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(54) Title: METHODS FOR IDENTIFYING A RECEPTOR FOR A LIGAND AND USES THEREOF

(57) Abstract: The present invention relates to a novel method for identifying pairs of receptors/ligands, transgenic animals useful for carrying out said method, and the use of ligands and/or modulators of the interaction between a ligand and its receptor in the food industry, fragrance industry, and health industry, for instance.

METHODS FOR IDENTIFYING A RECEPTOR FOR A LIGAND AND USES THEREOF

Field of the Invention

The present invention relates to the identification of chemoreceptors responding to defined chemical molecules or blend of chemical molecules, i.e. identifying pairs of receptor/ligand, and the application of the knowledge of such a relationship in any industry including food industry, fragrance industry, health industry.

Background of the Invention

Animals have evolved various types of chemosensory tools to perceive the outside world. Among these are the taste and the olfactory systems. Specialized chemosensors are expressed in these structures. Depending on the species, these may include for example G protein-coupled receptors (GPCRs) such as odorant receptors (ORs), vomeronasal receptors (VRs), trace amine receptors (TAARs), formyl peptide receptors (FPRs), T1R and T2R taste receptors, and non-GPCRs such as transient receptor potential (Trp) channels, guanylyl cyclases, and olfactory ionotropic receptors (IRs) (*Kaupp, 2010, Nature Rev. Neuroscience, 11: 188-200*). These sensors allow the animals to face the immense variety of external stimuli, in particular via their ORs, which allows them to detect and discriminate billions of different molecules. The gene repertoire encoding these receptors is diverse inside a given species, and is variable between species, both in terms of size and in terms of contents. In the mouse, for example, the OR repertoire reaches 1'250 members, which represents over 5% of its total number of genes. Every olfactory sensory neuron expresses a single olfactory receptor gene, which means that hundreds of functionally different populations of sensory neurons coexist in the nasal cavity. Each of these populations can be activated by various agonists, and each agonist can be recognized by various ORs. This leads to a combinatorial code, which allows discrimination between different blends.

In the last 30 years, in a process called deorphanization, ligands and antagonists have been assigned to a large fraction of GPCRs. This is to the exception of ORs, which remain orphans for their largest part. For example, over 90% of human ORs are still orphans (*Peterlin et al., 2014, J. Gen. Physiol., 143, 527-542*). Individual OR deorphanization is important, but even more interesting would be to provide a list of ORs that respond to a given chemical in a given species. Today, in mice or humans, not

a single odorant molecule is known for which an exhaustive list of cognate ORs has been defined. Such knowledge could be very valuable for understanding the combinatorial code at the base of the sense of smell, but could also have commercial applications. For example, the knowledge of the OR repertoire activated by a given odorant would be helpful for mimicking given olfactory stimuli, particularly those with positive hedonic values in humans (like chocolate or flowers). Alternatively this could allow the discovery of odorant antagonists blocking the perception of undesired odors or flavors (like unpleasant body odors or the smell of sewers). Taken as a whole, it would facilitate our ability to modulate specific chemosensory percepts.

The limited number of deorphanized ORs, to date, does not result from a lack of efforts, but rather from a lack of suitable assays. Most known olfactory agonist-receptor pairs were identified *in vitro*. These approaches involved the expression of rodent or human chemoreceptors in heterologous systems, including xenopus oocytes, yeasts, ovarian insect cells, baculoviruses, and native or engineered HEK and Hela cells (*Peterlin et al., 2014, supra*). These expression systems brought significant advances and allowed the deorphanization (that is to find at least one activating molecule) and characterization of 41 human and 95 mouse ORs. However, these non-native methodologies suffer from significant downsides. First, ORs produced *in vitro* are usually retained in the endoplasmic reticulum and thus fail to reach the cell membrane. Their fusion with segments of non-olfactory proteins is therefore often chosen for heterologous expression, possibly modifying their response profiles. Second, a complex nasal mucus containing odorant binding proteins, that represents the natural interface between receptors and their potential agonists, is absent *in vitro*. This is critical since this mucus plays an enzymatic role that chemically modifies many odorant molecules (*Nagashima and Touhara, 2010, J. Neurosci., 30, 16391 - 16398*). Third, the coupling of the OR to its native transduction cascade, which is usually not recapitulated in vitro, is known to affect receptor-odorant specificities (*Shirokova et al., 2005, J. Biol. Chem., 280, 11807 - 11815*). Finally, potential ligands are provided in liquid and not gaseous phase in vitro, making their concentrations difficult to relate to those present during natural ortho- and retronasal fluxes.

