Chem. Senses 32: 673–680, 2007
doi:10.1093/chemse/bjm033
Advance Access publication June 4, 2007

Distribution of Neurocalcin-Containing Neurons Reveals Sexual Dimorphism in the Mouse Olfactory Bulb

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Abstract

Olfactory sexual dimorphism has mainly been described in the vomeronasal system, in relation to reproductive behavior, while evidence of sexual dimorphism in the main olfactory bulb (OB) remains scarce. There are no data indicating sex-related differences in the neurochemistry of intrinsic olfactory elements. Neurocalcin (NC) is a calcium-binding protein that is expressed in specific neuronal populations of the central nervous system. Here we analyzed by immunohistochemistry the NC-containing neurons in the mouse main OB, comparing both their quantities and their locations between male and female animals. NC cell density was higher in males than in females in specific locations of the glomerular layer, the external plexiform layer, the mitral cell layer, and the internal plexiform layer. This divergence in the numbers of NC cells was especially patent in central rostrocaudal levels. The NC-containing neurons exhibiting sexual divergence were identified as both juxtaglomerular and short-axon cells. This is the first description of sexual dimorphism regarding neurons belonging to the mouse main OB. According to their distribution in the OB, neurocalcin-immunoreactive interneurons could reflect a sexually dimorphic regulation of specific odorants.

Key words: calcium-binding protein, immunohistochemistry, interneuron, neuroanatomy, rodent

Introduction

Over the past 20 years, some reports have shown that the olfactory system of adult rodents is sexually dimorphic (Guillamón et al. 1988; Collado et al. 1990, Segovia and Guillamón 1993; Peretto et al. 2001; Weruaga et al. 2001). Initially, sexual divergence was demonstrated in the accessory olfactory system, fundamentally related to reproductive behavior and pheromone-originated signal processing (Segovia and Guillamón 1993). Anatomical sexual differences have also been demonstrated in the rat in the accessory olfactory system of both the vomeronasal organ and the bed nucleus of the stria terminalis of the accessory olfactory system (Segovia and Guillamón 1982; Segovia et al. 1984, 1986; Guillamón et al. 1988; Collado et al. 1990, Peretto et al. 2001). Guillamón et al. (1988) have shown that female rats have more neurons than males in the lateral division of the bed nucleus of the stria terminalis, which belongs to the main olfactory pathway. Moreover, sexual dimorphism in specific glomeruli showing a strong cholinergic innervation (atypical glomeruli) in the mouse main olfactory bulb (OB) has been demonstrated (Weruaga et al. 2001). Analysis of the centrifugal innervation of rat atypical glomeruli demonstrates that serotonergic and cholinergic innervations are both higher and more constant in males than in females (Gómez et al. 2005). In sum, although there is evidence suggesting different types of odorant discrimination between males and females, different centrifugal control patterns in the OB, and sexual dimorphism in higher olfactory centers, together with strong evidence for such dimorphism in the accessory olfactory system, there are no data to confirm the existence of sexual dimorphism in the first relay station of the olfactory pathway, the OB. The distribution of neuronal types and synaptic relationships in the OB are well known, and hence, fine variations in the numbers of elements, connections, or neurochemical features may reflect marked differences in olfactory processing between the sexes.

Neuronal markers able to define subpopulations of interneurons within the OB are widespread and diverse, but some cytoplasmatic molecules are powerful tools for such a task because their immunohistochemical determination yields a Golgi-like staining of positive elements. Calcium-binding proteins have several calcium-binding domains able to maintain low intracellular calcium concentrations stable, thus

regulating specific neuronal functions (Rogers 1989a,1989b). Some of them are of special neuroanatomical interest because they are localized in specific neuronal subpopulations of the central nervous system, including the olfactory system (Baimbridge et al. 1982; García-Segura et al. 1984; Braun 1990; Celio 1990; Rogers et al. 1990). Antibodies against these proteins are also powerful neuroanatomical tools because they permit extraordinary histological resolution.

Neurocalcin (NC) is a calcium-binding protein with 3 EF-hand calcium-binding domains (Nakano et al. 1992; Terasawa et al. 1992). NC shares similarities with visilin and recoverin and has, therefore, been classified as a neural calcium-sensor protein (Terasawa et al. 1992), although its precise function in neurons is poorly understood (Kramer and Siegelbaum 1992). By immunoblot studies, the presence of NC in the OB, cerebrum, cerebellum, brainstem, spinal cord, retina, hypophysis, and adrenal glands has been demonstrated (Nakano et al. 1992; Bastianelli et al. 1993; Nakano et al. 1993; Hidaka and Okazaki 1993). In the olfactory system, NC is expressed by the olfactory epithelium, the main OB, the vomeronasal organ, and the accessory OB (Bastianelli et al. 1993, 1995; Iino et al. 1995; Porteros et al. 1996; Briñón et al. 1998).

Sexual dimorphism in NC expression has been found in the development of avian song nuclei, which are strongly dimorphic centers (Veney et al. 2003). In mammals, sexual divergences of calcium-binding proteins other than NC, such as calbindin D28-k or calretinin, have been described in several brain zones (Abe et al. 1990; Brager et al. 2000), but apparently, there are no studies addressing sex differences in NC expression. The aim of the present work was to analyze the differences between male and female NC distributions in a structure related to sexual behavior: the OB.

