Chem. Senses 32: 623–631, 2007

doi:10.1093/chemse/bjm032
Advance Access publication June 7, 2007

Expression of Trace Amine–Associated Receptors in the Grueneberg Ganglion

Joerg Fleischer, Karin Schwarzenbacher and Heinz Breer

Institute of Physiology, University of Hohenheim, Stuttgart, Germany

Correspondence to be sent to: Heinz Breer, University of Hohenheim, Institute of Physiology, Garbenstrasse 30, D-70599 Stuttgart, Germany. e-mail: breer@uni-hohenheim.de

Abstract

The Grueneberg ganglion (GG) in the vestibule of the anterior nasal cavity is considered as an olfactory subcompartment based on expression of the olfactory marker protein (OMP) and axonal projection to the olfactory bulb. Searching for olfactory receptors present in the GG, it has been observed recently that V2r83, a member of the V2R class of olfactory receptors, is expressed in numerous cells in the GG of mice. However, no other olfactory receptors have been found to be present in a considerable number of GG neurons so far. Here, we report that GG neurons express trace amine–associated receptors (TAARs) that have most recently been described as a novel class of olfactory receptors. It was observed that several TAAR subtypes are expressed by defined subpopulations of GG neurons distinct from the V2r83-positive cells. Analyzing the time course of TAAR expression during pre- and postnatal development revealed that TAARs are expressed by a substantial portion of GG neurons in late embryonic and neonatal stages, whereas in juveniles and adults, the number of TAAR-positive cells in the GG was significantly decreased.

Key words: G protein, olfaction, TAAR, vomeronasal receptor

Introduction

The detection of odorous and pheromonal compounds in the mammalian nose is mediated by distinct classes of G protein–coupled receptors (GPCRs) decorating the plasma membrane of chemosensory neurons that reside in the main olfactory epithelium (MOE), the vomeronasal organ (VNO), or the septal organ (SO). Besides these well-known olfactory subsystems, it has been found recently that the so-called Grueneberg ganglion (GG) located at the nasal vestibule of mice shares features characteristic for olfactory sensory neurons, most notably the expression of the olfactory marker protein (OMP) and axonal projection to the olfactory bulb (OB) of the brain. Therefore, it has been suggested that the GG constitutes a previously overlooked olfactory compartment (Fuss et al. 2005; Koos and Fraser 2005; Fleischer, Hass, et al. 2006; Roppolo et al. 2006; Storan and Key 2006). This concept received substantial support from the observation that a large portion of GG neurons expresses a particular GPCR termed V2r83 that belongs to the V2R class of olfactory receptors (Fleischer, Schwarzenbacher, et al. 2006). Based on the divergent projections of GG axons to different glomeruli in the OB (Fuss et al. 2005; Koos and Fraser 2005; Roppolo et al. 2006; Storan and Key 2006), it might be assumed that V2r83 is not the only olfactory receptor expressed in the GG. However, extensive experimental approaches to assess expression of further GPCRs belonging to the olfactory receptor classes of the odorant receptors (ORs) or vomeronasal receptors (V1Rs and V2Rs) strongly indicate that ORs and V1Rs are absent from the GG of postnatal mice (Fleischer, Schwarzenbacher, et al. 2006). Moreover, even for the V2Rs, only V2r83 appears to be present in GG neurons (our unpublished observations).

Most recently, a class of GPCRs designated as trace amine–associated receptors (TAARs) has been found to be expressed in the MOE (Liberles and Buck 2006). Although TAARs were originally considered to interact with so-called trace amines—compounds with a neuromodulatory potential (Borowsky et al. 2001; Lindemann and Hoener 2005)—the recent finding that murine TAARs are predominantly expressed in defined subsets of olfactory neurons in the MOE—similar to ORs—points to the notion that they are involved in chemosensory processes (Liberles and Buck 2006). Here, we report that several TAAR subtypes are expressed in distinct subpopulations of OMP-positive GG neurons.

Materials and methods

Mice

This study was performed on mice of wild-type strains, C57/BL6J or CD1, purchased from Charles River (Sulzfeld,

Germany). All experiments comply with the *Principles of animal care*, publication no. 85-23, revised 1985, of the National Institutes of Health and with the current laws of Germany.

Tissue preparation

Heads of mice were dissected in 1× phosphate-buffered saline (0.85% NaCl, 1.4 mM KH_2PO_4, 8 mM Na_2HPO_4, pH 7.4), embedded in Leica OCT Cryocompound "tissue-freezing medium" (Leica Microsystems, Bensheim, Germany) and quickly frozen on dry ice. Sections (12 μm) were cut on a CM3000 cryostat (Leica Microsystems) and adhered to Starfrost microslides (Knittel, Braunschweig, Germany) for in situ hybridization experiments or to PALM MembraneSlides (P.A.L.M. Microlaser Technologies, Bernried, Germany) for subsequent RNA isolation.

RNA isolation and cDNA synthesis

From cryosections mounted on PALM MembraneSlides, the GG was microdissected using a PALM MicroBeam system (P.A.L.M. Microlaser Technologies). Subsequent isolation of total RNA and synthesis of first-strand cDNA were carried out as described previously (Fleischer, Schwarzenbacher, et al. 2006). For preparation of cDNA from the MOE, the MOE was dissected and total RNA was isolated with the NucleoSpin RNA II kit (Macherey-Nagel, Dueren, Germany). First-strand cDNA synthesis was carried out as for the GG (see above). Mouse genomic DNA was kindly provided by Reiner Hoppe.