To circumvent some of the non-native downsides of heterologous expression, alternative approaches were taken. Efforts to develop *in silico* models have been made

(e.g. *Bavan et al., 2014, PLoS One 9, e92064*). Closer to physiological conditions, responses of olfactory sensory neurons expressing endogenous or exogenous ORs to chemicals were studied (*Araneda et al., 2000, Nat. Neurosci. 3, 1248-1255; Malnic et al., 1999, Cell 96, 713 - 723; Oka et al., 2006, Neuron, 52, 857-869).* Other methods based on gene-targeted mice in which defined olfactory sensory neurons were labeled, also proved successful for a handful of ORs. However, these methodologies based on sensory neurons involve *ex vivo* preparations or complex mouse surgeries, and most importantly, only allow deorphanization of one receptor at a time.

Therefore, there remains a need for a method allowing the rapid and easy identification of the receptors that respond to specific olfactory compounds *in vivo*, in particular those of special interest like malodor counteracting molecules or smell modulators. Such a method would also constitute a critical tool for large scale screening of agonists and antagonists.

Summary of the Invention

The inventors found that, unexpectedly, in mice and flies, following *in vivo* or *ex vivo* exposure to a chemical stimulus, olfactory sensory neurons which respond to this stimulus quickly modulate (upregulate or downregulate) the amount of transcripts corresponding to the olfactory receptor(s) they express. Based on these findings, the present invention provides a simple, fast and efficient method that allows the identification of receptors responding to specific chemical stimuli, based on alterations of mRNA expression. Although primarily illustrated with olfactory receptors/ligands, the present invention can be extended to other chemoreceptors/ligands pairs, to other species, and to transgenic species expressing specific chemoreceptors.

A first aspect of the invention provides a method of identifying at least one chemoreceptor for at least one ligand comprising the steps of:

a) providing a biological sample comprising cells expressing at least one chemoreceptor, wherein said biological sample (i) has been exposed to at least one test compound or (ii) was obtained from an animal that has been exposed to at least one test compound;

b) measuring a signal that is proportional to the level of transcription of at least one gene encoding a chemoreceptor in said biological sample,

c) comparing the level of signal determined in step b) to the level of signal determined in the same conditions with a negative control where the biological sample or animal has not been exposed to said at least one test compound;

wherein a difference between the level of signal determined in step b) and the level of signal determined in the same conditions with a negative control indicates that said at least one test compound constitutes a ligand for said at least one chemoreceptor and is able to bind and modulate the activity of said at least one chemoreceptor.

A second aspect of the invention relates to a method of identifying an agent able to modulate the binding of a ligand for its chemoreceptor based on the comparison in the level of transcription of a gene encoding said chemoreceptor in presence and absence of ligand and/or test agent.

A third aspect of the invention resides in transgenic non-human animal expressing at least five exogenous chemoreceptor genes.

A fourth aspect of the invention relates to isolated cells such as sensory cells and/or tissues such as tissues present in the olfactory system, extracted from said transgenic non-human animals.

A fifth and sixth aspects of the invention concerns a method for producing said transgenic non-human animals and the use thereof in the methods according to the invention.

A seventh aspect of the invention relates to a ligand binding to a chemoreceptor as well as agents modulating the binding of a ligand to its chemoreceptor, which can be identified by the methods of the invention, as well as compositions comprising said ligand and/or agents.

An eighth aspect of the invention provides a method for modulating the perception of at least one scent and/or at least one taste in a subject comprising the use of at least one ligand of at least one chemoreceptor involved in the perception of said scent and/or taste and/or at least one agent modulating the binding of a ligand to said chemoreceptor.

Other features and advantages of the invention will be apparent from the following detailed description.

Description of the figures

Figure 1: Olfactory receptor transcript modulation following neuronal activation. (a): Schematic representation of the protocol employed. (b)-(f): Olfactory receptor transcript levels following olfactory stimulation by acetophenone (b), heptanal (c), tetradecanal (d), lyral (e), ethyl isobutyrate (f), and vanillic acid (g). Olfactory receptor gene transcript levels were evaluated by RT-qPCR for each olfactory receptor gene, and the ratios between the values obtained in exposed versus control mice are shown. Olfactory receptors present in the left-side zone correspond to those which were previously shown to respond to the tested volatile. Those present in the right-hand zone were previously shown to be non-responsive to the chemicals. Each dot represents a single mouse. Medians are shown as black horizontal bars, and boxes extend from the 25^{th} and the 75^{th} percentile. The horizontal grey zone corresponds to values that are not considered as significantly modulated.

