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Sex differences in subcellular distribution of delta opioid receptors in the rat hippocampus in response to acute and chronic stress

Sanoara Mazid [a], Baila S. Hall [a, b], Shannon C. Odell [a, b], Khalifa Stafford [a], Andreina D. Dyer [a], Tracey A. Van Kempen [a, b], Jane Selegean [a], Bruce S. McEwen [c], Elizabeth M. Waters [c], Teresa A. Milner [a, b, c, *]

[a] Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, 407 East 61st Street, New York, NY 10065, United States
[b] Weill Cornell Graduate School of Medical Sciences, Weill Cornell Medicine, 1300 York Ave, New York, NY 10021, United States
[c] Harold and Margaret Milliken Hatch Laboratory of Neuroendocrinology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, United States

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ABSTRACT

Drug addiction requires associative learning processes that critically involve hippocampal circuits, including the opioid system. We recently found that acute and chronic stress, important regulators of addictive processes, affect hippocampal opioid levels and mu opioid receptor trafficking in a sexually dimorphic manner. Here, we examined whether acute and chronic stress similarly alters the levels and trafficking of hippocampal delta opioid receptors (DORs). Immediately after acute immobilization stress (AIS) or one-day after chronic immobilization stress (CIS), the brains of adult female and male rats were perfusion-fixed with aldehydes. The CA3b region and the dentate hilus of the dorsal hippocampus were quantitatively analyzed by light microscopy using DOR immunoperoxidase or dual label electron microscopy for DOR using silver intensified immunogold particles (SIG) and GABA using immunoperoxidase. At baseline, females compared to males had more DORs near the plasmalemma of pyramidal cell dendrites and about 3 times more DOR-labeled CA3 dendritic spines contacted by mossy fibers. In AIS females, near-plasmalemmal DOR-SIGs decreased in GABAergic hilar dendrites. However, in AIS males, near-plasmalemmal DOR-SIGs increased in CA3 pyramidal cell and hilar GABAergic dendrites and the percentage of CA3 dendritic spines contacted by mossy fibers increased to about half that seen in unstressed females. Conversely, after CIS, near-plasmalemmal DOR-SIGs increased in hilar GABA-labeled dendrites of females whereas in males plasmalemmal DOR-SIGs decreased in CA3 pyramidal cell dendrites and near-plasmalemmal DOR-SIGs decreased hilar GABA-labeled dendrites. As CIS in females, but not males, redistributed DOR-SIGs near the plasmalemmal of hilar GABAergic dendrites, a subsequent experiment examined the acute affect of oxycodone on the redistribution of DOR-SIGs in a separate cohort of CIS females. Plasmalemmal DOR-SIGs were significantly elevated on hilar interneuron dendrites one-hour after oxycodone (3 mg/kg, I.P.) administration compared to saline administration in CIS females. These data indicate that DORs redistribute within CA3 pyramidal cells and dentate hilar GABAergic interneurons in a sexually dimorphic manner that would promote activation and drug related learning in males after AIS and in females after CIS.

1. Introduction

Despite lower rates of drug use and abuse, women are more susceptible to several aspects of drug addiction than men. In particular, women often experience an accelerated course to addiction, shorter-drug free periods and higher levels of craving, and are more likely to relapse due to stressful events or depression (Becker and Hu, 2008; Becker et al., 2007; Fiorentine et al., 1997; Weiss et al., 1997). These processes require associative memory

* Corresponding author. Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, 407 East 61st Street, RM 307, New York, NY 10065, United States.
E-mail addresses: Smsanoarabio@gmail.com (S. Mazid), bah2012@med.cornell.edu (B.S. Hall), sho2004@med.cornell.edu (S.C. Odell), khalifa.stafford89@myhunter.cuny.edu (K. Stafford), adellgunz@gmail.com (A.D. Dyer), tvkempen@gmail.com (T.A. Van Kempen), jselegean@gmail.com (J. Selegean), mcewen@mail.rockefeller.edu (B.S. McEwen), elizabethwaters@mac.com (E.M. Waters), tmilner@med.cornell.edu (T.A. Milner).

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Abbreviations

ABC	avidin-biotin complex
AIS	acute immobilization stress
BSA	bovine serum albumin
CIS	chronic immobilization stress
CRF	corticotrophin releasing factor
CRF1	corticotrophin releasing factor receptor 1
DAB	diaminobenzidine
DE	diestrus phase
DOR	delta opioid receptor
E	estrus phase
EM	electron microscopy
GABA	gamma aminobutyric acid
IgG	immunoglobulin
ir	immunoreactivity
LM	light microscopy
LTP	long-term potentiation
NPY	neuropeptide Y
PE	proestrus phase
PARV	parvalbumin
PB	phosphate buffer
PBS	phosphate-buffered saline
ROI	region of interest
SIG	silver-intensified immunogold
SOM	somatostatin
TS	tris-buffered saline

