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# DIFFERENTIAL DENDRITIC REMODELING IN PRELIMBIC CORTEX OF MALE AND FEMALE RATS DURING RECOVERY FROM CHRONIC STRESS

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**Abstract—Chronic stress produces differential dendritic remodeling of pyramidal neurons in medial prefrontal cortex of male and female rats. In males, this dendritic remodeling is reversible. However, the timeline of recovery, as well as the potential for reversibility in females, is unknown. Here, we examined dendritic recovery of pyramidal neurons in layer II-II of prelimbic cortex in male and female rats following chronic restraint stress (3 h/day for 10 days). Dendritic morphology and spine density were analyzed immediately following the cessation of stress, or following a 7- or 10-day recovery period. Chronic stress produced apical dendritic retraction in males, which was coupled with a decrease in the density of stubby spine on apical dendrites. Further, following a 10-day recovery period, the morphology of neurons from stressed rats resembled that of unstressed rats. Male rats given a 7-day recovery period had apical dendritic outgrowth compared to unstressed rats. Immediately after cessation of stress, females showed only minimal dendritic remodeling. The morphology of neurons in stressed females resembled those of unstressed rats following only 7 days of recovery, at which time there was also a significant increase in stubby spine density. Males and females also showed different changes in baseline corticosterone concentrations during recovery. These findings not only indicate that dendritic remodeling in prelimbic cortex following chronic stress is different between males and females, but also suggest chronic stress induces differential hypothalamic–pituitary–adrenal axis dysregulation in males and females. These differences may have important implications for responses to subsequent stressors.** 

**Key words: prefrontal cortex, sex differences, corticosterone, dendritic morphology, spine density.**

## INTRODUCTION

Stress can disrupt a variety of cognitive and emotional behaviors (Holmes and Wellman, 2009), and can also precipitate or exacerbate several psychological disorders, including depression, posttraumatic stress disorder, and schizophrenia (Harder et al., 1980; Brown and Harris, 1989). Alterations in the structure and function of medial prefrontal cortex (mPFC) may be a key factor in the pathophysiology of many of these disorders (Harder et al., 1980; Weinberger et al., 1986; Milad et al., 2009), and mPFC modulates several behaviors that are disrupted by stress, including working memory (Hains et al., 2009; Mika et al., 2012), attentional-set shifting (Liston et al., 2006), fear conditioning (Conrad et al., 1999; Farrell et al., 2010), and the retrieval of extinction memory (Miracle et al., 2006; Wilber et al., 2011).

Further, there is evidence that the number of self-reported stressful life events is positively correlated with risk for depression (Risch et al., 2009) and PTSD (Lian et al., 2014), and alterations in prefrontal cortex volume are observed following repeated stressors, even in non-patient populations (Papagni et al., 2011). Thus, incomplete or aberrant recovery from stress may leave some individuals vulnerable to the deleterious effects of subsequent stressors.

The morphology of neurons in mPFC seems to be especially sensitive to the effects of stress. Indeed, both acute and chronic stresses profoundly alter the morphology of pyramidal neurons in the prelimbic (PL) region of the rodent mPFC. PL in rodents is structurally and functionally homologous to dorsal lateral PFC in humans (Uylings et al., 2003; Seamans et al., 2008), a region implicated in the cognitive deficits associated with stress-related psychopathologies (Sheline et al., 2010). In males, acute (Izquierdo et al., 2006), mild (Brown et al., 2005), and chronic stress (Cook and Wellman, 2004; Radley et al., 2004; Liu and Aghajanian, 2008) produce apical dendritic retraction in PL. In the case of chronic stress, this retraction is coupled with a decrease in spine density (Radley et al., 2006; Radley et al., 2008), whereas an increase in spine density is found following acute stress (Nava et al., 2015). These changes in dendritic morphology likely have important implications for neuronal function in PL, and therefore may contribute to stress-induced behavioral alterations.

Although corticolimbic morphology can undergo rapid and robust changes in response to stress, these changes are reversible in males. For example, chronic stress-induced dendritic remodeling in PL of males is

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*Abbreviations:* 0-d Rec, chronic stress plus no recovery period; 10-d Rec, chronic stress plus a 10-day recovery period; 7-d Rec, chronic stress plus a 7-day recovery period; ACTH, adrenocorticotropic hormone; CORT, corticosterone; CRH, corticotropin-releasing hormone; HPA, hypothalamicpituitary–adrenal; mPFC, medial prefrontal cortex; PL, prelimbic cortex; PTSD, posttraumatic stress disorder.

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reversible, with dendritic length resembling that of unstressed rats by 21 days after the cessation of stress (Radley et al., 2005; Bloss et al., 2010). There is also evidence that a shorter length of recovery time may be sufficient. For example, hippocampal neurons of male rats also undergo dendritic retraction following chronic stress, but following 10 days of recovery post-stress, this retraction is ameliorated (Conrad et al., 1999). Therefore, chronic stress-induced changes in mPFC neurons may be reversible in a shorter time than has previously been shown.

