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Research Report

Sex-specific 24-h profile of extracellular serotonin levels in the medial prefrontal cortex

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ABSTRACT

The medial prefrontal cortex (mPFC) controls emotional responses in many species, receiving serotonergic innervation from the dorsal and median raphe nucleus (DRN and MRN). To examine the sex difference in 24-h profiles of extracellular serotonin (5HT) levels in the mPFC, an in vivo microdialysis study was performed using intact male, diestrous female, and proestrous female rats. Dialysates were automatically collected by a microdialysis probe from the mPFC every 30 min for more than 24 h under freely moving conditions. The levels of 5HT in dialysates were quantified by high performance liquid chromatography. Extracellular 5HT levels exhibited episodic changes in the mPFC of both sexes of rats, with both diestrous and proestrous females exhibiting a clear diurnal change; the 5HT levels were high during the dark phase, but low during the light phase. In contrast, male rats exhibited relatively high 5HT levels throughout the day without significant diurnal changes. At mathematically analyzed trough, males showed higher 5HT levels than diestrous or proestrous females. The overall 24-h 5HT levels in males were significantly greater than proestrous females, but were not different from diestrous females. Further, stereological methods were used to examine the number of tryptophan hydroxylase (TrpH), but no sex differences in the number of TrpH immunoreactive cells in the DRN and MRN were observed. These results suggest that sex and/or the gonadal steroid environment may affect the 24-h profile of extracellular 5HT in the mPFC of rats without changes in the number of 5HT neurons in the DRN and MRN.

1. Introduction

The medial prefrontal cortex (mPFC) plays a pivotal role in emotional responses (Damasio, 1997; Uylings et al., 2003) which is characterized by similar connections in rats and humans (Öngür and Price, 2000; Quirk and Beer, 2006). Neurological patients with damage to the mPFC have impaired emotional responses (Damasio et al., 1994; Rolls et al., 1994). In nonhuman animals, lesions or inactivation of the mPFC facilitates affective behaviors, whereas stimulation of the mPFC suppresses them (Zbrozyna and Westwood, 1991; Morgan and LeDoux, 1995; Vouimba et al., 2000; Gerrits et al., 2003; Vidal-Gonzalez et al., 2006).

Serotonin (5HT) in the central nervous system is a powerful modulator of emotional processes. A large body of evidence implicates serotonergic neurotransmission dysfunction in the

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Abbreviations: ANOVA, analysis of variance; BLA, basolateral amygdala; DRN, dorsal raphe nucleus; mPFC, medial prefrontal cortex; MRN, median raphe nucleus; PBS, phosphate buffered saline; SSRI, selective serotonin reuptake inhibitors; 5HT, serotonin; TrpH, tryptophan hydroxylase

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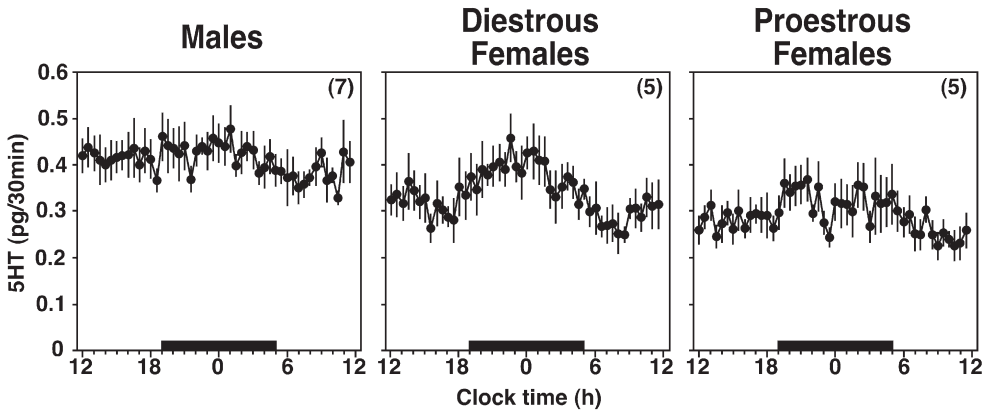

