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Extrasensory perception: Odorant and taste receptors beyond the nose and mouth

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ABSTRACT

G protein-coupled receptors (GPCRs) represent the largest family of transmembrane receptors and are prime therapeutic targets. The odorant and taste receptors account for over half of the GPCR repertoire, yet they are generally excluded from large-scale, drug candidate analyses. Accumulating molecular evidence indicates that the odorant and taste receptors are widely expressed throughout the body and functional beyond the oronasal cavity — with roles including nutrient sensing, autophagy, muscle regeneration, regulation of gut motility, protective airway reflexes, bronchodilation, and respiratory disease. Given this expanding array of actions, the restricted perception of these GPCRs as mere mediators of smell and taste is outdated. Moreover, delineation of the precise actions of odorant and taste GPCRs continues to be hampered by the relative paucity of selective and specific experimental tools, as well as the lack of defined receptor pharmacology. In this review, we summarize the evidence for expression and function of odorant and taste receptors in tissues beyond the nose and mouth, and we highlight their broad potential in physiology and pathophysiology.

Contents

Abbreviations: 16HBE cells, human bronchial epithelial cells; 3T3-L1 cells, mouse embryonic fibroblast (undifferentiated preadipocyte) cells; 7TM, seven transmembrane; ABCB1, ATP-binding cassette, sub-family B (MDR/TAP), member 1 (*ABCB1*); AC3, adenylyl cyclase type III (*ADYC3*); ASM, airway smooth muscle; BKCa, potassium large conductance calcium-activated channel, subfamily M, alpha member 1 (*KCNMA1*); BMSCs, bone marrow stromal cells; Caco-2 cells, human epithelial colorectal adenocarcinoma cells; cAMP, cyclic adenosine monophosphate; CCK, cholecystokinin; CNG, cyclic nucleotide-gated channel; DU145 cells, human prostate cancer cells; ELISA, enzyme-linked immunosorbent assay; EST, expressed sequence tag; FFA, free fatty acid; $G\alpha_{t3}$, G protein, alpha transducing 3 (*GNAT3*) or gustducin; $G\alpha_{olf}$, G protein, alpha activating activity polypeptide, olfactory type (*GNAL*); GIV3727, T2R competitive antagonist (4-(2,2,3-trimethylcyclopentyl) butanoic acid); GLP-1, glucagon-like peptide-1; GLUT2, solute carrier family 2 (facilitated glucose transporter), member 2 (*SLC2A2*); GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; H9C2 cells, rat cardiac myoblast cells; HCT116 cells, human colon carcinoma cells; HeLa cells, human cervical cancer cells; Hu-Tu 80 cells, human duodenum adenocarcinoma cells; HuCCT1 cells, human bile duct carcinoma cells; IHC, immunohistochemistry; IP_3R3, inositol 1,4,5-trisphosphate receptor, type 3; ISH, in situ hybridization; MIN6 cells, mouse insulinoma-derived pancreatic β-cells; N38 cells, embryonic mouse hypothalamic cell line N38; NB, northern blot; NCI-H716 cells, human enteroendocrine cells; OR, odorant (or olfactory) receptor; OSN, olfactory sensory neuron; PASMCs, pulmonary artery smooth muscle cells; RGS proteins, regulators of G protein signaling proteins; RT-PCR, reverse transcription-polymerase chain reaction; PepT1, solute carrier family 15 (oligopeptide transporter), member 1 (*Slc15a1*); PKA, cyclic AMP-dependent protein kinase A; PLCβ2, phospholipase C, beta 2; SGLT1, sodium/glucose co-transporter 1; siRNA, small interfering RNA; SNP, single nucleotide polymorphism; STC-1 cells, mouse intestine enteroendocrine cell line; T1R, taste receptor type 1; *TAS1*, taste receptor type 1 gene; T2R, taste receptor type 2; *TAS2*, taste receptor type 2 gene; Tg, transgenic (or gene-targeted mice) mice; TRPM5, transient receptor potential cation channel, subfamily M, member 5; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; VSMCs, vascular smooth muscle cells; WB, western blot.

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1. Introduction

G protein-coupled receptors (GPCRs) are seven transmembrane-spanning proteins that represent the largest receptor superfamily in the human genome (Lagerstrom & Schioth, 2008). GPCRs recognize and bind an array of sensory inputs and ligands, including photons, ions, bioamines, lipids, carbohydrates, peptides and proteins, as well as a diverse range of volatile compounds. Ligand-induced activation of GPCRs converts extracellular stimuli into intracellular signals, mediating diverse cellular and physiological responses, including the senses of smell, taste, and vision. Not surprisingly, mutations and modifications of GPCRs, G proteins and their regulatory partners are linked to dysfunction and disease (Drews, 2000; Hopkins & Groom, 2002; Wettschureck & Offermanns, 2005; Overington et al., 2006), and the importance of these receptors is reflected in the fact that 40% of drugs on the market target GPCRs.

The pioneering work of Buck and Axel identified that the sense of smell was mediated by a large family of GPCRs located in the olfactory epithelium (Buck & Axel, 1991). Indeed, with the sequencing of mammalian genomes, it is now clear that there are around 900 odorant GPCRs in humans (including pseudogenes) and ~1500 in rodents — these are by far the most prevalent subgroup of GPCRs in the vertebrate genomes (representing 3–5% of all encoded genes). In humans, there are 390 *bona fide* protein-coding odorant receptor genes (Olender et al., 2008), comprising the majority of the *Rhodopsin*/family A GPCRs. The dramatic expansion of the olfactory receptor gene family since the mammalian radiation, resulting from multiple gene duplications from a common primordial ancestor gene, points to the importance of odor discrimination in evolution and survival (Dryer, 2000).

The mammalian gustatory system is generally categorized into five basic taste qualities: sweet, umami, bitter, salty and sour, which together enable the assessment of nutritional value of food constituents. In the past two decades, the molecular mediators of sweet, umami and bitter tastes have been identified as families of GPCRs (referred to collectively as taste receptor type 1, T1R and taste receptor type 2, T2R).[1] The T1R family has 3 members within the *Glutamate*/family C GPCR group that form sweet and umami receptors, whereas the T2R family consists of 25 highly divergent GPCRs that mediate bitter taste. In addition, there is evidence that another taste quality related to lipid sensing is mediated via the free fatty acid (FFA) GPCR family.

Contemporaneously with the initial discovery of the GPCR mediators of olfaction and gustation, reports began to appear in the literature of odorant and taste receptor expression in tissues beyond the nose and mouth. These have predominantly been descriptive studies, for the most part relying on RT-PCR and microarray data without demonstrating either protein expression or function. However, they raised the intriguing possibility that these so-called 'chemosensory' GPCRs may subsume additional functions in multiple tissues. More recently, the field has advanced at pace with publications appearing on the function of odorant and taste receptors in the brain, skeletal muscle, the gastrointestinal tract and in the airways. These studies reinforce the idea that there remains novel and important biology to be discovered for these receptor families, with broader potential ramifications beyond the fragrance and food industries (Huang, 2005; Lagerstrom & Schioth, 2008).

In this review, we summarize the prevailing evidence for the expression of odorant and taste GPCRs in cells and tissues beyond the nose and mouth. We then highlight the putative function for these receptors in diverse physiological settings, ranging from nutrient sensing, autophagy, muscle regeneration, and regulation of gut motility to protective airway reflexes, bronchodilation and respiratory dysfunction and disease. We identify limitations in the field and discuss the currently available molecular and pharmacological toolkit for further investigation of these GPCRs in the nonchemosensory settings. Finally, we speculate on the widespread nature of the phenomenon and offer insights/predictions into the potential therapeutic utility for these GPCRs.

2. Odorant and taste GPCRs

2.1. The 'chemosensory' receptors comprise the largest GPCR families

The capacity to sense and respond to chemicals and factors in the surrounding environment is essential for life — for example, chemotaxis in simple organisms, such as the slime mold *Dictyostelium*; chemosensation in the worm *Caenorhabditis elegans*; and complex olfaction and taste in insects, fish, amphibians, reptiles, birds and mammals. These complex chemosensory systems enable the detection and discrimination of molecules of immense diversity, and provide the fundamental means to locate nutritious food and suitable mating partners and to avoid predators or ingesting toxic substances. In vertebrates, it is the olfactory and gustatory systems that facilitate the sensing of chemicals in the extracellular environment via large families of seven-transmembrane receptors.

There are six multigene families that are generally considered as 'chemosensory' GPCRs in the vertebrate genome: odorant receptor (OR), taste receptor types 1 and 2 (T1R and T2R), trace amine-associated receptor (TAAR) and vomeronasal receptor types 1 and 2 (V1R and V2R). The TAAR, V1R and V1R families encode pheromone GPCRs and are beyond the scope of this review (see Dulac & Axel, 1995; Herrada & Dulac, 1997; Liberles & Buck, 2006). However, the umbrella term 'chemosensory' can be misleading (and we will avoid using it) as the fundamental role of any GPCR/receptor is to sense chemicals in the extracellular environment. The term also evokes the narrow connotation for the function of the odorant/taste receptor families purely in the sensation of taste and smell, which is increasingly unlikely. In this review, we provide a more expansive conceptualization of the odorant and taste receptor genes, consistent with their broader functions in mammalian biology.

Accounting for more than half of the vertebrate GPCR repertoire, odorant and taste GPCRs represent two of the most heterogeneous and diverse of receptor families. These highly variable families arose from repeated random gene duplications of an ancestral gene or gene cluster, followed by adaptive and neutral mutations (Lancet & Ben-Arie, 1993; Dryer, 2000). This expansion explains the large differences in odorant and taste receptor repertoire between species, as well as the copy-number variation in these receptor genes within species (Nei et al., 2008). The genetic variation in these receptors also correlates with differences in human odor and taste perception/discrimination (Sandell & Breslin, 2006; Keller et al., 2007). In fact, odorant and taste receptor genes are among the most rapidly evolving and positively selected genes that are identified in comparative genomic analyses (Clark et al., 2003; Nielsen et al., 2005; Kosiol et al., 2008). This is undoubtedly a reflection on the need for organisms to respond and adapt to a variable chemical environment, but it provides an additional layer of intrigue when one considers the expression and function of these odorant and taste GPCRs beyond the classical cephalic sensory systems.

Although a comprehensive review of olfaction and taste is beyond the scope of this work, many excellent reviews can be found elsewhere (for olfaction/odorant receptors see (Mombaerts, 2004a; Nei et al., 2008; Spehr & Munger, 2009; Su et al., 2009; Touhara & Vosshall, 2009) and for taste (Chandrashekar et al., 2006; Bachmanov & Beauchamp, 2007; Yarmolinsky et al., 2009; Behrens & Meyerhof, 2011; Finger & Kinnamon, 2011)).

2.2. Odorant receptors and signaling

2.2.1. Odorant receptor genes

The OR family was originally identified based on sequence homology and the presence of conserved motifs within 7TM-spanning domains

[1] The receptor and gene nomenclature in this review where possible follows the guidelines set out by BPS/NC-IUPHAR (Alexander et al., 2011), except for the odorant GPCRs, which follow the HUGO and Mouse Genome Database (MGD) gene nomenclature (Eppig et al., 2012; HUGO Gene Nomenclature Committee (HGNC), 2013).

(Buck & Axel, 1991). ORs are single intron genes of around 1 kb in length and characteristically possess a hypervariable sequence region in the transmembrane segments, which constitutes the putative odorant binding pocket (Lagerstrom & Schioth, 2008). There are 390 functional ORs in humans (Olender et al., 2008) and ~1500 in rodents, with hundreds of additional putatively non-functional pseudogenes (Quignon et al., 2005; Nei et al., 2008).

The OR genes are distributed throughout the genome, residing in clusters of various sizes on nearly all chromosomes (except 20 and Y). ORs are highly variable at a sequence level, and are further grouped into 18 families and hundreds of subfamilies defined by their amino-acid identity (Lancet & Ben-Arie, 1993; Olender et al., 2008). While forming the largest group of GPCRs, the OR repertoire is still small in comparison to the number of potential odorants. To some extent, this is explained by the capacity of ORs to detect odorants in a 'combinatorial' manner, whereby one OR recognizes multiple odorants and one odorant is detected by multiple ORs (Malnic et al., 1999). Nevertheless, the vast majority of ORs are orphans, as the ligand(s) that bind and activate the receptor are not known. In fact, despite an enormous amount of research aimed at establishing ligand–OR pairs, only around 100 vertebrate ORs have been effectively deorphanized (Saito et al., 2009) (also reviewed in Mombaerts, 2004a). Beyond the obvious difficulties that this creates for the pharmacological description of these receptors in the non-olfactory setting, it may indicate that potential endogenously produced ligands exist (see Section 5 for further discussion).

2.2.2. Olfactory signal transduction

The identification of a multigene family of odorant GPCRs (Buck & Axel, 1991) unified the concept of a highly specialized olfactory signal transduction pathway (Fig. 1). This canonical pathway involves the coupling of odorant-bound receptor to a single type of G protein termed $G\alpha_{olf}$ (*GNAL*) (Jones & Reed, 1989) and activation of an olfactory isoform of adenylyl cyclase (AC3 or *ADCY3*) to generate the second messenger cyclic adenosine monophosphate (cAMP) (Sklar et al., 1986; Bakalyar & Reed, 1990). Elevated levels of cellular cAMP in turn activate cation-selective cyclic nucleotide-gated channels (CNG) that lead to increased permeability to Ca^{2+} ions (Dhallan et al., 1990), membrane depolarization and the generation of action potentials in olfactory neurons. Knockout studies later provided elegant genetic corroboration of the components of the olfactory pathway, as mice lacking each of $G\alpha_{olf}$ (Belluscio et al., 1998), AC3 (Wong et al., 2000) and CNG (Brunet et al., 1996) displayed profound anosmic phenotypes. More recently, a potassium-dependent Na^+/Ca^{2+} exchanger (NCKX4) has been implicated in shaping olfactory responses (Stephan et al., 2012), whereas the role and molecular identity of an olfactory Ca^{2+}-activated Cl^- channel have remained controversial (Billig et al., 2011).

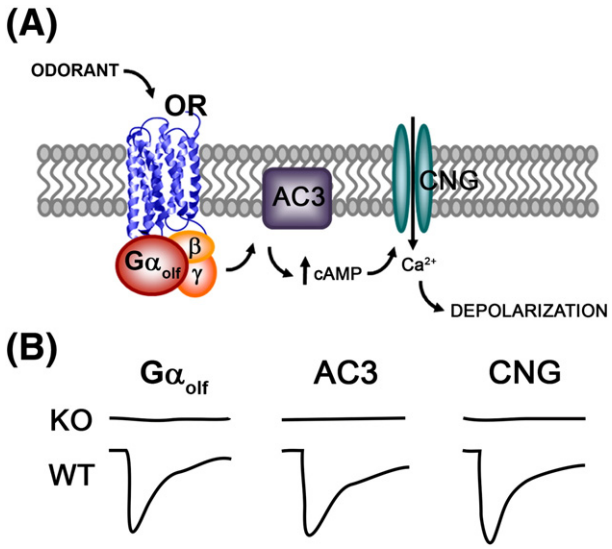

Fig. 1. The GPCR-mediated olfactory signaling cascade is highly specialized in the olfactory epithelium. **A.** The olfactory signaling pathway involves the coupling of ligand bound receptors to the olfactory isoform of the heterotrimeric G protein $G\alpha_{olf}$ and the activation of adenylyl cyclase (AC3) to generate cAMP as a second messenger. This leads to influx of Ca^{2+} via cyclic-nucleotide gated channels (CNG), membrane depolarization and the generation of action potentials in neurons. **B.** Knockout studies support the necessity of $G\alpha_{olf}$, AC3 and CNG in olfactory signaling, as seen in odorant-induced electroolfactogram responses.
Adapted with permission from Brunet et al. (1996), Wong et al. (1996), and Belluscio et al. (1998). GPCR ribbon structure modified from Pin et al. (2009).

2.3. Taste receptors and signaling

The sense of taste provides the ability to appraise potential food sources prior to ingestion. Aversive bitter and sour tastes can indicate the presence of toxins, strong acids and spoiled food; salty tastes usually imply the presence of sodium ions and ensure proper dietary electrolyte balance, whereas the appetitive tastes of sweet and umami (savory) signify energy-rich nutrients, including carbohydrates and amino acids (Bachmanov & Beauchamp, 2007). The presence of dietary lipids and free fatty acids is also perceived as fat taste, a perennially controversial research area that has only recently become accepted as an additional taste quality (Mattes, 2011). Although this repertoire of taste sensations seems modest, especially in contrast to the complexity of the olfactory system, it has satisfactorily accommodated the need to identify and distinguish key dietary components (Chandrashekar et al., 2006).

2.3.1. Taste receptor genes

In research conducted in the past two decades, the molecular candidates for the six (including fat taste) basic taste modalities and their associated signaling partners have been identified, with sweet, umami, bitter and fat taste mediated by GPCRs (Sawzdargo et al., 1997; Adler et al., 2000; Chandrashekar et al., 2000; Nelson et al., 2001, 2002). Comprising three members from within the *Glutamate*/family C group, the taste receptor type 1 (T1R; genes are designated *TAS1* in humans and *Tas1* in rodents) family forms heteromeric complexes to mediate sweet (T1R2–T1R3) and umami (T1R1–T1R3) tastes. The T1Rs are highly conserved and bind a range of sugars, artificial sweeteners and amino acids. Other amino acid sensing receptors have been identified in lingual tissue, including the metabotropic glutamate receptors ($mGlu_1$ and $mGlu_4$) (Chaudhari et al., 2000; Kusuhara et al., 2013), the calcium-sensing receptor (CaS) and GPRC6A, although their precise role in taste perception remains unclear (Wellendorph et al., 2009; Bystrova et al., 2010).

In contrast, the taste receptor type 2 (*TAS2*) gene family encodes a group of ~25 highly divergent GPCRs (designated T2Rs) that detect and respond to an incredibly broad range of structurally diverse aversive and toxic compounds (Meyerhof et al., 2010). These receptors are single exon genes that characteristically possess very short N-terminal extracellular domains. However, the *TAS2* receptors have been somewhat elusive in their classification, given that they lack most of the conserved features of other *Rhodopsin*/family A receptors, and have been designated as a distinct cluster of the *Frizzled/Taste 2* GPCR superfamily (Lagerstrom & Schioth, 2008).

