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# Distribution of the neuronal inputs to the ventral premammillary nucleus of male and female rats ☆

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ABSTRACT

The ventral premammillary nucleus (PMV) expresses dense collections of sex steroid receptors and receptors for metabolic cues, including leptin, insulin and ghrelin. The PMV responds to opposite sex odor stimulation and projects to areas involved in reproductive control, including direct innervation of gonadotropin releasing hormone neurons. Thus, the PMV is well positioned to integrate metabolic and reproductive cues, and control downstream targets that mediate reproductive function. In fact, lesions of PMV neurons blunt female reproductive function and maternal aggression. However, although the projections of PMV neurons have been well documented, little is known about the neuronal inputs received by PMV neurons. To fill this gap, we performed a systematic evaluation of the brain sites innervating the PMV neurons of male and female rats using the retrograde tracer subunit B of the cholera toxin (CTb). In general, we observed that males and females show a similar pattern of afferents. We also noticed that the PMV is preferentially innervated by neurons located in the forebrain, with very few projections coming from brainstem nuclei. The majority of inputs originated from the medial nucleus of the amygdala, the bed nucleus of the stria terminalis and the medial preoptic nucleus. A moderate to high density of afferents was also observed in the ventral subiculum, the arcuate nucleus and the ventrolateral subdivision of the ventromedial nucleus of the hypothalamus. Our findings strengthen the concept that the PMV is part of the vomeronasal system and integrates the brain circuitry controlling reproductive functions.



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## 1. Introduction

The ventral premammillary nucleus (PMV) is a small, compact group of neurons in the medial zone of the hypothalamus, located immediately caudal to the ventromedial nucleus of the hypothalamus and ventral to the dorsal premammillary nucleus (Canteras et al., 1992b; Donato and Elias, 2011). Initial studies using extensive electrolytic lesions of the premammillary area of rats suggested the involvement of the PMV in male conspecific aggression (van den Berg et al., 1983). These findings were later reinforced by neuroanatomical studies using Fos immunoreactivity as a marker of neuronal activation (Hoffman and Lyo, 2002). The PMV of male rats and hamsters shows increased Fos expression after aggressive conspecific encounters, but notably also after sexual behavior (Coolen et al., 1996; Kollack-Walker and Newman, 1995; Veening et al., 2005). In fact, conspecific opposite sex odors alone induce Fos expression in PMV neurons of rodents. These neurons coexpress nitric oxide synthases and cocaine- and amphetamine-regulated transcript (CART) (Cavalcante et al., 2006a; Donato et al., 2010; Yokosuka et al., 1999). We further demonstrated that CART mRNA is increased in the PMV of males following exposure to female odors and that PMV CART neurons project to areas controlling gonadotropin releasing hormone (GnRH) secretion (Cavalcante et al., 2006a; Rondini et al., 2004).

The PMV expresses high to moderate density of sex steroids receptors and projects to the sexually dimorphic circuitry, to nuclei of the vomeronasal system and reproductive control sites (Canteras et al., 1992b; Gautron et al., 2013; Merchenthaler et al., 2004; Rondini et al., 2004; Simerly et al., 1990). Therefore, PMV neurons may integrate external (odor) and internal (sex steroids) cues to modulate the downstream targets in neuroendocrine and behavioral responses. In agreement with this model, electrolytic lesions of the PMV abolished the luteinizing hormone (LH) rise induced by male odor or electrical stimulation of the medial nucleus of the amygdala (Beltramino and Taleisnik, 1985). Moreover, using excitotoxic lesions, we and others have shown that the PMV plays a role in female neuroendocrine regulation of estrous cycles, sexual behavior and maternal aggression (Donato et al., 2009, 2013; Motta et al., 2013).

The PMV also expresses receptors for several metabolic cues including leptin, insulin and ghrelin, and may represent a key integrative site for metabolic regulation (Donato and Elias, 2011; Elias et al., 2000; Elmquist et al., 1998; Frazao et al., 2014; Leshan et al., 2009; Zigman et al., 2006). Nutrition is fundamental to reproductive function (Frisch and McArthur, 1974; Hill et al., 2008; Kennedy and Mitra, 1963). States of negative energy balance decreases reproductive capacity and delays puberty. On the other hand, excess energy also disrupts fertility (Bluher and Mantzoros, 2004; Chan and Mantzoros, 2001). Leptin signaling-deficient mice and humans are obese and infertile, and the genetic deletion of insulin receptors in the brain disrupts fertility in mice (Bruning et al., 2000; Chua et al., 1996; Clement et al., 1998; Coleman, 1978; Farooqi et al., 2002; Tartaglia et al., 1995; Zhang et al., 1994). However, the exact brain sites involved in the metabolic control of reproduction are not completely known. We have shown that endogenous re-expression of leptin receptors only in the PMV of mice otherwise null for leptin receptor induces puberty and improves fertility (Donato et al., 2011). These findings indicate that the PMV plays a fundamental role in the integration of metabolism and reproduction. However, although the projections of PMV neurons have been well documented (Canteras et al., 1992b; Gautron et al., 2013; Leshan et al., 2009; Rondini et al., 2004), little is known about the neuronal inputs received by PMV neurons. To fill this gap and contribute to the understanding of the hypothalamic circuitry controlling complex physiological systems, we performed a systematic evaluation of the brain sites innervating the PMV neurons of male and female rats. In the present study, we chose to use a standard neuroanatomical tracer to acquire knowledge on the complete network of neuronal populations with the potential to modulate PMV output.

## 2. Results

Neurons projecting to the PMV from the entire brain were mapped using the cholera toxin b subunit (CTb) as a retrograde tracer. CTb is an efficient, allowing restricted injections combined with robust and consistent retrograde labeling with virtually no uptake from fibers of passage (Luppi et al., 1990). We observed that the projections to the PMV of male and female rats were predominantly ipsilateral with a few retrogradely labeled neurons in contralateral nuclei. The pattern of distribution of CTb immunoreactive (CTb-ir) neurons was virtually identical between sexes, with few differences in the density of retrogradely labeled cells in specific areas with apparent higher number of CTb-ir neurons in the female brain. Because injection sites were variable, quantification was not possible. Figs. 1 and 2 illustrate the injection sites of cases with CTb centered inside the boundaries of the PMV ($n=4$ males and $n=3$ females) and used in our mapping study. Fig. 3 shows examples of cases with injection sites restricted to the PMV (W282 male and W422 female). Injections outside the PMV were used as controls (see examples in Fig. 4). A comparative analysis of retrogradely labeled neurons in males and females is presented in Table 1. Abbreviations follow the nomenclature proposed by Swanson (1992).

### 2.1. *Telencephalon*

In the telencephalon, CTb-ir neurons were mainly concentrated in the medial and posterior amygdala, the bed nucleus of the stria terminalis and the lateral septal nucleus (Fig. 5, Fig. 7B–J). The medial prefrontal cortex, the bed nucleus of the olfactory accessory tract and the ventral subiculum also showed a consistent medium to small projections to the PMV (Figs. 7A, G and 8A–B).

The medial nucleus of the amygdala (MeA) comprises the major source of afferents to the PMV. CTb-ir neurons were spread through most of the MeA, with the highest concentration in the posterodorsal and posteroventral subdivisions (MeApd and MeApv, respectively, Figs. 5A–C, 7G–I). The posterior nucleus of the amygdala (PA) also showed a high