Material and methods

Animals

Five male and 5 virgin female mice (*Mus musculus*, B6 strain) were employed for this study. All animals were separated by sex at postnatal day P21 and analyzed at P90. They were kept in the animal facilities of the University of Salamanca housed in groups of 2–5 mice, at constant humidity and temperature, with a 12/12-h light cycle and were fed ad libitum. The care of the mice was performed according to the prescriptions of the directives of the European Communities Council (86/609/EEC) and current Spanish legislation (RD 1201/2005) for animal care and experimentation. The animals were deeply anesthetized with chloral hydrate (5% w/v in saline, 10 ml/kg body weight intraperitoneally) and killed by perfusion.

Tissue preparation

The animals were perfused through the ascending aorta, initially with heparinized saline and then for 15 min with a fixative made up of 4% (w/v) depolymerized paraformaldehyde and 0.2% (w/v) picric acid in 0.1 M phosphate buffer (PB), pH 7.3. The brains were removed and fixed in the same solution for 2 h and then washed in PB. Then, they were cryoprotected in 30% (w/v) sucrose in PB until they sank, frozen in liquid nitrogen, and stored at −80 °C until use. Six consecutive series of coronal sections 30 μm thick were obtained with a freezing-sliding microtome and then collected free-floating in PB.

Immunohistochemistry

The sections were washed in phosphate-buffered saline (PBS), pH 7.4, and incubated for 72 h at 4 °C in the primary antibody obtained from rabbit against bovine brain NC (a gift from Dr Nakano, Nagoya University, Japan). The primary antibody was diluted 1:5000 in PBS containing 0.3% Triton X-100 and 5% (w/v) goat serum (Vector Laboratories, Burlingame, CA). Then, the sections were rinsed in PBS and incubated for 90 min with 1:200 anti-rabbit secondary antibody (Vector Laboratories) and 0.3% Triton X-100 in PBS at room temperature. Subsequently, they were washed in PBS and incubated for 90 min with avidin–biotin peroxidase complex (Vector Laboratories) diluted 1:200 in PBS. The peroxidase was visualized by incubating the sections in 0.02% (w/v) 3,3′-diaminobenzidine and 0.003% H_2O_2 in 0.2 M Tris–HCl buffer. The reaction was controlled under the microscope when maximum contrast was reached. The sections were rinsed in PB, mounted on gelatin-coated slides, dehydrated in ethanol series, cleared in xylene, and coverslipped with Entellan (Merck, Darmstadt, Germany).

Analysis

Three OB areas were analyzed separately in order to properly compare the differences between sexes—the glomerular layer (GL), the external plexiform layer (EPL), and inframitral layers (IMLs)—because they contain well-known different interneurons. The IML included both the mitral cell layer and the internal plexiform layer because the neuronal types were the same in these 2 layers. The granule cell layer (GCL) was not analyzed because the NC-immunoreactive (NC-IR) granule cells were only faintly stained and could not be quantified reliably. For a better study and comparison of males and females, we followed the established patterns for the rodent OB anatomical subdivisions as previously described (Weruaga et al. 1999). We chose 5 equidistant rostrocaudal levels: rostral (I), centrorostral (II), central (III), centrocaudal (IV), and caudal (V; Figure 1). Further, coronal sections were divided into 4 quadrants: dorsal, lateral, ventral, and medial.

The Student *t*-test was employed to analyze the possible differences in the total quantity of NC-IR cells of the OB between males and females. Thus, the differences in the quantity of NC-IR neurons between males and females in each of the 3 layers (GL, EPL, IML) were analyzed. Because