Polymerase chain reaction amplification, cloning, and sequencing

Polymerase chain reaction (PCR) amplification was performed using Titanium Taq DNA polymerase (Clontech/Takara Bio Inc., Mountain View, CA). Thermal cycling (35 cycles) was carried out with the parameters recommended by the manufacturer (Clontech/Takara Bio Inc.). PCR products were cloned into pGem-T plasmid (Promega, Madison, WI) and were subjected to sequence analysis using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Dendrogram of TAARs

For the neighbor-joining dendrogram shown in Figure 1A, the deduced amino acid sequences of all intact murine TAAR-encoding genes (Lindemann et al. 2005) were aligned with ClustalW (Thompson et al. 1994). The dendrogram was then generated using the MEGA program (Kumar et al. 2001).

Design of oligonucleotide primers

To perform reverse transcriptase–PCR (RT-PCR) amplification of TAAR-encoding sequences, for each TAAR family, a specific pair of primers was designed. For families TAAR7 and TAAR8, which comprise several subtypes each, the coding nucleotide sequences of all putatively functional members (Lindemann et al. 2005) were aligned with ClustalW (Thompson et al. 1994) prior to the design of oligonucleotides matching to all relevant subtypes of family TAAR7 or TAAR8, respectively. In the following, all oligonucleotide primers are given according to the code of the International Union of Biochemistry. In primer names, "S" denotes the sense and "As" the antisense primer. To amplify sequences encoding TAARs of the different families, the following primers were used—TAAR1: TAAR1 S1 (5′-ctctggttggcaact taatag) and TAAR1 As1 (5′-gctcttctgaaccagggatag); TAAR2: TAAR2 S1 (5′-atggatcttgcccagagaatg) and TAAR2 As2 (5′-agagaaattcagaaacggatct); TAAR3: TAAR3 S1 (5′-gaagacttatccagctgtcca) and TAAR3 As1 (5′-ttgcaag tagagttgaagtac); TAAR4: TAAR4 S2 (5′-ccacttcaagcagctc cactc) and TAAR4 As1 (5′-cctgtgactatcatcctcag); TAAR5: TAAR5 S1 (5′-aggacagtccacccgctggc) and TAAR5 As1 (5′-agcaaaccagataaagatgtca); TAAR6: TAAR6 S1 (5′-cattt caagcagctgcactctc) and TAAR6 As1 (5′-gcatcaaytaatgaat caatgct); TAAR7: TAAR7 S1 (5′-ctggtgatgacatcaattcttc) and TAAR7 As1 (5′-tatggcagccatgaaaggagga); TAAR8: TAAR8 S1 (5′-acctcctggtggtgatttcagt) and TAAR8 As1 (5′-atcaaagggttcatggctgagt); and TAAR9: TAAR9 S1 (5′-gcctcgagccatcctctatg) and TAAR9 As1 (5′-gactttgccactca caataagt).

In situ hybridization

In situ hybridization and double fluorescence in situ hybridization experiments, including preparation of digoxigenin- or biotin-labeled RNA probes as well as subsequent microscopy and photography, were carried out as described previously (Schwarzenbacher et al. 2004; Fleischer, Schwarzenbacher, et al. 2006). Regarding TAAR7, based on the very high nucleotide sequence identities between the members of this family (>90%), only one antisense probe was used (generated from subtype 7D).

Quantitative analysis of in situ hybridization experiments

Hybridization with each antisense riboprobe (specific for distinct TAAR subtypes, V2r83 or OMP) was carried out on every section (12 μm thick) along the rostrocaudal axis of the GG, and the number of labeled cells was counted at high magnification. Values shown in Table 1 as well as in Figures 5 and 6 represent means of absolute cell numbers derived from 3 to 4 individuals of the same stage.

Results

As an initial step to explore whether TAARs are expressed in the GG, RT-PCR experiments were performed. Based on sequence homologies, the 16 murine genes encoding TAARs can be categorized into 9 families (TAAR1 through 9). With the exception of TAAR7 and TAAR8, these families