The genes encoding free fatty acid receptors (FFA_{1-4}), originally termed *GPR40*, *GPR43*, *GPR41* and *GPR120*, respond variously to short, medium and long-chain fatty acids. Within the taste research community, several recent publications have reinforced long standing anecdotal evidence that FFAs are the principal mediators of fat taste (Cartoni et al., 2010; Galindo et al., 2012). In recent years, there has also been considerable interest in FFA pharmacology and function due to the wide range of normal physiological processes and disease states in which fatty acids have been implicated. More detailed discussion of these GPCRs can be found in recent review articles (Stoddart et al., 2008; Talukdar et al., 2011; Smith, 2012). Similarly, the molecular candidates for sour, and the attractive low salt and aversive high salt tastes

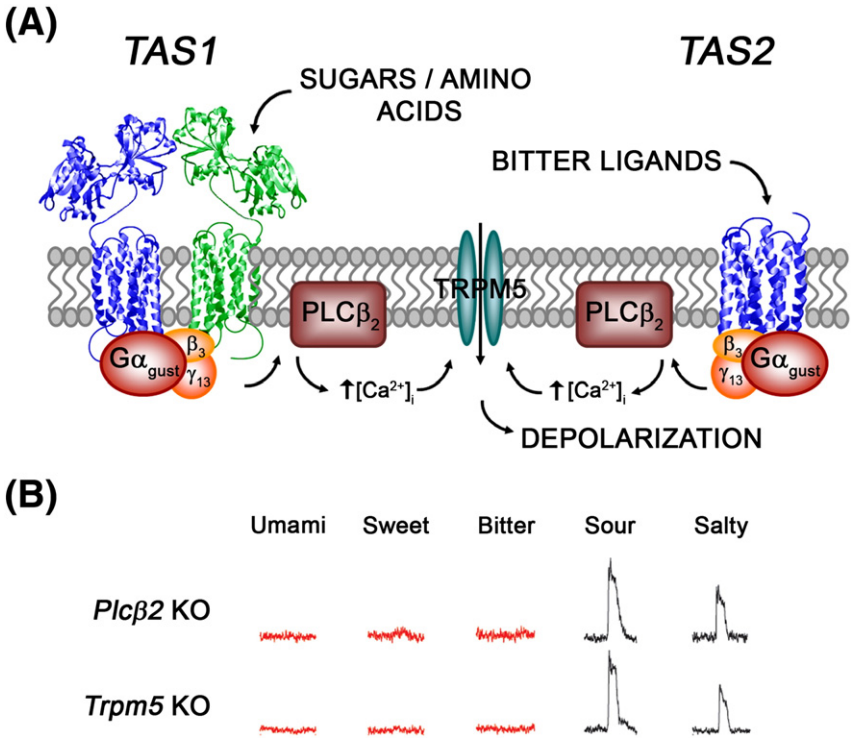

Fig. 2. The canonical T1R and T2R signal transduction cascades share common signaling molecules. Upon receptor activation, the βγ subunits activate PLCβ2, increasing $[Ca^{2+}]i$ from intracellular stores and resulting in ion flux through TRPM5 channels. Gustducin ($G\alpha_{t3}$) is generally considered to couple to T2Rs, although the evidence is less convincing for the T1Rs. Other proposed candidate α-subunits for sweet and umami tastes include $G\alpha_s$, $G\alpha_{i2}$, $G\alpha_t$, and $G\alpha_{14}$. **B.** This model of taste signaling has been supported by knockout studies for PLCβ2 or TRPM5. These mice displayed impaired responses to sweet, umami and bitter stimuli, without altered salty and sour taste responses.
Reproduced with permission from Chandrashekar et al. (2006). GPCR ribbon structure modified from Pin et al. (2009).

have been recently identified as ion channels, as detailed elsewhere (Huang et al., 2006; Chandrashekar et al., 2010; Oka et al., 2013).

2.3.2. Taste signal transduction

Despite the structural and functional differences between the T1R and T2R GPCRs, the canonical taste transduction cascade downstream of receptor activation shares common elements (Fig. 2). These include: the heterotrimeric G protein subunits, $G\alpha_{t3}$ (also known as gustducin or *GNAT3*), Gβ3 and Gγ13, a phospholipase C, PLCβ2, and a transient receptor potential ion channel, TRPM5 (Perez et al., 2002). The classical taste signaling paradigm holds that, following receptor activation, the βγ subunits dissociate from the α-subunit and activate PLCβ2, resulting in an inositol 1,4,5-trisphosphate receptor, type 3 (IP_3R3)-mediated increase in intracellular Ca^{2+} and the opening of TRPM5 channels to depolarize receptor cells (Huang et al., 1999; Yan et al., 2001; Huang & Roper, 2010). This model of taste signaling has been supported by knockout studies, with mice that lack either PLCβ2 or TRPM5 displaying impaired responses to sweet, umami and bitter stimuli (Zhang et al., 2003). However, the role of the Gα subunit in taste signaling has been more controversial. Gustducin/$G\alpha_{t3}$ was first identified as a taste-cell specific inhibitory G protein related to visual transducin (McLaughlin et al., 1992), and has been implicated in the transduction of sweet and bitter taste. Mice lacking gustducin display profoundly diminished, although not abolished, behavioral and electrophysiological responses to bitter, sweet and umami stimuli (Wong et al., 1996; Tomonari et al., 2012), raising the possibility of additional taste-associated G proteins. Indeed, other Gα subunits have been implicated in taste transduction (Kusakabe et al., 2000; He et al., 2002; Tizzano et al., 2008), albeit not as consistently as gustducin. In addition, although generally considered less important in taste signaling, gustducin can also activate a taste specific phosphodiesterase that decreases cAMP (Yan et al., 2001), in a manner functionally analogous to other $G\alpha_i$-subunits. Thus, by maintaining low levels of cyclic nucleotides, and consequently preventing the cAMP-dependent protein kinase A (PKA)-mediated dampening of the Ca^{2+} response, gustducin may help regulate taste cell responses (Clapp et al., 2008). Despite this controversy, it is important to note that gustducin is often used as a genetic marker to identify taste receptor-like cells and signaling in nongustatory tissues (see Section 3.1).

2.3.3. Taste receptor variants

Taste receptors are among the most rapidly evolving and highly polymorphic of GPCRs. In humans, this can account for the large differences in taste sensation that is observed across the population. For example, 47 unique single nucleotide polymorphisms (SNPs) have been identified in human T1Rs (Drayna, 2005), while mutations in the *TAS1R3* promoter (Fushan et al., 2009) and in the taste-specific G protein signaling components have been correlated to differences in sweet taste sensitivity (Fushan et al., 2010).

The *TAS2* family of receptors provides an extreme example of natural genetic diversity, displaying significantly more variation across human populations than in other gene families (Kim et al., 2005). *TAS2* genes contain on average 4.2 coding SNPs, the vast majority of which are nonsynonymous and encode amino acid substitutions (Drayna, 2005). In some cases, these *TAS2* receptor polymorphisms can have direct consequences for the functionality of the taste receptor itself, as determined by a combination of molecular, genetic and psychophysical approaches. The best characterized example is the T2R38 receptor, for which polymorphisms correlate with markedly different taste sensitivities to the bitter substance phenylthiocarbamide (Kim et al., 2003; Bufe et al., 2005). In brief, three commonly occurring amino acid polymorphisms (positions 49, 262 and 296) give rise to two frequent haplotypes: a taster (proline, alanine, and valine or PAV) and a non-taster (alanine, valine, and isoleucine or AVI). People who are homozygous for the taste allele perceive phenylthiocarbamide as intensely bitter, whereas the same concentration will be imperceptible to those with the non-functional allele. Based on molecular modeling and mutagenesis studies, it seems

that the valine at position 296 is important for the stabilization of the receptor, and plays a key role in receptor activation in tasters (Biarnés et al., 2010). More recently, the naturally occurring polymorphisms in *TAS2R38* (more specifically the non-taster haplotype) have been implicated in influencing food choice (Sandell & Breslin, 2006) and have been associated with increased alcohol intake (Duffy et al., 2004), adiposity (Tepper et al., 2008) and most recently with an increased susceptibility to respiratory tract infection (Lee et al., 2012). Similarly, other *TAS2* genes have been associated with increased susceptibility to colorectal cancer (Campa et al., 2010) and cardiovascular disease (Shiffman et al., 2008; Akao et al., 2012). Although the mechanisms by which these *TAS2* gene polymorphisms influence the pathophysiology of disease are mostly unknown, future assessments of the functionality of these receptor variants in tissues outside the sensory setting may have therapeutic implications.

3. Odorant and taste receptors beyond the nose and mouth

Undoubtedly, the common conception of these receptors has been shaped by their nomenclature, which inadvertently serves to reinforce the idea that odorant and taste GPCRs are only expressed in the tissues where they were first identified and after which they were named. Indeed, it was soon after their initial discovery that reports surfaced of so-called 'ectopic' expression of odorant or 'olfactory-like' receptors outside of the olfactory epithelium. Ectopic expression, which by definition occurs in 'an abnormal location or position within the body' is a universal phenomenon that extends beyond the OR and taste GPCR gene families, also extending to enzymes and neurotransmitters (Feldmesser et al., 2006). Even their designation as 'ectopic' seems imprudent, it is now evident that these odorant/taste GPCRs are found in diverse cells and tissues, where they are involved in additional processes beyond mediating the chemical senses of 'smell' and 'taste'. Moreover, despite the label 'odorant receptor' only a subset (~75%) of the odorant receptor repertoire is actually expressed in the olfactory epithelium, emphasizing the potentially misleading nature of the nomenclature (Zhang et al., 2007). As such, given that the odorant and taste receptors comprise over half of the entire human GPCR repertoire, we feel that it is more appropriate to simply consider them as GPCRs. In doing so, we evade constraining the nomenclature and limiting the connotations associated with these receptors, their putative endogenous ligands and their potential functions in mammalian biology.

3.1. Identification and characterization of odorant and taste GPCRs

Developing the necessary tools (i.e., antibodies) for the detection, and therefore characterization of the taste and odorant receptor proteins has been proven challenging, as it has for many GPCRs (Jensen et al., 2009; Herrera et al., 2013). Although there is an increasing number of commercially available antibodies, there are still very few selective and specific antibodies that have been reliably used for the odorant GPCRs, T1Rs receptors and even less for the T2Rs (Strotmann et al., 2004; Behrens et al., 2012; Meyer et al., 2012). Consequently, the evidence for expression of odorant and taste receptors in non-chemosensory tissues has almost exclusively relied on techniques aimed at detecting the messenger RNA for these proteins.

3.1.1. Reverse transcription polymerase chain reaction (RT-PCR)

Odorant receptors were initially identified on the basis of three assumptions: that they were a family of GPCRs; should be diverse in structure; and should be restricted in expression to the olfactory epithelium (Buck & Axel, 1991). The approach used degenerate primers for conserved regions within the GPCR transmembrane domains to clone numerous putative ORs from the rat olfactory epithelium. Analogous RT-PCR based cloning strategies were utilized to identify novel GPCRs, and soon after their description in the olfactory epithelium, OR transcripts were identified in many rodent and human tissues, including sperm (Parmentier et al., 1992; Vanderhaeghen et al., 1997a), heart (Drutel et al., 1995), spleen (Blache et al., 1998), erythroid cells (Feingold et al., 1999) and placenta (Itakura et al., 2006) (see Table 1). A pattern was likewise emerging for the other components of the olfactory signal transduction pathway. $G\alpha_{olf}$ was identified as an olfactory-specific G protein (Jones & Reed, 1989), yet transcripts have also been detected the heart and pancreas (Ferrand et al., 1999; Frayon et al., 1999). Moreover, $G\alpha_{olf}$ has also been reported to be critical in dopamine D_1 receptor and adenosine A_{2a} receptor signaling in the basal ganglia and striatum (Herve et al., 1993; Zhuang et al., 2000; Corvol et al., 2001). Similarly, AC3 is not specific to OSNs as originally reported, but is also expressed in the central nervous system, in the cardiovascular system, respiratory system and in the retina (Bakalyar & Reed, 1990; Xia et al., 1992; Jourdan et al., 2001). In many cases, these early reports seemed incidental, with many never followed up, and the authors demonstrated neither OR protein expression, nor a function outside of the olfactory setting. Yet almost universally, these studies speculated on the possibility that some predicted OR genes may not be odorant receptors per se, but may subsume other functions.

In a similar fashion, the T1R and T2R GPCRs have been identified in many tissues beyond the lingual palette in the decade since their discovery (Adler et al., 2000; Chandrashekar et al., 2000; Nelson et al., 2001, 2002) (see Table 2). Inevitably, given the general lack of good quality antibodies for detecting these receptors, these studies have also primarily relied on RT-PCR based techniques. The specific taste GPCRs identified and their corresponding cells and tissue types are detailed in Table 2. Nevertheless, the sheer number of studies identifying the expression of taste GPCRs outside of the mouth, particularly in tissues exposed to the external environment, such as the airways and gastrointestinal tract, has compelled their functional characterization.

3.1.2. Microarray

The advent of microarray technology was a boon for GPCR and olfactory research, allowing the analysis of OR gene expression across the entire family of receptors in a high-throughput manner not possible with a conventional candidate approach. Capitalizing on the wealth of computational data available following the sequencing of a number of mammalian genomes, custom-designed arrays were utilized to characterize the human and rodent OR expression profiles (Hakak et al., 2003; Zhang et al., 2004; Feldmesser et al., 2006; Zhang et al., 2007; De la Cruz et al., 2009) (see Table 1). These new arrays provided a significant advance over standard commercial arrays that only detected a small fraction of the OR repertoire, and were subject to cross-hybridization between OR genes. Importantly, these microarray expression profiling studies should be carefully interpreted, as the arrays are designed to compare levels of expression between samples or treatments (and not absolute gene expression) (Zhang et al., 2007). Nevertheless, the majority of OR genes were identified as specifically enriched in the olfactory epithelium, corroborating their function in olfaction.

However, a small subset of OR genes were detected in other tissues, with some of these expressed exclusively in non-olfactory tissues, consistent with previous reports using RT-PCR. In the human OR repertoire, 32 OR genes were enriched in tissues other than the olfactory epithelium, including 10 each in the lung and heart (Zhang et al., 2007). Given that this subset of OR genes were not detected in the olfactory epithelium, the authors questioned the functional annotations and nomenclature of these predicted human OR genes. Indeed, they suggested elsewhere that these may have been misclassified as ORs based on sequence similarity, but should be more properly considered as OR-like GPCRs with other putative functions (Zhang & Firestein, 2009). An alternative hypothesis was proposed by Feldmesser et al. (2006), whereby the extensive and heterogeneous expression of ORs outside of OSNs is a consequence of 'leaky' transcriptional regulation. This seems less likely, given the number of specific ORs that now have functions in non-olfactory tissues. Indeed, more recently, a subset of orthologous OR genes with conserved patterns of expression beyond the nose

Table 1
Evidence that odorant GPCRs and their associated signaling components are expressed and functional beyond the nose.

System	Tissue/s	Species	OR/s and signaling components identified[a]	Putative function	Technique/s utilized	Reference
Alimentary/ gastrointestinal	Tongue	Rat	Olr1867	–	RT-PCR, ISH	Abe et al., 1993
	Pancreas (β-cells)	Rat	$G\alpha_{olf}$, AC3	–	RT-PCR	Frayon et al., 1999
	Tongue	Human	OR1E1, OR8B8, OR5P3, OR8D1, OR8D2, OR10A5	–	RT-PCR	Gaudin et al., 2001
	Colon, liver	Mouse, rat	Olfr78, Olr59	–	NB	Yuan et al., 2001
	Tongue	Human	OR6Q1, OR10A4, OR7A5, OR2K2, OR5P2	–	RT-PCR	Durzynski et al., 2005
	Tongue	Mouse	Olfr20	–	RT-PCR	Gaudin et al., 2006
	Gut	Human	OR1A1, OR1G1, OR1E3, OR5D18	Odorant-mediated serotonin release	RT-PCR, Ca^{2+} imaging, serotonin ELISA	Braun et al., 2007
	Kidney	Mouse	Olfr78, Olfr90, Olfr1373, Olfr1392, Olfr1393, $G\alpha_{olf}$, AC3	Modulation of renin release and glomerular filtration rate	Microarray, RT-PCR, IHC ($G\alpha_{olf}$, AC3) Tg mice (AC3), renal function and blood pressure telemetry	Pluznick et al., 2009
	HeLa cells; HCT116 cells	Human	OR1A2, OR2A4	Cytokinesis	RT-PCR, WB/IHC (OR2A4), siRNA screen	X. Zhang et al., 2012
	Kidney	Mouse	Olfr78	Short chain fatty acids-induced renin release and blood pressure regulation	RT-PCR, luciferase-based cAMP reporter assay, Tg mice (Olfr78, GPR41), plasma rennin and blood pressure measurements	Pluznick et al., 2013
Cardiovascular/ pulmonary/ muscle	Heart	Rat	Olr1654	–	RT-PCR, ISH	Drutel et al., 1995
	Erythroid cells	Human	OR52A1	–	RT-PCR, RNase protection assay	Feingold et al., 1999
	Heart	Rat	Olr1654, $G\alpha_{olf}$, AC3	–	RT-PCR	Ferrand et al., 1999
	PASMC	Rat	AC3	–	IHC, WB (AC3)	Jourdan et al., 2001
	Skeletal muscle	Mouse	Olfr16 and 12 others, $G\alpha_{olf}$, AC3	Muscle regeneration; cell adhesion and migration	Microarray, RT-PCR, WB/IHC (Olfr16, $G\alpha_{olf}$, AC3)	Griffin et al., 2009
Brain and nervous system	Basal ganglia, striatum	Mouse	$G\alpha_{olf}$	–	WB	Herve et al., 1993; Corvol et al., 2001
	Brain	Rat	Olr59	–	RT-PCR, ISH	Raming et al., 1998
	Brain	Mouse	Olfr78		RT-PCR, ISH, X-gal staining, Tg mice (Olfr78)	Conzelmann et al., 2000
	Brain	Mouse; rat	Olfr78, Olr59	–	NB	Yuan et al., 2001
	Autonomic ganglia	Mouse	Olfr78	–	ISH, X-gal staining, Tg mice (Olfr78)	Weber et al., 2002
	Cerebral cortex	Mouse	Olfr151, Olfr49, Olfr15	–	RT-PCR, ISH, X-gal staining, Tg mice (Olfr151)	Otaki et al., 2004
	Frontal cortex	Human	OR2L13, OR1E1, OR2J3, OR52L1, OR11H, $G\alpha_{olf}$, AC3	–	Microarray, RT-qPCR, IHC (OR6K3)	Garcia-Esparcia et al., 2013

Immune	Spleen	Rat	Olr857	–	RT-PCR	Blache et al., 1998
Reproductive	Testis	Dog	OR1E2	–	RT-PCR, NB	Parmentier et al., 1992
	Testis	Dog	OR1E2	–	RNase protection assay, WB, IHC	Vanderhaeghen et al., 1993
	Testis	Rat	OD1, OD2	–	RT-PCR, WB, IHC	Walensky et al., 1995
	Testis	Mouse	Olfr16	–	RT-PCR, RNase protection assay	Asai et al., 1996
	Embryo	Chicken	OR45	–	ISH	Nef & Nef, 1997
	Testis	Human, dog, rat, mouse	Various ORs	–	RT-PCR, RNase protection assay	Vanderhaeghen et al., 1997a; Vanderhaeghen et al., 1997b
	Testis	Rat	Olr825, Olr1696	–	RNase protection assay, ISH	Walensky et al., 1998
	Prostate	Human	OR51E2	–	RT-PCR, NB, ISH	Xu et al., 2000
	Germ cells	Human	OR7E24	–	RT-PCR	Goto et al., 2001
	Prostate	Human	OR51E2	–	NB	Yuan et al., 2001
	Testis	Human	OR1D2, OR1E1	Chemotaxis	RT-PCR, Ca^{2+} imaging	Spehr et al., 2003
	Testis	Mouse	Olfr16	Chemosensing, sperm motility	RT-PCR, ISH, Ca^{2+} imaging, Tg mice (Olfr16)	Fukuda et al., 2004
	Testis	Mouse	Olfr16 and others	–	RT-PCR, ISH	Fukuda & Touhara, 2006
	Placenta	Rat	Olr1513, Olr1571, Olr1687, Olr1767, $G\alpha_{olf}$	–	RT-PCR	Itakura et al., 2006
	Prostate	Human	OR51E2	Inhibition of prostate cancer cell proliferation	RT-PCR, WB, Ca^{2+} imaging	Neuhaus et al., 2009
	Placenta	Mouse	Olfr154, Olfr433, Olfr520, Olfr1381	–	Microarray	Mao et al., 2010
	Testis	Human	OR1D2, OR4D1, OR7A5	Chemotaxis, Chemokinesis	RT-PCR, Ca^{2+} imaging	Veitinger et al., 2011
Various	Various	Cow	AC3	–	NB	Xia et al., 1992
	Various	Human	Many	–	Microarray	Hakak et al., 2003
	Various (testis, heart, lung)	Mouse	Many	–	Microarray	Zhang et al., 2004
	Various	Human, mouse	Many	–	Microarray, EST	Feldmesser et al., 2006
	Various (testis, lung, kidney, heart, liver)	Human	32 ORs expressed exclusively in non-olfactory tissues	–	Microarray, RT-PCR	Zhang et al., 2007
	Various (liver, lung, heart, testis)	Human, chimp	Many	–	Microarray	De la Cruz et al., 2009

[a] Gene nomenclature follows the BPS/NC-IUPHAR and HUGO gene nomenclature. For rodent GPCRs, MGD nomenclature is used.

