Hormones and Behavior 98 (2018) 145–158

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Oxytocin and the warm outer glow: Thermoregulatory deficits cause huddling abnormalities in oxytocin-deficient mouse pups

Christopher Harshaw[a,b,*], Joseph K. Leffel[b], Jeffrey R. Alberts[b]

[a] Department of Psychology, University of New Orleans, New Orleans, LA, United States
[b] Department of Psychological & Brain Sciences, Indiana University, Bloomington, IN, United States

ARTICLE INFO

Keywords:
Oxytocin
Huddling
Brown adipose tissue
Behavioral phenotyping
Mice

ABSTRACT

Oxytocin is a social and reproductive hormone that also plays critical roles in a range of homeostatic processes, including thermoregulation. Here, we examine the role of oxytocin (OT) as a mediator of brown adipose tissue (BAT) thermogenesis, cold-induced huddling, and thermotaxis in eight-day-old (PD8) OT 'knock out' (OTKO) mouse pups. We tested OTKO and wildtype (WT) pups in single- and mixed-genotype groups of six, exposing these to a period of ambient warmth (~35 °C) followed by a period of cold (~21.5 °C). Whether huddling exclusively with other OTKO or alongside WT pups, OTKO pups showed reduced BAT thermogenesis and were significantly cooler when cold-challenged. Huddles of OTKO pups were also significantly less cohesive than WT huddles during cooling, suggesting that thermoregulatory deficits contribute to contact abnormalities in OTKO pups. To further explore this issue, we examined thermotaxis in individuals and groups of four OTKO or WT pups placed on the cool end of a thermocline and permitted to freely locomote for 2 h. When tested individually, male OTKO pups displayed abnormal thermotaxis, taking significantly longer to move up the thermocline and settling upon significantly lower temperatures than WT pups during the 2 h test. OTKO mouse pups thus appear to have deficits in both thermogenesis and thermotaxis—the latter deficit being specific to males. Our results add to a growing body of work indicating that OT plays critical roles in thermoregulation and also highlight the entanglement of social and thermoregulatory processes in small mammals such as mice.

1. Introduction

Oxytocin (OT) is a nonapeptide hormone that plays prominent roles in social and reproductive behavior across a range of taxa (e.g., Carter et al., 2008; Donaldson and Young, 2008; Goodson and Bass, 2001; Keverne and Curley, 2004). In addition to these well-known functions, OT is now known to be involved in numerous non-social, homeostatic processes (cf. Lee et al., 2009), including ingestion (see Blevins et al., 2004; Leng et al., 2008), smooth muscle tone and peristalsis (e.g., Altura and Altura, 1984; Babygirija et al., 2010), fluid and electrolyte balance (e.g., Bernal et al., 2010), and metabolic and body temperature homeostasis (see Argiolas and Gessa, 1991; Blevins and Ho, 2013; Chaves et al., 2013).

Although interest in the social versus homeostatic functions of OT has fueled largely separate areas of research, it is unlikely that social and homeostatic processes are the output of entirely separate systems, particularly for highly social species (see Harshaw et al., 2017). This principle is particularly evident in species that modulate their gregariousness or sociability with fluctuations in ambient temperature and/or humidity. For example, in many species of rodents thermal energy (i.e., warmth) can be characterized as both an internal, physiological resource and a kind of 'social commodity' (see Haig, 2008; Harshaw et al., 2014). When challenged with cold, that is, mice and other rodents rely upon a combination of thermogenesis, heat conservation, and behavioral thermoregulation, involving positive thermotaxis (i.e., moving toward warmth) and huddling with conspecifics (Gordon, 2012, 1990; Leon, 1986; Satinoff, 1996). As an efficient means of reducing heat loss, huddling plays an important role in the social lives of many rodents and other small mammals (e.g., Alberts, 2006; Bautista et al., 2008; Harshaw and Alberts, 2012).