Figure 2: Olfactory receptor transcript modulation after *in vitro* ethylisobutyrate exposure. Olfactory receptor gene transcript levels were evaluated by RT-qPCR for each olfactory receptor gene, and the ratios between the values obtained in exposed versus control mice are shown. Olfactory receptors present in the left-side zone correspond to those which were previously shown to respond to the tested volatile. Those present in the right-hand zone were previously shown to be non-responsive to the chemicals.

Figure 3: Transcriptome-wide evaluation of olfactory receptor transcript downregulation after acetophenone exposure. Three mice were exposed per condition, and a library of OR genes was sequenced for each mouse. Black/grey/white rectangles indicate the levels of transcript reduction (from 0.1=90% reduction (black) to 1=0% reduction (white)) relative to the levels present in control mice. *Olfr* names in grey represent receptor genes whose products were previously shown to be responsive *in vitro* to acetophenone. *Olfr391ps*, *Olfr1025ps* and *Olfr1174ps* are considered by ENSEMBL as pseudogenes. However, following our own criteria based on sequence signatures, *Olfr391ps* and *Olfr1025ps* are considered herewith as potentially functional OR genes and *Olfr1174ps* as dubious. Right panel: RT-qPCRs of selected OR gene candidates after 5% acetophenone exposure.

Figure 4: Neurons whose transcripts are downregulated by odorant exposure are activated neurons. Receptor transcript-specific in situ hybridization followed by immunolabeling of the activity-dependent marker pS6 were performed. Quantification of pS6 coexpression with *Olfr983*, *Olfr171* and *Olfr145* after acetophenone or ethyl isobutyrate exposure.

Figure 5: Drosophila OR transcript levels are reduced after agonist stimulation. (a) Schematic representation of the protocol of the method of the invention used as described in Example 6. The amount of OR67c (b) and OR82a (c) mRNA from fly antennae as evaluated by qPCR after 5% ethyl lactate or 5% geranyl acetate exposure for 5 hours. Each dot represents pooled RNA from a vial containing 12 or 25 flies. p values: ***($p<0.001$), two-tailed Mann-Whitney U test between the tested OR gene and all control non-responsive receptor genes.

Figure 6: TAAR transcript modulation following neuronal activation. Transcript levels of olfactory receptor genes were evaluated in mice following olfactory stimulation during 48 hours by beta-phenylethylamine as described in Example 7. Olfactory receptor gene transcript levels were evaluated by RT-qPCR for each receptor gene, and the ratios between the values obtained in exposed versus control mice are shown. "TAAR" correspond to the olfactory receptor genes (trace amine receptor gene) previously shown to respond to beta-phenylethylamine and "olfr" correspond to the olfactory receptor genes which were previously shown to be non-responsive to the chemicals. Each dot represents a single mouse. Medians are shown as black horizontal bars, and boxes extend from the 25th and the 75th percentile. The horizontal grey zone corresponds to values that are not considered as significantly modulated.

Detailed description of the invention

As defined herewith, a "chemoreceptor", also called "chemosensor", is a sensory receptor that transduces a chemical signal into cellular responses. In more general terms, a chemoreceptor detects certain chemical stimuli in the environment. In vertebrates, chemoreceptors include odorant receptors (ORs), trace amine receptors (TAARs), vomeronasal type 1 (V1Rs), vomeronasal type 2 receptors (V2Rs), formyl peptide receptors (FPRs), transient receptor potential (Trp) channels, guanylyl cyclase D and G (GCD/G), taste type 1 receptors (T1Rs), taste type 2 receptors (T2Rs), endothelium non-voltage gated sodium channel (ENaC), polycistic kidney disease 2 like

1 (PKD2L1) channels. In insects, chemoreceptors include ionotropic 7TM ORs, 7TM GRs and ionotropic IRs (*Kaupp, 2010, Nature Rev. Neuroscience, 11: 188-200*). Chemoreceptors include, for instance, olfactory receptors from the olfactory system, taste (or gustatory) receptors from the gustatory system, Trp channels from the trigeminal system. The term "chemoreceptor" encompasses the polypeptides having the same amino acid sequence as those naturally found in animals, as well as any variant thereof that is biologically active, i.e. that functions as a chemoreceptor.