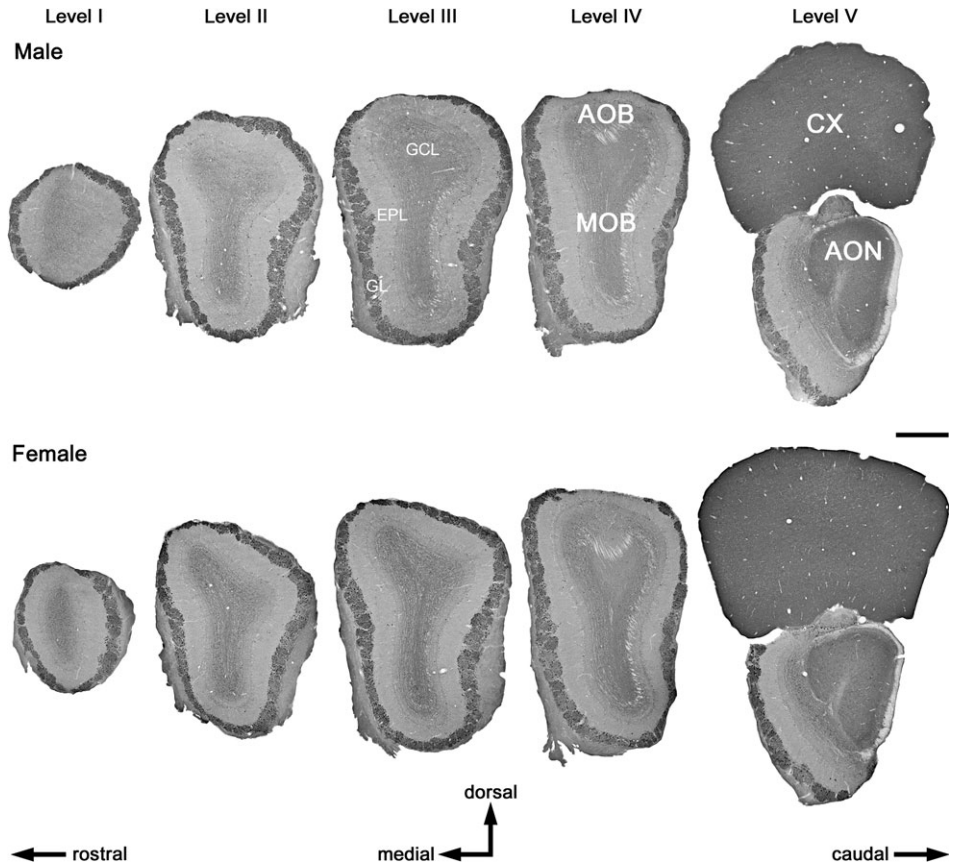

Figure 1 Rostrocaudal series of mouse OB coronal slices immunostained for NC. The slices in the top array belong to a male, whereas those at the bottom correspond to a female. The sections of each column correspond to equivalent rostrocaudal levels from the OBs of both sexes. At this magnification, NC staining is mainly detected in the GL, although a few positive neurons can also be distinguished in deeper layers. AON, anterior olfactory nucleus; CX, cortex; MOB, main olfactory bulb. Scale bar: 500 μm.

the Levene test yielded nonhomogeneous variance among samples, Kruskal–Wallis tests were employed. Thereafter, and depending on the homogeneity of the variance, the Mann–Whitney *U* test or Student *t*-test were applied to detect differences in each quadrant of each level between males and females.

Results

NC staining was distributed throughout the OB, except in the olfactory nerve fiber layer (Figure 1). A subpopulation of juxtaglomerular cells located around the glomeruli was strongly stained (Figure 2). As previously described (Briñón et al. 1998; Parrish-Aungst et al. 2007), in the rat and mouse OB, these NC-IR neurons mainly constitute a subpopulation of periglomerular cells, with small ovoid or spherical somata (Figure 2A,B). Very scarce cells with large fusiform or rounded somata, mainly located close to the EPL, were also stained. These neurons were identified as external tufted cells (Figure 2A,B). The main dendrites of both neuronal types were profusely branched inside the neighboring glomeruli.

In the EPL and IML, both positive somata and a weakly stained neuropil were observed, the former being identified as belonging to short-axon cells. These elements were scarce and were identified as: 1) van Gehuchten cells, which exhibited a rounded soma and very branched dendrites (Figure 2B); 2) horizontal cells, which showed elongated somatas parallel to the OB surface and prolongations extending horizontally within these layers (Figure 2C); and 3) vertical cells, which displayed elongated neuronal bodies located transversal to the OB surface while their neurites extended perpendicularly to the OB lamination (Figure 2C). The van Gehuchten cells were located close to the glomeruli, whereas the horizontal and vertical cells were seen close to the mitral cells (Figures 2 and 3). A sparse weakly stained subpopulation of granule cells was located in both the IML and the GCL. The GCL also showed a dense NC-positive neuropil, whereas the subependymal layer exhibited only sparse, weakly stained NC-positive processes.

The distribution pattern of NC-IR neurons in the EPL and IML comprised a higher number of positive cells in the OB of male as compared with female mice

