NEUROSCIENCE
RESEARCH ARTICLE
H. R. McAlinn et al. / Neuroscience 383 (2018) 98–113

# Sex Differences in the Subcellular Distribution of Corticotropin-Releasing Factor Receptor 1 in the Rat Hippocampus following Chronic Immobilization Stress

Helena R. McAlinn,[a] Batsheva Reich,[a] Natalina H. Contoreggi,[a] Renata Poulton Kamakura,[a] Andreina G. Dyer,[a] Bruce S. McEwen,[b] Elizabeth M. Waters[b] and Teresa A. Milner[a,b]*

[a] Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, New York, NY, USA

[b] Harold and Margaret Milliken Hatch Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY, USA

**Abstract—Corticotropin-releasing factor receptors (CRFR1) contribute to stress-induced adaptations in hippocampal structure and function that can affect learning and memory processes. Our prior studies showed that female rats with elevated estrogens compared to males have more plasmalemmal CRFR1 in CA1 pyramidal cells, suggesting a greater sensitivity to stress. Here, we examined the distribution of hippocampal CRFR1 following chronic immobilization stress (CIS) in female and male rats using immuno-electron microscopy. Without stress, total CRFR1 dendritic levels were higher in females in CA1 and in males in the hilus; moreover, plasmalemmal CRFR1 was elevated in pyramidal cell dendrites in CA1 in females and in CA3 in males. Following CIS, near-plasmalemmal CRFR1 increased in CA1 pyramidal cell dendrites in males but not to levels of control or CIS females. In CA3 and the hilus, CIS decreased cytoplasmic and total CRFR1 in dendrites in males only. These results suggest that in naive rats, CRF could induce a greater activation of CA1 pyramidal cells in females than males. Moreover, after CIS, which leads to even greater sex differences in CRFR1 by trafficking it to different subcellular compartments, CRF could enhance activation of CA1 pyramidal cells in males but to a lesser extent than either unstressed or CIS females. Additionally, CA3 pyramidal cells and inhibitory interneurons in males have heightened sensitivity to CRF, regardless of stress state. These sex differences in CRFR1 distribution and trafficking in the hippocampus may contribute to reported sex differences in hippocampus-dependent learning processes in baseline conditions and following chronic stress.** 

**Key words: electron microscopy, CA1/CA3 pyramidal cells, dentate gyrus, GABAergic interneurons, learning and memory, addiction.**

## INTRODUCTION

Drug addiction is a learning process that reinforces associations of drug use with reward and environmental cues with drug access (O'Brien et al., 1998; Crombag et al., 2008). Clinical studies in humans as well as in animal models found that females express higher sensitivity to craving and rates of relapse than males (Elman et al., 2001). Women are more susceptible to several aspects of addiction than men, including relapse due to stressful events or depression (Becker et al., 2017). The hippocampus is critically involved in 'cue' and 'context' learning important for drug craving and relapse (Hyman and Malenka, 2001; Nestler, 2002; Volkow et al., 2006). Following chronic stress, male rodents have impaired spatial learning and memory (McEwen, 1999; Sousa et al., 2000; Luine et al., 2007; McEwen and Milner, 2007) and undergo morphological changes and dendritic retraction in CA3 pyramidal cell dendrites (McEwen et al., 2016), suggesting that adaptive mechanisms of the hippocampus to chronic stress differ in females and males.

Evidence from other brain regions suggests that activation of corticotropin-releasing factor (CRF) can enhance the acute effects of drugs of abuse and potentiate neuroplasticity induced following drug withdrawal (Haass-Koffler and Bartlett, 2012). Additionally, CRF has been extensively implicated in drug relapse following extended periods of abstinence (Brown et al., 2009; Shalev et al., 2010; Logrip et al., 2011). In the adult

*Correspondence to: T. A. Milner, Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, 407 East 61st Street, RM 307, New York, NY 10065, USA. Fax: +1 (646)-962-0535.
E-mail address: tmilner@med.cornell.edu (T. A. Milner).

*Abbreviations:* AIS, acute immobilization stress; BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; CIS, chronic immobilization stress; CRF, corticotropin-releasing factor; CRFR1, corticotropin-releasing factor receptor 1; DG, dentate gyrus; DOR, delta opioid receptor; EM, electron microscopic; HPA, hypothalamic-pituitary–adrenal; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; NPY, neuropeptide Y; PB, phosphate buffer; PBS, phosphate-buffered saline; PKC, protein kinase C; SIG, silver-intensified immunogold; SOM, somatostatin; TS, tris-buffered saline.

https://doi.org/10.1016/j.neuroscience.2018.05.007
0306-4522/

rodent hippocampus, endogenous sources of CRF originate from local GABAergic interneurons, especially those containing parvalbumin and somatostatin (SOM) (Yan et al., 1998; Williams and Milner, 2011). Moreover, CRF receptor type 1 (CRFR1) is prominently located on pyramidal neurons and in GABAergic interneurons (Williams et al., 2011a; Chen et al., 2012; Tan et al., 2017). In response to short-term (minutes) stimulation, CRF released in the hippocampus excites synapses and enhances synaptic efficacy (Wang et al., 1998, 2000; Chen et al., 2012).