Table 2
Evidence that taste GPCRs and their associated signaling components are expressed and functional beyond the mouth.

System	Tissue/s	Species	TR/s and signaling components identified [a]	Putative function	Technique/s utilized	Reference
Alimentary/ gastrointestinal	Stomach, duodenum	Rat	$G\alpha_{t3}$	–	RT-PCR, IHC	Höfer et al., 1996
	Stomach, duodenum, STC-1 cells	Rat, mouse	T2Rs, $G\alpha_{t3}$, $G\alpha_{t}$	–	RT-PCR	Wu et al., 2002
	Pancreas, liver	Human	T1R3	–	IHC	Taniguchi, 2004
	Duodenum, jejunum, ileum STC-1 cells	Mouse	T1R2, T1R3, $G\alpha_{t3}$	–	RT-qPCR, WB (T1R2, T1R3, $G\alpha_{t3}$)	Dyer et al., 2005
	Stomach, Duodenum, STC-1 cells	Mouse, rat	Mouse T2Rs (Tas2r105, 134 and 138), rat T2Rs (Tas2r108, 134 and 138), $G\alpha_{t3}$	–	RT-PCR	Wu et al., 2005
	STC-1 cells	Mouse	T2Rs (Tas2r108, 135, 137, 138, 144), $G\alpha_{t3}$, $G\beta3$, $G\gamma13$, PLCβ2, TRPM5	CCK release	RT-PCR, Ca^{2+} imaging	Chen et al., 2006
	Colon, Gut Hu-Tu 80 cells, NCI-H716 cells	Human	T1R3, T2Rs (T2R3, 4, 5, 10, 13, 38, 39, 40, 42, 43, 44, 45, 46, 47, 49, 50 and 60), $G\alpha_{t3}$	–	RT-PCR, IHC ($G\alpha_{t3}$), Ca^{2+} imaging	Rozengurt et al., 2006
	Stomach, small intestine, colon	Human, mouse	T1Rs (T1R1, 2, 3), $G\alpha_{t3}$, $G\gamma13$, PLCβ2, TRPM5	–	RT-PCR, IHC (T1R1, T1R3, $G\alpha_{t3}$, $G\gamma13$, PLCβ2,), Tg mice (TRPM5)	Bezençon et al., 2007
	Duodenum, NCI-H716 cells	Human, mouse	T1R2, T1R3, $G\alpha_{t3}$, $G\beta3$, $G\gamma13$, PLCβ2, TRPM5	Regulation of GLP-1 secretion in gut	RT-PCR, WB/IHC (T1R2, T1R3, $G\alpha_{t3}$, $G\beta3$, $G\gamma13$, PLCβ2, TRPM5), siRNA ($G\alpha_{t3}$), Ca^{2+} imaging, Tg mice ($G\alpha_{t3}$), glucose gavage, hormone measurements	Jang et al., 2007
	Gut	Mouse	T1R2, T1R3, $G\alpha_{t3}$	Regulation of Na^{+}-glucose cotransporter 1 (SGLT1)	RT-PCR, ISH, IHC/WB (T1R2, T1R3, $G\alpha_{t3}$), Ca^{2+} imaging, glucose transport assay, Tg mice ($G\alpha_{t3}$, T1R3)	Margolskee et al., 2007
	Liver, HuCCT1 cells	Human	T1R1, T1R2, T1R3	–	RT-PCR, IHC (T1R2, T1R3)	Toyono et al., 2007
	Cecum, NCI-H716 cells	Human	T1R3, T2R7	–	RT-PCR, GLP-1 measurements, Ca^{2+} imaging, siRNA ($G\alpha_{t3}$)	Dotson et al., 2008
	STC-1 cells, small intestine	Mouse	Tas2r138	SREBP-mediated regulation of gut peptide secretion	RT-qPCR, ChIP-chip analysis, luciferase reporter assays, siRNA (Tas2r138), IHC/WB (Tas2r138), CCK ELISA, oral gavage	Jeon et al., 2008
	Duodenum	Rat	T1Rs (T1R1, 2, 3), $mGlu_1$, $mGlu_4$, CaS	Mucosal defense	RT-PCR, L-glutamate infusion, pH measurements, anion secretion	Akiba et al., 2009
	Colon	Human, rat	T2Rs (T2R1, 4 and 38), rat T2Rs (Tas2r119, 125, 138)	Anion secretion	RT-PCR, Ussing chamber experiments	Kaji et al., 2009
	Small intestine	Rat	T1Rs (T1R1, 2, 3), $G\alpha_{t3}$, $G\alpha_{t}$, PLCβ2	Reciprocal regulation of PepT1 and apical GLUT2 via PKC beta II	WB (T1R1, T1R2, T1R3, $G\alpha_{t3}$, $G\alpha_{t}$, PLCβ2), glucose absorption assay, amino acid transport	Mace et al., 2009
	Pancreatic islets, MIN6 cells	Mouse	T1R2, T1R3	Insulin secretion	RT-PCR, IHC (T1R2, T1R3), insulin secretion assay, Ca^{2+} imaging, cAMP assay	Nakagawa et al., 2009
	Duodenum, jejunum	Human, mouse	T1R2, T1R3, T1R2, T1R3, $G\alpha_{t3}$, TRPM5	Regulated by blood glucose in diabetes patients and in glucose-infused mice	RT-PCR, IHC ($G\alpha_{t3}$), glucose perfusion	Young et al., 2009
	Stomach	Mouse	T1R3	–	RT-PCR, ISH, IHC (T1R3)	Hass et al., 2010
	Small intestine	Pig	T1R2, T1R3, $G\alpha_{t3}$	Artificial sweetener-mediated upregulation of SGLT1	IHC (T1R2, T1R3, $G\alpha_{t3}$), feed supplementation	Moran et al., 2010
	Caco-2 cells, intestine	Human, mouse	$G\alpha_{t3}$, PLCβ2, TRPM5	ABCB1-induced efflux via CCK release	RT-PCR, siRNA (T2R38), oral gavage	Jeon et al., 2011
	Duodenal muscle strips, stomach	Mouse	$G\alpha_{t3}$, $G\alpha_{t}$, no evidence of TR	Food intake and emptying	Bitter agonist gavage, ghrelin measurements, Tg mice ($G\alpha_{t3}$)	Janssen et al., 2011
	Duodenum, jejunum	Mouse	T1R3	–	RT-qPCR, WB (T1R3, $G\alpha_{t3}$), germ-free mice	Swartz et al., 2011
	Stomach	Human	No evidence of TR	–	Infusion of T1R3 antagonist, hormone measurements	Gerspach et al., 2011
	Stomach	Pig	T1R3, $G\alpha_{t3}$, PLCβ2, TRPM5	–	RT-PCR, IHC (T1R3, $G\alpha_{t3}$, PLCβ2, TRPM5)	Widmayer et al., 2011
	Stomach, jejunum, colon	Pig		–	RT-qPCR	

			T2Rs (T2R1, 3, 7, 9, 10, 16 and 38) FFA_1, FFA_2 and FFA_4			Colombo et al., 2012
	Adipose tissue, stomach, small intestine, colon (and lung)	Human, Mouse	FFA_4	Dietary fat sensing and control of energy balance in humans and rodents	RT-qPCR, WB (FFA_4), GLP-1 secretion assay, Tg mice (FFA_4), high fat diet, in vivo metabolic studies	Ichimura et al., 2012
	Pancreatic MIN6 cells; H9C2 cells, HeLa cells	Mouse (tissue profile), rat, human	T1R1, T1R3, T1R1, T1R3	Direct sensing of amino acids and regulation of mTORC1 and autophagy	RT-qPCR, Ca^{2+} assays, WB (T1R3) siRNA (T1R1, T1R3), Tg mice (T1R3), amino acid analysis, autophagy assays	Wauson et al., 2012
	HeLa cells; DU145 cells	Human	T1R2; T2R13	Cytokinesis	siRNA screen, RT-qPCR	X. Zhang et al., 2012
	3T3-L1 cells	Mouse	T1R3	Negative regulatory role in adipogenesis	RT-qPCR, IHC (T1R3), siRNA (T1R3, $G\alpha_s$), cAMP assays	Masubuchi et al., 2013
Cardiovascular/ pulmonary/ muscle	Heart	Human, rat, mouse	Human T1R3, T2Rs (T2R3, 4, 5, 9, 10, 13, 14, 19, 20, 30, 31, 43, 45, 46, 50) Rodent T1R1, T1R3, T2Rs (Tas2r108, 120, 121, 126, 135, 137, 143)	Nutrient-sensing	RT-qPCR, ISH, Tg mice (T1R1)	Foster et al., 2013
	Kidney	Pig	T1R3	–	RT-PCR	Kiuchi et al., 2006
	Lung	Mouse	T1R3	–	NB	Max et al., 2001
	Nasal cavity	Mouse	Tas2r108, Tas2r119, $G\alpha_{t3}$, PLCβ2	Bitter-ligand induced trigeminal nerve response and respiratory depression	IHC ($G\alpha_{t3}$, PLCβ2), ISH, Tg mice ($G\alpha_{t3}$), nerve recordings, respiratory recordings	Finger et al., 2003
	Airways	Human	T2Rs (T2R1, 3, 4, 7, 8, 9, 10, 13, 14)	Motile cilia mediate cell-autonomous clearance of inhaled pathogens	Microarray, RT-PCR, IHC (T2R4, T2R43, T2R46, $G\alpha_{t3}$, PLCβ2), Ca^{2+} imaging, ciliary beat frequency assay	Shah et al., 2009
	Airway smooth muscle, trachea	Human, mouse	T2Rs (T2R1, 3, 4, 5, 8, 9, 10, 13, 14, 19, 20, 30, 31, 42, 45, 46, 50), $G\alpha_{t3}$	Relaxation of isolated ASM and dilation of airways; de creased airway obstruction in a mouse model of asthma	RT-qPCR, Ca^{2+} imaging, isolated trachea, single cell mechanics/membrane potentials, in vivo bronchoconstriction/airways resistance assay	Deshpande et al., 2010
	Nasal cavity	Mouse	No evidence of TR	Bacterial detection by 'taste signaling' in nasal chemosensory cells	Tg mice (TRPM5, $G\alpha_{t3}$), Ca^{2+} imaging, nerve recordings, respiratory recordings	Tizzano et al., 2010
	Trachea	Mouse	Tas2r105, Tas2r108	Bitter-ligand induced regulation of breathing	IHC ($G\alpha_{t3}$, PLCβ2), flow cytometry, RT-PCR, respiratory recordings	Krasteva et al., 2011
	Airway smooth muscle cells	Human, monkey	No evidence of TR	Agonist-promoted homologous desensitization of TR	Ca^{2+} assays, agonist-promoted desensitization assays, intact airway desensitization	Robinett et al., 2011
	Airways	Rat	T1Rs (T1R1, 2, 3) T2Rs (Tas2r13, 105, 107, 119, 121, 123, 126, 134), $G\alpha_{t3}$, PLCβ2, TRPM5	–	RT-PCR, IHC ($G\alpha_{t3}$, PLCβ2), ISH, Tg mice (T1R3, TRPM5)	Tizzano et al., 2011
	16HBE cells	Human	T2R38, T2R46	–	RT-PCR, Ca^{2+} mobilization, cAMP accumulation	Cohen et al., 2012
	Upper respiratory epithelium	Human	T2R38	NO-mediated increase in ciliary beat frequency/ mucous clearance and antibacterial effects in respiratory infection	IHC (T2R38), Ca^{2+} imaging, NO production, ciliary beat frequency assay, mucous clearance assay, bactericidal assay	Lee et al., 2012
	Respiratory epithelium	Mouse	Tas2r131	–	Tg mice (Tas2r131)	Voigt et al., 2012
	BMSCs, VSMCs	Human, rat	T2R46, Tas2r116, Tas2r143	–	Flow cytometry, IHC (T2R46), RT-PCR, Ca^{2+} imaging	Lund et al., 2013
	Airway smooth muscle	Mouse	Tas2r107, Tas2r108	Bitter tastant-induced bronchodilation	RT-PCR, IHC (Tas2r107), muscle contraction assay, Ca^{2+} imaging, patch-clamp recording, wire myography	Zhang et al., 2013
CNS	Brain	Mouse	T1R3	–	NB	Max et al., 2001

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Table 2 (*continued*)

System	Tissue/s	Species	TR/s and signaling components identified [a]	Putative function	Technique/s utilized	Reference
	Brain (numerous regions), N38 cells	Mouse	T1R1, T1R2 and T1R3, $G\alpha_{t3}$, Gγ13	Hypothalamic glucose sensing	RT-PCR, ISH, IHC (T1R2 and T1R3, $G\alpha_{t3}$), in vivo starvation and in vitro glucose deprivation	Ren et al., 2009
	Hippocampus	Rat	T1R2, T1R3, $G\alpha_{t3}$	–	WB, IHC (T1R2, T1R3, $G\alpha_{t3}$)	Shin et al., 2010
	Brain, C6 glial cells, primary neuronal cells	Rat	Tas2r108, Tas2r110 and Tas2r138	–	RT-PCR, IHC (Tas2r108), Ca^{2+} mobilization	Singh et al., 2011
	Brainstem, cerebellum	Rat	Tas2r119, $G\alpha_{t3}$, PLCβ2, TRPM5	–	RT-PCR, WB, IHC ($G\alpha_{t3}$, PLCβ2)	Dehkordi et al., 2012
	Vomeronasal organ	Mouse	Tas2r131	–	Tg mice (Tas2r131)	Voigt et al., 2012
	Frontal cortex	Human	T2R5, T2R50, T2R10 and T2R13	–	Microarray, RT-qPCR	Garcia-Esparcia et al., 2013
Reproductive	Testis	Mouse	Tas2r103, Tas2r140	–	RT-PCR	Matsunami et al., 2000
	Testis	Mouse	T1R3	–	RT-PCR	Kitagawa et al., 2001
	Testis	Mouse	T1R3	–	NB	Max et al., 2001
	Testis	Pig	T1R3	–	RT-PCR, ISH	Kiuchi et al., 2006
	Testis	Human, Mouse	Human T1R1, mouse T1R1 and T1R3	Regulation of basal Ca^{2+} and cAMP levels in spermatozoa	RT-PCR, IHC (T1R3), Tg mice (T1R1), TUNEL assay, sperm motility, Ca^{2+} imaging, cAMP accumulation	Meyer et al., 2012
	Testis	Mouse	T1R1, Tas2r131	–	Tg mice (T1R1, Tas2r131)	Voigt et al., 2012
	Testis	Mouse	Tas2r105, T1R3, $G\alpha_{t3}$, Gγ13, PLCβ2	Spermatogenesis	Tg mice (Tas2r105), IHC (PLCβ2), X-gal staining	Li & Zhou, 2012
	Testis	Mouse	T2Rs (Tas2r105, 106, 107, 108, 113, 117, 119, 125, 126)	–	RT-PCR, ISH, Ca^{2+} imaging, Tg mice ($G\alpha_{t3}$)	Xu et al., 2013
Immune	Thymus	Mouse	T1R3	–	NB	Max et al., 2001
	Lymphocytes	Pig	T1R3	–	RT-PCR, ISH	Kiuchi et al., 2006
	Macrophages Mature adipocytes	Mouse	FFA_4	Insulin sensitizing and antidiabetic effects via repression of macrophage-induced tissue inflammation	RT-PCR, WB (FFA_4), siRNA (FFA_4), cytokine ELISA, internalization assays, Tg mice (FFA_4), high fat diet, omega 3 supplementation, in vivo metabolic studies	Oh et al., 2010
	Leukocytes	Human	T2Rs (T2R4, 5, 10, 13, 14, 19, 20, 31, 45, 46 and 50)	Anti-inflammatory role in asthma	Microarray, RT-qPCR, cytokine ELISA	Orsmark-Pietras et al., 2013
	Thymus	Mouse	Tas2r131	–	Tg mice (Tas2r131)	Voigt et al., 2012

[a] Gene nomenclature follows the BPS/NC-IUPHAR and HUGO gene nomenclature. For rodent GPCRs, MGD nomenclature is used.

were shown to evolve under stronger evolutionary constraint than OR genes expressed exclusively in the olfactory epithelium (De la Cruz et al., 2009), again consistent with the notion that these receptors have additional functions.

Given that there are only ~30 taste GPCRs, microarrays have been less essential in gustatory research (Kusakabe et al., 2005; Bezencon et al., 2008; Shah et al., 2009). Taste GPCRs are often low abundance genes and appear below the limits of detection of arrays, compounded by the fact that arrays often are not optimized to detect taste GPCRs (in one case containing only 3 *TAS2* probes (Bezencon et al., 2008)). In any case, small scale RT-PCR screens have provided ample transcript data to lay the foundations for investigation of taste receptors beyond the mouth.

However, while it may be tempting to speculate on novel functions for these GPCRs based on the expression of transcripts alone (as many researchers have), these data are infinitely more compelling when combined with other approaches and the demonstration of a functional correlate.

3.1.3. Localization of odorant and taste GPCRs

The detection and localization of odorant and taste receptors, especially in cells and tissues outside of the oronasal milieu, have been proven difficult. Of course, there have been some success stories, where antibodies have been generated to detect specific ORs in testis and prostate (Vanderhaeghen et al., 1993; Neuhaus et al., 2009), skeletal muscle (Griffin et al., 2009) and in human cell lines (X. Zhang et al., 2012), yet these remain the minority. Likewise, several studies investigating taste GPCR function have used in-house antibodies for immunohistochemistry, most notably for T1R3 (Damak et al., 2003), though many of the commercially available antibodies do not appear specific (Meyer et al., 2012). Numerous commercial antibodies have also recently become available for the T2Rs that have been used to investigate their expression outside the gustatory system (Jeon et al., 2008; Shah et al., 2009; Deshpande et al., 2010). These data should be treated with a great deal of caution, especially as the antibodies have not been rigorously validated using taste tissue (discussed in Behrens et al., 2012). To date, only one specific human T2R antiserum has been identified (Behrens et al., 2012). Nonetheless, with careful validation, these antibodies may provide valuable information about the localization of odorant and taste GPCRs beyond the nose and mouth.

The chemosensory field has focused on in situ hybridization for the detection and localization of OR and taste receptor mRNA transcripts in the sensory setting (Vassar et al., 1993; Adler et al., 2000; Behrens et al., 2007) and in other tissues (e.g. Drutel et al., 1995; Nef & Nef, 1997; Finger et al., 2003; Fukuda et al., 2004; Hass et al., 2010) (Tables 1 and 2). The low abundance taste receptor transcripts are often pushing the limits of detection of in situ hybridization, so signal amplification techniques are frequently employed (Yang et al., 1999).

