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Chronic Methamphetamine Exposure Attenuates Neural Activation in Hypothalamic–Pituitary–Adrenal Axis-Associated Brain Regions in a Sex-specific Manner

Jason S. Jacobskind,[a] Zachary J. Rosinger,[a] Tiffany Gonzalez,[a] Kristen L. Zuloaga[b] and Damian G. Zuloaga[a]*

[a] *University at Albany, Department of Psychology, Albany, NY 12222, United States*

[b] *Albany Medical College, Department of Neuroscience & Experimental Therapeutics, Albany, NY 12208, United States*

Abstract—Sex differences in methamphetamine (MA) abuse and consequences of MA have been reported with females showing an increased addiction phenotype and withdrawal symptoms. One mechanism through which these effects might occur is via sex-specific alterations in the hypothalamic–pituitary–adrenal (HPA) axis and its associated brain regions. In this study, mice were administered MA (5 mg/kg) or saline for 10 consecutive days. During early withdrawal, anxiety-like behaviors were assessed in the open field, light/dark box, and elevated plus maze. At ten days of withdrawal, mice were injected with a final dose of MA (5 mg/kg) or saline. Chronic MA did not alter anxiety-like behaviors or corticosterone responses to a final dose of MA, although females showed elevated corticosterone responses compared to males. Chronic MA attenuated final MA-induced c-Fos in both sexes in the paraventricular hypothalamus (PVH), bed nucleus of the stria terminalis (BNST), cingulate cortex, central and basolateral amygdala. In CA1 and CA3 hippocampal areas, c-Fos attenuation by chronic MA occurred only in females. Within the PVH, final MA injection increased c-Fos to a greater extent in females compared to males regardless of prior MA exposure. Dual-labeling of c-Fos with glucocorticoid receptor revealed a specific attenuation of neural activation within this cell type in the PVH, central and basolateral amygdala, and BNST. Together these findings demonstrate that chronic MA can suppress subsequent activation of HPA axis-associated brain regions and cell phenotypes. Further, in select regions this reduction is sex-specific. These changes may contribute to reported sex differences in MA abuse patterns.

Key words: HPA axis, sex difference, methamphetamine, anxiety, immunohistochemistry.

INTRODUCTION

Methamphetamine (MA) is a highly addictive substance and is the most abused psychostimulant across the globe (Chomchai and Chomchai, 2015). The United Nations Office on Drug and Crime reported that from 2012 to 2015, prevalence rates of MA abuse among the United States' general population aged 15–64 increased from 0.5% to 0.8% (UNODC, 2017).

Individuals that chronically abuse MA experience a host of physiological and psychological effects including changes in mood behaviors such as anxiety and depression (London et al., 2004; Schep et al., 2010; Li et al., 2013).

MA has been shown to potently activate the hypothalamic–pituitary–adrenal (HPA) axis (Williams et al., 2000; Acevedo et al., 2008; Zhu et al., 2010; Tomita et al., 2013; Zuloaga et al., 2014, 2016), a neuroendocrine system associated with disorders of anxiety and depression (Naughton et al., 2014). Both psychological stressors and MA stimulate the release of corticotropin-releasing factor (CRF) from the paraventricular nucleus of the hypothalamus (PVH) into the anterior pituitary gland. CRF, acting in conjunction with arginine vasopressin, stimulates the production and subsequent secretion of adrenocorticotropic hormone (ACTH) into the bloodstream. This signals adrenal release of glucocorticoids (cortisol in humans, corticosterone in rodents), which circulate throughout the body to regulate numerous functions including the "fight or flight response". In regions of the brain including the hippocampus, hypothalamus, and cerebral cortex, the binding of glucocorticoids to the glucocorticoid receptor (GR) induces negative feedback on the HPA axis, halting further secretion of CRF and

*Corresponding author. Address: Dept. of Psychology, University at Albany, 1400 Washington Ave, Albany, NY 12222, United States. Fax: +1-518-442-4867.
E-mail address: dzuloaga@albany.edu (D. G. Zuloaga).

Abbreviations: ACTH, adrenocorticotropic hormone; BNST, bed nucleus of the stria terminalis; Cart, cocaine and amphetamine related transcript; CRF, corticotropin-releasing factor; GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenal axis; MA, methamphetamine; PVH, hypothalamus; PVH, paraventricular hypothalamus.

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ACTH (Herman et al., 2012, 2016). Although stressors and MA both activate the HPA axis, their effects are not equal. In rats, exposure to psychological stressors increased blood corticosterone levels to a lesser extent than did a moderate dose of MA, suggesting that MA is a more potent stimulator of glucocorticoid release (Grace et al., 2008).

