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CHRONIC STRESS ALTERS NEURAL ACTIVITY IN MEDIAL PREFRONTAL CORTEX DURING RETRIEVAL OF EXTINCTION

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Abstract—Chronic restraint stress produces morphological changes in medial prefrontal cortex and disrupts a prefrontally mediated behavior, retrieval of extinction. To assess potential physiological correlates of these alterations, we compared neural activity in infralimbic and prelimbic cortex of unstressed versus stressed rats during fear conditioning and extinction. After implantation of microwire bundles into infralimbic or prelimbic cortex, rats were either unstressed or stressed via placement in a plastic restrainer (3 h/day for 1 week). Rats then underwent fear conditioning and extinction while activity of neurons in infralimbic or prelimbic cortex was recorded. Percent freezing and neural activity were assessed during all phases of training. Chronic stress enhanced freezing during acquisition of conditioned fear, and altered both prelimbic and infralimbic activity during this phase. Stress did not alter initial extinction or conditioned stimulus (CS)-related activity during this phase. However, stress impaired retrieval of extinction assessed 24 h later, and this was accompanied by alterations in neuronal activity in both prelimbic and infralimbic cortex. In prelimbic cortex, unstressed rats showed decreased activity in response to CS presentation, whereas stressed rats showed no change. In infralimbic cortex, neurons in unstressed rats exhibited increased firing in response to the CS, whereas stressed rats showed no increase in infralimbic firing during the tone. Finally, CS-related firing in infralimbic but not prelimbic cortex was correlated with extinction retrieval. Thus, the stress-induced alteration of neuronal activity in infralimbic cortex may be responsible for the stress-induced deficit in retrieval of extinction.

Key words: stress, medial prefrontal cortex, infralimbic cortex, prelimbic cortex, extracellular recording, extinction retrieval.

Stress can precipitate or exacerbate many psychological disorders, most notably depression, schizophrenia, and posttraumatic stress disorder (e.g., Brown and Harris, 1989; Ventura et al., 1989), and can also disrupt cognitive and emotional behavior (Holmes and Wellman, 2009). Prefrontal cortex has been implicated in many stress-related disorders (Baxter et al., 1989; Drevets et al., 1992; Carter et al., 2001; Takahashi et al., 2004), is involved in many of the cognitive processes that are influenced by chronic stress (e.g., Dias et al., 1996), and is a target for the hormones involved in the stress response (Meaney and Aitken, 1985).

Chronic stress produces profound changes in the morphology of neurons in both the infralimbic and prelimbic regions of medial prefrontal cortex (mPFC) of male rats (Cook and Wellman, 2004; Izquierdo et al., 2006; Radley et al., 2006). Interestingly, dendritic morphology of mPFC appears to be exquisitely sensitive to stress: Just one week of brief daily restraint reduces mPFC apical dendritic branch number and length (Brown et al., 2005).

Exposure to chronic stress also produces deficits in retrieval of extinction of cued fear conditioning (Miracle et al., 2006; Garcia et al., 2008; Baran et al., 2009; Farrell et al., 2010), a behavior mediated by the infralimbic region of mPFC (Quirk et al., 2000). This suggests that chronic stress may compromise the function of mPFC. Indeed, acute stress produces sustained activation of infralimbic neurons (Jackson and Moghaddam, 2006) and impairs the induction of long term potentiation (LTP) in prelimbic cortex (Maroun and Richter-Levin, 2003); and chronic stress attenuates 5-HT-induced excitatory post-synaptic potentials (EPSP) in prelimbic neurons (Liu and Aghajanian, 2008) and impairs induction of LTP in infralimbic cortex (Goldwater et al., 2009). However, no studies to date have assessed the effects of chronic stress on neuronal physiology in mPFC in behaving animals. Likewise, the relationship between stress-induced changes in neuronal firing in mPFC and stress-induced changes in fear conditioning and extinction is unknown. Therefore, we assessed the effects of chronic stress on activity of mPFC neurons during fear conditioning and extinction.

Previous studies suggest different roles for prelimbic (PL) and infralimbic cortex (IL) in expression of cued fear conditioning and acquisition and retrieval of extinction. For instance, lesions of dorsal mPFC facilitate reactivity to the conditioned stimulus (CS) (Morgan and LeDoux, 1995), neurons in PL respond to the CS during fear conditioning (Baeg et al., 2001), temporary inactivation of PL disrupts the expression of conditioned fear (Corcoran and Quirk, 2007), stimulation of PL increases expression of conditioned fear and retards its extinction (Vidal-Gonzalez et al., 2006), and activity in PL is associated with increased freezing during extinction (Burgos-Robles et al., 2009). Thus, PL may contribute to expression of conditioned fear. On the other hand, IL appears to play a critical role in

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Abbreviations: AHP, afterhypolarizations; CS, conditioned stimulus; EPSP, excitatory post-synaptic potentials; IL, infralimbic cortex; LTP, long term potentiation; mPFC, medial prefrontal cortex; NMDA, N-methyl-D-aspartic acid; PL, prelimbic cortex; PSTH, peri-stimulus time histograms; US, unconditioned stimulus.

