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Research paper

Selective deletion of the oxytocin gene remodels the number and shape of dendritic spines in the medial amygdala of males with and without sexual experience

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ABSTRACT

Oxytocin has central actions that modulate synaptic plasticity and the occurrence of social behavior in rodents. The posterodorsal medial amygdala (MePD) composes a sexually dimorphic neural circuit for the display of male sexual behavior. Local dendritic spines are notably plastic and affected by context-dependent social stimuli. Here, we examined the effects of the selective deletion of the OT gene (OTKO) in the number and shape of Golgi-impregnated dendritic spines in the MePD of näive and sexually experienced (SexExp) male mice (n = 6 each group). Compared to the control wild-type mice (WT), OTKO näive mice did not differ in the density of dendritic spines, but there was a significant and more intense reduction in the number of spines in the WT/SexExp (~40%) than in the OTKO/SexExp (~25%). This structural change had a spine-specific feature. That is, sexual experience induced a decrease in the number of thin (~50%) and mushroom-like spines (~35%) at the same time that increased (~30%) the number of stubby/wide spines. In addition, the OTKO/SexExp animals have more thin and mushroom spines than the WT/SexExp ones (~25% and 55%, respectively; $p < 0.01$ in all cases). In conjunction, these novel data indicate that OT participates in the spine remodeling, synaptic refinement, and social stimuli-dependent plasticity in the MePD of male mice.

1. Introduction

The modulation of synaptic plasticity and the occurrence of social behaviors, including maternal care, affiliation, and sexual behavior, involve the oxytocinergic action in the bed nucleus the of the stria terminalis (BNST), the medial preoptic area (MPOA), the ventromedial hypothalamus (VMH), and the medial nucleus of the amygdala (MeA) in rodents [1–4]. Among these areas, the MeA forms part of the "extended amygdala" [5] and its sexually dimorphic posterodorsal subdvision (MePD) integrates olfactory and pheromonal stimuli [6,7] with neural gonadal hormone effects to control the reproductive behavior of male and female rats [reviewed in 8].

The density and shape of dendritic spines in the rat MePD can be affected by circulating levels of sex steroids or by experimental manipulations, such as castration and gonadal hormone replacement therapy [9,10]. For example, the density of proximal dendritic spines is higher in males than in females in proestrous, estrous or metaestrous [9]. Castration of adult males, irrespective of brain hemisphere, decreased the dendritic spine density in the MePD, but induced different effects on each spine type studied [10]. I.e., compared to control groups, castration reduced the number of thin, mushroom-like, and ramified spines, but increased the number of stubby/wide ones [10]. These data are relevant because dendritic spines are specialized postsynaptic elements that receive most inputs from excitatory axons [11,12]. Spines can dynamically modulate synaptic strength and plasticity [13] and their shapes can be related with functional features. Thin spines with a narrow neck can impose a higher resistance for the voltage coupling of the spine with the parent dendrite [14], a finding that does not occur for stubby/wide spines [15]. Mushroom shaped spines have a large head and the head diameter is related with the extension of the postsynaptic density and the type of glutamatergic receptor to be activated [16].

It was recently reported that oxytocin (OT) is an important synaptic modulator in the MePD of mice [2,17]. The selective deletion of the OT gene in knockout mice (OTKO) decreased the display of female sexual behavior [17], a finding that also showed a selective involvement of the

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different types of dendritic spines in the MePD [2]. On the other hand, male mice lacking the OT gene did not exhibit an evident sexual behavior deficit [18,19]. This does not mean that OT is not important for the central modulation of this behavior. Microinjection of OT into the lateral ventricles stimulates copulation in male rats [20], an effect that can be blocked by the administration of an OT receptor (OTR) antagonist [20,21]. Oxytocin is one of the neuropeptides that control penile erection and sexual motivation [22]. Furthermore, sexual experience is associated with the increased OTR expression in the MPOA of male rats [23].

Interestingly, the MePD participates in the central control of penile erection [24], the initiation of copulatory behavior [25], and for experience-dependent changes in electrophysiological responses induced by a receptive female [26]. Because OT can modulate both the synaptic plasticity and reproductive behavior, we studied the effects of the selective deletion of the OT gene in the number and shape of dendritic spines in the MePD of male mice with and without sexual experience.

2. Material and methods

The animals were the offspring of a backcrossed stock from Dr. W. Scott Young (B6; 129S-Oxttm1Wsy/J; NIMH, USA; [cf.2,17]). All animals were littermates from heterozygous breeders (C57BL/6 mice). Genotyping was described in details elsewhere [17,27]. Adult males (N = 24), 5–8 months old and weighing 25–35 g, were housed in groups with free access to food and water. All subjects were maintained in a temperature-controlled room (22 ± 1 °C) on a 12:12 light–dark cycle with the lights off at 5 p.m. All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (8th edition, 2011) and approved by the local Ethics Committee (UFCSPA, Brazil, protocol no. 920/09 and 130/13).

Males were randomly divided in 4 groups (n = 6 in each group): 2 groups of sexually naïve mice [wild-type (WT/Naïve) and OTKO (OTKO/Naïve)] and 2 groups of sexually experienced mice [WT (WT/SexExp) and OTKO (OTKO/SexExp)]. The animals that were sexually experienced were placed during 3 weeks with sexually receptive females, which became pregnant [adapted from 28].

