutamate and acetylcholine release (Bagley & Moghaddam, 1997; Jedema & Moghaddam, 1994; Mark, Rada, & Shors, 1996; Moghaddam, 1993). Dendritic morphology of mPFC appears to be particularly sensitive to chronic stress: either six (Radley et al., 2004) or three hours (Cook & Wellman, 2004) of restraint per day for three weeks results in retraction of apical dendrites of layer II–III pyramidal neurons in mPFC. This effect occurs with as little as one week of brief daily restraint (Brown, Henning, & Wellman, 2005), suggesting that the morphology of mPFC is exquisitely sensitive to stress.

Given that the geometry of the dendritic arbor (e.g., dendritic branching patterns, distribution, and overall shape) determines many functional properties of neurons (e.g., Grudt & Perl, 2002; Koch & Segev, 2000; Lu, Inokuchi, McLachlan, Li, & Higashi, 2001; Mainen & Sejnowksi, 1996; Rall et al., 1992), the pronounced stress-induced dendritic changes in mPFC likely result in important functional changes that may have consequences for the behaviors mediated by mPFC. To begin to examine the functional significance of chronic stress effects in mPFC, we assessed the effects of chronic restraint stress on a prefrontally mediated behavior, extinction of conditioned fear. Lesions of mPFC impair extinction learning (Morgan & LeDoux, 1995; Quirk, Russo, Barron, & Lebron, 2000); in addition, electrophysiological data have demonstrated that firing of neurons in ventral mPFC is correlated with memory for fear extinction (Milad & Quirk, 2002), and consolidation of extinction is

* Corresponding author. Fax: +1 812 855 4691.
E-mail address: wellmanc@indiana.edu (C.L. Wellman).

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impaired by blockade of protein synthesis in ventral mPFC (Santini, Ge, Ren, de Ortiz, & Quirk, 2004).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (175–200 g, 48–50 days old at initiation of experiment; Harlan, Indianapolis, IN), were individually housed in a vivarium with a 12:12 h light/dark cycle (lights on at 7 AM) and ambient temperature of 23–25 °C. To motivate rats for bar pressing, weights were gradually reduced to 85% of free-feeding weight; rats were then maintained at this weight with weekly increases allowed for normally occurring weight gain. All experimental procedures occurred between 9:30 AM and 5:00 PM. All experimental procedures were approved by the Bloomington Institutional Animal Care and Use Committee and were conducted in accordance with USPHS and NIH guidelines.

2.2. Bar press training

To obtain a baseline level of activity against which to measure freezing, rats were trained to bar press for food reinforcement (see Quirk et al., 2000). Each rat was placed in an operant chamber within a sound-attenuating cabinet (Med Associates, St. Albans, VT). The chamber contained one operant lever on either side of a food receptacle, a house light on the opposite wall, a cue light over each lever, and a floor consisting of metal rods. The house light and the cue light over the reinforced lever were illuminated throughout each session. Rats were shaped to press the left lever for a food pellet reinforcer (BioServ pellets, Holton Industries, Frenchtown, NJ); shaping lasted 1–2 sessions, after which the reinforcement schedule was gradually reduced over several days from FR-1 to VI-60. Computer-based operant software (MedPCIV; Med Associates, St. Albans, VT) controlled pellet delivery.

2.3. Restraint stress

Following the final bar press training session, rats were randomly assigned to either stressed ($n = 8$) or unstressed conditions ($n = 8$). Stressed rats were placed in a small plastic restrainer for 3 h per day for 1 week, a manipulation that produces significant increases in plasma corticosterone levels (Cook & Wellman, 2004). Because the food restriction required for maintenance of bar pressing prevented the use of weight data as a verification of the stress manipulation, a separate group of rats ($N = 8$) with ad lib access to food was restrained for 3 h per day for 1 week. Weight data from these rats were compared to that of unstressed controls ($N = 8$) using a t test.