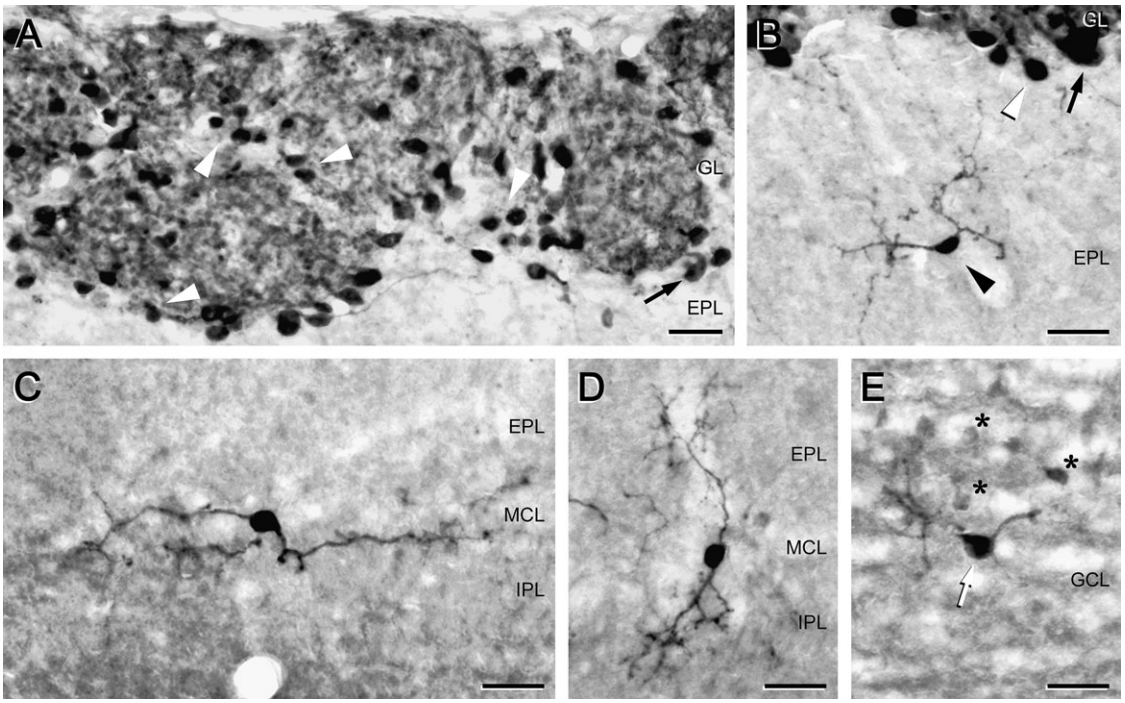

Figure 2 Coronal sections of the mouse OB showing different neuronal types immunostained for NC. The NC-IR juxtaglomerular cells in the GL show strongly stained somata and are mainly periglomerular cells (white arrowhead, **A** and **B**). A few external tufted cells are also stained in this layer (black arrow, A and B). These neurons have profusely branched dendrites inside the nearest glomeruli. Van Gehuchten cells exhibit very branched dendrites departing from a very rounded soma and are located in the EPL near to the GL (black arrowhead, B). In the MCL and IPL, horizontal cells have prolongations that extend horizontally to the OB lamination **(C)**, whereas the neurites of vertical cells run perpendicularly to the OB surface **(D)**. These neuronal types are located close to the mitral cells. Deep short-axon cells are located in the inner zone of the GCL (white arrow, **E**) and exhibit larger and intensely stained somata as compared with those of granule cells (black asterisk, E). IPL, internal plexiform layer; MCL, mitral cell layer; Scale bar: 25 μm.

(3241.0 ± 32.0 vs. 2736.5 ± 69.4, mean ± standard errors of the mean (SEM), $P < 0.005$).

The distribution of NC-IR neurons in the GL, EPL and IML in each of the quadrants of the levels analyzed differed between males and females. The GL only showed differences between the sexes in the dorsal ($P < 0.01$) and lateral quadrants ($P < 0.05$, Figure 4A) of level II, where males exhibited more NC-IR cells than females. In the EPL (Figure 4B), the number of NC-IR neurons was greater in males than in females in the medial quadrant of levels II and III ($P < 0.05$), whereas in level IV, the amount of NC-IR cells was higher in the ventral quadrant of male animals than in that of female mice ($P < 0.05$). Analysis of the IML (Figure 4C) revealed that the males had more NC-IR cells than females in the lateral quadrant of level II ($P < 0.05$), whereas in level III this difference was significant in the lateral quadrant ($P < 0.01$). Additionally, in level IV, the number of NC-IR cells present in the male OB was greater than in the females in the dorsal ($P < 0.01$), ventral, and medial quadrants ($P < 0.05$). Finally, in level V, the females exhibited more NC-IR neurons than males in the ventral quadrant ($P < 0.05$). In contrast, males had more positive cells than females in the medial quadrant of this level ($P < 0.05$).

Discussion

In the present study, we report a sex difference in the number of NC-IR interneurons in the GL, EPL, and IML of the mouse OB. Such neurons are more numerous in males than in females, but this divergence does not persist throughout the whole OB. In each of the layers studied, the mice exhibit divergences in the distribution of NC-IR neurons among the quadrants, and these differences are not equal among the 5 studied levels. The difference in the number of NC-IR neurons between males and females was very evident, and the SEM were low, even if the estrous cycle was not controlled. Accordingly, the female hormonal cycle may not affect the NC phenotype in the OB at all, or only to a slight extent. This is the first time that a sex difference in an interneuronal subpopulation and in the expression of a calcium-binding protein have been observed in the mouse main OB, even though different sexual dimorphisms in olfactory function have been described previously (Guillamón et al. 1988; Collado et al. 1990, Segovia and Guillamón 1993; Peretto et al. 2001; Weruaga et al. 2001; Gómez et al. 2005, 2007).

The accessory olfactory bulb (AOB) is one of the first olfactory region in which sexual dimorphism was demonstrated (Segovia et al. 1984). The AOB of males shows a greater

