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Sex Differences and Estrous Cycle Changes in Synaptic Plasticity-related microRNA in the Rat Medial Amygdala

Mauro Mozael Hirsch, [a,b] **Janaína Brusco,** [c,d] **Tamara Vaccaro,** [a] **Rogerio Margis,** [b,e] **Jorge E. Moreira,** [c,d] **Carmem Gottfried** [a,b*] **and Alberto A. Rasia-Filho** [f*]

[a] *Research Group in Neuroglial Plasticity, Department of Biochemistry, Federal University of Rio Grande do Sul, Porto Alegre, RS 90035-003, Brazil*

[b] *National Institute of Science and Technology on Neuroimmunomodulation, INCT-NIM/CNPq. Oswaldo Cruz Foundation, Rio de Janeiro, Brazil*

[c] *Department of Pathology and Forensic Medicine, Ribeirão Preto School of Medicine, University of São Paulo, Ribeirão Preto, SP 14049-900, Brazil*

[d] *Department of Neurology, Neuroscience and Behavior, Ribeirão Preto School of Medicine, University of São Paulo, Ribeirão Preto, SP 14049-900, Brazil*

[e] *Center of Biotechnology and PPGBCM, Laboratory of Genomes and Plant Populations, Federal University of Rio Grande do Sul – UFRGS, Porto Alegre, RS, Brazil*

[f] *Department of Basic Sciences/Physiology, Federal University of Health Sciences, Porto Alegre, RS 90050-170, Brazil*

Abstract—The posterodorsal medial amygdala (MePD) is a sex steroid-sensitive and sexually dimorphic subcortical area that dynamically modulates social behaviors in rats. As different microRNA (miRNA) can act as post-transcriptional regulators of synaptic processing, we addressed changes that occur in miRNA expression in the MePD of males and females along the estrous cycle. The expression of miR25-3p, miR132-3p, miR138-5p, miR181a-5p, miR195-5p, and miR199a-5p, involved in neuronal cytoskeleton remodeling and synaptic plasticity, were evaluated by RT-qPCR. We found that the expression of miR138-5p was higher in males than in females along the different phases of the estrous cycle. Males also showed higher levels of miR-181a when compared to females in diestrus and estrus. On the other hand, when compared to females in proestrus, males presented lower levels of miR132-3p and miR199a-5p. The expression of miR25-3p was higher in diestrus females than in proestrus females. In addition, diestrus females showed higher values of miR25-3p, miR181a-5p, and miR195-5p when compared to estrus females. These miRNA expression profiles indicate a variable and fine-tuned protein regulation in the adult MePD. It is likely that these miRNA can be involved in structural and functional synaptic features and plasticity characteristic of males and cycling females and for the MePD regulation of mammalian reproduction.

Key words: extended amygdala, neural plasticity, sexual dimorphism, estrous cycle, noncoding RNA.

INTRODUCTION

The posterodorsal medial amygdala (MePD) is a plastic subcortical area with one of the highest androgen, estrogen (ER-α and ER-β), and progesterone receptor (PR) expression in the rat forebrain (Simerly et al., 1990; Gréco et al., 2001; De Vries and Simerly, 2002). Sex differences were found in the neuronal and glial shape, synaptic processing, and function of the rat MePD (reviewed in Rasia-Filho et al., 2012a,b and references therein), some of them with hemispheric specialization (Johnson et al., 2008; Morris et al., 2008; Brusco et al., 2014). Interestingly, adult males have a higher density of dendritic spines, specialized postsynaptic elements involved in the excitatory transmission, than females in proestrus and estrus (Rasia-Filho et al., 2004, 2012a). More inhibitory contacts are made directly on dendritic shafts on the right than on the left MePD of proestrus females or in the other phases of the estrous cycle (Brusco et al., 2014).

In addition to be a gonadal steroid-responsive area, the MePD responds to chemosensorial olfactory/pheromonal cues (Meredith and Westberry, 2004; Dhungel et al., 2011; Petrulis, 2013) and genitosensorial stimuli (Lehmann et al., 2005) to modulate the activity of

*Corresponding authors. Address: UFCSPA, DCBS/Physiology, Sarmento Leite 245, Porto Alegre, RS 90050-170, Brazil (A. A. Rasia-Filho). UFRGS, Department of Biochemistry/Research Group in Neuroglial Plasticity, Ramiro Barcelos 2600, Porto Alegre, RS 90035-003, Brazil (C. Gottfried).
E-mail addresses: cgottfried@ufrgs.br (C. Gottfried), aarf@ufcspa.edu.br, rasiafilho@pq.cnpq.br (A. A. Rasia-Filho).
Abbreviations: cDNA, complementary DNA; Ct, crossing threshold; ER-α, estrogen receptor α; ER-β, estrogen receptor β; MePD, posterodorsal medial amygdala; miRNA, microRNA; PR, progesterone receptor.

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interconnected areas in the social behavior brain network (Newman, 1999; Rasia-Filho et al., 2012a,b; Brusco et al., 2014; Zancan et al., 2015). In males, the MePD projects to hypothalamic areas that modulate sexual behavior, mainly the occurrence of intromission and ejaculation (Dong et al., 2001; Petrovich et al., 2001; Choi et al., 2005; Rasia-Filho et al., 2012b). In females, the MePD projects to hypothalamic nuclei that control the neuroendocrine secretion of GnRH, the occurrence of puberty, sexual receptivity, and maternal behavior (Simerly, 2004; Rasia-Filho et al., 2012b; Li et al., 2015).