As used herewith the term "variants" of a chemoreceptor encompasses polypeptides that have a high degree of similarity or a high degree of identity with the amino acid sequence of a chemoreceptor found in nature (e.g. a human chemoreceptor or a murine chemoreceptor) and which are biologically active, i.e. said polypeptides function as chemoreceptors and transduce a chemical signal into cellular responses. In particular, the term "variants" of a chemoreceptor encompasses polypeptides substantially homologous to a reference polypeptide (e.g. defined by a specific amino acid sequence) including orthologous polypeptides found in different species, or an isoform, a mutant, or fragment thereof, e.g. which have an amino acid sequence different from that of the reference polypeptide because of one or more deletions, insertions or substitutions. Substantially homologous means a variant amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the reference amino acid sequence. The term "variant" also applies to the nucleic acid sequence encoding a chemoreceptor. Applied to a nucleic acid sequence, substantially homologous means a variant nucleic acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the reference nucleic acid sequence. The "percentage of identity" between two amino acid sequences or between two nucleic acid sequences can be determined by visual inspection and/or mathematical calculation, or more easily by comparing sequence information using known computer programs used for sequence analyses such as Clustal package version 1.83.

As used herewith, "G protein-coupled receptor proteins (GPCRs)", also known as "seven-transmembrane domain receptors", "7TM receptors", "heptahelical receptors", "serpentine receptors", and "G protein-linked receptors (GPLR)", designate a large protein family of receptors that sense molecules outside the cell and activate, inside the

cell, signal transductions pathways and, ultimately, cellular responses. GPCRs are found in eukaryotes, including yeast and animals. The ligands that bind and activate these receptors include light-sensitive compounds, odors, pheromones, hormones, and neurotransmitters, and vary in size from small molecules to peptides to large proteins.

As used herewith, the terms “olfactory receptors“ designate the receptors expressed in the cell membranes of olfactory sensory neurons responsible for the detection of chemical cues. Activated olfactory receptors are the initial player in a signal transduction cascade which ultimately produces a nerve impulse which is transmitted to the brain. Most of these receptors are members of the GPCR superfamily. The olfactory receptors form a multigene family consisting of about 400 potentially functional genes in humans and about 1250 genes in mice. Olfactory receptors are generally categorized, in mammals, into several receptor families including odorant receptors (ORs), vomeronasal receptors (V1Rs and V2Rs), trace amine-associated receptors (TAARs), formyl peptide receptors (FPRs), and the membrane guanyl cyclase GC-D.

“Olfactory sensory neurons” (OSNs) designate herewith highly specialized chemosensory cells found in the nasal compartments constituting the olfactory system including the main olfactory epithelium (MOE), the vomeronasal organ (VNO), the septal organ (SO) and the Grueneberg ganglion (GG). Olfactory sensory neurons allow the perception of odors and pheromones.

As used herewith, the terms “gustatory receptors“ include T1Rs, T2Rs, ENaC, PKD2L1 and GRs.

“Gustatory sensory cells” or "taste receptor cells" designate cells expressing gustatory receptors such as T1Rs, T2Rs, ENaC, PKD2L1 or GRs.

The term "ligand" or "chemical stimulus" as used herein refers to a molecule that can bind a chemoreceptor and modulate (activate or inhibit) the function of said chemoreceptor. It follows that a ligand can modulate the downstream signaling activities of its cognate chemoreceptor (e.g a GPCR) and/or the global olfactory response in the specific case of an olfactory receptor. When a molecule activates a chemoreceptor, it is qualified as “agonist” of said receptor. When a molecule inhibits the activation by an agonist of its cognate chemoreceptor, said molecule is qualified as “antagonist” of said receptor. A ligand for a chemoreceptor can be a molecule of various chemical structures including a peptide, a polypeptide (including an antibody or

antigen-binding fragment thereof), a lipid, a carbohydrate, a nucleic acid, a small organic or non-organic molecule including but not limited to an odorant, a fragrance compound and a pheromone, a molecule from a synthetic or natural source, from a chemical or peptide library for instance. A chemical stimulus that can modulate the function of an olfactory receptor is called an "olfactory stimulus".