The coexpression of the olfactory and gustatory signal transduction molecules has been offered as evidence of a functional receptor cascade outside the sensory setting. A case-in point is the 'olfactory-specific' adenylyl cyclase (AC3), which has been localized to numerous additional tissues (Pluznick et al., 2009) (Table 1). Equally, gustducin has been considered as a genetic marker of taste receptor expressing cells (McLaughlin et al., 1992), yet it was already detected in stomach (Höfer et al., 1996) before the discovery of taste GPCRs in the gustatory papillae. The literature describing olfactory and taste signaling components in other tissues has expanded dramatically in subsequent years, in contrast to the receptors, aided by the availability of selective antibodies. Similarly, the generation of numerous transgenic mice has been incredibly useful, whereby the promoter of a gene of interest (for example gustducin or TRPM5) drives the expression of a reporter, such as a fluorescent protein or lacZ (Wong et al., 1999; Bozza et al., 2002; Clapp et al., 2006). These reporter mice have accelerated research into taste receptors beyond the oral cavity, especially their delineation in the gastrointestinal tract, and in the respiratory and reproductive tissues (see Section 4 and Table 2).

3.1.4. Novel approaches to investigate odorant and taste GPCRs

It is no surprise that the most compelling evidence for the novel functions of odorant and taste GPCRs results from a combination of in vitro and in vivo approaches. By necessity, these have included the standard techniques detailed above, often complemented with informative transgenic mouse models (see Section 4). Meanwhile, the development and application of cutting-edge technologies, such as high-throughput quantitative expression profiling using RT-qPCR arrays and next generation sequencing, will further aid the investigation of odorant and taste GPCRs in non-sensory tissues (Khan et al., 2011; Snead & Insel, 2012). A case-in point is the recent open-access publication of the Illumina Human BodyMap 2.0 project. This enormously useful data set contains transcriptome data from 16 human tissue types (adrenal, adipose, brain, breast, colon, heart, kidney, liver, lung, lymph, ovary, prostate, skeletal muscle, testes, thyroid, and white blood cells), providing a resource for interrogating the expression of any annotated transcript. The data set has recently been curated by Flegel et al. (2013), and it beautifully demonstrates in an unbiased and comparable manner that different subsets of odorant and taste GPCRs are found in all of the human tissues sequenced. The field now must use these resources/technologies as a platform for accelerating the novel biology related to odorant and taste GPCRs.

3.2. Odorant and taste GPCR regulation

The regulation of expression and tissue distribution of odorant and taste GPCRs is of obvious importance to their potential functionality, yet there remain many gaps in our understanding. Here, we outline what is known about the transcriptional regulation and modulation of these receptors in the sensory tissues, and appraise the evidence that they are regulated at the transcript and functional level in other cells and tissues.

3.2.1. Transcriptional regulation

In the decades since their discovery, the transcriptional regulation of the large odorant receptor family has attracted much interest, especially as an olfactory sensory neuron has the unique ability to selectively express only one type of OR (Mombaerts, 2004b). Neurons expressing the same OR then converge to the same glomerulus in the olfactory bulb to convey odorant information to the brain. Thus, the monogenic and monoallelic OR expression pattern helps to imbue the odorant selectivity on the neuron, as well as establishing its identity within the olfactory system (Nguyen et al., 2007). Despite its fundamental importance to olfactory function, the mechanisms controlling the expression and regulation of ORs remain unclear. Numerous studies have highlighted the potential role of feedback mechanisms (Serizawa et al., 2003) and allelic exclusion (Chess et al., 1994), of putative OR-specific transcription factors and regulatory motifs (Hirota & Mombaerts, 2004; Michaloski et al., 2006; McIntyre et al., 2008), and of the probability of OR gene choice for a given OSN (Khan et al., 2011). More recently, next-generation sequencing approaches have enabled more comprehensive OR promoter analyses (Clowney et al., 2011), as well as investigation of the heterochromatin methylation profile leading to OR silencing (Magklara et al., 2011).

The processes underlying taste GPCR regulation are also poorly understood. Taste coding appears simple, with the receptors for bitter, sweet, umami, and sour expressed in different subsets of cells in the taste buds, implying the tuning of these cells to a single taste modality (Yarmolinsky et al., 2009). Less is known about the precise promoters and transcription factors involved in the regulation of taste GPCRs, although several groups have created transgenic mice incorporating the putative promoter/enhancer region (an ~10 kb fragment upstream of the taste receptor) to demonstrate transgene expression specifically in taste receptor-expressing cells (Mueller et al., 2005; Damak et al., 2008; Ohmoto et al., 2008, 2010).

Nonetheless, beyond passing reference, it is worth emphasizing that there has been little examination of the genetic elements and tissue-

specific factors regulating odorant/taste receptor expression beyond the nose and mouth. This will be of significant interest for future studies, given the widespread expression of different subsets of these receptors in human tissues.

3.2.2. Modulation of olfactory and gustatory response

There is extensive anecdotal and experimental evidence that suggests that the olfactory and gustatory response can be modulated by the endocrine system (Martin et al., 2009). The olfactory signaling response is subject to modulation by circulating hormones, such as adrenaline (Kawai et al., 1999) and also those involved in energy homeostasis, including the orexigenic peptides, ghrelin and neuropeptide Y (Martin et al., 2009; Tong et al., 2011; Trellakis et al., 2011). This implies that the olfactory epithelium is not simply transducing extracellular signals, but is intimately tied to the central nervous system and to the endocrine system (Firestein & Menini, 1999).

Similarly, there is emergent evidence that the taste response can be modulated by numerous hormones, including cannabinoids (Yoshida et al., 2010), adenosine (Dando et al., 2012; Kataoka et al., 2012) and most recently angiotensin II (Shigemura et al., 2013). These studies have understandably focused on the modulation of the olfactory and gustatory response in the naso-oropharynx, but may take on more significance when viewed in the context of odorant and taste GPCR expression throughout the body. This is clearly reflected in the putative roles for taste receptors throughout the gastrointestinal tract (Section 4.2).

3.2.3. Perturbations of odorant/taste GPCRs in physiology and disease

Numerous recent studies have reported the regulation of odorant and taste GPCRs in diverse physiological and pathological settings. For example, a number of specific ORs were differentially regulated during myogenesis and in muscle regeneration (Griffin et al., 2009), whereas odorant receptors may be developmentally regulated during spermatogenesis (Fukuda & Touhara, 2006). Meanwhile, T1R3 is upregulated at the transcript and protein level in skeletal muscle upon starvation (Wauson et al., 2012), as well as in mice lacking gut microflora (Swartz et al., 2011) and in a rat model of cerebral ischemia (Shin et al., 2010). These data should be cautiously interpreted, as there remain concerns over the specificity of the antibodies (discussed above). Another study showed that T2R stimulation promoted the secretion of both cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), and that *TAS2* gene expression can be regulated by dietary sterols (Jeon et al., 2008). In addition, we have recently shown that a subset of cardiac-expressed *Tas2* genes are developmentally down-regulated in adult rodents but are upregulated upon starvation, suggesting a potential novel role in cardiac nutrient sensing (Foster et al., 2013).

3.2.4. Odorant and taste GPCR regulation and internalization

In addition to the transcriptional regulation, GPCR function is dynamically regulated at the post-translational level. The classical paradigm of GPCR signaling holds that following ligand activation, the initial receptor signaling is rapidly desensitized by a series of events including receptor phosphorylation, arrestin recruitment and internalization (Pierce et al., 2002; Shukla et al., 2011). These processes enable the exquisite temporal regulation of the GPCR-mediated signal. With regard to the odorant receptors, this process shares much in common with other *Rhodopsin*/family A GPCRs. After prolonged odorant exposure, odorant receptor desensitization occurs via G protein-coupled receptor kinase (GRK)-dependent phosphorylation (Dawson et al., 1993; Peppel et al., 1997), followed by β-arrestin2-mediated clathrin-dependent internalization (Mashukova et al., 2006).

In contrast, until recently, there has been negligible interest in the delineation of taste GPCR pharmacology and receptor regulation. To date, there have been preliminary reports implicating N-glycosylation (Reichling et al., 2008), GRK-mediated desensitization (Robinett et al., 2011) and regulators of G protein signaling (RGS) proteins in T2R function (Cohen et al., 2012). We would argue that the field needs to focus considerable effort on these downstream regulatory processes, as a means to a more complete understanding of taste receptor pharmacology and physiology.

4. Novel functions for odorant and taste GPCRs

Although there is an ever-increasing literature describing extensive odorant and taste GPCR expression outside the classical sensory organs, a functional role in these tissues is often lacking and/or remains controversial. Here, we highlight the best characterized, novel functions for these receptors in diverse physiological settings, most notably in the reproductive, gastrointestinal, and respiratory systems.

4.1. Reproductive system

A number of early studies identified a novel role for specific ORs expressed in testis and sperm, in both humans and mice (Spehr et al., 2003; Fukuda et al., 2004). These ORs couple to distinct signal transduction pathways, resulting in specialized flagellar motion patterns and sperm chemotaxis. Indeed, in a follow-up work, the authors propose that the OR may play essentially the same role in both sperm and the nose, given that in psychophysical and physiological experiments, humans displayed attraction to the OR agonist ligand bourgeonal, consistent with in vitro chemotaxis assays (Spehr et al., 2004; Vosshall, 2004). An additional study elucidated a potential role for a specific OR (OR51E2) in the inhibition of prostate cancer cell proliferation (Neuhaus et al., 2009).

Similarly, there have been several preliminary reports of taste receptor expression and function in sperm, where they have been implicated in regulating Ca^{2+} and cAMP levels and potentially in spermatogenesis (Li & Zhou, 2012; Meyer et al., 2012).

4.2. Gastrointestinal system

Some compounds that are detected by the gustatory system as taste stimuli also need to be detected inside the body for homeostatic regulation. It is essential that numerous tissues including the kidney, pancreas, gut and brain, can detect and regulate nutrients, such as sodium, pH, amino acids and glucose. In gut, somewhat intuitively, the taste GPCRs have been proposed as organic nutrient sensors that can also regulate hormone release and gut function (Wellendorph et al., 2009).

The GPCRs for sweet, umami and bitter stimuli have roles throughout various parts of the alimentary canal, as reviewed by Behrens and Meyerhof (2011). Briefly, T1Rs regulate the secretion of GLP-1 from enteroendocrine cells, as well as incretin secretion and the modulation of glucose homeostasis via regulation of a sodium-dependent glucose transporter (Jang et al., 2007; Margolskee et al., 2007). L-Glutamate may also influence mucosal defense processes, purportedly via the umami taste receptor (Akiba et al., 2009). It has since been shown that L-glutamate and glucose activate T1Rs to reciprocally modulate the apical glucose transporter (GLUT2) and the oligopeptide transporter (PepT1). These findings suggest that there may be a wider taste receptor-regulated transport network to control energy homeostasis (Mace et al., 2009). Indeed, sweet and umami taste receptors are present in the hypothalamus, which is the main organ in the control of food intake, and are upregulated in mice as a result of feed deprivation (Ren et al., 2009). Consequently, T1Rs have been proposed as potential therapeutic targets for the treatment of metabolic disease and type 2 diabetes (Dotson et al., 2010). However, the widespread expression and the apparent complexity of the in vivo signaling pathways in the gastrointestinal tract are both important considerations in determining the suitability of T1Rs as pharmacological candidates.

The T2Rs have also been implicated in gastrointestinal modulation of hormone secretion, albeit often in cultured cell systems where their physiological relevance remains uncertain (Table 2). In many cases, direct evidence for the T2R-dependent effects is lacking. For example,

bitter tastants have been shown to increase intracellular Ca^{2+} in both human and mouse enteroendocrine cells leading to the secretion of gut hormones, including CCK and GLP-1 (Chen et al., 2006; Dotson et al., 2008; Jeon et al., 2008). In addition, a putative T2R ligand evokes prostaglandin-regulated anion secretion in the large intestine and modulates gastric emptying (Kaji et al., 2009; Janssen et al., 2011). Food-derived odorants present in the gut lumen may also stimulate serotonin release via olfactory receptors present in human enterochromaffin cells (Braun et al., 2007), although this awaits further validation. These studies collectively raise the possibility of targeting the previously unappreciated odorant/taste GPCRs in the gastrointestinal tract, potentially for development of novel therapeutics for obesity, diabetes, and malabsorption syndromes (Dotson et al., 2010).

4.3. Respiratory system

In the respiratory system, T2Rs have been implicated in distinct roles associated with several different cell types. In a population of solitary chemosensory cells in the nasal respiratory epithelium, T2Rs are activated by irritants, such as bitter substances and xenobiotics, to activate trigeminal nerve fibers and regulate protective airway reflexes (Finger et al., 2003; Tizzano et al., 2010). Similarly, in the trachea *TAS2* receptor-expressing cells respond to the bitter compound cycloheximide, resulting in a decrease in respiratory rate (Krasteva et al., 2011). Human respiratory epithelial cells are activated in response to bitter tasting compounds to increase the beat frequency in motile cilia, providing a mechanism both to sense noxious substances and initiate a cell-autonomous clearance of inhaled pathogens (Shah et al., 2009). Most recently, Lee and colleagues linked a well-studied polymorphism in the *TAS2R38* gene (encoding the 'non-taster' or AVI phenotype, see Section 2.3) to an increased susceptibility to respiratory infection. In this setting, T2R38 is activated by quorum-sensing molecules secreted from bacteria, contributing to a nitric oxide-mediated endogenous defense response (Lee et al., 2012).

In addition, T2Rs have been identified in human and mouse airway smooth muscle, where they have been proposed as potential novel therapeutic targets for the treatment of asthma (Deshpande et al., 2010). Administration of bitter ligands elicited an increase in intracellular calcium, but paradoxically produced smooth muscle relaxation in mouse trachea (with greater efficacy than the β-adrenergic receptor agonist isoproterenol) and reduced airway obstruction in a mouse model of asthma. This striking demonstration of a possible pathophysiological function for T2R activation in a non-taste tissue has nevertheless provoked controversy within the field (Sanderson & Madison, 2010; Belvisi et al., 2011; Morice et al., 2011; An et al., 2012; C.H. Zhang et al., 2012). Importantly, the very high concentrations of bitter ligands used to elicit the bronchodilation raise the ever-present possibility of non-specific drug actions, nor were the responses directly attributed to the T2Rs using knockdown or knockout studies. Additionally, the proposed mechanism for airway relaxation, via intracellular calcium-dependent activation of large-conductance Ca^{2+}-activated K+ (BKCa) channels, has been questioned, with Zhang et al. (2013) recently proposing an alternative bitter tastant-dependent Gβγ effect on the voltage-dependent Ca^{2+} channels.

Undoubtedly, T2Rs offer potentially attractive targets for the treatment of asthma and chronic obstructive pulmonary disease. The ongoing controversy suggests that there is still much mechanistic work to be done and serves to emphasize the confounding differences in the genetics and pharmacology of human and rodent GPCRs.

4.4. Kidney

Odorant receptors may play an important role in regulating fundamental aspects of renal function (Pluznick et al., 2009). Six odorant receptors (as well as $G\alpha_{olf}$ and AC3) were initially identified in extracts of whole kidney. $G\alpha_{olf}$ and AC3 were co-localized by immunocytochemistry in the distal nephron/macula densa and $AC3^{-/-}$ mice showed defects in renin secretion and glomerular filtration rate. In a recent follow-up study, an OR knockout (Olfr78), along with another GPCR (Gpr41) responded to gut microbiota-derived short chain fatty acids to regulate renin secretion and blood pressure (Pluznick et al., 2013).

4.5. Skeletal and cardiac muscle

Microarray approaches revealed the presence and induction of specific odorant receptors during myogenesis of proliferating myoblasts in vitro and muscle regeneration and repair in vivo (Griffin et al., 2009). In this study, the researchers successfully employed a soluble synthetic ligand (lyral), previously identified as a selective agonist for the same OR implicated in sperm chemotaxis (Olfr16, also designated MOR23). This study highlights the utility of a pharmacological approach to functionally characterize odorant/taste GPCRs to expose the underlying biology.

Similarly, we have recently described the presence of taste receptors in rodent and human heart (Foster et al., 2013). We originally performed a microarray to identify genes modulated during angiotensin II-induced cardiac hypertrophy and observed the expression/regulation of several odorant receptors (unpublished observations) as well as taste receptors that we subsequently confirmed using a combination of RT-qPCR and histological approaches (Foster et al., 2013). We identified that a subset of taste receptors are present in rodent heart and are regulated upon nutrient deprivation. Moreover, our novel description of a number of *TAS2* GPCRs in failing human heart, notably at comparable abundance to the classically-targeted angiotensin (AT_1) and β_1-adrenergic receptors, foreshadows a previously unappreciated role in heart.

4.6. Additional functions for odorant/taste GPCRs

The free fatty acid receptor FFA_4 (previously GPR120) provides an example of a GPCR that subsumes several important physiological functions, in addition to a role in tasting lipids/fats. In response to omega-3 fatty acid stimulation, FFA_4 reduces macrophage-induced tissue inflammation, thereby mediating potent anti-diabetic effects in vivo (Oh et al., 2010). FFA_4 expression in adipose tissue is significantly higher in obese individuals and plays a key role in dietary fat sensing and control of energy balance in rodents and humans (Ichimura et al., 2012). Meanwhile, the T1R1/T1R3 is a direct sensor of amino acid availability, which regulates the autophagic pathway via the mammalian target of rapamycin complex 1 (mTORC1) (Wauson et al., 2012). Finally, the potential role for an OR (OR2A4) and TRs (T1R2 and T2R13) in cytokinesis was recently reported (X. Zhang et al., 2012).

Under this apparent weight of evidence, the initial narrow conception of odorant/taste GPCRs as solely mediators of olfaction and gustation has been strongly challenged. Indeed, in addition to the functions described above, the widely expressed T2Rs may mediate off-target effects associated with numerous bitter-tasting pharmaceuticals (Clark et al., 2012). The array of functions beyond the nose and mouth will undoubtedly grow over time, necessitating a change in our view of the potential utility of odorant and taste GPCRs in physiology and disease.

5. Limitations of the field

The recent description of novel odorant/taste receptor functions in skeletal muscle, brain, the respiratory tract and the gastrointestinal system should compel the field to further investigate the biology of these GPCRs in diverse tissues. It is apparent, especially in comparison to other therapeutically-targeted GPCRs and receptor systems, that the detailed characterization and receptor pharmacology of these GPCRs are significantly less mature. Of course, this is understandable given

that odorant and taste receptors have only relatively recently been considered in tissues beyond the classical sensory setting.

5.1. Identification of ligands

The elucidation of novel functions for these receptors beyond the nose and mouth has been complicated by several factors. The large and diverse odorant/taste GPCR families have posed practical problems in terms of a candidate approach, as have the difficulties of unequivocally detecting the receptors in their endogenous settings. However, most importantly from the pharmacological standpoint, the identification of potent and selective ligands for ORs or T2Rs has been challenging (reviewed in Kato & Touhara, 2009; Behrens & Meyerhof, 2013). In both receptor families, individual receptors can bind multiple ligands, and a given ligand may bind multiple receptors and other intracellular targets. There is also pronounced variation in the apparent ligand specificity, where some ORs and T2Rs are activated by a single compound and others seem to be broadly tuned to a spectrum of ligands.

In addition, studies aimed at deorphanizing the odorant and taste receptors have used libraries of diverse compounds with known odorant or taste qualities (Saito et al., 2009; Meyerhof et al., 2010), thereby inadvertently biasing towards receptor-dependent effects (i.e. trying to identify the receptor that detects a given odorant/tastant). That many of these compounds have other known molecular targets (e.g., channels, transporters) and, in some cases, current clinical applications, is often overlooked. This is an important consideration, which may influence or confound the interpretation of some of the experimentally-derived data, especially beyond the oronasal cavity. For example, in human psychophysical tests, chloroquine and cycloheximide taste bitter — yet chloroquine is a known lysosomal inhibitor that is used as an antimalarial drug, with reported anti-inflammatory and possible immunosuppressive effects, whereas cycloheximide is a commonly used protein synthesis inhibitor. Similarly, one of the most bitter substances known, denatonium benzoate, also acts on an ATP-sensitive potassium channel (Straub et al., 2003).