Genetic and epigenetic factors are involved in the regulation of the development of structural and functional sex differences in the brain (Woolley, 1998; Morgan and Bale, 2012; McCarthy and Nugent, 2015; McCarthy et al., 2015). By acting as post-transcriptional regulators of diverse cellular processes, regulatory microRNAs (miRNA) can impact the final expression of various genes simultaneously (Im and Kenny, 2012; Morgan and Bale, 2012; McCarthy and Nugent, 2015). miRNAs are small nucleotide sequences (19–25 bases) of noncoding RNA well conserved along evolution involved with stability and translation of specific-targeted mRNA (Ambros, 2004; Hansen et al., 2010; Morgan and Bale, 2012; Wang and El Naqa, 2008). miRNA bind to the 3′UTR or coding regions of mRNA and reversibly mediate either RNA degradation or translation inhibition (Cougot et al., 2008; Filipowicz et al., 2008; Bartel, 2009; Davis-Dusenbery and Hata, 2010). These molecules are known to be developmentally regulated (Davis-Dusenbery and Hata, 2010) and selectively adjust sex differences (McCarthy et al., 2015) to fine-tune protein expression in specific regions of the rat brain, including the amygdala (Olsen et al., 2009).

Various miRNA modulate protein synthesis at different neuronal sites depending on its availability and activation, and the specific embryonic or postnatal period (Cougot et al., 2008). For example, miRNA regulate number and shape of dendritic spines, modulating synaptic strength and plasticity (Banerjee et al., 2009; Edbauer et al., 2010; Hansen et al., 2010; Broderick and Zamore, 2011). In dissociated cortical and hippocampal neuronal culture of rats at embryonic day 18, some miRNA regulate synaptic protein synthesis and dendritic spine morphogenesis (Siegel et al., 2009) or negatively regulate the development and size of dendritic spines (Schratt et al., 2006). Other studies also showed the importance of some brain-enriched miRNA on synaptic plasticity and dendritic spine development and function (Yu et al., 2008; Rajasethupathy et al., 2009; Earls et al., 2012; Saba et al., 2012; Tsujimura et al., 2015; Chen et al., 2017). These miRNA actions add another level of complexity to synaptic plasticity, since the translation of mRNA can remain inhibited until neurons are exposed to appropriate stimuli, such as neurotrophic factors or neurotransmitter release (Schratt et al., 2006). It is likely that gonadal hormones can also induce modulatory effects on miRNA expression and activity in sexually dimorphic areas of the rat brain.

Here, we studied the expression of miR25-3p, miR132-3p, miR138-5p, miR181a-5p, miR195-5p, and miR199a-5p in the MePD of adult male and cycling female rats. These miRNA were already involved in the neuronal cytoskeletal remodeling and synaptic plasticity of neurons (see Wayman et al., 2008; Yu et al., 2008; Banerjee et al., 2009; Olsen et al., 2009; Rajasethupathy et al., 2009; Siegel et al., 2009; Edbauer et al., 2010; Hansen et al., 2010; Earls et al., 2012; Im and Kenny, 2012; Saba et al., 2012; Tsujimura et al., 2015; Chen et al., 2017). We found that miRNA expressions in the MePD are dependent on sex and specific phases of the estrus cycle.

EXPERIMENTAL PROCEDURE

Animals

Adult male Wistar rats (3-month-old, weighing 300–390 g) and age-matched females (weighing 180–240 g) were housed in groups with free access to food and water, room temperature around 21 °C, and 12-h light/dark cycle (lights on at 6:00). Vaginal smears were obtained daily and examined under light microscopy. Estrous cycle was monitored along 3 weeks. Only regularly cycling females were included and studied in the diestrus, proestrus, and estrus phases.

All attempts were made to minimize the number and suffering of animals. All experimental procedures were approved by the Ethical Committee of the São Paulo University, School of Medicine at Ribeirão Preto (Protocol No. 045/2008), and according to the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85–23, revised 1985, USA).

MePD dissection

Decapitation was performed under anesthesia with isoflurane (0.0125%; Baxter, San Juan, Puerto Rico). Brains were quickly removed and placed in a Petri dish on ice. MePD samples from the right hemisphere were dissected under a stereomicroscope. Sex-specific differences in morphological features can occur in the MePD of both hemispheres in adult males and females (Arpini et al., 2010; Zancan et al., 2017), but the right hemisphere was selected for further study due to the marked and selective estrous cycle effects on synaptic plasticity previously reported by Brusco et al. (2014). The position of the optic tract laterally and the stria terminalis dorsally served as anatomical references to identify the MePD in the ventral forebrain (de Olmos et al., 2004; Paxinos and Watson, 1998; Fig. 1). Considering that the MePD is a small structure, approximately 800 μm × 600 μm, samples were pooled together from three different animals to provide enough material for further analysis. Four experimental groups were tested: males (n = 12), and females in diestrus (n = 18), proestrus (n = 18), and estrus (n = 12). Tissue was quickly stored (in less than 2 min after decapitation) in 200 μl of Trizol (Invitrogen, Waltham, MA, USA) and placed at −80 °C until RNA extraction (Chomczynski, 1993).