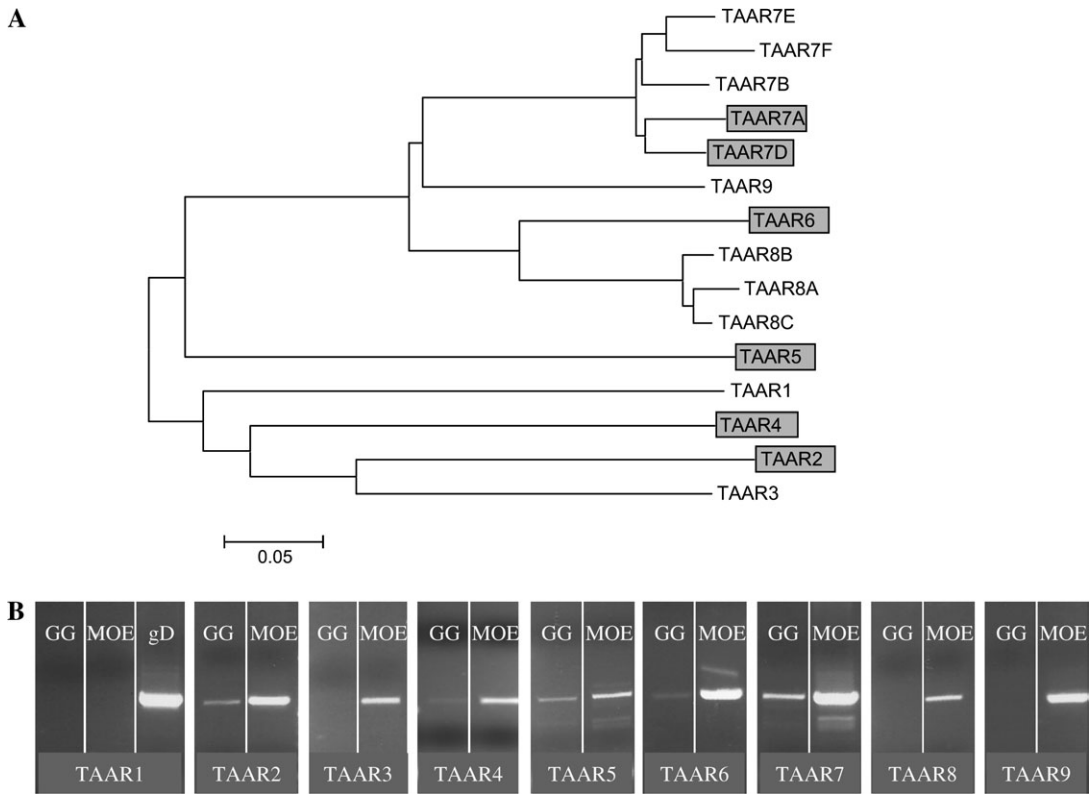

Figure 1 Unraveling the repertoire of TAARs expressed in the GG by RT-PCR. **(A)** Dendrogram of the deduced amino acid sequences of all putatively functional mouse TAARs according to Lindemann et al. (2005). TAAR7C, which is considered to be a pseudogene (Lindemann et al. 2005), has not been included. The scale bar denotes a sequence diversity of 5%. The 6 TAAR subtypes for which PCR amplification from GG cDNA of postnatal stages was obtained (see Figure 1B) are boxed. **(B)** In RT-PCR approaches with specific primer pairs for each of the TAAR families, no amplicons of the expected molecular weight were obtained from GG cDNA of several days old postnatal animals for TAAR1, TAAR3, TAAR8, and TAAR9. In control experiments, using the same primers, such PCR products were easily amplified from MOE cDNA (or genomic DNA [gD] in the case of TAAR1). With primers for TAAR2, TAAR4, TAAR5, TAAR6, and TAAR7, PCR products of the predicted size were amplified from GG and MOE cDNA, respectively.

Table 1 Number of cells expressing distinct receptor subtypes in the GG of neonates

	TAAR2	TAAR4	TAAR5	TAAR6	TAAR7	V2r83	OMP
Stained GG cells	4 (±1)	2 (±2)	4 (±1)	53 (±3)	56 (±6)	653 (±32)	785 (±10)
% of GG cells	0.5	0.3	0.5	6.8	7.1	83.2	100

Coronal sections through the GG at P0/P1 were assessed by in situ hybridization with antisense probes specific for distinct TAAR subtypes, OMP, or the vomeronasal receptor V2r83, respectively. For each riboprobe, all stained cells on every section along the rostrocaudal extent of the GG were counted; means of values derived from 3 to 4 animals are shown (the standard deviation is given). The bottom line indicates the percentage of GG cells expressing TAARs or V2r83; the number of OMP-positive cells was taken as total number of GG neurons (100%).

comprise one member only (Figure 1A). For each of these families, a specific primer pair was designed matching to all members of the corresponding family. With these primers, for TAAR2 through TAAR9, PCR products of the expected size were amplified from MOE cDNA in control experiments (Figure 1B). For TAAR1, consistent with a recent report (Liberles and Buck 2006), no amplification product was obtained from MOE cDNA with the specific primer pair; however, an amplicon of the expected size was amplified from mouse genomic DNA (Figure 1B). Using cDNA prepared from the GG of early postnatal animals as template, PCR amplification was observed for TAAR2, TAAR4, TAAR5, TAAR6, and TAAR7; for the other TAARs, no PCR products of the predicted size were obtained (Figure 1B). Subsequent cloning and sequencing of these 5 different amplicons revealed that they indeed encoded the above-mentioned TAAR subtypes. For TAAR7, a family comprising several members, only 2 different PCR products were identified—one coded for TAAR7A and the other one for TAAR7D. Thus, only a subset of the TAAR7 family members seems to be expressed in the GG; this view is supported by the finding that in PCR experiments using the same

primers and MOE cDNA as template, also other subtypes of the TAAR7 family were amplified (data not shown). Taken together, 6 different TAAR subtypes (TAAR2, TAAR4, TAAR5, TAAR6, TAAR7A, and TAAR7D) were found to be expressed in the GG of mice by RT-PCR approaches. To investigate expression of these TAAR subtypes in the GG in more detail, RNA probes were generated and used for in situ hybridization experiments. For TAAR7A and TAAR7D, based on the very high identity of their coding sequences (~93%), a riboprobe was prepared from a TAAR7D-encoding fragment only (referred to as TAAR7 probe). Performing in situ hybridization on tissue sections through the GG of neonates (postnatal day 0 or 1 [P0/P1]) with antisense probes specific for TAAR2, TAAR4, TAAR5, TAAR6, or TAAR7, subpopulations of cells were intensely stained (Figure 2). By contrast, the corresponding sense probes did not label cells in the GG (data not shown). In situ hybridization also revealed striking differences in the number of GG neurons expressing a given TAAR subtype (Figure 2). Although antisense probes for TAAR2, TAAR4, and TAAR5 stained only about 2–4 cells, probes specific for TAAR6 or TAAR7 labeled approximately 55 GG neurons per newborn animal (Table 1). By means of in situ hybridization with an OMP-specific antisense riboprobe, approximately 785 OMP-positive GG neurons were counted in neonates (Table 1). To examine whether TAARs are present in OMP-positive GG neurons, double labeling experiments were performed with probes for OMP, TAAR6, and TAAR7. These experiments demonstrated that TAARs are expressed in subsets of OMP-positive GG neurons (Figure 3A–F). To investigate whether the different TAAR subtypes are expressed in overlapping or in distinct populations of GG cells, double labeling in situ hybridization experiments were conducted. The results indicate that different TAAR subtypes are expressed in nonoverlapping subsets of GG neurons (Figure 3G–R). Thus, taken together, it can be concluded that approximately 15% of the OMP-positive GG cells in neonates express distinct TAAR subtypes (Table 1).