Another confounding issue when examining the putative T2R-dependent functions of bitter ligands is that very high concentrations (sometimes in the millimolar range) are often used. This might be appropriate when interrogating T1Rs (as has been argued for the fatty acid receptors), because the physiological levels of these ligands are usually in that range. In contrast, the ligands for the T2Rs often have low micromolar EC_{50} values (Meyerhof et al., 2010) and some have known cytotoxicity, increasing the likelihood of non-specific effects with such high concentrations. In framing the physiological relevance of putative T2R-ligand pairings, these discrepancies need to be closely considered.

We also do not know the source of potential, activating ligands for these odorant and taste GPCRs in tissues outside of the nose and mouth. Intuitively, the gut-expressed odorant/taste GPCRs could be activated by compounds in food, and this idea has been extended to apply to the T2Rs (Kaji et al., 2009). At this juncture, the best candidates for activating T2Rs outside the gustatory system are inhaled irritants and toxins in the airways (Tizzano et al., 2011; Lee et al., 2012). Although, we might argue that ingested molecules, be they toxins or food components, via absorption in the gut, could enter the bloodstream at sufficient concentrations to target peripheral odorant/taste receptors.

5.2. Endogenous odorant and taste receptor agonists

The known repertoire of odorant/taste receptor ligands is predominantly a synthetic and/or xenobiotic compound. Given that the receptors are broadly expressed throughout the body, an alternative hypothesis posits that there may be endogenously produced activating ligand(s) for these GPCRs. The identification of such putative endogenous agonists (e.g. circulating or tissue specific locally-produced factors) may provide clues to delineate the scope of additional odorant and taste receptor functions. In this regard, Kazushige Touhara and colleagues have demonstrated that exocrine glands release peptides that serve as sex-specific reproductive cues through their action on pheromone receptors (Kimoto et al., 2005; Haga et al., 2010). More recently, they have used fractionation approaches to screen various crude tissue extracts for naturally-occurring OR ligands, whereby a fatty acid metabolite was identified that functions as a physiological ligand for an odorant receptor (Yoshikawa et al., 2013). Moreover, this metabolite had not been included in any previous collection of synthetic odorants used in OR ligand screens. Indeed, it is possible that the effectively 'orphan' odorant receptors and T2Rs may be activated by endogenous metabolites (recently reviewed in Blad et al., 2012). In this regard, metabolite ligands have been associated with multiple, other receptor systems and may have potential therapeutic applications for the treatment of pain and a variety of metabolic diseases (Sotnikova et al., 2009; Patwardhan et al., 2010; Waku et al., 2010; Deng et al., 2012; Wootten et al., 2012).

It is worth reiterating that the ligand profiles for the T1R family of taste receptors have been better characterized. T1Rs are members of the *Glutamate*/family C GPCR family, which includes receptors activated broadly by nutrients including amino acids, proteolytic degradation products, ions, carbohydrates, or free fatty acids (Wellendorph et al., 2009). A combination of heterologous expression and in vivo approaches has demonstrated that the T1R2/T1R3 'sweet receptor' is activated by a range of sweet tasting (or attractive to rodents) including sugars, artificial sweeteners, D-amino acids and glycine, and sweet proteins (Behrens & Meyerhof, 2011). Importantly, pronounced species differences have been revealed by these studies, primarily that some of the artificial sweeteners and sweet proteins fail to elicit a response from the rodent sweet receptor. Similarly, the T1R1/T1R3 'umami receptor' preferentially binds L-glutamate (supported by human psychophysical studies on umami taste), whereas the rodent receptor responds to most L-amino acids (Nelson et al., 2002). In addition to their apparent promiscuity with respect to ligands, a potential problem for the development of therapeutics to target these GPCRs, especially for the treatment of metabolic disorders and diabetes, remains their widespread tissue expression (Wauson et al., 2012).

5.3. Odorant/taste receptor antagonists

There are various taste/odorant GPCR-modifying compounds that are of keen interest as tools for the further functional characterization of these receptors outside the nose and mouth (Table 3). Over the years, a number of putative antagonists have been reported for T1Rs, the best characterized of which is the small molecule allosteric compound lactisole (reviewed in Sigoillot et al., 2012). Used extensively as a research tool to investigate sweet taste mechanisms and approved for use in the United States as a food additive, lactisole and other sweet taste inhibitors have recently been reconceived as potential therapeutics for metabolic diseases. In addition, the development of novel allosteric modulators has been driven by research programs from within the food and beverage industries (Servant et al., 2010), and may be of further use in the unraveling and understanding of the full biological roles for these GPCRs.

With the growing evidence that T2Rs are expressed in tissues throughout the body, the few reports of T2R antagonism may take on increased significance beyond their impacts on bitter taste (Slack et al., 2010; Brockhoff et al., 2011). Similarly, as the odorant receptors comprise by far the largest subset of *Rhodopsin*/family A GPCRs, traditionally the most successfully-targeted receptors, earlier reports of putative OR antagonists are of considerable interest (Spehr et al., 2003; Oka et al., 2004b). Ultimately, the utility of these antagonists in the elucidation of odorant/taste receptor function outside of the oronasal setting may be limited by their receptor-specificity, and will require further experimental validation in these tissues.

Table 3
Putative odorant/taste GPCR modulators and antagonists.

Targeted GPCR(s)	Compound name	Description/source	Mechanism of action	Reference
Sweet taste[a]	Gymnemic acid	Plant derived (*Gymnema sylvestre*)	Unknown	Kurihara, 1969; Liu et al., 1992
Sweet taste[a]	Ziziphin	Plant derived (*Ziziphus jujube*)	Unknown	Meiselman et al., 1976
Sweet taste[a]	Gurmarin	Plant derived (*Gymnema sylvestre*)	Unknown	Ninomiya & Imoto, 1995; Margolskee et al., 2007
Sweet taste, bitter taste[a]	Zinc sulfate	–	Unknown	Keast, 2003; Keast et al., 2004
Sweet taste, bitter taste[a]	Riboflavin-binding protein	Chicken egg white	Unknown	Maehashi et al., 2007; Maehashi et al., 2008
T1R3	Lactisole (±2-(4-methoxyphenoxy) propanoic acid)	Coffee beans	Binds allosteric site of T1R3 TM domains	Schiffman et al., 1999; Xu et al., 2004
T1R2/T1R3[b]	Acesulfame K	Artificial sweetener	Unknown	Galindo-Cuspinera et al., 2006
T1R2/T1R3[b]	Saccharin	Artificial sweetener	Binds allosteric site of T1R3 TM domains	Galindo-Cuspinera et al., 2006
T1R2/T1R3	Amiloride	ENaC inhibitor	Binds allosteric site of T1R3 TM domains	Imada et al., 2010
T1R3	Organoauxins (e.g. 2-(2,4-dichlorophenoxy) propanoic acid) Fibrates (clofibric acid, gemfibrozil, bezafibric acid)	Herbicides Lipid-lowering drugs	Binds allosteric site of T1R3 TM domains	Maillet et al., 2009
Bitter taste[a]	Various	Various	Unknown	Ley, 2008
Multiple T2Rs (T2R4, 7, 31, 40, 43, 49)	GIV3727 (4-(2,2,3-trimethylcyclopentyl) butanoic acid)	Small synthetic molecule screen	Competitive orthosteric antagonist	Slack et al., 2010
T2R46 (also 30, 40) T2R46 (also 30, 31, 43)	3β-Hydroxydihydrocostunolide 3β-Hydroxypelenolide	Plant-derived (*Artemisia absinthium*/*Artemisia arborescens*)	Competitive orthosteric antagonist	Brockhoff et al., 2011
T2R31	Flavonoids (e.g. sakuranetin, 6-methoxysakuranetin, jaceosidin)	Plant-derived (*Eriodictyon californicum*)	Unknown	Fletcher et al., 2011
T2R10	Enterodiol	Mammalian lignan (plant metabolite)	Unknown	Ley et al., 2012
Olr226	Citral (3,7-dimethyl-2,6-octadienal)	Plant derived odorant	Partial antagonist	Araneda et al., 2000
OR1D2	Undecanal	Synthetic aliphatic aldehyde	Unknown	Spehr et al., 2003
Olfr16	Oxidatively-dimerized isoeugenol	Isoeugenol derivative (oxidized breakdown product)	Competitive antagonist	Oka et al., 2004a
Olfr16	Methyl isoeugenol Isosafrol	Odorant compounds	Unknown	Oka et al., 2004b
OR1G1	1-Hexanol, hexanal, cyclohexanone	Odorant compounds	Competitive antagonist	Sanz et al., 2005
Olfr544, Olfr586	Octanoic acid	Odorant compound	Competitive antagonist	Shirokova et al., 2005
Olfr544, Olfr545	C12 dicarboxylic acid	Odorant compound	Unknown	Abaffy et al., 2007
Olr226	Octanal analogs	Synthetic compounds	Unknown	Peterlin et al., 2008

[a] No specific GPCR target identified.
[b] Inhibits sweet taste at high concentrations.

5.4. Heterologous expression systems in odorant and taste research

The enormous research effort to deorphanize and interrogate the ligand-binding properties of odorant and taste GPCRs has been also hampered by their poor cell-surface expression in heterologous expression systems. Several approaches have been utilized to circumvent this problem, including the use of several different model systems and the coexpression of putative accessory proteins (Krautwurst et al., 1998; Zhao et al., 1998; Saito et al., 2004; Shirokova et al., 2005; Von Dannecker et al., 2006; Zhuang & Matsunami, 2008). In the case of the taste GPCRs (most notably the T2Rs), where the G protein-coupling properties have been more controversial, the majority of receptor–ligand pairing data have come from approaches where promiscuous and chimeric G proteins have been employed (Chandrashekar et al., 2000; Ueda et al., 2003; Meyerhof et al., 2010). Although not representative of the endogenous TR-mediated signaling pathway, these assays have led to the identification of many potential agonist-ligands by coupling the ligand-mediated receptor activation to a robust readout (intracellular Ca^{2+} mobilization). Nevertheless, in other cells and tissues, where the protein complement and therefore signal-transduction pathways may differ, it is possible that odorant and taste GPCRs could couple to other G protein-dependent and independent pathways. In this regard, the investigation of central concepts in GPCR signaling, such as biased agonism, receptor crosstalk and desensitization, offers new opportunities for researchers in the field. At present, with the exception of OR internalization (see Section 3.2), only passing interest has been shown in these facets of odorant and taste GPCR pharmacology (Wu et al., 2005; Robinett et al., 2011; Cohen et al., 2012).

Thus, the elucidation of novel functions for odorant and taste GPCRs beyond the classical sensory milieu provides many challenges. There are still gaps in our understanding of the complex ligand binding properties of these GPCRs, which may have implications for their study in other tissues. This is especially true for odorant and taste receptors, where one receptor may bind multiple ligands, and one ligand may bind to multiple receptors. As there is apparent functional redundancy imbued in odorant and taste receptor signaling, the use of traditional genetic approaches, such as knockout mice, to delineate function is fraught. In any case, there are important and, in some cases, pronounced species differences in sequence and functional GPCR properties. Accordingly, it is possible/probable that basic discoveries in model systems, particularly in rodents, may not be directly transferrable to the human setting. These GPCRs are also highly polymorphic in human populations, which may have ramifications for determining disease prevalence and susceptibility. However, thanks to continuous technological advances and a great deal of persistence from the field, the lists of novel functions for odorant and taste GPCRs continue to grow.

6. Concluding remarks and future opportunities

The simple fact that there are so many odorant and taste GPCR genes, and that these are expressed in unique subsets in a multitude of tissues suggests that researchers are just scratching the surface of a new field of biology. The rapid advances in genomics and next generation sequencing approaches now mean that the unambiguous annotation of members of these gene families for all tissues is within reach (for example, see Flegel et al., 2013). We will soon be able to garner a wealth of information across populations of individuals to establish the breadth of haplotype variation and establish full tissue distribution (and copy number) data for all receptors in with perturbations (aging, stress, food) and in disease states.

There is little doubt that heterologous cell-based expression systems will continue to aid in identification/deorphanization of novel agonist and antagonist ligands for odorant and taste GPCRs. It is likely that further endogenous ligands will be identified, that will in turn facilitate the uncovering of information on the regulation of receptor function and physiology. As mentioned earlier, the use of standard genetic approaches such as knockout mice to study OR and TR function may not be as informative as in other systems, due to the overlapping ligand-binding profiles and potential functional redundancy of the receptors. However, the genomic organization and clustering of odorant and taste GPCRs may prove beneficial in enabling the simultaneous ablation of multiple receptors. Regardless, there are marked species differences between rodents and humans, in terms of their sequence homology, ligand-binding profiles and receptor functionality that must be considered (Mueller et al., 2005; Fushan et al., 2009). Hence, in order to avoid focusing on rodent specific phenomenology, studying the human receptors and tissues would be ideal. For most researchers, the difficulty in attaining human tissues has made this impractical. However, the rapid progress in tissue engineering technologies may provide one viable opportunity, especially for studies on the gastrointestinal tract and heart (Eschenhagen et al., 2012; Li & Clevers, 2012).

Given that odorant and taste GPCRs are among the most polymorphic gene families, obtaining receptor genotype information will also be a necessary component of further research. Where polymorphisms result in non-functional receptor variants, as has been described in a number of recent studies on T2Rs (Soranzo et al., 2005; Pronin et al., 2007; Lee et al., 2012), there is even the exciting possibility to investigate odorant/taste GPCR function in what is effectively a human knockout individual.

The future study of odorant and taste GPCRs in cells and tissues outside of the nose and mouth will undoubtedly benefit from a rigorous interdisciplinary pharmacological approach, using tools that have been applied to numerous other well characterized *Rhodopsin*/family A and *Glutamate*/family C GPCRs. That specific odorant and taste GPCR have already been implicated in processes as diverse as muscle regeneration, blood pressure maintenance, gastrointestinal nutrient regulation, bronchodilation and in cytokinesis, suggests that other odorant and taste GPCRs will have further profound physiological/pathophysiological roles. The description of these receptors in therapeutically important organs, such as the lung and heart, emphasizes the great potential for the delineation of hitherto undiscovered biology.

Conflict of interest statement

The authors declare there are none.

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References

Abaffy, T., Malhotra, A., & Luetje, C. W. (2007). The molecular basis for ligand specificity in a mouse olfactory receptor: a network of functionally important residues. *J Biol Chem 282*, 1216–1224.

Abe, K., Kusakabe, Y., Tanemura, K., Emori, Y., & Arai, S. (1993). Primary structure and cell-type-specific expression of a gustatory G protein-coupled receptor-related to olfactory receptors. *J Biol Chem 268*, 12033–12039.

Adler, E., Hoon, M.A., Mueller, K. L., Chandrashekar, J., Ryba, N. J. P., & Zuker, C. S. (2000). A novel family of mammalian taste receptors. *Cell 100*, 693–702.

Akao, H., Polisecki, E., Kajinami, K., Trompet, S., Robertson, M., Ford, I., et al. (2012). KIF6, LPA, TAS2R50, and VAMP8 genetic variation, low density lipoprotein cholesterol lowering response to pravastatin, and heart disease risk reduction in the elderly. *Atherosclerosis 220*, 456–462.

Akiba, Y., Watanabe, C., Mizumori, M., & Kaunitz, J.D. (2009). Luminal L-glutamate enhances duodenal mucosal defense mechanisms via multiple glutamate receptors in rats. *Am J Physiol Gastrointest Liver Physiol 297*, G781–G791.

Alexander, S. P., Mathie, A., & Peters, J. A. (2011). Guide to Receptors and Channels (GRAC), 5th edition. *Br J Pharmacol 164*(Suppl. 1), S1–S324.

An, S. S., Robinett, K. S., Deshpande, D. A., Wang, W. C., & Liggett, S. B. (2012). Reply to: activation of BK channels may not be required for bitter tastant-induced bronchodilation. *Nat Med 18*, 650–651.

Araneda, R. C., Kini, A.D., & Firestein, S. (2000). The molecular receptive range of an odorant receptor. *Nat Neurosci 3*, 1248–1255.

Asai, H., Kasai, H., Matsuda, Y., Yamazaki, N., Nagawa, F., Sakano, H., et al. (1996). Genomic structure and transcription of a murine odorant receptor gene: differential initiation of transcription in the olfactory and testicular cells. *Biochem Biophys Res Commun 221*, 240–247.

Bachmanov, A. A., & Beauchamp, G. K. (2007). Taste receptor genes. *Annu Rev Nutr 27*, 389–414.

Bakalyar, H. A., & Reed, R. R. (1990). Identification of a specialized adenylyl cyclase that may mediate odorant detection. *Science 250*, 1403–1406.

Behrens, M., Born, S., Redel, U., Voigt, N., Schuh, V., Raguse, J. -D., et al. (2012). Immunohistochemical detection of TAS2R38 protein in human taste cells. *PLoS One 7*, e40304.

Behrens, M., Foerster, S., Staehler, F., Raguse, J.D., & Meyerhof, W. (2007). Gustatory expression pattern of the human TAS2R bitter receptor gene family reveals a heterogenous population of bitter responsive taste receptor cells. *J Neurosci 27*, 12630–12640.

Behrens, M., & Meyerhof, W. (2011). Gustatory and extragustatory functions of mammalian taste receptors. *Physiol Behav 105*, 4–13.

Behrens, M., & Meyerhof, W. (2013). Bitter taste receptor research comes of age: from characterization to modulation of TAS2Rs. *Semin Cell Dev Biol 24*, 215–221.

Belluscio, L., Gold, G. H., Nemes, A., & Axel, R. (1998). Mice deficient in G(olf) are anosmic. *Neuron 20*, 69–81.

Belvisi, M. G., Dale, N., Birrell, M.A., & Canning, B. J. (2011). Bronchodilator activity of bitter tastants in human tissue. *Nat Med 17*, 776–778.

Bezencon, C., Furholz, A., Raymond, F., Mansourian, R., Metairon, S., Le Coutre, J., et al. (2008). Murine intestinal cells expressing Trpm5 are mostly brush cells and express markers of neuronal and inflammatory cells. *J Comp Neurol 509*, 514–525.

Bezençon, C., le Coutre, J., & Damak, S. (2007). Taste-signaling proteins are coexpressed in solitary intestinal epithelial cells. *Chem Senses 32*, 41–49.

Biarnés, X., Marchiori, A., Giorgetti, A., Lanzara, C., Gasparini, P., Carloni, P., et al. (2010). Insights into the binding of phenyltiocarbamide (PTC) agonist to its target human TAS2R38 bitter receptor. *PLoS One 5*, e12394.

Billig, G. M., Pal, B., Fidzinski, P., & Jentsch, T. J. (2011). Ca^{2+}-activated Cl^{-} currents are dispensable for olfaction. *Nat Neurosci 14*, 763–769.

Blache, P., Gros, L., Salazar, G., & Bataille, D. (1998). Cloning and tissue distribution of a new rat olfactory receptor-like (OL2). *Biochem Biophys Res Commun 242*, 669–672.

Blad, C. C., Tang, C., & Offermanns, S. (2012). G protein-coupled receptors for energy metabolites as new therapeutic targets. *Nat Rev Drug Discov 11*, 603–619.

Bozza, T., Feinstein, P., Zheng, C., & Mombaerts, P. (2002). Odorant receptor expression defines functional units in the mouse olfactory system. *J Neurosci 22*, 3033–3043.

Braun, T., Voland, P., Kunz, L., Prinz, C., & Gratzl, M. (2007). Enterochromaffin cells of the human gut: sensors for spices and odorants. *Gastroenterology 132*, 1890–1901.

Brockhoff, A., Behrens, M., Roudnitzky, N., Appendino, G., Avonto, C., & Meyerhof, W. (2011). Receptor agonism and antagonism of dietary bitter compounds. *J Neurosci 31*, 14775–14782.

