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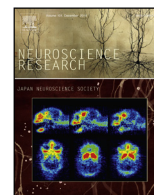

Rapid Communication

Differently shaped spines increase in the posterodorsal medial amygdala of oxytocin knockout female mice

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ABSTRACT

The posterodorsal medial amygdala (MePD) is a sexually dimorphic area in the social behavior neural network, with high concentration of oxytocin (OT) receptors. Wild type (WT) and OT knockout (OTKO) females were studied in proestrus, and Golgi-impregnated spines in the MePD were classified. Results show that the OTKO group has increased density of thin, mushroom, and stubby/wide spines when compared to the WT ($p < 0.01$ in all cases). These data indicate that OT is an important synaptic modulator in the MePD, a finding that is likely involved with the display of the female sexual behavior.

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1. Introduction

Oxytocin (OT) has been implicated in the modulation of social behaviors (Choleris et al., 2009), including parental, affiliative, aggressive, social memory, pair bonding, and sexual display in rats and mice (Russell et al., 2003; Neumann, 2008; Lee et al., 2009). OT has sexually dimorphic effects. Although the selective deletion of the OT gene in knockout mice (OTKO) does not impair the reproductive behavior in males (Lazzari et al., 2013), OT in females is important to coordinate the onset of puberty and the cyclic gonadatrophin releasing hormone (GnRH) secretion, mainly acting in the hypothalamic ventromedial nucleus (VMH) and in the medial preoptic area (MPOA) (Becker et al., 2013 and references therein), areas that also control the display of lordosis behavior during mating (Kow and Pfaff, 1998).

Recently, we found that OTKO females in proestrus decreased sexual behavior and had a significantly higher density of dendritic spines in the posterodorsal medial amygdala (MePD) compared to wild-type (WT) mice (Becker et al., 2013). The MePD has one of the highest expressions of receptors for gonadal hormones in the brain, has a notable concentration of OT receptors, and is a nodal point in the social behavior neural network (Veinante and Freund-Mercier, 1997; Newman, 1999; De Olmos et al., 2004; Rasia-Filho et al., 2012a; Becker et al., 2013; Quagliotto et al., 2014 and references therein). Indeed, differences between males and females were found in the MePD volume, neuron and glial cells number, spatial orientation of the dendritic branches, density of dendritic spines, synaptic connectivity, neurotransmitter binding sites, and local expression of various neuropeptides (reviewed in Rasia-Filho et al., 2012a,b and references therein) It is noteworthy that dendritic spines are specialized postsynaptic elements that have crucial properties for synaptic formation, strength, and plasticity (Bourne and Harris, 2007; Rochefort and Konnerth, 2012). The density of dendritic spines in the MePD of female rats changes in the course of hours to few days and is lower in the proestrus and estrus (Rasia-Filho et al., 2004). This indicates a cyclic, variable synaptic input processing in the MePD that, acting in hypothalamic integrated circuits, is likely involved with the timely neuroendocrine secretion and reproductive behavior display of females (Rasia-Filho et al., 2012a,b; Brusco et al., 2014). This process is evidently impaired in OTKO mice (Becker et al., 2013).

Although there are relevant data that support the idea of a direct structure-function coupling to differently shaped dendritic spines (Bourne and Harris, 2007; Kasai et al., 2010), this issue has not reached a definite consensus. Rather, it is likely that the labile or the

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stable aspect of a dendritic spine depends on the specific synaptic demand and the function at each single spine level and brain region studied (Nimchinsky et al., 2002; Segal, 2010; Spruston et al., 2013). Here, we add new data to the previously reported increase in the local density of dendritic spines in the MePD of OTKO female mice by testing if this effect can be attributed to a specific type of spine. If so, a subpopulation of dendritic spines could be mainly altered by the specific synaptic demand change in the MePD following the selective deletion of the OT gene and the disrupted oxytocinergic transmission.

2. Materials and methods

We have employed the same methodological procedure described in Becker et al. (2013). That is, the mice were the offspring of a backcrossed stock obtained from Dr. W. Scott Young (B6; 129S-Oxttm1Wsy/J; NIMH, USA). All animals were littermates from heterozygous breeders (C57BL/6 mice). Genotyping was described in details previously (Becker et al., 2013).

Adult females ($N = 12$, weighing 25–35 g, and 5–8 months old) were housed in ventilated transparent acrylic cages (37 cm × 24 cm × 24 cm) and grouped with up to 4 same-sex individuals under room temperature at $22 \pm 1\,^{\circ}$C and a 12:12 light–dark cycle. The mice had access to food and water ad libitum. All procedures were conducted in accordance with the national and international regulations for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, reviewed 1985, USA) and were approved by the local Ethics Committee (UFCSPA, Brazil, protocol no. 920/09).

Both WT and OTKO females ($n = 6$ in each group) had their estrous cycle phase determined daily (in the late afternoon) according to cytological criteria of the vaginal smears examined under light microscopy. The phases of the estrous cycle were monitored along 2 weeks and regularly cycling females in proestrus were used in this study.