Recently, we have shown that the majority of OMP-positive GG neurons expresses V2R receptor subtype V2r83 as well as the G protein G_i (Fleischer, Schwarzenbacher, et al. 2006). Therefore, as a next step, attempts were made to determine whether TAARs are coexpressed with V2r83 and G_i in the

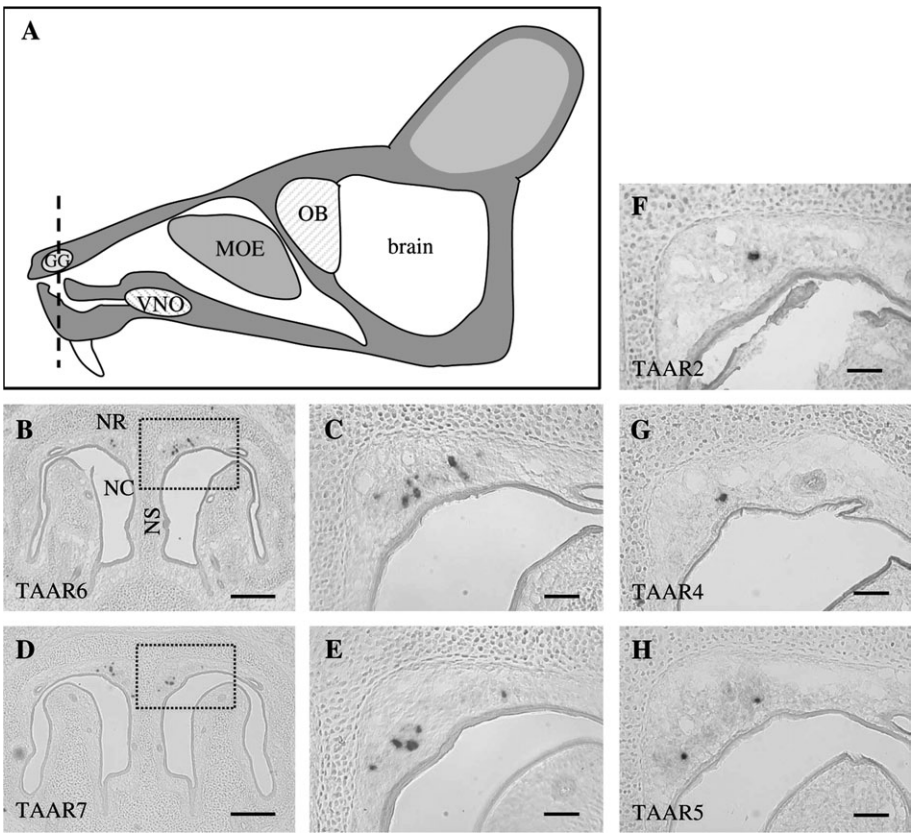

Figure 2 Topographic expression pattern of different TAAR subtypes in the GG. **(A)** Schematic representation of a sagittal section through the head of a mouse. The positions of the GG, the VNO, the MOE, and the OB are indicated. The broken line denotes the coronal section plane shown in (B–H). **(B–H)** In situ hybridization on coronal sections through the anterior nasal region of a P0 mouse with antisense probes specific for TAAR6 (B–C), TAAR7 (D–E), TAAR2 (F), TAAR4 (G), and TAAR5 (H) labels subpopulations of cells in the GG which is confined by the nasal septum (NS), the nasal cavity (NC), and the nasal roof (NR). (C, E) Higher magnification of the boxed area in B or D, respectively. Scale bars: B, D = 200 μm; C, E–H = 50 μm.