Brunet, L. J., Gold, G. H., & Ngai, J. (1996). General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. *Neuron 17*, 681–693.

Buck, L., & Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell 65*, 175–187.

Bufe, B., Breslin, P. A. S., Kuhn, C., Reed, D. R., Tharp, C. D., Slack, J. P., et al. (2005). The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. *Curr Biol 15*, 322–327.

Bystrova, M. F., Romanov, R. A., Rogachevskaja, O. A., Churbanov, G. D., & Kolesnikov, S. S. (2010). Functional expression of the extracellular-Ca^{2+}-sensing receptor in mouse taste cells. *J Cell Sci 123*, 972–982.

Campa, D., Vodicka, P., Pardini, B., Naccarati, A., Carrai, M., Vodickova, L., et al. (2010). A gene-wide investigation on polymorphisms in the taste receptor 2R14 (TAS2R14) and susceptibility to colorectal cancer. *BMC Med Genet 11*.

Cartoni, C., Yasumatsu, K., Ohkuri, T., Shigemura, N., Yoshida, R., Godinot, N., et al. (2010). Taste preference for fatty acids is mediated by GPR40 and GPR120. *J Neurosci 30*, 8376–8382.

Chandrashekar, J., Hoon, M.A., Ryba, N. J., & Zuker, C. S. (2006). The receptors and cells for mammalian taste. *Nature 444*, 288–294.

Chandrashekar, J., Kuhn, C., Oka, Y., Yarmolinsky, D. A., Hummler, E., Ryba, N. J. P., et al. (2010). The cells and peripheral representation of sodium taste in mice. *Nature 464*, 297–301.

Chandrashekar, J., Mueller, K. L., Hoon, M.A., Adler, E., Feng, L., Guo, W., et al. (2000). T2Rs function as bitter taste receptors. *Cell 100*, 703–711.

Chaudhari, N., Landin, A.M., & Roper, S. D. (2000). A metabotropic glutamate receptor variant functions as a taste receptor. *Nat Neurosci 3*, 113–119.

Chen, M. C., Wu, S. V., Reeve, J. R., & Rozengurt, E. (2006). Bitter stimuli induce Ca^{2+} signaling and CCK release in enteroendocrine STC-1 cells: role of L-type voltage-sensitive Ca^{2+} channels. *Am J Physiol Cell Physiol 291*, C726–C739.

Chess, A., Simon, I., Cedar, H., & Axel, R. (1994). Allelic inactivation regulates olfactory receptor gene-expression. *Cell 78*, 823–834.

Clapp, T. R., Medler, K. F., Damak, S., Margolskee, R. F., & Kinnamon, S.C. (2006). Mouse taste cells with G protein-coupled taste receptors lack voltage-gated calcium channels and SNAP-25. *BMC Biol 4*.

Clapp, T. R., Trubey, K. R., Vandenbeuch, A., Stone, L. M., Margolskee, R. F., Chaudhari, N., et al. (2008). Tonic activity of Galpha-gustducin regulates taste cell responsivity. *FEBS Lett 582*, 3783–3787.

Clark, A. G., Glanowski, S., Nielsen, R., Thomas, P. D., Kejariwal, A., Todd, M.A., et al. (2003). Inferring nonneutral evolution from human–chimp–mouse orthologous gene trios. *Science 302*, 1960–1963.

Clark, A. A., Liggett, S. B., & Munger, S. D. (2012). Extraoral bitter taste receptors as mediators of off-target drug effects. *FASEB J 26*, 4827–4831.

Clowney, E. J., Magklara, A., Colquitt, B.M., Pathak, N., Lane, R. P., & Lomvardas, S. (2011). High-throughput mapping of the promoters of the mouse olfactory receptor genes reveals a new type of mammalian promoter and provides insight into olfactory receptor gene regulation. *Genome Res 21*, 1249–1259.

Cohen, S. P., Buckley, B. K., Kosloff, M., Garland, A. L., Bosch, D. E., Cheng, G., Jr., et al. (2012). Regulator of G-protein signaling-21 (RGS21) is an inhibitor of bitter gustatory signaling found in lingual and airway epithelia. *J Biol Chem 287*, 41706–41719.

Colombo, M., Trevisi, P., Gandolfi, G., & Bosi, P. (2012). Assessment of the presence of chemosensing receptors based on bitter and fat taste in the gastrointestinal tract of young pig. *J Anim Sci 90*(Suppl. 4), 128–130.

Conzelmann, S., Levai, O., Bode, B., Eisel, U., Raming, K., Breer, H., et al. (2000). A novel brain receptor is expressed in a distinct population of olfactory sensory neurons. *Eur J Neurosci 12*, 3926–3934.

Corvol, J. C., Studler, J. M., Schonn, J. S., Girault, J. A., & Herve, D. (2001). G alpha(olf) is necessary for coupling D1 and A2a receptors to adenylyl cyclase in the striatum. *J Neurochem 76*, 1585–1588.

Damak, S., Mosinger, B., & Margolskee, R. F. (2008). Transsynaptic transport of wheat germ agglutinin expressed in a subset of type II taste cells of transgenic mice. *BMC Neurosci 9*, 96.

Damak, S., Rong, M. Q., Yasumatsu, K., Kokrashvili, Z., Varadarajan, V., Zou, S. Y., et al. (2003). Detection of sweet and umami taste in the absence of taste receptor T1r3. *Science 301*, 850–853.

Dando, R., Dvoryanchikov, G., Pereira, E., Chaudhari, N., & Roper, S. D. (2012). Adenosine enhances sweet taste through A2B receptors in the taste bud. *J Neurosci 32*, 322–330.

Dawson, T. M., Arriza, J. L., Jaworsky, D. E., Borisy, F. F., Attramadal, H., Lefkowitz, R. J., et al. (1993). Beta-adrenergic receptor kinase-2 and beta-arrestin-2 as mediators of odorant-induced desensitization. *Science 259*, 825–829.

De la Cruz, O., Blekhman, R., Zhang, X., Nicolae, D., Firestein, S., & Gilad, Y. (2009). A signature of evolutionary constraint on a subset of ectopically expressed olfactory receptor genes. *Mol Biol Evol 26*, 491–494.

Dehkordi, O., Rose, J. E., Fatemi, M., Allard, J. S., Balan, K. V., Young, J. K., et al. (2012). Neuronal expression of bitter taste receptors and downstream signaling molecules in the rat brainstem. *Brain Res 1475*, 1–10.

Deng, H., Hu, H., & Fang, Y. (2012). Multiple tyrosine metabolites are GPR35 agonists. *Sci Rep 2*.

Deshpande, D. A., Wang, W. C. H., McIlmoyle, E. L., Robinett, K. S., Schillinger, R. M., An, S. S., et al. (2010). Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. *Nat Med 16*, 1299–1304.

Dhallan, R. S., Yau, K. -W., Schrader, K. A., & Reed, R. R. (1990). Primary structure and functional expression of a cyclic nucleotide-activated channel from olfactory neurons. *Nature 347*, 184–187.

Dotson, C. D., Vigues, S., Steinle, N. I., & Munger, S. D. (2010). T1R and T2R receptors: the modulation of incretin hormones and potential targets for the treatment of type 2 diabetes mellitus. *Curr Opin Investig Drugs 11*, 447–454.

Dotson, C. D., Zhang, L., Xu, H., Shin, Y. K., Vigues, S., Ott, S. H., et al. (2008). Bitter taste receptors influence glucose homeostasis. *PLoS One 3*, e3974.

Drayna, D. (2005). Human taste genetics. *Annu Rev Genomics Hum Genet 6*, 217–235.

Drews, J. (2000). Drug discovery: a historical perspective. *Science 287*, 1960–1964.

Drutel, G., Arrang, J. M., Diaz, J., Wisnewsky, C., Schwartz, K., & Schwartz, J. C. (1995). Cloning of OL1, a putative olfactory receptor and its expression in the developing rat heart. *Receptors Channels 3*, 33–40.

Dryer, L. (2000). Evolution of odorant receptors. *Bioessays 22*, 803–810.

Duffy, V. B., Davidson, A.C., Kidd, J. R., Kidd, K. K., Speed, W. C., Pakstis, A. J., et al. (2004). Bitter receptor gene (TAS2R38), 6-n-propylthiouracil (PROP) bitterness and alcohol intake. *Alcohol Clin Exp Res 28*, 1629–1637.

Dulac, C., & Axel, R. (1995). A novel family of genes encoding putative pheromone receptors in mammals. *Cell 83*, 195–206.

Durzynski, L., Gaudin, J. C., Myga, M., Szydlowski, J., Gozdzicka-Jozefiak, A., & Haertle, T. (2005). Olfactory-like receptor cDNAs are present in human lingual cDNA libraries. *Biochem Biophys Res Commun 333*, 264–272.

Dyer, J., Salmon, K. S., Zibrik, L., & Shirazi-Beechey, S. P. (2005). Expression of sweet taste receptors of the T1R family in the intestinal tract and enteroendocrine cells. *Biochem Soc Trans 33*, 302–305.

Eppig, J. T., Blake, J. A., Bult, C. J., Kadin, J. A., & Richardson, J. E. (2012). The Mouse Genome Database (MGD): comprehensive resource for genetics and genomics of the laboratory mouse. *Nucleic Acids Res 40*, D881–D886.

Eschenhagen, T., Eder, A., Vollert, I., & Hansen, A. (2012). Physiological aspects of cardiac tissue engineering. *Am J Physiol Heart Circ Physiol 303*, H133–H143.

Feingold, E. A., Penny, L. A., Nienhuis, A. W., & Forget, B. G. (1999). An olfactory receptor gene is located in the extended human beta-globin gene cluster and is expressed in erythroid cells. *Genomics 61*, 15–23.

Feldmesser, E., Olender, T., Khen, M., Yanai, I., Ophir, R., & Lancet, D. (2006). Widespread ectopic expression of olfactory receptor genes. *BMC Genomics 7*.

Ferrand, N., Pessah, M., Frayon, S., Marais, J., & Garel, J. M. (1999). Olfactory receptors, Golf alpha and adenylyl cyclase mRNA expressions in the rat heart during ontogenic development. *J Mol Cell Cardiol 31*, 1137–1142.

Finger, T. E., Böttger, B., Hansen, A., Anderson, K. T., Alimohammadi, H., & Silver, W. L. (2003). Solitary chemoreceptor cells in the nasal cavity serve as sentinels of respiration. *Proc Natl Acad Sci U S A 100*, 8981–8986.

Finger, T. E., & Kinnamon, S.C. (2011). Taste isn't just for taste buds anymore. *F1000 Biol Rep 3*, 20.

Firestein, S., & Menini, A. (1999). The smell of adrenaline. *Nat Neurosci 2*, 106–108.

Flegel, C., Manteniotis, S., Osthold, S., Hatt, H., & Gisselmann, G. (2013). Expression profile of ectopic olfactory receptors determined by deep sequencing. *PLoS One 8*, e55368.

Fletcher, J. N., Kinghorn, A.D., Slack, J. P., McCluskey, T. S., Odley, A., & Jia, Z. (2011). In vitro evaluation of flavonoids from *Eriodictyon californicum* for antagonist activity against the bitterness receptor hTAS2R31. *J Agric Food Chem 59*, 13117–13121.

Foster, S. R., Porrello, E. R., Purdue, B., Chan, H. -W., Voigt, A., Frenzel, S., et al. (2013). Expression, regulation and putative nutrient-sensing function of taste GPCRs in the heart. *PLoS One 8*, e64579.

Frayon, S., Pessah, M., Giroix, M. H., Mercan, D., Boissard, C., Malaisse, W. J., et al. (1999). G alpha olf identification by RT-PCR in purified normal pancreatic B cells and in islets from rat models of non-insulin-dependent diabetes. *Biochem Biophys Res Commun 254*, 269–272.

Fukuda, N., & Touhara, K. (2006). Developmental expression patterns of testicular olfactory receptor genes during mouse spermatogenesis. *Genes Cells 11*, 71–81.

Fukuda, N., Yomogida, K., Okabe, M., & Touhara, K. (2004). Functional characterization of a mouse testicular olfactory receptor and its role in chemosensing and in regulation of sperm motility. *J Cell Sci 117*, 5835–5845.

Fushan, A. A., Simons, C. T., Slack, J. P., & Drayna, D. (2010). Association between common variation in genes encoding sweet taste signaling components and human sucrose perception. *Chem Senses 35*, 579–592.

Fushan, A. A., Simons, C. T., Slack, J. P., Manichaikul, A., & Drayna, D. (2009). Allelic polymorphism within the TAS1R3 promoter is associated with human taste sensitivity to sucrose. *Curr Biol 19*, 1288–1293.

Galindo, M. M., Voigt, N., Stein, J., van Lengerich, J., Raguse, J.D., Hofmann, T., et al. (2012). G protein-coupled receptors in human fat taste perception. *Chem Senses 37*, 123–139.

Galindo-Cuspinera, V., Winnig, M., Bufe, B., Meyerhof, W., & Breslin, P. A. (2006). A TAS1R receptor-based explanation of sweet 'water-taste'. *Nature 441*, 354–357.

Garcia-Esparcia, P., Schluter, A., Carmona, M., Moreno, J., Ansoleaga, B., Torrejon-Escribano, B., et al. (2013). Functional genomics reveals dysregulation of cortical olfactory receptors in Parkinson disease: novel putative chemoreceptors in the human brain. *J Neuropathol Exp Neurol 72*, 524–539.

Gaudin, J. -C., Breuils, L., & Haertlé, T. (2001). New GPCRs from a human lingual cDNA library. *Chem Senses 26*, 1157–1166.

Gaudin, J. -C., Breuils, L., & Haertlé, T. (2006). Mouse orthologs of human olfactory-like receptors expressed in the tongue. *Gene 381*, 42–48.

Gerspach, A.C., Steinert, R. E., Schönenberger, L., Graber-Maier, A., & Beglinger, C. (2011). The role of the gut sweet taste receptor in regulating GLP-1, PYY and CCK release in humans. *Am J Physiol Endocrinol Metab 301*, E317–E325.

Goto, T., Salpekar, A., & Monk, M. (2001). Expression of a testis-specific member of the olfactory receptor gene family in human primordial germ cells. *Mol Hum Reprod 7*, 553–558.

Griffin, C. A., Kafadar, K. A., & Pavlath, G. K. (2009). MOR23 promotes muscle regeneration and regulates cell adhesion and migration. *Dev Cell 17*, 649–661.

Haga, S., Hattori, T., Sato, T., Sato, K., Matsuda, S., Kobayakawa, R., et al. (2010). The male mouse pheromone ESP1 enhances female sexual receptive behaviour through a specific vomeronasal receptor. *Nature 466*, 118–122.

Hakak, Y., Shrestha, D., Goegel, M. C., Behan, D. P., & Chalmers, D. T. (2003). Global analysis of G-protein-coupled receptor signaling in human tissues. *FEBS Lett 550*, 11–17.

Hass, N., Schwarzenbacher, K., & Breer, H. (2010). T1R3 is expressed in brush cells and ghrelin-producing cells of murine stomach. *Cell Tissue Res 339*, 493–504.

He, W., Danilova, V., Zou, S., Hellekant, G., Max, M., Margolskee, R. F., et al. (2002). Partial rescue of taste responses of alpha-gustducin null mice by transgenic expression of alpha-transducin. *Chem Senses 27*, 719–727.

Herrada, G., & Dulac, C. (1997). A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. *Cell 90*, 763–773.

Herrera, M., Sparks, M.A., Alfonso-Pecchio, A.R., Harrison-Bernard, L. M., & Coffman, T. M. (2013). Lack of specificity of commercial antibodies leads to misidentification of angiotensin type 1 receptor protein. *Hypertension 61*, 253–258.

Herve, D., Levi-Strauss, M., Marey-Semper, I., Verney, C., Tassin, J., Glowinski, J., et al. (1993). G(olf) and Gs in rat basal ganglia: possible involvement of G(olf) in the coupling of dopamine D1 receptor with adenylyl cyclase. *J Neurosci 13*, 2237–2248.

Hirota, J., & Mombaerts, P. (2004). The LIM-homeodomain protein Lhx2 is required for complete development of mouse olfactory sensory neurons. *Proc Natl Acad Sci U S A 101*, 8751–8755.

Höfer, D., Püschel, B., & Drenckhahn, D. (1996). Taste receptor-like cells in the rat gut identified by expression of alpha-gustducin. *Proc Natl Acad Sci U S A 93*, 6631–6634.

Hopkins, A. L., & Groom, C. R. (2002). The druggable genome. *Nat Rev Drug Discov 1*, 727–730.

Huang, E. S. (2005). Predicting ligands for orphan GPCRs. *Drug Discov Today 10*, 69–73.

Huang, A. L., Chen, X., Hoon, M.A., Chandrashekar, J., Guo, W., Trankner, D., et al. (2006). The cells and logic for mammalian sour taste detection. *Nature 442*, 934–938.

Huang, Y. A., & Roper, S. D. (2010). Intracellular Ca(2+) and TRPM5-mediated membrane depolarization produce ATP secretion from taste receptor cells. *J Physiol 588*, 2343–2350.

Huang, L. Q., Shanker, Y. G., Dubauskaite, J., Zheng, J. Z., Yan, W. T., Rosenzweig, S., et al. (1999). Ggamma13 colocalizes with gustducin in taste receptor cells and mediates IP3 responses to bitter denatonium. *Nat Neurosci 2*, 1055–1062.

HUGO Gene Nomenclature Committee (HGNC) (). HGNC Database. Last modified on Jan 2013. Accessed on 07/05/13. www.genenames.org

Ichimura, A., Hirasawa, A., Poulain-Godefroy, O., Bonnefond, A., Hara, T., Yengo, L., et al. (2012). Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. *Nature 483*, 350–354.

Imada, T., Misaka, T., Fujiwara, S., Okada, S., Fukuda, Y., & Abe, K. (2010). Amiloride reduces the sweet taste intensity by inhibiting the human sweet taste receptor. *Biochem Biophys Res Commun 397*, 220–225.

Itakura, S., Ohno, K., Ueki, T., Sato, K., & Kanayama, N. (2006). Expression of Golf in the rat placenta: possible implication in olfactory receptor transduction. *Placenta 27*, 103–108.

Jang, H. J., Kokrashvili, Z., Theodorakis, M. J., Carlson, O. D., Kim, B. J., Zhou, J., et al. (2007). Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc Natl Acad Sci U S A 104*, 15069–15074.

Janssen, S., Laermans, J., Verhulst, P. J., Thijs, T., Tack, J., & Depoortere, I. (2011). Bitter taste receptors and {alpha}-gustducin regulate the secretion of ghrelin with functional effects on food intake and gastric emptying. *Proc Natl Acad Sci U S A 108*, 2094–2099.

Jensen, B. C., Swigart, P.M., & Simpson, P. C. (2009). Ten commercial antibodies for alpha-1-adrenergic receptor subtypes are nonspecific. *Naunyn Schmiedebergs Arch Pharmacol 379*, 409–412.

Jeon, T. I., Seo, Y. K., & Osborne, T. F. (2011). Gut bitter taste receptor signaling induces ABCB1 through a mechanism involving CCK. *Biochem J 438*, 33–37.

Jeon, T. I., Zhu, B., Larson, J. L., & Osborne, T. F. (2008). SREBP-2 regulates gut peptide secretion through intestinal bitter taste receptor signaling in mice. *J Clin Invest 118*, 3693–3700.

Jones, D. T., & Reed, R. R. (1989). Golf — an olfactory neuron specific G-protein involved in odorant signal transduction. *Science 244*, 790–795.