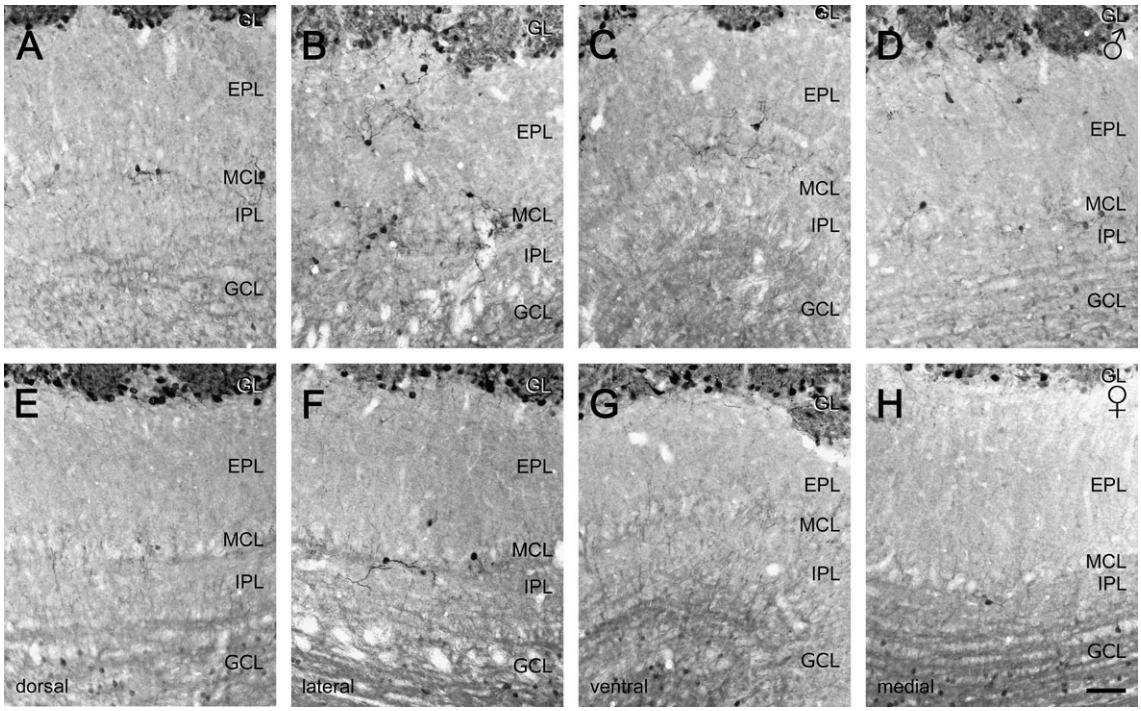

Figure 3 Coronal sections of the mouse OB belonging to level III immunostained for NC; **(A–D)** are from a male mouse, whereas **(E–H)** are from a female animal. (A) and (E) correspond to the dorsal, (B) and (F) to the lateral, (C) and (G) to the ventral, and (D) and (H) to the medial quadrants. In males, the density of NC-positive interneurons is higher than in females in the EPL, the MCL and the IPL. This sexual dimorphism is more evident in the lateral and medial quadrants. IPL, internal plexiform layer; MCL, mitral cell layer. Scale bar: 50 μm.

volume and a higher number of neurons than that of female rats (Segovia et al. 1984, 1986; Valencia et al. 1986). This structure is mainly related to reproductive behavior and the processing of pheromone-originated signals (for a review, see Segovia and Guillamón 1993; Guillamón and Segovia 1997). Although the vomeronasal system mediates pheromonal responses, evidence has been reported that the olfactory system could also participate in pheromone detection in rodents (Restrepo et al. 2004). In the rabbit, elimination of the olfactory epithelium with $ZnSO_4$ affects suckling behavior in pups, whereas removal of the vomeronasal organ does not have any detectable effect (Hudson and Distel 1986). Keverne (2004) proposed that AOB stimulation would be required to couple the motor components of mating activity in a sex-specific way but, in parallel, that OB activation would be necessary to attract males to estrous females. These data suggest that apart from the accessory olfactory system, the main olfactory system (including the OB) is indeed involved in reproductive behaviors. It, therefore, seems feasible to reason that sexual variation in the neurochemical organization of the OB and divergences in reproductive behavior could be linked.

There is a considerable body of neuroanatomical and physiological evidence to suggest that olfactory information processing is carried out in the OB by vertical units that would include specific receptor neurons in the olfactory epithelium, concrete glomeruli for a narrow range of odorants, mitral cells, and centrifugal projections that innervate these structures (for a review, see Mori et al. 1999; Mombaerts 2001). These ''columns'' of specific olfactory information would include not only projection neurons but also interneurons, such as periglomerular, granule, and short-axon cells. These neurons would modulate olfactory input at different levels: the interneurons in the GL and EPL would fine-tune the principal cells of the OB via their lateral dendrites, whereas the short-axon cells of the deep layers would mainly contact other interneurons (Toida et al. 1994, 1996; Crespo et al. 2001, 2002; Gracia-Llanes et al. 2003; Hamilton et al. 2005, Kosaka and Kosaka 2007). Accordingly, odorants would activate specific sets of glomeruli localized in topographically fixed positions in the OB (for a review, see Mori et al. 1998, 1999; Mombaerts 2004; Komiyama and Luo 2006) and hence both the projecting neurons and interneurons belonging to those functional units. The short-axon cells would modulate the olfactory information by inhibiting other ''columns'' in order to diminish the noise produced by nonspecific cell activations (Sharp et al. 1975; Jourdan et al. 1980; Guthrie et al. 1993; Johnson et al. 1998; Mori et al. 1998; Aungst et al. 2003; Leon and Johnson 2003; Komiyama and Luo 2006).

Calcium regulation is necessary for both neuronal structure and function, including transcription process and neurotransmitter release, to be modified (see Burgoyne