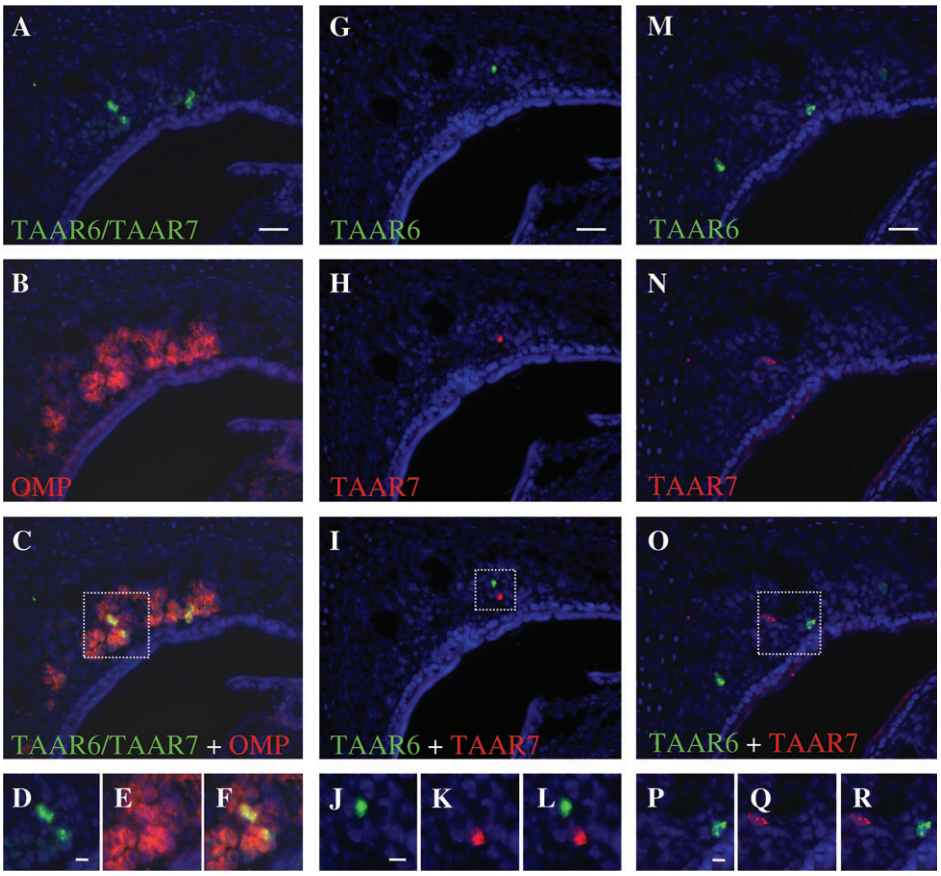

Figure 3 Expression of different TAAR subtypes in nonoverlapping subsets of OMP-positive GG neurons. **(A–C)** Double fluorescent in situ hybridization on coronal sections through the GG using antisense RNA probes for TAAR6 and TAAR7 (green) as well as OMP (red). The merged image (C) demonstrates expression of these TAAR subtypes in OMP-positive GG neurons. **(D–F)** Higher magnification of the boxed area in C. **(G–I, M–O)** Two-color in situ hybridization with antisense riboprobes for TAAR6 (green) and TAAR7 (red). The overlay (I, O) shows that these TAAR subtypes are expressed by different subsets of GG cells. **(J–L, P–R)** Higher magnification of the boxed area in (I) and (O). Sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, blue). Scale bars: A–C, G–I, M–O = 20 μm; D–F, J–L, P–R = 5 μm.

GG. In neonates, counting the total number of GG cells expressing TAARs (119) and the number of GG cells expressing V2r83 (653) revealed that the aggregate of both populations is almost identical to the number of OMP-positive GG neurons (785; Table 1). This observation suggests that TAARs and V2r83 are expressed in different GG cell types. In fact, subsequent double labeling in situ hybridization with probes for V2r83 as well as for TAAR6 and TAAR7 confirmed this notion: no indication for coexpression of TAARs and V2r83 was found (Figure 4A–L), suggesting that V2r83 and TAARs are expressed by nonoverlapping subsets of GG cells. Double staining experiments with probes for TAARs as well as for the G protein G_i revealed that TAAR-positive GG cells coexpress G_i (Figure 4M–R). Based on these observations, it can be concluded that besides the V2r83-positive cells, the GG comprises a second population of neurons that expresses TAARs; both populations express the G protein G_i.

Following the time course of TAAR expression during postnatal development, it was found that the number of TAAR-positive GG neurons was remarkably reduced in 6- to 8-week-old adult mice, in which we observed a total of only approximately 33 TAAR-expressing GG cells compared with approximately 119 in neonates (Figures 5 and 6M). By contrast, the number of OMP-positive GG neurons in adults (~688 cells per individual) versus neonates (~785 cells) was decreased by about 12% only (Figure 6M). To determine when this reduction in the number of TAAR-positive GG cells occurs during postnatal life, expression of TAARs in the GG of individuals from postnatal day (P7) was examined by in situ hybridization. In these animals, a total of approximately 42 TAAR-positive cells were counted (Figures 5 and 6M), indicating that the low number of TAAR-expressing GG neurons in adults was already reached at stage P7. To monitor TAAR expression during prenatal development, embryonic stage E17.5 (=1.5 days prior to birth) was chosen because the GG cannot be unambiguously identified before E15 to E16 (Fuss et al. 2005; Fleischer, Hass, et al. 2006). At E17.5, by in situ hybridization experiments, the number of TAAR-expressing cells was

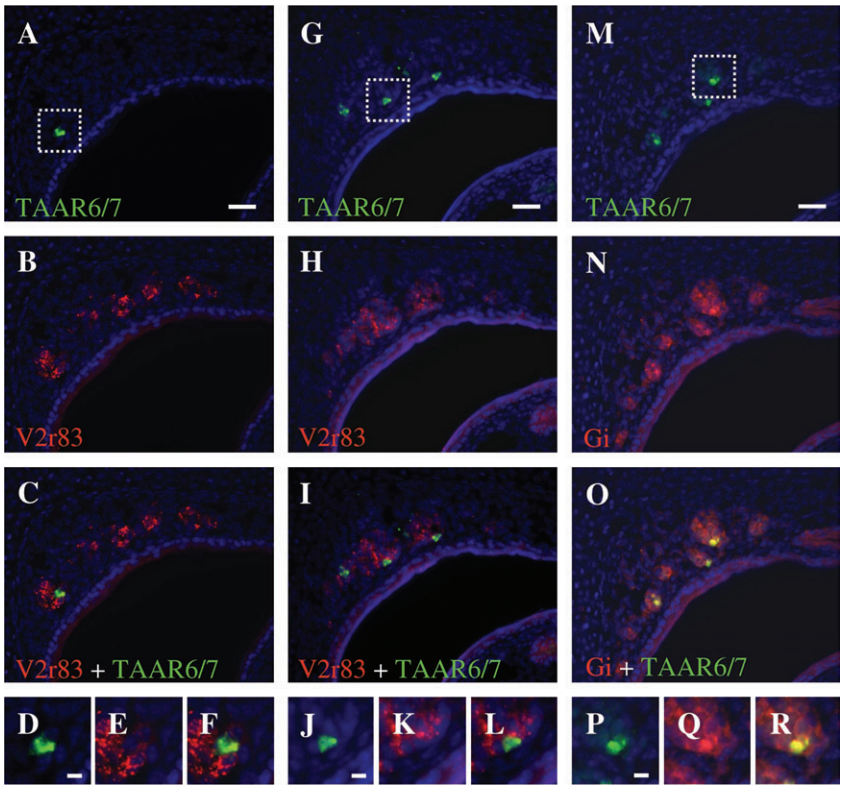

Figure 4 TAAR-expressing GG neurons are distinct from V2r83-positive cells but coexpress the signaling element G_i. **(A–C, G–I)** Double fluorescent in situ hybridization on coronal sections revealed that GG neurons stained by a mixture of RNA antisense probes for TAAR6 and TAAR7 (green) were not labeled by a V2r83-specific riboprobe (red). **(D–F, J–L)** Higher magnification of the boxed area in (A) and (G). **(M–O)** GG neurons stained by a mixture of RNA antisense probes for TAAR6 and TAAR7 (green) hybridized to a G_i-specific probe (red). **(P–R)** Higher magnification of the boxed area in (M). Sections were counterstained with 4′,6-diamidino-2-phenylindole (blue). Scale bars: A–C, G–I, M–O = 20 μm; D–F, J–L, P–R = 5 μm.