At the end of this period, the mice of the four experimental groups were submitted to the Golgi method. The methodological procedure described here was previously employed by [2,17]. Briefly, males were deeply anesthetized with intraperitoneal injections of ketamine (80 mg/kg) and xylazine (10 mg/kg), brains were perfused and fixed with 4% paraformaldehyde and 2% picric acid in 0.1 M phosphate buffer solution (pH = 7.4), sectioned coronally (150-μm thick) using a Vibratome™ (Leica, Germany), immersed in 3% potassium dichromate and, afterwards, in 1.5% silver nitrate solutions (Merck, Germany). Sections were rinsed in distilled water, dehydrated in an ascending series of alcohols, cleared with xylene, mounted on slides, and covered with synthetic balsam and coverslips. Both hemispheres were studied.

The selected brain sections were approximately 1.46–1.94 mm posterior to the bregma [29] where the MePD is edged laterally by the optic tract and the "molecular layer", and ventrally by the stria terminalis ([5]; Fig. 1). The including criteria for neuronal selection were: (a) be undoubtedly located within the boundaries of the MePD; (b) 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 [17]. Neurons whose proximal dendrites fulfilled these above mentioned criteria had their spines drawn along 40 μm [based on 30] through the z-axis focal planes using a camera lucida (at 2000x; i.e., 100 x oil-immersion objective lens and 20 x ocular lens) coupled to an optic microscope (Olympus BX-41, Japan). For each male, 8 different dendrites (one per neuron) were studied per mouse. Spine density was defined as the number of spines per unit length of dendritic segment (in μm). Three-dimensional dendritic lengths were measured from the same microscopic images (400x; Olympus BX-61, Japan) after the selected dendrites had been captured by a high-resolution digital camera (CCD DP72, Japan) and measured using the Image Pro Plus 7.0 computer software (Media Cybernetics, USA).

The three differently shaped and most common dendritic spines in the MePD were identified and counted in the above mentioned experimental groups. The morphological features of the spine head and neck allowed spine classification as thin, mushroom or stubby/wide one [31]. The number of each type of spine was counted along the z-axis on the same dendritic segments evaluated for the density of spines.

Mean values were calculated for each animal. Data were tested for normal distribution and homodedasticity using the Kolmogorov-Smirnov test and the Bartlett test, respectively. The overall density of spines was compared between groups by a two-way analysis of variance (ANOVA) test followed by the Bonferroni test. The number of each type of spine per experimental group was submitted to an one-way ANOVA and the Tukey post hoc test. The statistical level of significance was set as $P \leq 0.05$. We used the Prism statistical software (GraphPad, USA).

3. Results

Representative microscopic images of a long Golgi-impregnated spiny dendrites from the MePD and camera lucida drawings for the dendritic spines of each experimental group are shown in Fig. 2A and 2B, respectively. Minimum and maximum ranges (spines/dendritic μm) in the MePD were 1.77 − 2.10 (WT/Naïve), 2.00 − 2.12 (OTKO/Naïve), 0.92 − 1.35 (WT/SexExp), and 1.27 − 1.50 (OTKO/SexExp; Fig. 2C).

The density of dendritic spines in the MePD showed a statistically significant difference between groups due to the selective deletion of the OT gene [$F(1,20) = 23.70$, $P < 0.01$], sexual experience [$F(1,20) = 306.85$, $P < 0.01$] and the interaction of these two factors [$F(1,20) = 4.58$, $P = 0.04$]. The post hoc test showed that both sexually experienced groups had a significant decrease in the density of dendritic spines in the MePD than the naïve groups ($P < 0.01$). No difference was found between WT/Naïve and OTKO/Naïve mice ($P > 0.05$), but

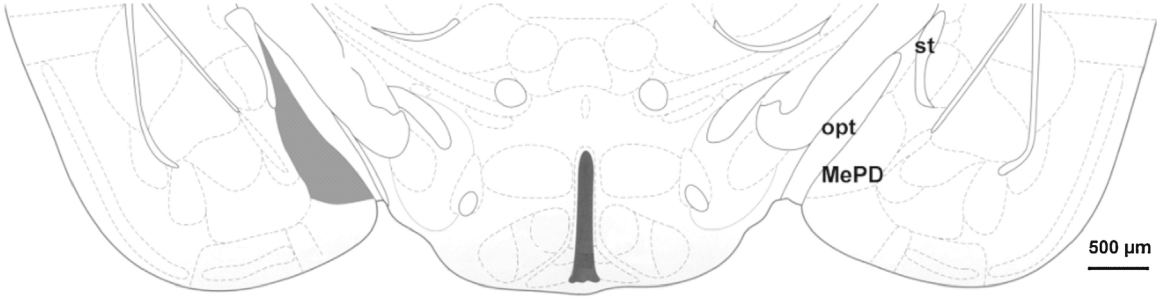

Fig. 1. Schematic diagram of the ventral part of a coronal slice showing the posterodorsal medial amygdala (MePD) in the mice forebrain and from where part of the present data was obtained (in this case, 1.70 mm posterior to the bregma). Gray filled area indicates the MePD location. MePV, posteroventral medial amygdala; opt, optic tract; st, stria terminalis. Scale bar = 500 μm. Adapted from the atlas of Franklin and Paxinos [29].