2.4. Fear conditioning and extinction

One day after the final day of restraint, rats were placed in the operant chambers for a final session of bar press training (VI-60 schedule). During all subsequent phases of training and testing, rats were allowed to bar press for pellets on a VI-60 schedule. Fear conditioning and extinction took place over the following 2 days using a procedure similar to that of Quirk et al. (2000). On day 1, rats were placed in the operant chambers and underwent fear conditioning. After a 3-min acclimation period, rats received five habituation trials consisting of presentation of a 30-s tone (4.5-kHz, 80 db). Rats then underwent fear conditioning, consisting of seven pairings of the tone CS with a footshock US (500-ms, 0.5 mA) co-terminating with the CS. Rats were then returned to their home cages for 1 h, after which they were returned to the chambers and given extinction trials consisting of tone alone. To ensure comparable levels of extinction learning across both groups, on day 1 extinction trials continued until the rat exhibited less than 10% (3 s) freezing on four consecutive trials. The following day, rats were given another 15 extinction trials. For all phases of conditioning and extinction, variable intertrial intervals averaged 4 min, and computer-based operant software (MedPC-IV; Med Associates, St. Albans, VT) controlled the delivery of tones and shocks. For all trials, the duration of freezing (defined as the absence of any visible movements except that due to breathing) during the tone was measured with a digital stopwatch by an observer blind to experimental conditions. Percent freezing (seconds spent freezing/30 s) during habituation, fear conditioning, extinction on day 1, and extinction on day 2 was calculated and compared across groups using two-way repeated-measures ANOVAS (group × trial) followed by Bonferroni post hoc comparisons. In addition, trials to criterion on extinction days 1 and 2 were calculated and compared across groups using two-way repeated-measures ANOVAS (group × extinction session) followed by Bonferroni post hoc comparisons.

3. Results

One week of daily restraint stress significantly attenuated weight gain. By day seven of restraint, average weight of unstressed rats increased to $119.93 \pm 3.45\%$ of their starting weight, whereas in stressed rats, average weight increased to only $107.30 \pm 3.69\%$ of their starting weight ($t(14) = -7.07$, $p < .05$).

To rule out potential confounds due to differences in activity level between stressed and unstressed groups, average bar presses per min on the days immediately preceding and following chronic restraint were compared across groups using two-way repeated-measures ANOVA (Day × Group). Although there was no main effect of group on freezing ($F(1,14) = 1.11$, $p > .05$), a main effect of day was present ($F(1,14) = 50.61$, $p < .05$), with both stressed and unstressed rats pressing at a lower rate following the week of restraint. Although a significant interaction of day and group was present ($F(1,14) = 5.47$, $p < .05$), planned comparisons revealed that rate of bar pressing did not differ between unstressed and stressed rats on either the last day of bar press training (Unstressed mean $= 10.23 \pm 0.84$ presses/min, Stressed mean $= 10.03 \pm 0.90$ presses/min; Bonferroni comparison, $p > .05$) or after the final day of restraint (Unstressed mean $= 8.34 \pm 0.61$ presses/min, Stressed mean $= 6.31 \pm 0.82$ bar presses per min; Bonferroni comparison, $p > .05$). Thus, although a trend towards decreased activity in the stressed rats was present, there were no significant differences in activity level across groups.

One week of daily restraint did not significantly influence unconditioned responding to tone alone (Fig. 1A). During the habituation phase, there was no main effect of group on freezing ($F(1,56) = 1.33$, $p > .05$) and no interaction of group and trial ($F(4,56) = 0.82$, $p > .05$). Likewise, chronic restraint stress did not significantly alter acquisition of the conditioned fear response (Fig. 1B). Overall, percent freezing varied significantly across trials ($F(6,84) = 10.30$, $p < .05$), with both groups acquiring the conditioned fear response. No effect of group ($F(1,84) = 2.23$, $p > .05$) or interaction of group and trial ($F(6,84) = 0.91$, $p > .05$) was present. Thus, by the last acquisition trial, the two groups showed equivalent learning. To further explore the possibility that stressed rats may have acquired the conditioned fear response more rapidly, acquisition data were further analyzed by performing separate analyses on trials 1–4 (before unstressed rats reached