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Research Report

Sexual dimorphism in the vomeronasal system of the rabbit

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ABSTRACT

Studies have shown that the vomeronasal system (VNS), an olfactory neural network that participates in the control of reproductive physiology and behavior, is sexually dimorphic in the rat. These works have also shown two main characteristics of brain sexual dimorphism: (a) dimorphism appears in neural networks related to reproduction and (b) it can present two morphological patterns: one in which males present greater morphological measures than females (male > female) and another in which the opposite is true (female > male). The present work extends the hypothesis to the rabbit, as a representative species of Lagomorpha. In addition, the locus coeruleus (LC), which is known to send rich noradrenergic projections to VNS structures, was also studied. Sex differences were found in: (a) the number of mitral, and dark and light granule cells (female > male) of the accessory olfactory bulb (AOB); (b) the medial amygdala (Me) and its dorsal (Med) and ventral (Mev) subdivisions, males showing greater values than females in volume and number of neurons, while in the posteromedial cortical amygdala (PMCo or C_3), females show greater density of neurons than males and (c) the posteromedial division of the bed nucleus of the stria terminalis (BSTMP) in which males have more neurons than females. No sex differences were seen in the bed nucleus of the accessory olfactory tract (BAOT) and the LC. These results evidence that, as it was observed in rodents, sex differences are also seen in the VNS of Lagomorpha and that these sex differences present the two morphological patterns seen in Rodentia. Differences between orders are discussed with respect to the species-specific physiological and behavioral peculiarities.

1. Introduction

The dual olfactory system hypothesis, which proposes the existence of two separate olfactory pathways: the main and vomeronasal pathways (Scalia and Winans, 1975, 1976; Winans and Scalia, 1970), has generated a huge amount of research and has helped us to understand important aspects of vertebrate reproductive behaviors. Starting with Powers and Winans (1975) who demonstrated that the vomeronasal organ (VNO) participates in the control of copulatory behavior of male hamsters, many studies have appeared in the literature suggesting that the VNS mediates the action of pheromones implicated in the expression of masculine and feminine behavior, maternal behavior and physiological primer

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pheromone mechanisms that influence puberty and estrous cycle (see for review Del Cerro, 1998; Halpern, 1987; Halpern and Martinez-Marcos, 2003; Wysocki, 1979).

At the beginning of the eighties, and working with rats, we found that the rat VNO is a sexually dimorphic chemosensory structure differentiated by gonadal hormones early after birth (Segovia and Guillamon, 1982) and we suggested that the whole vomeronasal system (VNS) could be sexually dimorphic (see for review Guillamon and Segovia, 1993, 1996, 1997; Segovia and Guillamon, 1986, 1993, 1996, Segovia et al., 1999). The structures that receive vomeronasal input, such as the medial amygdala (Me), medial preoptic area (MPA), the ventromedial hypothalamus (VMH) and premammillary nucleus (PMV) have androgen and estrogen receptors (Simerly et al., 1990) and present sexual dimorphism (Bleier et al., 1982a; Dörner, 1976; Gorski et al., 1978, 1980; Matsumoto and Arai, 1983; Nishizuka and Arai, 1981, 1983; see also for review Arnold and Gorski, 1984; Guillamon and Segovia, 1993, 1996, 1997; Segovia and Guillamon, 1986, 1993, 1996; Segovia et al., 1999). As it occurs in the SDN-POA (Döhler et al., 1986), we have demonstrated, also in the rat, the existence of sex differences, controlled by sex steroids shortly after birth, in other structures of the vomeronasal pathway like the AOB (Segovia et al., 1984, 1986; Valencia et al., 1986), the BAOT (Collado et al., 1990, 1993, 1998), the BST (Del Abril et al., 1987; Guillamon et al., 1988a) and the C_3 (Vinader-Caerols et al., 1998, 2000).

Sex differences in structures that receive VNO input have been found in several species; for instance, differences in the sexually dimorphic nucleus of the medial preoptic area (SDN-POA) were reported first in rats (Gorski et al., 1978, 1980; see for review Arnold and Gorski, 1984) and confirmed in hamsters (Greenough et al., 1977), polygamous montane voles (Shapiro et al., 1991), gerbils (Yahr et al., 1994), ferrets (Cherry et al., 1990), guinea pigs (Bleier et al., 1982b; Hines et al., 1985), sheep (Roselli et al., 2004), rhesus monkeys (Byne, 1998), humans (Hofman and Swaab, 1989; Swaab and Fliers, 1985) and also in quails (Viglietti-Panzica et al., 1986) and doves (Steimer and Hutchison, 1990). Similarly, sex differences in the medial region of the BST have been reported in some species of birds (Panzica et al., 2001) as well as in Wistar and Long Evans rats (Del Abril et al., 1987; Garcia-Falgueras et al., 2005; Guillamon et al., 1988a), guinea pigs (Hines et al., 1985) and humans (Allen and Gorski, 1990; Zhou et al., 1995). All these findings support the hypothesis that the VNS might be a sexually dimorphic network in vertebrates.