and motivational incentives (Koob and Volkow, 2010) that critically involve hippocampal output relayed directly or indirectly to the mesolimbic reward system (Luo et al., 2011; Vorel et al., 2001). This system is a predominant direct target of abused drugs, including opioid receptor agonists, such as the prescription medication oxycodone. Intrinsic hippocampal circuitry supports spatial and episodic memory acquisition processes essential for associating a drug of abuse with a particular place and set of events (i.e., drug-related learning) (Berke and Hyman, 2000; Kilts et al., 2001; Risinger and Oakes, 1995; Volkow et al., 2006). Notably, the opioid system in the CA3 region has been implicated in visual-spatial pattern completion (Kesner and Warthen, 2010), an important component of context associative learning.

Within the hippocampus, the opioid peptide, enkephalin, is contained in the mossy fiber pathway and lateral perforant path (Drake et al., 2007). Enkephalins as well as exogenous opiates (e.g., morphine) predominantly affect excitability and long-term potentiation (LTP) of CA3 pyramidal cells indirectly via activation of μ opioid receptors (MORs) and δ opioid receptors (DORs) which result in inhibition of inhibitory GABAergic interneurons (i.e., disinhibition) (Commons and Milner, 1995; Derrick et al., 1992; Drake et al., 2007; Witter, 1993; Xie and Lewis, 1991). Additionally, enkephalins and exogenous opiates can directly inhibit DORs present on CA3 pyramidal cells (Bao et al., 2007). Our light and electron microscopic studies have demonstrated sex differences in the hippocampal opioid system. Notably, at elevated estrogen states, compared to low estrogen states and males, enkephalins, MORs, and DORs are subcellularly positioned to enhance excitability and learning processes (McEwen and Milner, 2017; Torres-Reveron et al., 2008, 2009; Williams et al., 2011b). Moreover, females in high estrogen states compared to males have a lower baseline transmission in the mossy fiber-CA3 pathway that is regulated by MORs and, unlike males, exhibit a LTP evoked by low frequency stimulation of the mossy fibers that is regulated by DORs.

Drug addiction, particularly relapse, is often provoked by stress (reviewed by (Bruchas et al., 2008; Shalev et al., 2000)). Stress has powerful influences on the addictive processes in both males and females (Koob, 2008). However, females have a heightened sensitivity to stress (Becker et al., 2007) and enhanced cognitive performance following chronic stress (Luine et al., 2007) that may contribute to their accelerated course of addiction, particularly to opioid analgesics (Elman et al., 2001; Hu et al., 2004; Lynch et al., 2000; Robbins et al., 1999). Our recent anatomical studies have shown notable sex differences in the hippocampal opioid system in response to acute and chronic immobilization stress (AIS and CIS, respectively). In particular, our studies suggest that the opioid system of females, regardless of estrogen state, is "primed" for even greater excitation of CA3 pyramidal cells after CIS. After CIS females do not display the atrophy of CA3 pyramidal cell dendrites and the loss of parvalbumin (PARV)-containing GABA interneurons seen in males (McEwen, 1999; Milner et al., 2013; Vyas et al., 2002). Instead, in CIS females, enkephalin levels in mossy fibers are elevated and the subcellular distribution of MORs in hippocampal PARV interneurons resembles that seen in unstressed females at high estrogen states (Milner et al., 2013; Pierce et al., 2014). However, whether DORs also redistribute differently within hippocampal neurons in females and males following AIS and CIS is not known.

Previous light and electron microscopic studies suggest that stress could impact the levels and subcellular distribution of hippocampal DORs in a sexually dimorphic manner (Williams et al., 2011a, 2011b; Williams and Milner, 2011). DOR-immunoreactivity (DOR-ir) is colocalized in neurons containing the stress neurohormone corticotrophin releasing factor (CRF) and its receptor (Williams et al., 2011a; Williams and Milner, 2011). Our electron microscopic studies have demonstrated that females in high estrogen states exhibit internalization and trafficking of DORs towards the soma of CA1 pyramidal cells; this is not seen in females in low estrogen states in males (Williams et al., 2011b). Moreover, females at high estrogen states have elevated levels of phosphorylated DORs in CA2/3 pyramidal cells compared to females at low estrogen states and males, which then is reduced following AIS (Burstein et al., 2013). As DOR phosphorylation parallels the uncoupling and internalization of DORs (Pradhan et al., 2009), this suggests that CA2/3 neurons in females at high estrogen states may be more sensitive to opioid agonists.

Thus, this study sought to determine the levels and trafficking of DORs in the hippocampus following stress, and further if this differs in females and males. For this, dual labeling electron microscopic immunocytochemistry examined the distribution of DORs in CA3 pyramidal cells and GABAergic interneurons in the dentate hilus after AIS and CIS.

2. Materials and methods

2.1. Animals

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. Adult male (~275–325 gm) and female