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retrieval of extinction: although there is some debate (Garcia et al., 2006), several laboratories have now shown that lesions of IL impair retrieval of extinction (Quirk et al., 2000; Baran et al., 2010; Farrell et al., 2010). Further, neuronal firing in this region increases in response to the CS during retrieval of extinction (Milad and Quirk, 2002), and stimulation of IL facilitates retrieval of extinction (Milad et al., 2004). Therefore, we assessed potential stress-induced changes in neuronal activity in each region.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (175–200 g, approximately 50 days old on arrival; Harlan, Indianapolis, IN, USA), were individually housed in a vivarium with a 12:12 h light/dark cycle (lights on at 6:30 AM) and ambient temperature of 23–25 °C. To motivate rats for bar pressing, weights were gradually reduced to 85% of free-feeding weight. As in previous studies (Quirk et al., 2000; Miracle et al., 2006; Farrell et al., 2010), rats were then maintained at this weight, with weekly increases allowing for normally occurring weight gain, throughout the duration of the experiment. All experimental procedures occurred between 7:00 AM and 5:00 PM. All experimental procedures were carried out in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and approved by the Bloomington Institutional Animal Care and Use Committee.

Bar press training

When rats had reached their target weight behavioral training and testing commenced. To obtain a baseline level of activity against which to measure freezing, rats were trained to bar press for food reinforcement (Quirk et al., 2000; Miracle et al., 2006; Farrell et al., 2010). Each rat was placed in an operant chamber within a sound-attenuating cabinet (Med Associates, St. Albans, VT, USA). The chamber contained one operant lever on the left side of a food receptacle, an illuminated cue light over the lever, and a floor consisting of metal rods. Rats were shaped to press the lever for a food pellet reinforcer (BioServ pellets, Holton Industries, Frenchtown, NJ, USA); shaping lasted one to two sessions, after which the reinforcement schedule was gradually reduced over 4 days from FR-1 to VI-60. As in previous studies (Quirk et al., 2000; Miracle et al., 2006; Farrell et al., 2010), during all subsequent phases of training and testing, rats were allowed to bar press for pellets on a VI-60 schedule. Computer-based operant software (MedPCIV; Med Associates, St. Albans, VT, USA) controlled pellet delivery.

Surgery

After bar press training was complete (at approximately 60 days of age), electrode bundles were implanted unilaterally into either PL or IL. Unilateral implantation was used to minimize electrode track damage to the dorsal mPFC, as such damage may potentiate conditioned freezing (Milad and Quirk, 2002; Milad et al., 2004). Each electrode bundle consisted of one external stainless steel groundwire plus either one, two, or four pairs of 25 μm formvar insulated, stainless steel microwires (California Fine Wire, Grover Beach, CA, USA) twisted together to form stereotrodes. All wires were friction fitted to gold pins and attached to a plastic connector (Omnetics Connector; Minneapolis, MN, USA) with epoxy adhesive. Rats were anesthetized with ketamine (74 mg/kg), xylazine (3.7 mg/kg), and acepromazine (0.74 mg/kg) and placed in a stereotaxic instrument (Kopf) with the incisor bar set so that bregma and lambda were in the same horizontal plane. The scalp was incised and retracted, a hole was drilled at 2.7 mm anterior and 0.5 mm lateral to bregma, and the underlying dura carefully retracted. Three additional holes were drilled for stainless steel anchoring screws, and the ground wire was secured to two of the anchoring screws. Electrode bundles were lowered to either 3.5 mm (PL) or 4.3 mm (IL) ventral to the skull, and cemented to the skull and anchoring screws with dental acrylic.

Restraint stress

Approximately 7 days after surgery, rats were randomly assigned to either unstressed (n=19) or stressed conditions (n=17). Stressed rats were placed in a small plastic semi-cylindrical restrainer (6.35 cm dia×15.24 cm l, modified so the tail piece locks into place; Braintree Scientific, Braintree, MA, USA) for 3 h per day for 1 week, a manipulation that produces significant increases in plasma corticosterone levels (Cook and Wellman, 2004) and has been shown to impair retrieval of extinction (Miracle et al., 2006; Farrell et al., 2010).

Fear conditioning and extinction

Within 24 h of the final day of restraint, rats were placed in the operant chamber for a final session of bar press training (VI-60 schedule) and the headstage connector was plugged into a tether for acquisition of electrophysiological data while allowing the rat to move freely. 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 a 30-s tone (4.5-kHz, 80 db). Habituation trials were included to mitigate ceiling effects (Baran et al., 2009), and because previous studies describing the role of neural activity in IL (Milad and Quirk, 2002; Milad et al., 2004; Burgos-Robles et al., 2007) and PL (Burgos-Robles et al., 2009) in fear conditioning and extinction, as well as stress effects on extinction retrieval (Miracle et al., 2006; Baran et al., 2009; Farrell et al., 2010) have utilized habituation trials. Rats then underwent fear conditioning, consisting of seven pairings of the tone CS with a footshock unconditioned stimulus (US; 500-ms, 0.5 mA) co-terminating with the tone 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. As previously described (Miracle et al., 2006), 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 (i.e., criterion). The following day, rats were given another 15 CS-alone trials to assess retrieval of extinction. For all phases of conditioning and extinction, variable intertrial intervals averaged 4 min. Computer-based operant software (Spike2; CED, London, UK) controlled the delivery of tones and shocks. For all trials, the duration of freezing (defined as the absence of any visible movement except that due to breathing) during the tone was measured with a digital stopwatch by an observer blind to experimental condition, and expressed as percent freezing (seconds spent freezing/30 s) during habituation, fear conditioning, extinction on day 1 (initial extinction), and extinction on day 2 (retrieval of extinction).

Single-unit recording

Neural activity was acquired and stored with a Power 1401 625 kHz data acquisition system and computer running Spike2 software (CED, London, UK) connected to a Lynx 8 amplifier (Neuralynx, Bozeman, MT, USA). Data were amplified (20 kHz), band-pass filtered (0.3–9 kHz), and continuously sampled (20 kHz) throughout fear conditioning and extinction for subsequent anal-