Stress initiates a cascade of events in the hypothalamic–pituitary–adrenal (HPA) axis that leads to the release of glucocorticoids from the adrenal cortex (Smith and Vale, 2006). Depending on duration, stress can have different effects on CRF-mediated hippocampal neuroplasticity processes important for learning and memory (Smith and Vale, 2006; Regev and Baram, 2014). In response to high-frequency stimulation (e.g., stress), CRF contained in dense-core vesicles (Williams and Milner, 2011) is released from axon terminals (Chen et al., 2012) and, like other peptides, can act in a paracrine manner on CRFRs on neighboring cells (Thureson-Klein and Klein, 1990; Herkenham, 1991). Following 1 h of acute immobilization stress (AIS), CRF released from male mouse hippocampal nerve terminals can mediate the persistence of long-term potentiation (LTP) population spikes which are essential for enhanced context-dependent fear learning (Blank et al., 2002, 2003). In contrast, early life stress or chronic social stress can result in hippocampus-dependent memory deficits that are ameliorated in CRFR1 forebrain knockout mice (Wang et al., 2011a,b).

Our previous studies demonstrate that CRF and opioid systems in the hippocampus are closely linked and sex, as well as the hormonal milieu, can alter this relationship. In particular, CRF and delta opioid receptor (DOR) immunoreactivities colocalize in interneurons throughout the rat hippocampus, and proestrus/estrus (high estrogen) females have fewer CRF/DOR interneurons in the hilus of the dentate gyrus (DG) compared to males (Williams and Milner, 2011). However, proestrus female rats compared to males have greater numbers of terminals containing CRF alone in the DG (Williams and Milner, 2011). Moreover, our previous electron microscopic (EM) studies in rats demonstrated that although proestrus females and males had comparable levels of CRFR1 in DOR-containing CA1 pyramidal cell dendrites, proestrus females had increased density of CRFR1 on the plasma membrane (Williams et al., 2011a). Together, these results suggest that females could have elevated sensitivity to CRF at baseline states. However, a systematic evaluation of the subcellular distribution of CRFR1 in the hippocampus of females and males comparing different subregions is lacking.

Additionally, our recent EM studies found that chronic immobilization stress (CIS) affects the subcellular distribution of DORs within CA3 pyramidal cells and DG hilar interneurons in a way that could promote excitation and learning processes in females but not males (Mazid et al., 2016). However, whether CIS alters the subcellular distribution of hippocampal CRFR1 in female and male rats is unknown. Thus, this study used immunoEM to examine sex differences in the subcellular distribution of CRFR1 in CA1 and CA3 pyramidal cells as well as in DG interneurons at baseline states and following CIS.

## EXPERIMENTAL PROCEDURES

### Animals

Male and female Sprague–Dawley rats ($N = 24$) from Charles River Laboratories (Wilmington, MA; https://www.criver.com/products-services/find-model/sas-sd?region=3611) were 2–3 months old upon arrival (males weighed 275–325 g and females weighed 225–250 g). Animals were pair-housed in cages with a 12-h light/dark cycle (lights on 0600–1800) and *ad libitum* access to water and food. The rats used in this study were the same as those used in our previous studies (Milner et al., 2013; Pierce et al., 2014; Mazid et al., 2016). All procedures were approved by the Rockefeller University and Weill Cornell Medicine Institutional Animal Care and Use Committees and were in accordance with the 2011 Eighth edition of the National Institutes of Health guidelines for the Care and Use of Laboratory Animals.

### Estrous cycle determination

The study included only female rats that had two consecutive, regular, 4–5 day estrous cycles. One week after the rats arrived and were acclimated, estrous cycle stage was determined using vaginal smear cytology (Turner and Bagnara, 1971). Mock estrous cycling on male rats was performed at the same time to control for handling differences. Estrous cycle stage was verified by uterine weight and radioimmunoassay of plasma serum estradiol levels from blood samples from the heart directly before the perfusion procedure. The females used in this study were all diestrus II, the stage in which estrogens and progestins are lowest. This stage was chosen so that we could make direct comparisons with our previous studies examining the effect of CIS on DOR trafficking in the hippocampus (Mazid et al., 2016).

### Chronic immobilization stress

Rats were randomly assigned to the unstressed control or CIS experimental groups. To minimize unwanted stress on the control rats, they were housed in a neighboring room to the CIS rats. All CIS procedures were performed between 9:00 a.m. and 1:00 p.m. daily. Rats were subjected to CIS for 10 consecutive days (Lucas et al., 2007; Shansky et al., 2010). For this, rats were placed in plastic cone-shaped polyethylene bags with a small apical hole and a Kotex mini-pad underneath them for urine collection. The rats were placed with their nose at the hole of the bag, sealed in with tape and left for 30 min undisturbed. The rats were anesthetized and perfused 1 day after the final CIS session. Control rats were