The prevalence of stress-linked disorders differs between men and women, with women being twice as likely to develop major depression and posttraumatic stress disorder as men (Solomon and Herman, 2009). Given this difference in susceptibility, it is unsurprising that stress can have divergent effects on behaviors modulated by mPFC, as well as dendritic morphology in males and females. For example, while chronic stress disrupts temporal order recognition memory in males, females show no deficits (Wei et al., 2014). Additionally, whereas acquisition of conditioned fear is enhanced in male rats following chronic stress (Conrad et al., 1999; Farrell et al., 2010), there is evidence that females show impairment in fear acquisition following stress (Baran et al., 2009). Further, in contrast to the dendritic retraction following chronic stress observed in males, females show apical dendritic outgrowth in PL (Garrett and Wellman, 2009). The potential for reversibility in females has yet to be examined. Therefore, to further characterize the process of dendritic recovery in mPFC, we assessed dendritic morphology in PL of male and female rats immediately following chronic restraint stress, as well as after 7 and 10 days of post-stress "recovery." We focused on PL in the present study, as the majority of studies examining stress effects on dendritic morphology have focused on this region of mPFC, where chronic stress-induced dendritic retraction has been robustly demonstrated in males (e.g., Cook and Wellman, 2004; Radley et al., 2005; Garrett and Wellman, 2009).

## EXPERIMENTAL PROCEDURES

### Subjects and stressors

Male and female Sprague–Dawley rats (approximately 68 days of age at start; Harlan, Indianapolis, IN; N = 73) were group-housed (3 per cage) in standard laboratory cages (48 cm × 20 cm × 26 cm), with ambient temperature 23–25 °C, free access to food and water, and a 12:12 light/dark cycle (lights on at 0800 h). Rats were either left unstressed or subjected to chronic restraint stress for 10 days, and were given a recovery period of 0, 7, or 10 days, resulting in 8 groups (Fig. 1A): unstressed males (n = 11) and females (n = 12), 0-d Rec males (n = 8) and females (n = 8), 7-d Rec males (n = 7) and females (n = 9), and 10-d Rec males (n = 9) and females (n = 9). All rats were weighed daily throughout the stress procedure. Immediately after weighing, unstressed rats were returned to their home cages and left undisturbed for 3 h in a separate room. Stressed rats were placed in semi-cylindrical restrainers (6.35 cm diameter × 15.24 cm length, modified so the tail piece locks into place; Braintree Scientific) for 3 h in their home cages, a manipulation that produces significant increases in plasma corticosterone levels (Cook and Wellman, 2004). All rats within a cage were assigned to the same experimental group, and all stressed rats underwent restraint simultaneously. Rats were left undisturbed during the recovery period. All procedures were conducted between 8:00 am and 8:00 pm (i.e., during the light phase), were in accordance with NIH Guidelines, and were approved by the Bloomington Animal Care and Use Committee.

### Estrous phase characterization

On the day of perfusion, vaginal lavages were performed and exfoliate cytology was examined immediately under light microscopy. Estrous phase was determined based on the morphology of cells present (Garrett and Wellman, 2009). Due to the small number of rats in proestrus (n = 3) and estrus (n = 2) compared to diestrus (n = 33), we did not analyze our data relative to estrous phase.

### Corticosterone EIA

Immediately prior to perfusion, blood was collected via cardiac puncture and allowed to clot at room temperature for 30 min followed by centrifugation at 13,000 rpm for 5 min to obtain serum. Corticosterone was measured via a commercially available EIA kit (Enzo Life Sciences, Plymouth Meeting, PA) that shows low crossreactivity with other major steroid hormones. Samples were diluted (1:20) with assay buffer and run in duplicates according to instructions provided by the manufacturer. The sensitivity of the assay was 27 pg/mL, and intra-assay variation was 1.77% and 2.62% for each plate.

### Histology and dendritic analysis

Brains were processed using a modification of Glaser and van der Loos' Golgi stain, as described previously (Glaser and Van der Loos, 1981; Martin and Wellman, 2011). On the final day of either stress or recovery, rats were deeply anesthetized with urethane and transcardially perfused with saline. To verify the stress manipulation, adrenal glands were removed and weighed. Brains were removed and immersed in Golgi-Cox solution for 14 days and then moved to 30% sucrose in saline (Gibb and Kolb, 1998). Brains were sectioned at 200 μm on a vibratome (Campden Instruments, MA752). Sections were mounted, alkalinized, developed in Dektol (Kodak), fixed in Ilford rapid fixer, dehydrated in a graded series of ethanols, cleared in xylenes, and coverslipped (Wellman, 2016).

Pyramidal neurons in layer II-III of prelimbic cortex were reconstructed (Fig. 1B). Prelimbic cortex is readily identified by its position on the medial wall of the rostral cortex, and its location dorsal to infralimbic cortex, which is markedly thinner and has fewer, less well-defined layers (Zilles and Wree, 1995), and ventral to



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