The "single-section" Golgi method was performed as already reported (Becker et al., 2013). Briefly, females were deeply anesthetized with intraperitoneal injections of ketamine (80 mg/kg) and xylazine (10 mg/kg). Tissue fixation was obtained after rapid transcardial perfusion with 4% paraformaldehyde and 2% picric acid in 0.1 M phosphate buffer solution (pH 7.4). The brains were sectioned coronally (150-μm thick) using a vibratome (Leica, Germany), and put in solutions of 3% potassium dichromate (Merck, Germany) and 1.5% silver nitrate (Merck, Germany) for 24 h and 48 h, respectively. The MePD was studied from 1.46 to 1.94 mm posterior to the bregma, laterally to the optic tract and the "molecular layer", and ventrally to the stria terminalis, according to the atlas of Franklin and Paxinos (1997).

The including criteria for the study of MePD neurons were: (a) to be undoubtedly located within the boundaries of the intended area in both hemispheres; (b) to be relatively isolated from neighboring impregnated cells to avoid "tangled" dendrites; (c) dendrites should have well-impregnated and defined borders; and (d) spines should be clearly distinguishable from the background [6]. Proximal dendrites that fulfilled these criteria had their spines drawn along the different focal planes in "z" using a camera lucida (2000×; i.e., 100× oil-immersion objective lens and 20× ocular lens) coupled to an optic microscope (Olympus BX-41, Japan). For each female, 8 different dendrites were studied with 1 dendrite per sampled neuron. The 3 main differently shaped spines found in the MePD were identified and counted from these samples. That is, the morphological features of the spine head and neck allowed spine classification as thin, mushroom or stubby/wide (as in Brusco et al., 2010). In principle, a salient round broadening on a dendritic shaft was not considered a stubby spine. Other spine shapes (ramified or atypical) were not usually seen. After this procedure, three-dimensional dendritic lengths were measured from the same microscopic images (400×; Olympus BX-61, Japan) and the images of the selected dendrites were captured by a high-resolution digital camera (CCD DP72, Japan) and measured using the Image Pro Plus 7.0 computer software (Media Cybernetics, USA). Dendritic length sampled varied from 30 to 60 μm in both groups [mean ± standard deviation (SD) values of 41 ± 5 μm and 39 ± 7 μm for WT and OTKO mice, respectively]. The density of each type of spine was defined as the number of spines per unit length of dendritic segment (in μm; Rasia-Filho et al., 2004).

We calculated the mean values for the densities of each type of dendritic spine for each animal and respective groups. Data were compared using a two-way ANOVA for repeated measures followed by the Bonferroni test. The statistical level of significance was set as $p < 0.05$. Data were analyzed using Prism software (GraphPad, USA).

3. Results

Representative images of Golgi-impregnated spiny neurons in the MePD of WT and OTKO females are shown in Fig. 1 (top).

Thin spines were more commonly (∼51%) found in both groups. Minimum to maximum ranges for the MePD spine density of the different spine shapes observed in the WT and the OTKO groups were, respectively: 0.7–1.2 and 0.9–1.9 for thin spines/dendritic μm, 0.3–0.5 and 0.5–1.0 for mushroom spines/dendritic μm, and 0.2–0.6 and 0.3–0.8 for stubby/wide spines/dendritic μm.

Mean ± standard deviation data for the density of each type of dendritic spine are shown in Fig. 1 (bottom). There were highly significant differences between the studied groups [$F(1,30) = 112.13$, $p < 0.01$], the spine types [$F(2,30) = 1229.99$, $p < 0.01$], and the interaction of these two factors [$F(2,30) = 25.58$, $p < 0.01$]. The post hoc comparisons showed that the OTKO group had a significant increase in the proximal density of thin ($p < 0.01$), mushroom ($p < 0.01$), and stubby/wide ($p < 0.01$) spines when compared to WT group. The increase in thin and stubby/wide spines was up to 16% and 20%, respectively, whereas mushroom spines were 65% higher in OTKO mice.

4. Discussion

Our present results reinforce that OTKO females in proestrus have a higher number of proximal dendritic spines than WT mice (cf. Becker et al., 2013) and add the new finding that this spine density increase in OTKO mice involves differently shaped spines. Thin spines represent half of all observed shapes in both groups, but the number of thin, mushroom, and stubby/wide spines was consistently higher in proximal dendrites of OTKO mice than in WT females. Notably, these data also indicate that mushroom spines were the type that showed more intense effects following the selective deletion of the OT gene and the altered oxytocinergic transmission to and/or within the MePD. These results were consistently found in the MePD since results obtained from randomly Golgi-impregnated neurons showed a low variability, as assessed by standard deviation values and the high statistical level of significance reached.

Therefore, there is an altered local synaptic processing in the MePD involving both the number and the shape of dendritic spines in OTKO mice. From data obtained in rats, most MePD axo-spine contacts are excitatory (Brusco et al., 2014), and the number of spines is normally affected by fluctuations in ovarian steroids (e.g., reducing 35% from diestrus to proestrus; Rasia-Filho et al., 2004, 2012a). These latter finding highlights the proportion of MePD spines that show labile excitatory inputs and are dynamically

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