Jourdan, K. B., Mason, N. A., Long, L., Philips, P. G., Wilkins, M. R., & Morrell, N. W. (2001). Characterization of adenylyl cyclase isoforms in rat peripheral pulmonary arteries. *Am J Physiol Lung Cell Mol Physiol 280*, L1359–L1369.

Kaji, I., Karaki, S., Fukami, Y., Terasaki, M., & Kuwahara, A. (2009). Secretory effects of a luminal bitter tastant and expressions of bitter taste receptors, T2Rs, in the human and rat large intestine. *Am J Physiol Gastrointest Liver Physiol 296*, G971–G981.

Kataoka, S., Baquero, A., Yang, D., Shultz, N., Vandenbeuch, A., Ravid, K., et al. (2012). A2BR adenosine receptor modulates sweet taste in circumvallate taste buds. *PLoS One 7*, e30032.

Kato, A., & Touhara, K. (2009). Mammalian olfactory receptors: pharmacology, G protein coupling and desensitization. *Cell Mol Life Sci 66*, 3743–3753.

Kawai, F., Kurahashi, T., & Kaneko, A. (1999). Adrenaline enhances odorant contrast by modulating signal encoding in olfactory receptor cells. *Nat Neurosci 2*, 133–138.

Keast, R. S. (2003). The effect of zinc on human taste perception. *J Food Sci 68*, 1871–1877.

Keast, R. S., Canty, T. M., & Breslin, P. A. (2004). Oral zinc sulfate solutions inhibit sweet taste perception. *Chem Senses 29*, 513–521.

Keller, A., Zhuang, H., Chi, Q., Vosshall, L. B., & Matsunami, H. (2007). Genetic variation in a human odorant receptor alters odour perception. *Nature 449*, 468–472.

Khan, M., Vaes, E., & Mombaerts, P. (2011). Regulation of the probability of mouse odorant receptor gene choice. *Cell 147*, 907–921.

Kim, U. K., Jorgenson, E., Coon, H., Leppert, M., Risch, N., & Drayna, D. (2003). Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. *Science 299*, 1221–1225.

Kim, U., Wooding, S., Ricci, D., Jorde, L. B., & Drayna, D. (2005). Worldwide haplotype diversity and coding sequence variation at human bitter taste receptor loci. *Hum Mutat 26*, 199–204.

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

Kitagawa, M., Kusakabe, Y., Miura, H., Ninomiya, Y., & Hino, A. (2001). Molecular genetic identification of a candidate receptor gene for sweet taste. *Biochem Biophys Res Commun 283*, 236–242.

Kiuchi, S., Yamada, T., Kiyokawa, N., Saito, T., Fujimoto, J., & Yasue, H. (2006). Genomic structure of swine taste receptor family 1 member 3, TAS1R3, and its expression in tissues. *Cytogenet Genome Res 115*, 51–61.

Kosiol, C., Vinař, T., da Fonseca, R. R., Hubisz, M. J., Bustamante, C. D., Nielsen, R., et al. (2008). Patterns of positive selection in six mammalian genomes. *PLoS Genet 4*, e1000144.

Krasteva, G., Canning, B. J., Hartmann, P., Veres, T. Z., Papadakis, T., Muhlfeld, C., et al. (2011). Cholinergic chemosensory cells in the trachea regulate breathing. *Proc Natl Acad Sci U S A 108*, 9478–9483.

Krautwurst, D., Yau, K. W., & Reed, R. R. (1998). Identification of ligands for olfactory receptors by functional expression of a receptor library. *Cell 95*, 917–926.

Kurihara, Y. (1969). Antisweet activity of gymnemic acid A1 and its derivatives. *Life Sci 8*, 537–543.

Kusakabe, Y., Kim, M. -R., Miura, H., Shindo, Y., Ninomiya, Y., & Hino, A. (2005). Regional expression patterns of T1r family in the mouse tongue. *Chem Senses 30*, i23–i24.

Kusakabe, Y., Yasuoka, A., Asano-Miyoshi, M., Iwabuchi, K., Matsumoto, I., Arai, S., et al. (2000). Comprehensive study on G protein alpha-subunits in taste bud cells, with special reference to the occurrence of G alpha i2 as a major G alpha species. *Chem Senses 25*, 525–531.

Kusuhara, Y., Yoshida, R., Ohkuri, T., Yasumatsu, K., Voigt, A., Hubner, S., et al. (2013). Taste responses in mice lacking taste receptor subunit T1R1. *J Physiol 591*, 1967–1985.

Lagerstrom, M. C., & Schioth, H. B. (2008). Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat Rev Drug Discov 7*, 339–357.

Lancet, D., & Ben-Arie, N. (1993). Olfactory receptors. *Curr Biol 3*, 668–674.

Lee, R. J., Xiong, G., Kofonow, J. M., Chen, B., Lysenko, A., Jiang, P., et al. (2012). T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. *J Clin Invest 122*, 4145–4159.

Ley, J. (2008). Masking bitter taste by molecules. *Chemosens Percept 1*, 58–77.

Ley, J. P., Dessoy, M., Paetz, S., Blings, M., Hoffmann-Lücke, P., Reichelt, K. V., et al. (2012). Identification of enterodiol as a masker for caffeine bitterness by using a pharmacophore model based on structural analogues of homoeriodictyol. *J Agric Food Chem 60*, 6303–6311.

Li, V. S., & Clevers, H. (2012). In vitro expansion and transplantation of intestinal crypt stem cells. *Gastroenterology 143*, 30–34.

Li, F., & Zhou, M. (2012). Depletion of bitter taste transduction leads to massive spermatid loss in transgenic mice. *Mol Hum Reprod 18*, 289–297.

Liberles, S. D., & Buck, L. B. (2006). A second class of chemosensory receptors in the olfactory epithelium. *Nature 442*, 645–650.

Liu, H. M., Kiuchi, F., & Tsuda, Y. (1992). Isolation and structure elucidation of gymnemic acids, antisweet principles of *Gymnema sylvestre*. *Chem Pharm Bull (Tokyo) 40*, 1366–1375.

Lund, T. C., Kobs, A. J., Kramer, A., Nyquist, M., Kuroki, M. T., Osborn, J., et al. (2013). Bone marrow stromal and vascular smooth muscle cells have chemosensory capacity via bitter taste receptor expression. *PLoS One 8*, e58945.

Mace, O. J., Lister, N., Morgan, E., Shepherd, E., Affleck, J., Helliwell, P., et al. (2009). An energy supply network of nutrient absorption coordinated by calcium and T1R taste receptors in rat small intestine. *J Physiol 587*, 195–210.

Maehashi, K., Matano, M., Kondo, A., Yamamoto, Y., & Udaka, S. (2007). Riboflavin-binding protein exhibits selective sweet suppression toward protein sweeteners. *Chem Senses 32*, 183–190.

Maehashi, K., Matano, M., Nonaka, M., Udaka, S., & Yamamoto, Y. (2008). Riboflavin-binding protein is a novel bitter inhibitor. *Chem Senses 33*, 57–63.

Magklara, A., Yen, A., Colquitt, Bradley M., Clowney, E. J., Allen, W., Markenscoff-Papadimitriou, E., et al. (2011). An epigenetic signature for monoallelic olfactory receptor expression. *Cell 145*, 555–570.

Maillet, E. L., Margolskee, R. F., & Mosinger, B. (2009). Phenoxy herbicides and fibrates potently inhibit the human chemosensory receptor subunit T1R3. *J Med Chem 52*, 6931–6935.

Malnic, B., Hirono, J., Sato, T., & Buck, L. B. (1999). Combinatorial receptor codes for odors. *Cell 96*, 713–723.

Mao, J., Zhang, X., Sieli, P. T., Falduto, M. T., Torres, K. E., & Rosenfeld, C. S. (2010). Contrasting effects of different maternal diets on sexually dimorphic gene expression in the murine placenta. *Proc Natl Acad Sci U S A 107*, 5557–5562.

Margolskee, R. F., Dyer, J., Kokrashvili, Z., Salmon, K. S. H., Ilegems, E., Daly, K., et al. (2007). T1R3 and gustducin in gut sense sugars to regulate expression of Na^+-glucose cotransporter 1. *Proc Natl Acad Sci U S A 104*, 15075–15080.

Martin, B., Maudsley, S., White, C. M., & Egan, J. M. (2009). Hormones in the naso-oropharynx: endocrine modulation of taste and smell. *Trends Endocrinol Metab 20*, 163–170.

Mashukova, A., Spehr, M., Hatt, H., & Neuhaus, E. M. (2006). Beta-arrestin2-mediated internalization of mammalian odorant receptors. *J Neurosci 26*, 9902–9912.

Masubuchi, Y., Nakagawa, Y., Ma, J., Sasaki, T., Kitamura, T., Yamamoto, Y., et al. (2013). A novel regulatory function of sweet taste-sensing receptor in adipogenic differentiation of 3T3-L1 cells. *PLoS One 8*, e54500.

Matsunami, H., Montmayeur, J., & Buck, L. (2000). A family of candidate taste receptors in human and mouse. *Nature 404*, 601–604.

Mattes, R. D. (2011). Accumulating evidence supports a taste component for free fatty acids in humans. *Physiol Behav 104*, 624–631.

Max, M., Shanker, Y. G., Huang, L. Q., Rong, M., Liu, Z., Campagne, F., et al. (2001). Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac. *Nat Genet 28*, 58–63.

McIntyre, J. C., Bose, S.C., Stromberg, A. J., & McClintock, T. S. (2008). Emx2 stimulates odorant receptor gene expression. *Chem Senses 33*, 825–837.

McLaughlin, S. K., McKinnon, P. J., & Margolskee, R. F. (1992). Gustducin is a taste-cell-specific G-protein closely related to the transducins. *Nature 357*, 563–569.

Meiselman, H. L., Halpern, B. P., & Dateo, G. P. (1976). Reduction of sweetness judgements by extracts from the leaves of *Ziziphus jujuba*. *Physiol Behav 17*, 313–317.

Meyer, D., Voigt, A., Widmayer, P., Borth, H., Huebner, S., Breit, A., et al. (2012). Expression of Tas1 taste receptors in mammalian spermatozoa: functional role of Tas1r1 in regulating basal Ca^{2+} and cAMP concentrations in spermatozoa. *PLoS One 7*, e32354.

Meyerhof, W., Batram, C., Kuhn, C., Brockhoff, A., Chudoba, E., Bufe, B., et al. (2010). The molecular receptive ranges of human TAS2R bitter taste receptors. *Chem Senses 35*, 157–170.

Michaloski, J. S., Galante, P. A., & Malnic, B. (2006). Identification of potential regulatory motifs in odorant receptor genes by analysis of promoter sequences. *Genome Res 16*, 1091–1098.

Mombaerts, P. (2004a). Genes and ligands for odorant, vomeronasal and taste receptors. *Nat Rev Neurosci 5*, 263–278.

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

Moran, A. W., Al-Rammahi, M.A., Arora, D. K., Batchelor, D. J., Coulter, E. A., Daly, K., et al. (2010). Expression of Na^+/glucose co-transporter 1 (SGLT1) is enhanced by supplementation of the diet of weaning piglets with artificial sweeteners. *Br J Nutr 104*, 637–646.

Morice, A. H., Bennett, R. T., Chaudhry, M.A., Cowen, M. E., Griffin, S.C., & Loubani, M. (2011). Effect of bitter tastants on human bronchi. *Nat Med 17*, 775.

Mueller, K. L., Hoon, M.A., Erlenbach, I., Chandrashekar, J., Zuker, C. S., & Ryba, N. J. P. (2005). The receptors and coding logic for bitter taste. *Nature 434*, 225–229.

Nakagawa, Y., Nagasawa, M., Yamada, S., Hara, A., Mogami, H., Nikolaev, V. O., et al. (2009). Sweet taste receptor expressed in pancreatic beta-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. *PLoS One 4*, e5106.

Nef, S., & Nef, P. (1997). Olfaction: transient expression of a putative odorant receptor in the avian notochord. *Proc Natl Acad Sci U S A 94*, 4766–4771.

Nei, M., Niimura, Y., & Nozawa, M. (2008). The evolution of animal chemosensory receptor gene repertoires: roles of chance and necessity. *Nat Rev Genet 9*, 951–963.

Nelson, G., Chandrashekar, J., Hoon, M.A., Feng, L. X., Zhao, G., Ryba, N. J. P., et al. (2002). An amino-acid taste receptor. *Nature 416*, 199–202.

Nelson, G., Hoon, M.A., Chandrashekar, J., Zhang, Y. F., Ryba, N. J. P., & Zuker, C. S. (2001). Mammalian sweet taste receptors. *Cell 106*, 381–390.

Neuhaus, E. M., Zhang, W. Y., Gelis, L., Deng, Y., Noldus, J., & Hatt, H. (2009). Activation of an olfactory receptor inhibits proliferation of prostate cancer cells. *J Biol Chem 284*, 16218–16225.

Nguyen, M. Q., Zhou, Z., Marks, C. A., Ryba, N. J., & Belluscio, L. (2007). Prominent roles for odorant receptor coding sequences in allelic exclusion. *Cell 131*, 1009–1017.

Nielsen, R., Bustamante, C., Clark, A. G., Glanowski, S., Sackton, T. B., Hubisz, M. J., et al. (2005). A scan for positively selected genes in the genomes of humans and chimpanzees. *PLoS Biol 3*, e170.

Ninomiya, Y., & Imoto, T. (1995). Gurmarin inhibition of sweet taste responses in mice. *Am J Physiol 268*, R1019–R1025.

Oh, D. Y., Talukdar, S., Bae, E. J., Imamura, T., Morinaga, H., Fan, W., et al. (2010). GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell 142*, 687–698.

Ohmoto, M., Maeda, N., Abe, K., Yoshihara, Y., & Matsumoto, I. (2010). Genetic tracing of the neural pathway for bitter taste in t2r5-WGA transgenic mice. *Biochem Biophys Res Commun 400*, 734–738.

Ohmoto, M., Matsumoto, I., Yasuoka, A., Yoshihara, Y., & Abe, K. (2008). Genetic tracing of the gustatory and trigeminal neural pathways originating from T1R3-expressing taste receptor cells and solitary chemoreceptor cells. *Mol Cell Neurosci 38*, 505–517.

Oka, Y., Butnaru, M., von Buchholtz, L., Ryba, N. J. P., & Zuker, C. S. (2013). High salt recruits aversive taste pathways. *Nature 494*, 472–475.

Oka, Y., Nakamura, A., Watanabe, H., & Touhara, K. (2004). An odorant derivative as an antagonist for an olfactory receptor. *Chem Senses 29*, 815–822.

Oka, Y., Omura, M., Kataoka, H., & Touhara, K. (2004). Olfactory receptor antagonism between odorants. *EMBO J 23*, 120–126.

Olender, T., Lancet, D., & Nebert, D. W. (2008). Update on the olfactory receptor (OR) gene superfamily. *Hum Genomics 3*, 87–97.

Orsmark-Pietras, C., James, A., Konradsen, J. R., Nordlund, B., Söderhäll, C., Pulkkinen, V., et al. (2013). Transcriptome analysis reveals upregulation of bitter taste receptors in severe asthmatics. *Eur Respir J 42*, 65–78.

Otaki, J. M., Yamamoto, H., & Firestein, S. (2004). Odorant receptor expression in the mouse cerebral cortex. *J Neurobiol 58*, 315–327.

Overington, J. P., Al-Lazikani, B., & Hopkins, A. L. (2006). Opinion — how many drug targets are there? *Nat Rev Drug Discov 5*, 993–996.

Parmentier, M., Libert, F., Schurmans, S., Schiffmann, S., Lefort, A., Eggerickx, D., et al. (1992). Expression of members of the putative olfactory receptor gene family in mammalian germ cells. *Nature 355*, 453–455.

Patwardhan, A.M., Akopian, A. N., Ruparel, N.B., Diogenes, A., Weintraub, S. T., Uhlson, C., et al. (2010). Heat generates oxidized linoleic acid metabolites that activate TRPV1 and produce pain in rodents. *J Clin Invest 120*, 1617–1626.

Peppel, K., Boekhoff, I., McDonald, P., Breer, H., Caron, M. G., & Lefkowitz, R. J. (1997). G protein-coupled receptor kinase 3 (GRK3) gene disruption leads to loss of odorant receptor desensitization. *J Biol Chem 272*, 25425–25428.

Perez, C. A., Huang, L. Q., Rong, M. Q., Kozak, J. A., Preuss, A. K., Zhang, H. L., et al. (2002). A transient receptor potential channel expressed in taste receptor cells. *Nat Neurosci 5*, 1169–1176.

Peterlin, Z., Li, Y. D., Sun, G. X., Shah, R., Firestein, S., & Ryan, K. (2008). The importance of odorant conformation to the binding and activation of a representative olfactory receptor. *Chem Biol 15*, 1317–1327.

Pierce, K. L., Premont, R. T., & Lefkowitz, R. J. (2002). Seven-transmembrane receptors. *Nat Rev Mol Cell Biol 3*, 639–650.

Pin, J. -P., Acher, F., Conn, P. J., Duvoisin, R., Ferraguti, F., Flor, P. J., et al. (). Metabotropic glutamate receptors, introductory chapter. Last modified on 13/10/2009. Accessed on 07/05/2013. IUPHAR database (IUPHAR-DB). http://www.iuphar-db.org/DATABASE/FamilyIntroductionForward?familyId=40

Pluznick, J. L., Protzko, R. J., Gevorgyan, H., Peterlin, Z., Sipos, A., Han, J., et al. (2013). Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proc Natl Acad Sci U S A 110*, 4410–4415.

Pluznick, J. L., Zou, D. J., Zhang, X. H., Yan, Q. S., Rodriguez-Gil, D. J., Eisner, C., et al. (2009). Functional expression of the olfactory signaling system in the kidney. *Proc Natl Acad Sci U S A 106*, 2059–2064.

Pronin, A. N., Xu, H., Tang, H., Zhang, L., Li, Q., & Li, X. (2007). Specific alleles of bitter receptor genes influence human sensitivity to the bitterness of aloin and saccharin. *Curr Biol 17*, 1403–1408.

Quignon, P., Giraud, M., Rimbault, M., Lavigne, P., Tacher, S., Morin, E., et al. (2005). The dog and rat olfactory receptor repertoires. *Genome Biol 6*.

Raming, K., Konzelmann, S., & Breer, H. (1998). Identification of a novel G-protein coupled receptor expressed in distinct brain regions and a defined olfactory zone. *Receptors Channels 6*, 141–151.

Reichling, C., Meyerhof, W., & Behrens, M. (2008). Functions of human bitter taste receptors depend on N-glycosylation. *J Neurochem 106*, 1138–1148.

Ren, X., Zhou, L., Terwilliger, R., Newton, S. S., & de Araujo, I. E. (2009). Sweet taste signaling functions as a hypothalamic glucose sensor. *Front Integr Neurosci 3*, 12.

Robinett, K. S., Deshpande, D. A., Malone, M. M., & Liggett, S. B. (2011). Agonist-promoted homologous desensitization of human airway smooth muscle bitter taste receptors. *Am J Respir Cell Mol Biol 45*, 1069–1074.

Rozengurt, N., Wu, S. V., Chen, M. C., Huang, C., Sternini, C., & Rozengurt, E. (2006). Colocalization of the alpha-subunit of gustducin with PYY and GLP-1 in L cells of human colon. *Am J Physiol Gastrointest Liver Physiol 291*, G792–G802.

Saito, H., Chi, Q., Zhuang, H., Matsunami, H., & Mainland, J.D. (2009). Odor coding by a mammalian receptor repertoire. *Sci Signal 2*, ra9.

Saito, H., Kubota, M., Roberts, R. W., Chi, Q. Y., & Matsunami, H. (2004). RTP family members induce functional expression of mammalian odorant receptors. *Cell 119*, 679–691.