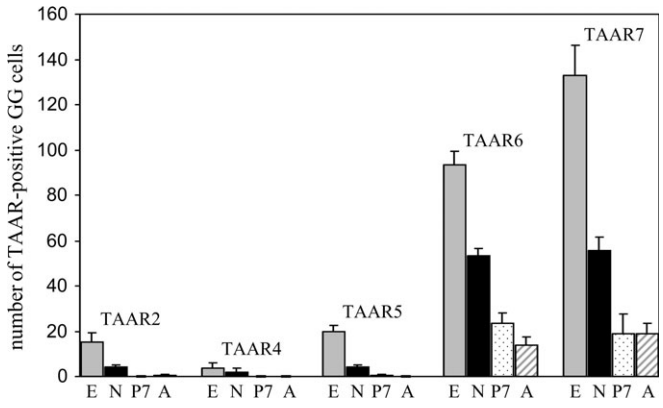

Figure 5 Age-dependent expression of TAARs in the GG. The number of GG cells expressing distinct TAAR subtypes as determined by in situ hybridization is comparatively high in the late embryonic stage E17.5 (E, gray bars) before undergoing a considerable and continuous reduction in neonates (N, P0/P1, black bars), youngsters (P7, dotted bars), and adults (A, 6–8 weeks old, striped bars). The *y* axis indicates the number of GG cells per animal expressing a given TAAR subtype. Data are means of 3–4 individuals (the standard deviation is given).

determined as 265, thus, clearly higher than at postnatal stages (Figures 5 and 6M). In summary, these findings indicate that the high number of GG neurons expressing TAARs during the late prenatal phase declines by about 55% in neonatal and by about 88% in adult animals (Figure 6M). To approach the question whether a predominantly prenatal expression is also realized for V2r83 receptor, the number of V2r83-positive GG neurons was determined at various stages by in situ hybridization. The results indicate that the number of V2r83-expressing cells in the GG is also high in perinatal stages and significantly reduced in adults (Figure 6M). In contrast to TAARs, the number of V2r83-expressing neurons seems to peak at birth (Figure 6I–M). In this regard, it is important to note that the total number of GG neurons—as determined by in situ hybridization using an OMP-specific antisense riboprobe—also declines, however, with a much more moderate rate (Figure 6M). Therefore, in adults, the number of OMP-positive GG neurons (~688 cells) is almost 5 times larger than the total number of GG cells expressing V2r83 or TAAR subtypes (~144 cells), respectively.

Discussion

Based on expression of the OMP and axonal projection to the OB, the GG in the vestibule of the nasal cavity is considered as an olfactory subsystem although it apparently lacks