Rats and rabbits present substantial differences in their reproductive physiology and behavior. Female rats are spontaneous ovulators and female rabbits are reflex ovulators. Moreover, there are important differences between rats and rabbits in copulatory patterns. Male rats behave with multiple intromissions that end in one ejaculation and a postejaculatory period, while in rabbits ejaculation occurs in almost every intromission and the postejaculatory period is very short (Dewsbury, 1972). Females of both species, spontaneously or most commonly after the appropriate hormonal treatment, show male like copulatory patterns called pseudomale behavior (Morali and Beyer, 1992; Morali et al., 2003). With respect to this behavior, rats are isomorphic while rabbits are dimorphic (Morali and Beyer, 1992; Morali et al., 2003).

The participation of the VNS in the control of maternal behavior in rodents is well known. Most VNS structures exert a tonic inhibition (Del Cerro, 1998) on the MPOA, a structure that receives VNS input and facilitates the expression of maternal behavior (Del Cerro, 1998; Numan, 1994). With respect to rabbits, to our knowledge, there is only one study in the literature that performed by Gonzalez-Mariscal et al. (2004). These authors demonstrated a tonic inhibitory action of the AOB over the expression of maternal behavior in virgin rabbits and a stimulation of maternal responsiveness by ovarian hormones following AOB lesions.

Taking into account the differences in the reproductive physiology and behaviors above described between rats and rabbit species and in order to support the hypothesis that the VNS might be sexually dimorphic in mammals, this work studies the possible existence of sex differences in the AOB, BAOT, BST and C_3 in the rabbit. Moreover, the locus coeruleus (LC), which is sexually dimorphic in some strains of rats (Garcia-Falgueras et al., 2005; Guillamon et al., 1988b; Luque et al., 1992; Pinos et al., 2001) and sends rich noradrenergic projections to the AOB (Shipley et al., 1985), is also studied.

2. Results

2.1. Accessory olfactory bulb

The AOB is displayed in Fig. 1. The rabbit AOB is an ovoid structure, dorsocaudally embedded in the main olfactory bulb (MOB). It has five well differentiated layers: glomerular, external plexiform, mitral cells, internal plexiform and granular (Fig. 1). Four main characteristics can be detected: (a) glomerules are round shaped and well differentiated, (b) the mitral cell layer is not stratified, (c) bundles of fibers from the internal plexus invade the granular layer giving it an indented form and (d) light and dark granules can be distinguished in the granular layer.

There were sex differences in the overall volume ($t_8 = 2.52$, $P < 0.03$), since males had larger volume than female rabbits (Table 1). However, it may be considered as an technical artifact, since sex differences were not seen in the volume of the glomerular, external plexiform, mitral, internal plexiform and

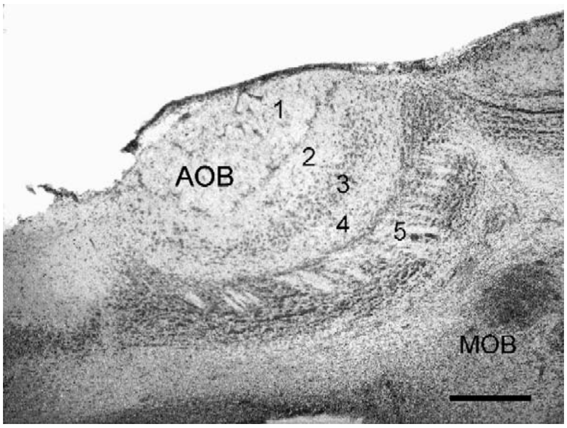

Fig. 1 – Photomicrograph illustrating the morphology of the rabbit (male) accessory olfactory bulb (AOB): (1) glomerular layer, (2) external plexus, (3) mitral cell layer, (4) internal plexus and (5) granular layer; 4×, scale bar = 300 μm; MOB: main olfactory bulb.