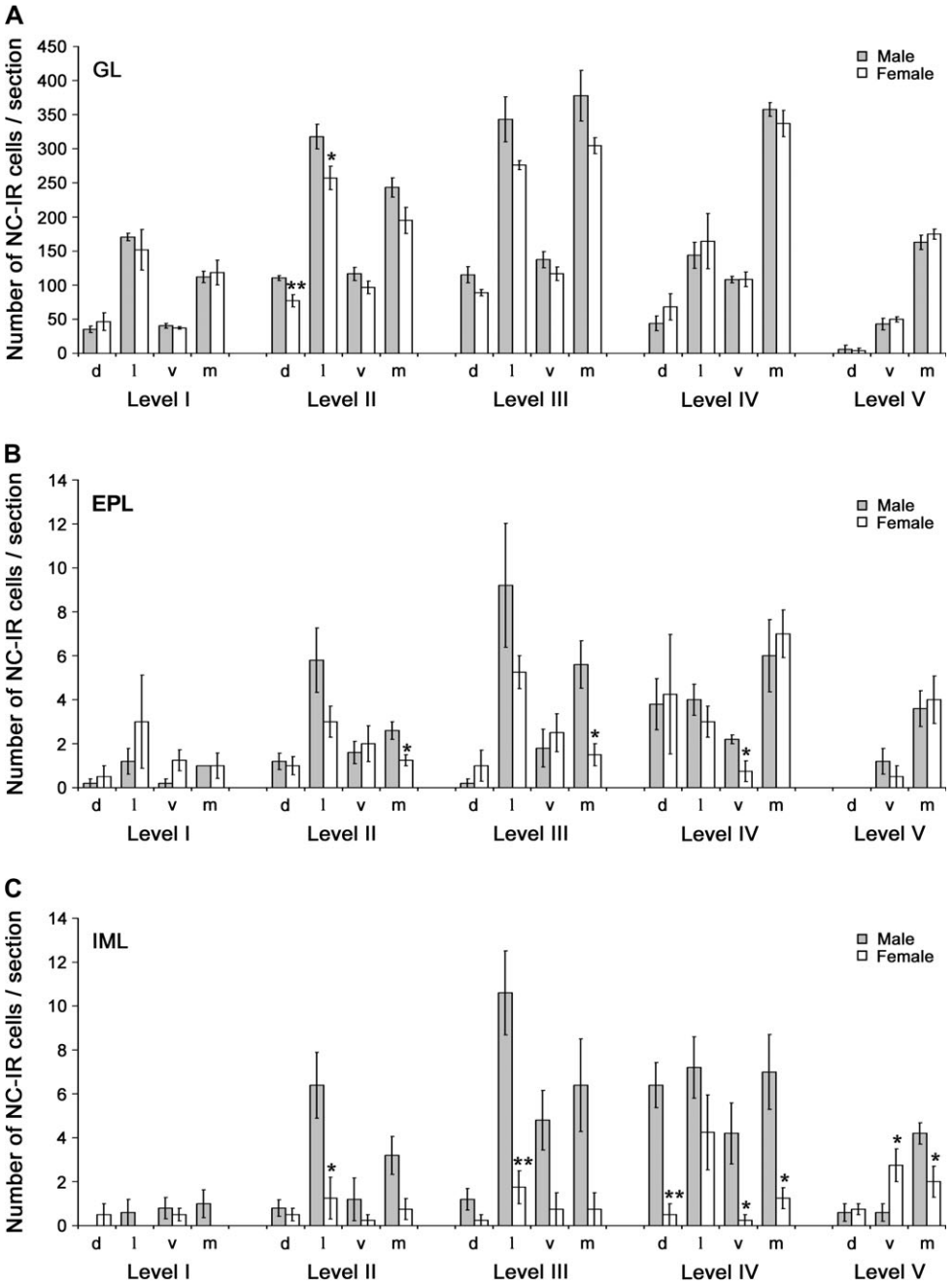

Figure 4 Differences in the number of NC-IR cells in the mouse OB between males and females. **(A–C)** show the number of NC-IR cells in the GL (A), EPL (B), and IML (C) inside each level in the dorsal, lateral, ventral, and medial quadrants. Shaded columns correspond to males and open ones to females. Values represent means ± SEM. The statistical analysis employed the Student *t*-test or the Mann–Whitney *U* test, depending on the homogeneity of the variance. $*P < 0.05$; $**P < 0.01$. d, dorsal; l, lateral; m, medial; and v, ventral.

and Morgan 1995; Braunewell et al. 1999). Moreover, recent studies have demonstrated that NC interacts specifically with clathrin heavy chain, α- and β-tubulin, and β-actin (Ivings et al. 2002). These observations suggest that NC could be involved in the control of the traffic of clathrin-coated vesicles and in cytoskeletal alterations, and hence in the regulation of neuronal activity.

In light of the present findings, it could be speculated that some of the NC-positive populations of the central levels of the OB would regulate specific olfactory information, processing specific odorants in a sexually dimorphic way. Whether these olfactory inputs trigger sexual or reproductive behavior remains unknown because the map of odorants in the olfactory system of rodents is far from complete.

Acknowledgements

The authors would like to thank Dr Nakano for kindly providing the NC antiserum. This work was supported by the Ministerio de Educación y Ciencia (SAF2006-05705), Ministerio de Sanidad y Consumo (PNSD 132/06), Junta de Castilla y León, Centre for Regenerative Medicine and Cell Therapy of Castilla y León and Federación de Cajas de Ahorro de Castilla y León. The authors express their gratitude to N. Skinner for revising the English version of the article.

References

Abe H, Amano O, Yamakuni T, Takahashi Y, Kondo H. 1990. Localization of spot 35-calbindin (rat cerebellar calbindin) in the anterior pituitary of the rat: developmental and sexual differences. Arch Histol Cytol. 53:585–591.

Aungst JL, Heyward PM, Puche AC, Karnup SV, Hayar A, Szabo G, Shipley MT. 2003. Centre-surround inhibition among olfactory bulb glomeruli. Nature. 426:623–629.

Baimbridge KG, Miller JJ, Parkes CO. 1982. Calcium-binding protein distribution in the rat brain. Brain Res. 239:519–525.

Bastianelli E, Okazaki K, Hidaka H, Pochet R. 1993. Neurocalcin immunoreactivity in rat olfactory bulb. Neurosci Lett. 161:165–168.

Bastianelli E, Polans AS, Hidaka H, Pochet R. 1995. Differential distribution of six calcium-binding proteins in the rat olfactory epithelium during postnatal development and adulthood. J Comp Neurol. 354:395–409.