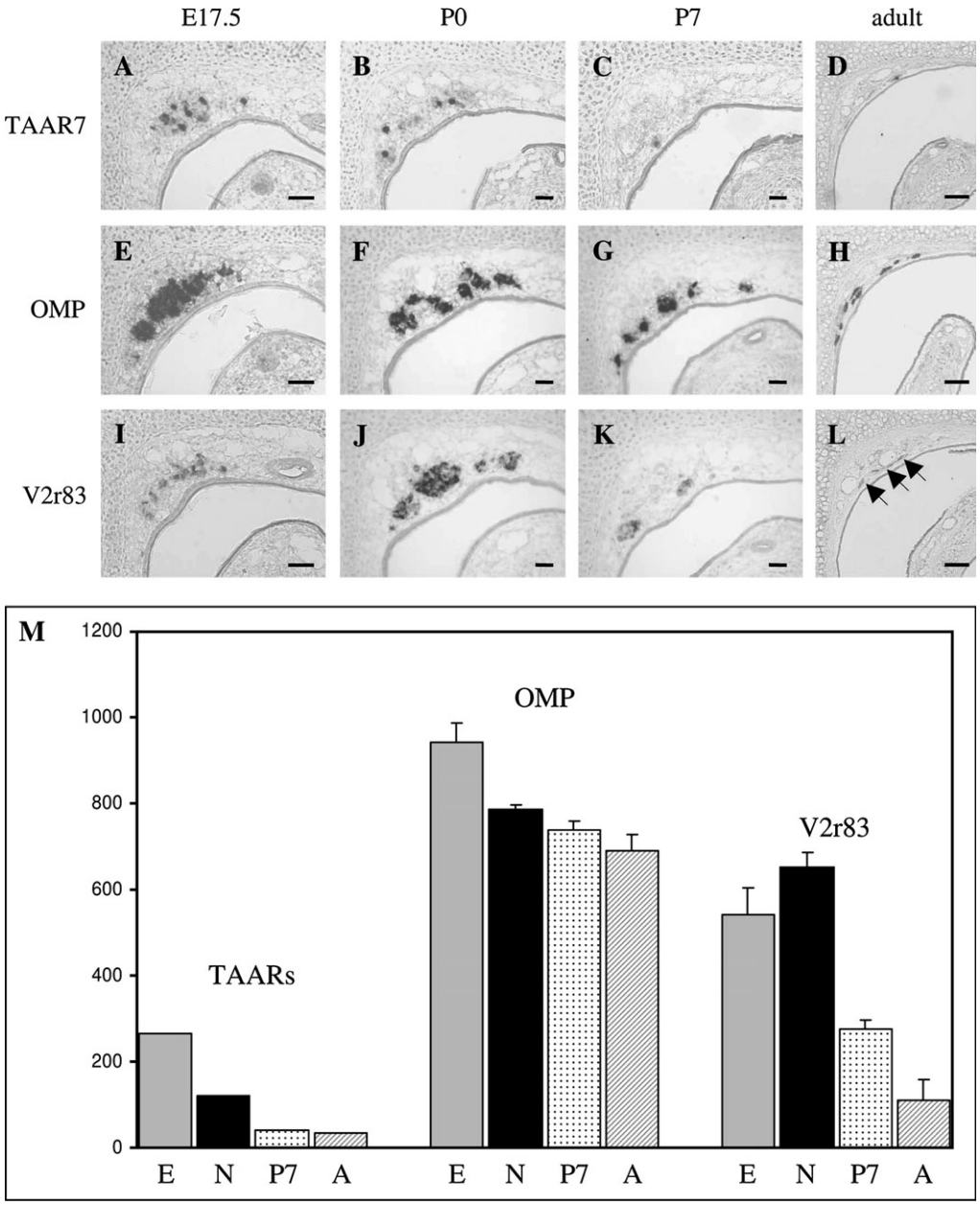

Figure 6 Expression levels of OMP and V2r83 in the GG vary between different stages. **(A–L)** Time course of TAAR7, OMP, and V2r83 expression analyzed by in situ hybridization on coronal sections through the GG at different stages. For TAARs (as exemplarily depicted for TAAR7 in A–D) and OMP (E–H), expression peaks in late embryonic stages (A, E). By contrast, for V2r83 (I–L), the highest level of expression was observed in neonates (J). In adults—consistent with our previous observations (Fleischer, Hass, et al. 2006)—the GG cells are not arranged any more in ganglia-shaped clusters but form a rather thin layer instead (H, L; the V2r83-positive cells in L are indicated by arrows). Moreover, the extent of the GG in the anterior–posterior axis is increased in adults; thus, GG cells are found on a larger number of consecutive coronal sections. Please note the varying scale bars. **(M)** Quantification of GG cells expressing TAARs, OMP, or V2r83, respectively. The *y* axis denotes the number of GG cells per animal expressing TAARs, OMP, or V2r83 as determined by in situ hybridization for stage E17.5 (E, gray bars) as well as for neonates (N, postnatal day 0 or 1, black bars), youngsters (P7, dotted bars), and adults (A, 6 to 8 weeks old, striped bars). For TAARs, the numbers represent the aggregate of TAAR-positive GG cells as derived from the numbers displayed in Figure 5; therefore, no standard deviation (SD) is given. For OMP and V2r83, data are means of 3 individuals (the SD is indicated). Scale bars: A–C, E–G, I–K = 20 μm; D, H, L = 50 μm.

direct access to the lumen of the nasal cavity (Fuss et al. 2005; Koos and Fraser 2005; Fleischer, Hass, et al. 2006; Roppolo et al. 2006; Storan and Key 2006). This concept received substantial support from the finding that V2r83, a member of the V2R receptor family, is expressed in GG neurons (Fleischer, Schwarzenbacher, et al. 2006). In search for further olfactory receptor types in GG neurons, potential expression of TAARs was probed due to the recent finding that TAARs are a novel group of olfactory receptors (Liberles and Buck 2006). The results of the present study demonstrate that

several TAAR subtypes are indeed expressed in defined subsets of GG neurons. Thus, in spite of its small size, the GG appears to be composed of different populations of cells: although a large subpopulation of GG neurons expresses receptor V2r83, which is also present in the VNO (Fleischer, Schwarzenbacher, et al. 2006), other GG neurons express distinct TAAR receptor types that are absent from the VNO but present in subsets of cells in the MOE (Liberles and Buck 2006). Recent studies indicate that V2R-expressing cells in the VNO respond to peptides and/or small proteins and that the predicted ligand binding domains of V2Rs are tuned to interact with α-carboxyl/amino groups, characteristic of amino acids, peptides, and proteins (Leinders-Zufall et al. 2004; Kimoto et al. 2005; Silvotti et al. 2005). TAAR receptors are activated by small volatile amines, and although some of their identified ligands are derivatives of certain amino acids, TAARs are not activated by these amino acids (Liberles and Buck 2006). Thus, a picture emerges, suggesting that the V2R receptor and the TAAR receptors expressed in the GG have a nonoverlapping but rather complementary spectrum of ligands. Therefore, it can be assumed that expression of TAARs in the GG does not only render the receptor repertoire more complex than initially thought but also increases the spectrum of ligands that may be perceived by the GG. The observation that only one receptor type appears to be expressed in a given GG neuron suggests that each cell is specifically tuned to a presumably limited subset of ligands, implying that the GG—similar to other olfactory subcompartments—does not only enable detection of divergent chemical compounds but also allows to discriminate between them. This notion is also supported by the finding that cells in the GG project their axons to a series of different glomeruli in the OB (Fuss et al. 2005; Koos and Fraser 2005; Roppolo et al. 2006; Storan and Key 2006).

In view of the widespread distribution of TAARs in sensory neurons of the MOE (Liberles and Buck 2006), it is unclear why they are also expressed in the GG. This is reminiscent of the SO that expresses OR subtypes also found in the MOE (Kaluza et al. 2004; Tian and Ma 2004). Concerning the septal organ, a few glomeruli in the OB are innervated exclusively by axons from neurons in the septal organ, whereas cells in the MOE with the same OR types project to other glomeruli (Levai and Strotmann 2003). Accordingly, it is conceivable that axons from GG neurons expressing TAARs may project to glomeruli in the OB that are different from glomeruli innervated by TAAR-expressing cells in the MOE. As a consequence, responses from TAAR-positive cells in the GG versus the MOE may be differently processed in higher brain centers.