Heat produced by brown adipose tissue (BAT) thermogenesis appears to make a critical contribution to huddling. When BAT is pharmacologically inactivated, for example, huddles of infant rats are markedly less cohesive—a result that suggests that the thermal attractiveness of pups to each other drives the formation and maintenance of huddles (Sokoloff and Blumberg, 2001). Differences in BAT activation have also been shown to drive spontaneous *assortment* within huddles, whereby warmer individuals preferentially contact each other, while

* Corresponding author at: Department of Psychology, Geology/Psychology Building, 2000 Lakeshore Drive, New Orleans, LA 70148, United States.
E-mail address: charshaw@uno.edu (C. Harshaw).

https://doi.org/10.1016/j.yhbeh.2017.12.007
Received 3 February 2017; Received in revised form 18 November 2017; Accepted 20 December 2017
Available online 04 January 2018
0018-506X/

cooler individuals are driven to the periphery (Harshaw et al., 2014; Sokoloff and Blumberg, 2001). Situated in the natural ecological niche—i.e., a group of huddling individuals—BAT can thus be seen as both a thermal and "social" effector (Harshaw et al., 2014).

Interestingly, OT manipulations have been shown to result in significant shifts in huddling, in species as diverse as rats (*Rattus norvegicus*; Alberts, 2006; Kojima and Alberts, 2011), mice (Arakawa et al., 2015), meadow voles (*Microtus pennsylvanicus*; Beery and Zucker, 2010), naked mole rats (*Heterocephalus glaber*; Mooney et al., 2014), and marmoset monkeys (*Callithrix penicillata*; Smith et al., 2010). There has also long been evidence that central OT acts as an endogenous *pyrogen* or producer of metabolic heat (e.g., Lipton and Glyn, 1980; Mason et al., 1986), though the mechanism(s) underlying this effect were unclear.[1] Recent studies have shed a great deal of light on this question. For example, when exposed to a 5 °C cold challenge, adult male OT 'knockout' (OTKO) and OT-receptor (OTR) KO mice have difficulty maintaining core temperature and lose significantly more body heat than wildtype (WT) mice (Kasahara et al., 2013, 2007; Takayanagi et al., 2008). A number of abnormalities suggestive of reduced BAT activation (e.g., shifts in beta-adrenergic receptor expression; accumulation of lipids) have also been identified in the BAT of adult OTR KO mice (Kasahara et al., 2013, 2015). When OTR is restored to the DMN/VMN of the hypothalamus via injection of an OTR viral vector, OTR KO mice nevertheless show full recovery of cold-induced BAT thermogenesis (Kasahara et al., 2013). A recent study moreover demonstrated that ablating OT neurons[2] resulted in both reduced BAT activation and impaired peripheral vasoconstriction (i.e., heat retention) in response to cold (Xi et al., 2017). Thus, a picture has emerged in which central OT must be seen as a regulator of multiple components of the homeostatic response to cold in rodents, including BAT thermogenesis and thermolysis via peripheral vasoconstriction (e.g., Deis et al., 1963; Kasahara et al., 2015, 2013, 2007; Lin et al., 1983; Xi et al., 2017).

Given the importance of BAT thermogenesis as an organizer of cold-induced huddling, mice lacking a functional OT system may experience significant disruption to a core feature of their social lives beginning shortly after birth. More specifically, if OTKO and OTR KO pups have impaired BAT thermogenesis, they are likely to be less *attractive* to each other and thus experience disruption to the typical, spontaneous emergence of huddling during periods of maternal absence from the nest (e.g., Harshaw et al., 2014; Sokoloff and Blumberg, 2001). Variation in early behavior within the huddle has also been shown to predict variation in later social and emotional phenotypes in rabbits and rats (e.g., Reyes-Meza et al., 2011; Rödel and Meyer, 2011), suggesting that this issue may also be important for interpreting the many studies in which adult OTKO and OTR KO mice display differences in social-emotional behavior compared to WT conspecifics (e.g., Ferguson et al., 2000; Mantella et al., 2003; Winslow et al., 2000). Here, we present an initial exploration of these questions in OTKO mice, examining the effects of oxytocin deficiency on huddling behavior, BAT thermogenesis, and thermotaxic behavior during early development. Based on findings in adult OTKO mice, we hypothesized that OTKO mouse pups would show reduced BAT thermogenesis and impaired huddling ability relative to WT pups when challenged with cold. Additionally, we hypothesized that the normally positive correlation between the thermal status of individual pups and the number of contacts they receive while huddling (see Harshaw et al., 2014) would be absent or reduced in OTKO pups. Lastly, we hypothesized that thermoregulatory differences would be sex-specific, given known sex differences in nonapeptide hormones and receptors (e.g., Dumais and Veenema, 2016; Tamborski et al., 2016).