Brager DH, Sickel MJ, McCarthy MM. 2000. Developmental sex differences in calbindin-D(28K) and calretinin immunoreactivity in the neonatal rat hypothalamus. J Neurobiol. 42:315–322.

Braun K. 1990. Calcium-binding proteins in avian and mammalian central nervous system: localization, development and possible functions. Prog Histochem Cytochem. 21:1–64.

Braunewell KH, Gundelfinger ED. 1999. Intracellular neuronal calcium sensor proteins: a family of EF-hand calcium-binding proteins in search of a function. Cell Tissue Res. 295:1–12.

Briñón JG, Arévalo R, Crespo C, Bravo IG, Okazaki K, Hidaka H, Aijón J, Alonso JR. 1998. Neurocalcin immunoreactivity in the rat main olfactory bulb. Brain Res. 795:204–214.

Burgoyne RD, Morgan A. 1995. Ca^{2+} and secretory-vesicle dynamics. Trends Neurosci. 18:191–196.

Celio MR. 1990. Calbindin D-28k and parvalbumin in the rat nervous system. Neuroscience. 35:375–475.

Collado P, Guillamón A, Valencia A, Segovia S. 1990. Sexual dimorphism in the bed nucleus of the accessory olfactory tract in the rat. Dev Brain Res. 56:263–268.

Crespo C, Blasco-Ibáñez JM, Marqués-Marí AI, Martínez-Guijarro FJ. 2001. Parvalbumin-containing interneurons do not innervate granule cells in the olfactory bulb. Neuroreport. 12:2553–2556.

Crespo C, Blasco-Ibáñez JM, Marqués-Marí AI, Alonso JR, Briñón JG, Martínez-Guijarro FJ. 2002. Vasoactive intestinal polypeptide-containing elements in the olfactory bulb of the hedgehog (*Erinaceus europaeus*). J Chem Neuroanat. 24:49–63.

García-Segura LM, Baetens D, Roth J, Norman AW, Orci L. 1984. Immunohistochemical mapping of calcium-binding protein immunoreactivity in the rat central nervous system. Brain Res. 296:75–86.

Gómez C, Briñón JG, Barbado MV, Weruaga E, Valero J, Alonso JR. 2005. Heterogeneous targeting of centrifugal inputs to the glomerular layer of the main olfactory bulb. J Chem Neuroanat. 29:238–254.

Gómez C, Briñón JG, Valero J, Recio JS, Murias AR, Curto GG, Orio L, Colado MI, Alonso JR. 2007. Sex differences in catechol contents in the olfactory bulb of control and unilaterally deprived rats. Eur J Neurosci. 25:1517–1528.

Gracia-Llanes FJ, Crespo C, Blasco-Ibáñez JM, Marqués-Marí AI, Martínez-Guijarro FJ. 2003. VIP-containing deep short-axon cells of the olfactory bulb innervate interneurons different from granule cells. Eur J Neurosci. 18:1751–1763.

Guillamón A, Segovia S. 1997. Sex differences in the vomeronasal system. Brain Res Bull. 44:377–382.

Guillamón A, Segovia S, del Abril A. 1988. Early effects of gonadal steroids on the neuron number in the medial posterior region and the lateral division of the bed nucleus of the stria terminalis in the rat. Dev Brain Res. 44:281–290.

Guthrie KM, Anderson AJ, Leon M, Gall C. 1993. Odor-induced increases in c-fos mRNA expression reveal an anatomical "unit" for odor processing in olfactory bulb. Proc Natl Acad Sci USA. 90:3329–3333.

Hamilton KA, Heinbockel T, Ennis M, Szabó G, Erdélyi F, Hayar A. 2005. Properties of external plexiform layer interneurons in mouse olfactory bulb slices. Neuroscience. 133:819–829.

Hidaka H, Okazaki K. 1993. Neurocalcin family: a novel calcium-binding protein abundant in bovine central nervous system. Neurosci Res. 16:73–77.

Hudson R, Distel H. 1986. Pheromonal release of suckling in rabbits does not depend on the vomeronasal organ. Physiol Behav. 37:123–128.

Iino S, Kobayashi S, Okazaki K, Hidaka H. 1995. Neurocalcin-immunoreactive receptor cells in the rat olfactory epithelium and vomeronasal organ. Neurosci Lett. 191:91–94.

Ivings L, Pennington SR, Jenkins R, Weiss JL, Burgoyne RD. 2002. Identification of Ca^{2+}-dependent binding partners for the neuronal calcium sensor protein neurocalcin δ: interaction with actin, clathrin and tubulin. Biochem J. 363:599–608.

Johnson BA, Woo CC, Leon M. 1998. Spatial coding of odorant features in the glomerular layer of the rat olfactory bulb. J Comp Neurol. 393:457–471.

Jourdan F, Duveau A, Astic L, Holley A. 1980. Spatial distribution of [^{14}C]2-deoxyglucose uptake in the olfactory bulbs of rats stimulated with two different odours. Brain Res. 188:139–154.

Keverne EB. 2004. Importance of olfactory and vomeronasal systems for male sexual function. Physiol Behav. 83:177–187.

Komiyama T, Luo L. 2006. Development of wiring specificity in the olfactory system. Curr Opin Neurobiol. 16:67–73.