The observation that the number of TAAR-expressing GG cells was much higher during the perinatal phase of development than in adults suggests that the GG may be particularly relevant for perinatal stages. This view is supported by the finding that the number of V2r83-expressing cells as well as the number of OMP-positive neurons in the GG was also particularly high in perinatal animals. Due to this developmental expression pattern, it can be speculated that the GG neurons expressing TAARs or V2r83 may be important for interactions between the pup and its mother because newborn nestlings are almost exclusively in contact with their mother. Therefore, it is conceivable that the GG might be associated with behaviors such as nipple finding, induction of suckling, or filial imprinting. This notion receives support by GG axonal projection to so-called ''necklace glomeruli'' (Fuss et al. 2005; Koos and Fraser 2005; Roppolo et al. 2006; Storan and Key 2006) because previous studies have indicated that at least some necklace glomeruli are involved in suckling behavior, a pheromone-induced response of rodent pups (Teicher et al. 1980; Greer et al. 1982). However, the relevance of the GG may not be confined to these early stages: as the number of OMP-expressing GG cells remains comparatively high during postnatal life, even adults are endowed with a robust GG. Moreover, the morphology of the GG undergoes a significant change during postnatal development: although in perinatal stages, the GG neurons are arranged in ganglion-like structures, in adults, they form a filiform layer underlying the thin epithelium (Figure 6E–L) (see also Fleischer, Hass, et al. 2006). These obvious morphological changes suggest that the GG might also be of relevance for adults; however, the functional implications of these anatomical rearrangements remain elusive.

The striking decline in the number of GG neurons expressing TAARs or V2r83 in postnatal stages could either be due to cell death or termination of receptor expression. Based on the observation that GG neurons are obviously not replaced by newly generated cells (Fuss et al. 2005; Ropollo et al. 2006), the only slight reduction in the number of OMP-positive GG cells suggests that GG neurons terminate expression of TAARs and V2r83 or reduce it to a level that can no longer be detected by analytical procedures.

Acknowledgements

We would like to thank Kerstin Bach, Maria-Isabell Burry, and Selina Kern for excellent technical assistance. We are indebted to Reiner Hoppe for providing mouse genomic DNA and Jürgen Krieger for help with the MEGA program. This work was supported by the Deutsche Forschungsgemeinschaft.

References

Borowsky B, Adham N, Jones KA, Raddatz R, Artymyshyn R, Ogozalek KL, Durkin MM, Lakhlani PP, Bonini JA, Pathirana S, et al. 2001. Trace amines: identification of a family of mammalian G protein-coupled receptors. Proc Natl Acad Sci USA. 98:8966–8971.

Fleischer J, Hass N, Schwarzenbacher K, Besser S, Breer H. 2006. A novel population of neuronal cells expressing the olfactory marker protein (OMP) in the anterior/dorsal region of the nasal cavity. Histochem Cell Biol. 125: 337–349.

Fleischer J, Schwarzenbacher K, Besser S, Hass N, Breer H. 2006. Olfactory receptors and signalling elements in the Grueneberg ganglion. J Neurochem. 98:543–554.

Fuss SH, Omura M, Mombaerts P. 2005. The Grueneberg ganglion of the mouse projects axons to glomeruli in the olfactory bulb. Eur J Neurosci. 22:2649–2664.

Greer CA, Stewart WB, Teicher MH, Shepherd GM. 1982. Functional development of the olfactory bulb and a unique glomerular complex in the neonatal rat. J Neurosci. 2:1744–1759.

Kaluza JF, Gussing F, Bohm S, Breer H, Strotmann J. 2004. Olfactory receptors in the mouse septal organ. J Neurosci Res. 76:442–452.

Kimoto H, Haga S, Sato K, Touhara K. 2005. Sex-specific peptides from exocrine glands stimulate mouse vomeronasal sensory neurons. Nature. 437:898–901.

Koos DS, Fraser SE. 2005. The Grueneberg ganglion projects to the olfactory bulb. Neuroreport. 16:1929–1932.

Kumar S, Tamura K, Jakobsen IB, Nei M. 2001. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics. 17:1244–1245.

Leinders-Zufall T, Brennan P, Widmayer P, Chandramani PS, Maul-Pavicic A, Jager M, Li XH, Breer H, Zufall F, Boehm T. 2004. MHC class I peptides as chemosensory signals in the vomeronasal organ. Science. 306:1033–1037.

Levai O, Strotmann J. 2003. Projection pattern of nerve fibers from the septal organ: DiI-tracing studies with transgenic OMP mice. Histochem Cell Biol. 120:483–492.

Liberles SD, Buck LB. 2006. A second class of chemosensory receptors in the olfactory epithelium. Nature. 442:645–650.

Lindemann L, Ebeling M, Kratochwil NA, Bunzow JR, Grandy DK, Hoener MC. 2005. Trace amine-associated receptors form structurally and functionally distinct subfamilies of novel G protein-coupled receptors. Genomics. 85:372–385.

Lindemann L, Hoener MC. 2005. A renaissance in trace amines inspired by a novel GPCR family. Trends Pharmacol Sci. 26:274–281.

Roppolo D, Ribaud V, Jungo VP, Luscher C, Rodriguez I. 2006. Projection of the Gruneberg ganglion to the mouse olfactory bulb. Eur J Neurosci. 23:2887–2894.

Schwarzenbacher K, Fleischer J, Breer H, Conzelmann S. 2004. Expression of olfactory receptors in the cribriform mesenchyme during prenatal development. Gene Expr Patterns. 4:543–552.

Silvotti L, Giannini G, Tirindelli R. 2005. The vomeronasal receptor V2R2 does not require escort molecules for expression in heterologous systems. Chem Senses. 30:1–8.

Storan MJ, Key B. 2006. Septal organ of Gruneberg is part of the olfactory system. J Comp Neurol. 494:834–844.

Teicher MH, Stewart WB, Kauer JS, Shepherd GM. 1980. Suckling pheromone stimulation of a modified glomerular region in the developing rat olfactory bulb revealed by the 2-deoxyglucose method. Brain Res. 194:530–535.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673–4680.

Tian H, Ma M. 2004. Molecular organization of the olfactory septal organ. J Neurosci. 24:8383–8390.

Accepted April 4, 2007