Fig. 1 – Sex-specific 24-h profiles of extracellular 5HT levels in the mPFC. Diestrous and proestrous female rats exhibited a significant diurnal change in 5HT levels, while male rats did not exhibit this rhythm. Male rats had greater overall 5HT levels than proestrous female rats ($P<0.01$). Each data point on the graph represents the mean ± SEM. Horizontal black bars represent the dark phase.

pathogenesis of affective disorders including depression and anxiety (Dubovsky and Thomas, 1995; Griebel, 1995; Meyer et al., 1999; Alexandre et al., 2006). One of the main targets of the serotonergic system is the mPFC. Approximately 60% of neurons in the mPFC express $5HT_{1A}$ and/or $5HT_{2A}$ receptor mRNA (Amargós-Bosch et al., 2004), while GABAergic interneurons express $5HT_3$ receptor mRNA (Puig et al., 2004). Increases in 5HT levels in the rat mPFC have been observed following exposure to a variety of fear- and anxiety-related stimuli, producing an anxiolytic or antidepressant effect (Petty and Sherman, 1993; Yoshioka et al., 1995; Hashimoto et al., 1999; Bland et al., 2003).

Since the circulating gonadal hormone influences 5HT synthesis, receptor property, and neuronal activity, endogenous 5HT release and/or related emotional responses may show clear sex difference. In fact, we found that female rats exhibited lower extracellular 5HT levels in the basolateral amygdala (BLA) than male rats (Mitsushima et al., 2006). In behavioral studies, female rats showed longer immobility in tail suspension task (Goel and Bale, 2008). Since the amygdala is a downstream structure of the mPFC in emotional regulation (Rosenkranz et al., 2003; Quirk and Beer, 2006; Akirav and Maroun, 2007), we hypothesized sex difference in extracellular 5HT levels in the mPFC and/or the 24-h profiles in freely behaving rats. Since the mPFC receives serotonergic innervation from the dorsal and median raphe nucleus (DRN and MRN), we examined possible sex difference in the number of serotonergic neurons in those areas using immunocytochemistry for tryptophan hydroxylase (TrpH).

2. Results

2.1. *Extracellular levels of 5HT in the mPFC*

The 24-h profiles of extracellular 5HT in the mPFC of male, proestrous female, and diestrous female rats were obtained by averaging the values from corresponding times (Fig. 1). Two-way analysis of variance ANOVA with repeated measures demonstrated a significant difference between groups ($F_{(2,658)}=4.516$, $P<0.05$) and within time points ($F_{(47,658)}=3.227$, $P<0.001$), but no significant interaction was observed ($F_{(94,658)}=1.085$, $P>0.05$). In post-hoc ANOVA, the overall extracellular levels of

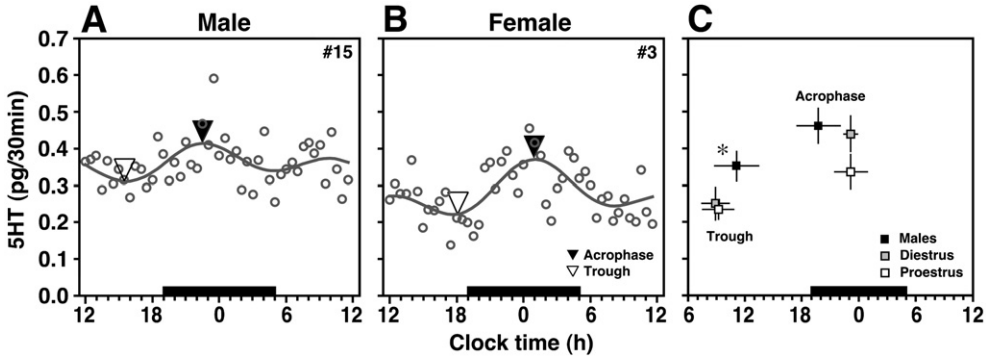

Fig. 2 – The acrophase and trough of 5HT levels in the mPFC. Representative cases in male (A) and female rats (B). The data was fit to a double-cosinor curve (grey line). Mean acrophase and trough of extracellular 5HT levels (C). The trough of extracellular 5HT levels in male rats was significantly greater than of that in both groups of female rats (*$P<0.05$). Horizontal black bars represent the dark phase. Data is the mean ± SEM.