Kosaka T, Kosaka K. 2007. Heterogeneity of nitric oxide synthase-containing neurons in the mouse main olfactory bulb. Neurosci Res. 57:165–178.

Kramer RH, Siegelbaum SA. 1992. Intracellular Ca^{2+} regulates the sensitivity of cyclic nucleotide-gated channels in olfactory receptor neurons. Neuron. 9:897–906.

Leon M, Johnson BA. 2003. Olfactory coding in the mammalian olfactory bulb. Brain Res Rev. 42:23–32.

Mombaerts P. 2001. How smell develops. Nat Neurosci. 4:1192–1198.

Mombaerts P. 2004. Odorant receptor gene choice in olfactory sensory neurons: the one receptor-one neuron hypothesis revisited. Curr Opin Neurobiol. 14:31–36.

Mori K, Nagao H, Sasaki YF. 1998. Computation of molecular information in mammalian olfactory systems. Network. 9:R79–R102.

Mori K, Nagao H, Yoshihara Y. 1999. The olfactory bulb: coding and processing of odor molecule information. Science. 286:711–715.

Nakano A, Terasawa M, Watanabe M, Okazaki K, Inoue S, Kato M, Nimura Y, Usuda N, Morita T, Hidaka H. 1993. Distinct regional localization of neurocalcin, a Ca^{2+}-binding protein, in the bovine adrenal gland. J Endocrinol. 138:283–290.

Nakano A, Terasawa M, Watanabe M, Usuda N, Morita T, Hidaka H. 1992. Neurocalcin, a novel calcium binding protein with three EF-hand domains, expressed in retinal amacrine cells and ganglion cells. Biochem Biophys Res Commun. 186:1207–1211.

Parrish-Aungst S, Shipley MT, Erdelyi F, Szabo G, Puche AC. 2007. Quantitative analysis of neuronal diversity in the mouse olfactory bulb. J Comp Neurol. 501:825–836.

Peretto P, Giachino C, Panzica GC, Fasolo A. 2001. Sexually dimorphic neurogenesis is topographically matched with the anterior accessory olfactory bulb of the adult rat. Cell Tissue Res. 306:385–389.

Porteros A, Briñón JG, Crespo C, Okazaki K, Hidaka H, Aijón J, Alonso JR. 1996. Neurocalcin immunoreactivity in the rat accessory olfactory bulb. Brain Res. 729:82–89.

Restrepo D, Arellano J, Oliva AM, Schaefer ML, Lin W. 2004. Emerging views on the distinct but related roles of the main and accessory olfactory systems in responsiveness to chemosensory signals in mice. Horm Behav. 46:247–256.

Rogers JH. 1989a. Two calcium-binding proteins mark many chick sensory neurons. Neuroscience. 31:697–709.

Rogers JH. 1989b. Immunoreactivity for calretinin and other calcium-binding proteins in cerebellum. Neuroscience. 31:711–721.

Rogers JH, Khan M, Ellis J. 1990. Calretinin and other CaBPs in the nervous system. Adv Exp Med Biol. 269:195–203.

Segovia S, Guillamón A. 1982. Effects of sex steroids on the development of the vomeronasal organ in the rat. Brain Res. 281:209–212.

Segovia S, Guillamón A. 1993. Sexual dimorphism in the vomeronasal pathway and sex differences in reproductive behaviors. Brain Res Rev. 18:51–74.

Segovia S, Orensanz LM, Valencia A, Guillamón A. 1984. Effects of sex steroids on the development of the accessory olfactory bulb in the rat: a volumetric study. Brain Res. 318:312–314.

Segovia S, Valencia A, Cales JM, Guillamón A. 1986. Effects of sex steroids on the development of two granule cell subpopulations in the rat accessory olfactory bulb. Brain Res. 395:283–286.

Sharp FR, Kauer JS, Shepherd GM. 1975. Local sites of activity-related glucose metabolism in rat olfactory bulb during olfactory stimulation. Brain Res. 98:596–600.

Terasawa M, Nakano A, Kobayashi R, Hidaka H. 1992. Neurocalcin: a novel calcium-binding protein from bovine brain. J Biol Chem. 267:19596–19599.

Toida K, Kosaka K, Heizmann CW, Kosaka T. 1994. Synaptic contacts between mitral/tufted cells and GABAergic neurons containing calcium-binding protein parvalbumin in the rat olfactory bulb, with special reference to reciprocal synapses between them. Brain Res. 650: 347–352.

Toida K, Kosaka K, Heizmann CW, Kosaka T. 1996. Electron microscopic serial-sectioning/reconstruction study of parvalbumin-containing neurons in the external plexiform layer of the rat olfactory bulb. Neuroscience. 72:449–466.

Valencia A, Segovia S, Guillamón A. 1986. Effects of sex steroids on the development of the accessory olfactory bulb mitral cells in the rat. Brain Res. 389:287–290.

Veney SL, Peabody C, Smith GW, Wade J. 2003. Sexually dimorphic neurocalcin expression in the developing zebra finch telencephalon. J Neurobiol. 56:372–386.

Weruaga E, Briñón JG, Barbado V, Aijón J, Alonso JR. 1999. A standardized model for the anatomical division of the rodent olfactory bulb. Eur J Anat. 3:27–34.

Weruaga E, Briñón JG, Porteros A, Arévalo R, Aijón J, Alonso JR. 2001. A sexually dimorphic group of atypical glomeruli in the mouse olfactory bulb. Chem Senses. 26:7–15.

Accepted April 12, 2007