Repeated elevations in glucocorticoids can have deleterious effects on the brain, and have been shown to produce lasting changes in basal and stress induced function of brain regions associated with the HPA axis (Girotti et al., 2006; Santos et al., 2014). Increased cell death (Zuloaga et al., 2011), decreased cell proliferation (Kim et al., 2004), and dendritic remodeling (Kim et al., 2014) have been observed following exposure to high levels of glucocorticoids. In regions contributing to HPA axis function, these adverse effects of chronic glucocorticoid exposure are manifested as behavioral and cognitive deficits such as major depression disorder, anxiety, addiction, and impaired learning and memory (Raber, 1998; Shoener et al., 2006; Fernández-Guasti et al., 2012; Nawata et al., 2012; van Boxelaere et al., 2017). Humans with a history of chronic MA abuse display dysregulated levels of glucocorticoids and ACTH during abstinence (Carson et al., 2012; Li et al., 2013). In addition, chronic MA abuse is associated with lasting alterations in mood, including elevated anxiety and depression (London et al., 2004; Li et al., 2013; Jang et al., 2013). These findings have been further substantiated in rodent models using behavioral tests of anxiety (Nawata et al., 2012; Georgiou et al., 2016). Importantly, elevations in anxiety and depression are key contributors to drug relapse (Koob et al., 2014). MA has also been shown to induce lasting alterations in HPA axis/stress associated with the HPA axis including CRF, arginine vasopressin, and GR (Lowy and Novotney, 1994; Nawata et al., 2012; Zuloaga et al., 2013, 2015; Cadet et al., 2014; Georgiou et al., 2016). Our recent studies have further demonstrated that compared to mice that received a single dose of MA, adult male mice exposed to repeated doses of MA had reduced neuronal activation in brain regions that regulate the HPA axis (Zuloaga et al., 2016). These changes may underlie development of anxiety/depressive symptoms reported during MA withdrawal (Zuloaga et al., 2015).

Several studies have found sex differences in patterns of MA abuse in both humans and rodents. Women begin using MA at an earlier age than men (Dluzen and Liu, 2008) and have a shorter duration from first use to regular use (Brecht et al., 2004). Furthermore, women are more dependent on and committed to MA, whereas men tend to use other drugs when MA is unavailable (Brecht et al., 2004). Women have also been reported to show greater overall withdrawal symptoms including elevated anxiety (Su et al., 2017; Rungnirundorn et al., 2016). In rats, females exhibit greater MA seeking and reinstatement following a period of withdrawal (Reichel et al., 2012). In response to acute MA, corticosterone levels in females are also elevated to a greater extent than in males (Zuloaga et al., 2014; Jacobskind et al., 2017). These elevations over a prolonged period of time may exacerbate negative effects of MA in females and contribute to their greater MA addiction phenotype (Zuloaga et al., 2015).

In the present study we hypothesized that in response to chronic exposure to MA, males and females would exhibit differential alterations in anxiety-like behaviors during withdrawal, as well as differences in neural activation patterns within HPA axis-associated brain regions. This knowledge will be critical for understanding the neural mechanisms that underlie sexually dimorphic effects of MA and patterns of abuse.

EXPERIMENTAL PROCEDURES

Animals

C57BL/6J male and female mice (N = 68, 34/sex) were purchased from Jax Laboratories (Bar Harbor, Maine), and were maintained on a 12/12 light/dark cycle with lights on at 7:00 am. Rodent chow and water were available *ad libitum*. Mice were singly housed for at least one week prior to testing and randomly assigned to treatment groups. At the start of testing, animals were 8–9 weeks of age. All experimental procedures were carried out between 8:00 am and 12:00 pm. Procedures were approved by the University at Albany Institutional Animal Care and Use Committee and were in accordance with National Institutes of Health guidelines.

Drug administration

Singly housed mice were given a once daily i.p. injection of vehicle (saline) or 5 mg/kg d-Methamphetamine (weight of salt, Sigma Chemical Co., St Louis, MO, USA) dissolved in saline for 10 consecutive days. Injection volume varied between 80 and 130 µl depending on the weight of the mouse. This dosing regimen was chosen based on our previous report indicating it is effective in attenuating c-Fos in select brain regions in males (Zuloaga et al., 2016). This treatment paradigm has not been shown to induce locomotor sensitization in mice.

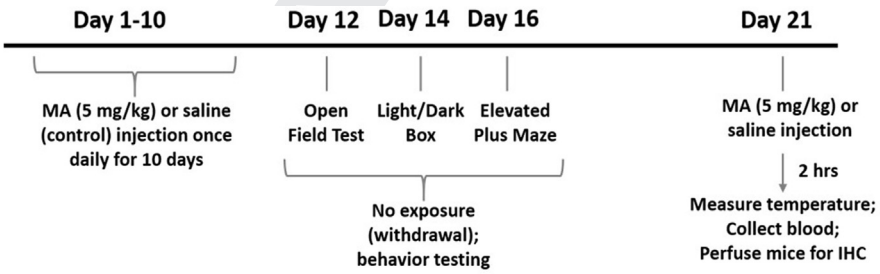

Fig. 1. Experimental timeline.

Behavior testing

To study lasting effects of chronic MA exposure, animals (N = 16/sex for