2. Methods and materials

2.1. Animals and housing

All animals were derived from B6;129S-Oxt$^{tm1Wsy/J}$ heterozygous stock purchased from Jackson Labs (Bar Harbor, Maine) and bred in the Animal Behavior Laboratory at Indiana University. The F1 offspring were genotyped (Transnetyx, Cordova, TN) shortly after weaning and housed thereafter in same-sex groups, separated by genotype (Oxt$^{+/+}$ or Oxt$^{-/-}$). Males and females of each genotype were then bred to produce homozygous litters (F2). Pups born to Oxt$^{-/-}$ dams were immediately fostered to actively lactating Oxt$^{+/-}$ or Oxt$^{+/+}$ dams, to ensure their survival (Winslow et al., 2000). All mice were born and reared in standard mouse cages (30 × 13 × 19 cm) with food and water available ad libitum. The colony was maintained on 14:10 h light/dark cycle (lights on at 0700 h) at 22 ± 2 °C.

All animal care and procedures were approved by the Bloomington Institutional Animal Care and Use Committee (BIACUC) at Indiana University (IU #12-024) and were conducted in accordance with both international standards and the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Care was taken at every step to minimize pain and discomfort to animals.

2.2. Tests and apparatus

2.2.1. Huddling and activity

Huddling tests involved simultaneous testing of six Postnatal Day 8 (PD8; day of birth = PD0) pups: either three male-female sibling pairs, drawn from the same litter and thus of the same genotype (Exp. 1) or three same-sex OTKO-WT pairs, drawn from two different litters (Exp. 3). PD8 pups were employed because pups of this age show robust huddling and locomotor competence, whereas fur development impedes thermography after PD8 (Harshaw et al., 2014; Harshaw and Alberts, 2012). PD8 pups drawn from different litters do not huddle preferentially with littermates over same-age non-littermates and thus do not spontaneously assort by litter during huddling tests (Harshaw et al., *unpublished data*).

Pups were weight-matched within-pairs, to minimize advantage due to weight (see Bautista et al., 2010; Rödel et al., 2008). In Exp. 1, the average weight difference within male-female pairs was 0.00 ± 0.03 g for both genotypes, with pups from OTKO litters being heavier, on average, than pups from WT litters (5.35 ± 0.06 vs. 5.04 ± 0.05 g; $t_{90.2} = 4.08$, $d = -0.83$, $p < 0.0001$). In Exp. 3 the average difference within mixed-genotype OTKO-WT pairs was 0.04 ± 0.02 g, with OTKO pups slightly outweighing WT pups (4.76 vs. 4.72 g; $t_{41} = -2.28$, $d = -0.35$, $p < 0.03$). Although congruent with reports of lower energy expenditure and obesity proneness in OTKO and OTR KO mice (Camerino, 2009; Kasahara et al., 2013; Nishimori et al., 2008; Takayanagi et al., 2008), this difference was likely also influenced by the smaller litter size of OTKO compared to WT litters (8.4 vs. 9 pups, across both experiments; $t_{37.6} = 1.33$, $d = -0.40$, $p = 0.193$).

2.2.1.1. Apparatus. All huddling tests were performed within a double-walled glass chamber (height = 30 cm; dia = 15.2 cm) on a circular platform (dia = 11.25 cm), 21.5 cm from the chamber's upper edge. The platform was constructed of 1.27 cm Styrofoam insulation (Dow Chemical Company), circled by a polyethylene mesh wall (height = 15 cm), to prevent pups from making contact with the glass wall of the chamber, covered with a circular piece of clear plastic sheeting, permitting cleaning between sessions. Ambient temperature (T_a) was controlled by circulating either chilled or heated water through its walls. An ICI 7320 P-Series infrared thermal imaging camera (Infrared Cameras Inc., Beaumont, TX) and Sony DXC-

[1] Although a number of studies have shown that peripheral administration of large doses of OT generally triggers a *hypothermic* rather than pyrogenic response (e.g., Murzenok et al., 1989; Ring et al., 2006), Hicks et al. (2014) demonstrated that such OT-induced hypothermia is due to the action of OT on AVP (V_{1A}) rather than OT receptors.

[2] Via injection of diphtheria toxin in mice expressing both an OT promoter for *cre* expression and a *cre*-inducible diphtheria toxin receptor (Xi et al., 2017).