Sandell, M.A., & Breslin, P. A. S. (2006). Variability in a taste-receptor gene determines whether we taste toxins in food. *Curr Biol 16*, R792–R794.

Sanderson, M. J., & Madison, J. M. (2010). Bitter treats for better breathing. *Nat Med 16*, 1190–1191.

Sanz, G., Schlegel, C., Pernollet, J. C., & Briand, L. (2005). Comparison of odorant specificity of two human olfactory receptors from different phylogenetic classes and evidence for antagonism. *Chem Senses 30*, 69–80.

Sawzdargo, M., George, S. R., Nguyen, T., Xu, S., Kolakowski, L. F., & O'Dowd, B. F. (1997). A cluster of four novel human G protein-coupled receptor genes occurring in close proximity to CD22 gene on chromosome 19q13.1. *Biochem Biophys Res Commun 239*, 543–547.

Schiffman, S. S., Booth, B. J., Sattely-Miller, E. A., Graham, B. G., & Gibes, K. M. (1999). Selective inhibition of sweetness by the sodium salt of +/−2-(4-methoxyphenoxy) propanoic acid. *Chem Senses 24*, 439–447.

Serizawa, S., Miyamichi, K., Nakatani, H., Suzuki, M., Saito, M., Yoshihara, Y., et al. (2003). Negative feedback regulation ensures the one receptor-one olfactory neuron rule in mouse. *Science 302*, 2088–2094.

Servant, G., Tachdjian, C., Tang, X. Q., Werner, S., Zhang, F., Li, X., et al. (2010). Positive allosteric modulators of the human sweet taste receptor enhance sweet taste. *Proc Natl Acad Sci U S A 107*, 4746–4751.

Shah, A. S., Ben-Shahar, Y., Moninger, T. O., Kline, J. N., & Welsh, M. J. (2009). Motile cilia of human airway epithelia are chemosensory. *Science 325*, 1131–1134.

Shiffman, D., O'Meara, E. S., Bare, L. A., Rowland, C. M., Louie, J. Z., Arellano, A.R., et al. (2008). Association of gene variants with incident myocardial infarction in the cardiovascular health study. *Arterioscler Thromb Vasc Biol 28*, 173–179.

Shigemura, N., Iwata, S., Yasumatsu, K., Ohkuri, T., Horio, N., Sanematsu, K., et al. (2013). Angiotensin II modulates salty and sweet taste sensitivities. *J Neurosci 33*, 6267–6277.

Shin, Y. -J., Park, J. -H., Choi, J. -S., Chun, M. -H., Moon, Y., & Lee, M. -Y. (2010). Enhanced expression of the sweet taste receptors and alpha-gustducin in reactive astrocytes of the rat hippocampus following ischemic injury. *Neurochem Res 35*, 1628–1634.

Shirokova, E., Schmiedeberg, K., Bedner, P., Niessen, H., Willecke, K., Raguse, J.D., et al. (2005). Identification of specific ligands for orphan olfactory receptors — G protein-dependent agonism and antagonisms of odorants. *J Biol Chem 280*, 11807–11815.

Shukla, A. K., Xiao, K., & Lefkowitz, R. J. (2011). Emerging paradigms of beta-arrestin-dependent seven transmembrane receptor signaling. *Trends Biochem Sci 36*, 457–469.

Sigoillot, M., Brockhoff, A., Meyerhof, W., & Briand, L. (2012). Sweet-taste-suppressing compounds: current knowledge and perspectives of application. *Appl Microbiol Biotechnol 96*, 619–630.

Singh, N., Vrontakis, M., Parkinson, F., & Chelikani, P. (2011). Functional bitter taste receptors are expressed in brain cells. *Biochem Biophys Res Commun 406*, 146–151.

Sklar, P. B., Anholt, R. R., & Snyder, S. H. (1986). The odorant-sensitive adenylate cyclase of olfactory receptor cells. Differential stimulation by distinct classes of odorants. *J Biol Chem 261*, 15538–15543.

Slack, J. P., Brockhoff, A., Batram, C., Menzel, S., Sonnabend, C., Born, S., et al. (2010). Modulation of bitter taste perception by a small molecule hTAS2R antagonist. *Curr Biol 20*, 1104–1109.

Smith, N. J. (2012). Low affinity GPCRs for metabolic intermediates: challenges for pharmacologists. *Front Endocrinol (Lausanne) 3*, 1.

Snead, A. N., & Insel, P. A. (2012). Defining the cellular repertoire of GPCRs identifies a profibrotic role for the most highly expressed receptor, protease-activated receptor 1, in cardiac fibroblasts. *FASEB J 26*, 4540–4547.

Soranzo, N., Bufe, B., Sabeti, P. C., Wilson, J. F., Weale, M. E., Marguerie, R., et al. (2005). Positive selection on a high-sensitivity allele of the human bitter-taste receptor TAS2R16. *Curr Biol 15*, 1257–1265.

Sotnikova, T. D., Caron, M. G., & Gainetdinov, R. R. (2009). Trace amine-associated receptors as emerging therapeutic targets. *Mol Pharmacol 76*, 229–235.

Spehr, M., Gisselmann, G., Poplawski, A., Riffell, J. A., Wetzel, C. H., Zimmer, R. K., et al. (2003). Identification of a testicular odorant receptor mediating human sperm chemotaxis. *Science 299*, 2054–2058.

Spehr, M., & Munger, S. D. (2009). Olfactory receptors: G protein-coupled receptors and beyond. *J Neurochem 109*, 1570–1583.

Spehr, M., Schwane, K., Heilmann, S., Gisselmann, G., Hummel, T., & Hatt, H. (2004). Dual capacity of a human olfactory receptor. *Curr Biol 14*, R832–R833.

Stephan, A.B., Tobochnik, S., Dibattista, M., Wall, C. M., Reisert, J., & Zhao, H. (2012). The Na^+/Ca^{2+} exchanger NCKX4 governs termination and adaptation of the mammalian olfactory response. *Nat Neurosci 15*, 131–137.

Stoddart, L. A., Smith, N. J., & Milligan, G. (2008). International Union of Pharmacology. LXXI. Free fatty acid receptors FFA1, -2, and -3: pharmacology and pathophysiological functions. *Pharmacol Rev 60*, 405–417.

Straub, S. G., Mulvaney-Musa, J., Yajima, H., Weiland, G. A., & Sharp, G. W. G. (2003). Stimulation of insulin secretion by denatonium, one of the most bitter-tasting substances known. *Diabetes 52*, 356–364.

Strotmann, J., Levai, O., Fleischer, J., Schwarzenbacher, K., & Breer, H. (2004). Olfactory receptor proteins in axonal processes of chemosensory neurons. *J Neurosci 24*, 7754–7761.

Su, C. Y., Menuz, K., & Carlson, J. R. (2009). Olfactory perception: receptors, cells, and circuits. *Cell 139*, 45–59.

Swartz, T. D., Duca, F. A., de Wouters, T., Sakar, Y., & Covasa, M. (2011). Up-regulation of intestinal type 1 taste receptor 3 and sodium glucose luminal transporter-1 expression and increased sucrose intake in mice lacking gut microbiota. *Br J Nutr 1–10*.

Talukdar, S., Olefsky, J. M., & Osborn, O. (2011). Targeting GPR120 and other fatty acid-sensing GPCRs ameliorates insulin resistance and inflammatory diseases. *Trends Pharmacol Sci 32*, 543–550.

Taniguchi, K. (2004). Expression of the sweet receptor protein, T1R3, in the human liver and pancreas. *J Vet Med Sci 66*, 1311–1314.

Tepper, B. J., Koelliker, Y., Zhao, L., Ullrich, N. V., Lanzara, C., d'Adamo, P., et al. (2008). Variation in the bitter-taste receptor gene TAS2R38, and adiposity in a genetically isolated population in southern Italy. *Obesity 16*, 2289–2295.

Tizzano, M., Cristofoletti, M., Sbarbati, A., & Finger, T. (2011). Expression of taste receptors in solitary chemosensory cells of rodent airways. *BMC Pulm Med 11*, 3.

Tizzano, M., Dvoryanchikov, G., Barrows, J. K., Kim, S., Chaudhari, N., & Finger, T. E. (2008). Expression of Galpha14 in sweet-transducing taste cells of the posterior tongue. *BMC Neurosci 9*.

Tizzano, M., Gulbransen, B.D., Vandenbeuch, A., Clapp, T. R., Herman, J. P., Sibhatu, H. M., et al. (2010). Nasal chemosensory cells use bitter taste signaling to detect irritants and bacterial signals. *Proc Natl Acad Sci U S A 107*, 3210–3215.

Tomonari, H., Miura, H., Nakayama, A., Matsumura, E., Ooki, M., Ninomiya, Y., et al. (2012). Galpha-gustducin is extensively coexpressed with sweet and bitter taste receptors in both the soft palate and fungiform papillae but has a different functional significance. *Chem Senses 37*, 241–251.

Tong, J., Mannea, E., Aimé, P., Pfluger, P. T., Yi, C. -X., Castaneda, T. R., et al. (2011). Ghrelin enhances olfactory sensitivity and exploratory sniffing in rodents and humans. *J Neurosci 31*, 5841–5846.

Touhara, K., & Vosshall, L. B. (2009). Sensing odorants and pheromones with chemosensory receptors. *Annu Rev Physiol 71*, 307–332.

Toyono, T., Seta, Y., Kataoka, S., & Toyoshima, K. (2007). CCAAT/enhancer-binding protein beta regulates expression of human T1R3 taste receptor gene in the bile duct carcinoma cell line, HuCCT1. *Biochim Biophys Acta 1769*, 641–648.

Trellakis, S., Tagay, S., Fischer, C., Rydleuskaya, A., Scherag, A., Bruderek, K., et al. (2011). Ghrelin, leptin and adiponectin as possible predictors of the hedonic value of odors. *Regul Pept 167*, 112–117.

Ueda, T., Ugawa, S., Yamamura, H., Imaizumi, Y., & Shimada, S. (2003). Functional interaction between T2R taste receptors and G-protein α subunits expressed in taste receptor cells. *J Neurosci 23*, 7376–7380.

Vanderhaeghen, P., Schurmans, S., Vassart, G., & Parmentier, M. (1993). Olfactory receptors are displayed on dog mature sperm cells. *J Cell Biol 123*, 1441–1452.

Vanderhaeghen, P., Schurmans, S., Vassart, G., & Parmentier, M. (1997a). Molecular cloning and chromosomal mapping of olfactory receptor genes expressed in the male germ line: evidence for their wide distribution in the human genome. *Biochem Biophys Res Commun 237*, 283–287.

Vanderhaeghen, P., Schurmans, S., Vassart, G., & Parmentier, M. (1997b). Specific repertoire of olfactory receptor genes in the male germ cells of several mammalian species. *Genomics 39*, 239–246.

Vassar, R., Ngai, J., & Axel, R. (1993). Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. *Cell 74*, 309–318.

Veitinger, T., Riffell, J. R., Veitinger, S., Nascimento, J. M., Triller, A., Chandsawangbhuwana, C., et al. (2011). Chemosensory Ca^{2+} dynamics correlate with diverse behavioral phenotypes in human sperm. *J Biol Chem 286*, 17311–17325.

Voigt, A., Hubner, S., Lossow, K., Hermans-Borgmeyer, I., Boehm, U., & Meyerhof, W. (2012). Genetic labeling of Tas1r1 and Tas2r131 taste receptor cells in mice. *Chem Senses 37*, 897–911.

Von Dannecker, L. E., Mercadante, A. F., & Malnic, B. (2006). Ric-8B promotes functional expression of odorant receptors. *Proc Natl Acad Sci U S A 103*, 9310–9314.

Vosshall, L. B. (2004). Olfaction: attracting both sperm and the nose. *Curr Biol 14*, R918–R920.

Waku, T., Shiraki, T., Oyama, T., Maebara, K., Nakamori, R., & Morikawa, K. (2010). The nuclear receptor PPARgamma individually responds to serotonin- and fatty acid-metabolites. *EMBO J 29*, 3395–3407.

Walensky, L. D., Roskams, A. J., Lefkowitz, R. J., Snyder, S. H., & Ronnett, G. V. (1995). Odorant receptors and desensitization proteins colocalize in mammalian sperm. *Mol Med 1*, 130–141.

Walensky, L. D., Ruat, M., Bakin, R. E., Blackshaw, S., Ronnett, G. V., & Snyder, S. H. (1998). Two novel odorant receptor families expressed in spermatids undergo 5′-splicing. *J Biol Chem 273*, 9378–9387.

Wauson, E. M., Zaganjor, E., Lee, A. Y., Guerra, M. L., Ghosh, A.B., Bookout, A. L., et al. (2012). The G protein-coupled taste receptor T1R1/T1R3 regulates mTORC1 and autophagy. *Mol Cell 47*, 851–862.

Weber, M., Pehl, U., Breer, H., & Strotmann, J. (2002). Olfactory receptor expressed in ganglia of the autonomic nervous system. *J Neurosci Res 68*, 176–184.

Wellendorph, P., Johansen, L. D., & Bräuner-Osborne, H. (2009). Molecular pharmacology of promiscuous seven transmembrane receptors sensing organic nutrients. *Mol Pharmacol 76*, 453–465.

Wettschureck, N., & Offermanns, S. (2005). Mammalian G proteins and their cell type specific functions. *Physiol Rev 85*, 1159–1204.

Widmayer, P., Breer, H., & Hass, N. (2011). Candidate chemosensory cells in the porcine stomach. *Histochem Cell Biol 136*, 37–45.

Wong, G. T., Gannon, K. S., & Margolskee, R. F. (1996). Transduction of bitter and sweet taste by gustducin. *Nature 381*, 796–800.

Wong, G. T., Ruiz-Avila, L., & Margolskee, R. F. (1999). Directing gene expression to gustducin-positive taste receptor cells. *J Neurosci 19*, 5802–5809.

Wong, S. T., Trinh, K., Hacker, B., Chan, G. C. K., Lowe, G., Gaggar, A., et al. (2000). Disruption of the type III adenylyl cyclase gene leads to peripheral and behavioral anosmia in transgenic mice. *Neuron 27*, 487–497.

Wootten, D., Savage, E. E., Valant, C., May, L. T., Sloop, K. W., Ficorilli, J., et al. (2012). Allosteric modulation of endogenous metabolites as an avenue for drug discovery. *Mol Pharmacol 82*, 281–290.

Wu, S. V., Chen, M. C., & Rozengurt, E. (2005). Genomic organization, expression, and function of bitter taste receptors (T2R) in mouse and rat. *Physiol Genomics 22*, 139–149.

Wu, S. V., Rozengurt, N., Yang, M., Young, S. H., Sinnett-Smith, J., & Rozengurt, E. (2002). Expression of bitter taste receptors of the T2R family in the gastrointestinal tract and enteroendocrine STC-1 cells. *Proc Natl Acad Sci U S A 99*, 2392–2397.

Xia, Z., Choi, E. J., Wang, F., & Storm, D. R. (1992). The type III calcium/calmodulin-sensitive adenylyl cyclase is not specific to olfactory sensory neurons. *Neurosci Lett 144*, 169–173.

Xu, J., Cao, J., Iguchi, N., Riethmacher, D., & Huang, L. (2013). Functional characterization of bitter-taste receptors expressed in mammalian testis. *Mol Hum Reprod 19*, 17–28.

Xu, L. L., Stackhouse, B. G., Florence, K., Zhang, W., Shanmugam, N., Sesterhenn, I. A., et al. (2000). PSGR, a novel prostate-specific gene with homology to a G protein-coupled receptor, is overexpressed in prostate cancer. *Cancer Res 60*, 6568–6572.

Xu, H., Staszewski, L., Tang, H. X., Adler, E., Zoller, M., & Li, X. D. (2004). Different functional roles of T1R subunits in the heteromeric taste receptors. *Proc Natl Acad Sci U S A 101*, 14258–14263.

Yan, W. T., Sunavala, G., Rosenzweig, S., Dasso, M., Brand, J. G., & Spielman, A. I. (2001). Bitter taste transduced by PLC-beta(2)-dependent rise in IP3 and alpha-gustducin-dependent fall in cyclic nucleotides. *Am J Physiol Cell Physiol 280*, C742–C751.

Yang, H., Wanner, I. B., Roper, S. D., & Chaudhari, N. (1999). An optimized method for in situ hybridization with signal amplification that allows the detection of rare mRNAs. *J Histochem Cytochem 47*, 431–445.

Yarmolinsky, D. A., Zuker, C. S., & Ryba, N. J. P. (2009). Common sense about taste: from mammals to insects. *Cell 139*, 234–244.

Yoshida, R., Ohkuri, T., Jyotaki, M., Yasuo, T., Horio, N., Yasumatsu, K., et al. (2010). Endocannabinoids selectively enhance sweet taste. *Proc Natl Acad Sci U S A 107*, 935–939.

Yoshikawa, K., Nakagawa, H., Mori, N., Watanabe, H., & Touhara, K. (2013). An unsaturated aliphatic alcohol as a natural ligand for a mouse odorant receptor. *Nat Chem Biol 9*, 160–162.

Young, R. L., Sutherland, K., Pezos, N., Brierley, S. M., Horowitz, M., Rayner, C. K., et al. (2009). Expression of taste molecules in the upper gastrointestinal tract in humans with and without type 2 diabetes. *Gut 58*, 337–346.

Yuan, T. T. -T., Toy, P., McClary, J. A., Lin, R. J., Miyamoto, N. G., & Kretschmer, P. J. (2001). Cloning and genetic characterization of an evolutionarily conserved human olfactory receptor that is differentially expressed across species. *Gene 278*, 41–51.

Zhang, X., Bedigian, A. V., Wang, W., & Eggert, U. S. (2012b). G protein-coupled receptors participate in cytokinesis. *Cytoskeleton (Hoboken) 69*, 810–818.

Zhang, C. H., Chen, C., Lifshitz, L. M., Fogarty, K. E., Zhu, M. S., & ZhuGe, R. (2012a). Activation of BK channels may not be required for bitter tastant-induced bronchodilation. *Nat Med 18*, 648–650 (author reply 650-641).

Zhang, X. H., De la Cruz, O., Pinto, J. M., Nicolae, D., Firestein, S., & Gilad, Y. (2007). Characterizing the expression of the human olfactory receptor gene family using a novel DNA microarray. *Genome Biol 8*.

Zhang, X., & Firestein, S. (2009). Genomics of olfactory receptors. *Results Probl Cell Differ 47*, 25–36.

Zhang, Y., Hoon, M.A., Chandrashekar, J., Mueller, K. L., Cook, B., Wu, D., et al. (2003). Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell 112*, 293–301.

Zhang, C. -H., Lifshitz, L. M., Uy, K. F., Ikebe, M., Fogarty, K. E., & ZhuGe, R. (2013). The cellular and molecular basis of bitter tastant-induced bronchodilation. *PLoS Biol 11*, e1001501.

Zhang, X. M., Rogers, M., Tian, H. K., Zhang, X. H., Zou, D. J., Jian, L., et al. (2004). High-throughput microarray detection of olfactory receptor gene expression in the mouse. *Proc Natl Acad Sci U S A 101*, 14168–14173.

Zhao, H. Q., Ivic, L., Otaki, J. M., Hashimoto, M., Mikoshiba, K., & Firestein, S. (1998). Functional expression of a mammalian odorant receptor. *Science 279*, 237–242.

Zhuang, X. X., Belluscio, L., & Hen, R. (2000). G(olf)alpha mediates dopamine D1 receptor signaling. *J Neurosci 20*, RC91.

Zhuang, H., & Matsunami, H. (2008). Evaluating cell-surface expression and measuring activation of mammalian odorant receptors in heterologous cells. *Nat Protoc 3*, 1402–1413.