The term "olfactory stimulus" or "odorant" as used herein comprises any molecule, or group of molecules, volatile or not, aqueous soluble or not, that could interact with an olfactory receptor system such as an olfactory receptor *in vivo* or an olfactory receptor *in vitro* expressed in a cell or a tissue. The sources and the identity of pleasant and unpleasant odorants are very diverse. Olfactory stimuli can be molecules such as alkanes, esters, linear terpenes, cyclic terpenes, aromatic, amines, alcohols, aldehydes, ketones, lactones, thiols, gases. Examples of olfactory stimuli include unpleasant body odors such as those found in breath (methanethiol, hydrogen sulfide, dimethyl sulfide, etc), on the feet (propanoic acid, isovaleric acid, etc), or on the armpits ((E)-3-methyl-2-hexenoic acid, (S)-3-methyl-3-sulfanylhexan-1-ol, 3-hydroxy-3-methylhexanoic acid, propionic acid, androstenone, etc).

The terms “agonist” and “antagonist” of a chemoreceptor refer herewith to an agent that modulates (activates and inhibits, respectively) the function of said chemoreceptor and, thus, the downstream signaling activities related to said chemoreceptor and/or, in the case of a ligand binding an olfactory receptor for instance, a global olfactory response related to said olfactory receptor. The agonist and antagonist of a chemoreceptor can act by modulating (enhancing and inhibiting, respectively) the binding of a ligand for its chemoreceptor. Said agonist and said antagonist can be of various natures including a peptide, a polypeptide, an antibody or antigen-binding fragment thereof, a lipid, a carbohydrate, a nucleic acid, a small organic or non-organic molecule including but not limited to an odorant, a fragrance compound and a pheromone, a molecule from a synthetic or natural source, from a chemical or peptide library for instance.

The term "transgene" or “exogenous gene” as used herein refers to a foreign gene that is placed into one or more of the cells of an organism (called a “transgenic organism”) by introducing said foreign gene by way of human intervention, such as by microinjection, electric shock or by infection with a recombinant virus, into newly fertilized eggs, germ cells or early embryos, for instance. Thus, one or more of the cells of a transgenic

animal contains at least one foreign gene (the transgene), which is part of the genetic material of this transgenic animal. It is advantageous that the transgene is contained in the transgenic animal's germ line such that it can be transmitted to the animal's offspring. The term "foreign gene" refers to any nucleic acid (e.g. gene sequence) that is introduced into the genome of an animal by experimental manipulations. The foreign gene generally encompasses the gene sequence of a gene (e.g. of a chemoreceptor such as an olfactory receptor) from a different animal species than the transgenic animal expressing said transgene, e.g. the foreign gene can be a human olfactory receptor gene expressed in a transgenic mouse. A foreign gene may also include a gene sequence found in that animal as long as the introduced gene does not reside in the same location as does the naturally-occurring gene. A foreign gene can also be an "autologous gene" defined as encompassing variants (e.g., polymorphisms or mutants) of the naturally occurring gene.

Methods of identifying ligands of chemoreceptors and/or agents modulating the effect of a ligand on its chemoreceptor

In a first aspect, the invention provides a method of deorphanizing a chemoreceptor, i.e. identifying at least one ligand of at least one chemoreceptor and, thus, identifying the members of at least one ligand/chemoreceptor pair.

Typically, the binding of a ligand to its chemoreceptor generates the downstream signaling activities related to said chemoreceptor and/or, in the case of a ligand binding an olfactory receptor, a global olfactory response related to said olfactory receptor.

In one embodiment, the invention provides a method of identifying at least one chemoreceptor for at least one ligand comprising the steps of:

a) providing a biological sample comprising cells expressing at least one chemoreceptor, wherein said biological sample (i) has been exposed to at least one test compound or (ii) was obtained from an animal that has been exposed to at least one test compound;

b) measuring a signal that is proportional to the level of transcription of at least one gene encoding a chemoreceptor in said biological sample,

c) comparing the level of signal determined in step b) to the level of signal determined in the same conditions with a negative control where the biological sample or animal has not been exposed to said at least one test compound;

wherein a difference between the level of signal determined in step b) and the level of signal determined in the same conditions with a negative control indicates that said at least one test compound constitutes a ligand for said at least one chemoreceptor and is able to bind and modulate the activity of said at least one chemoreceptor.

According to one embodiment of the method of the invention, when the level of signal determined in step b) in the biological sample after exposure to at least one test compound is lower than the level of signal determined in the same conditions with a negative control without exposure to a test compound, this indicates that said at least one test compound constitutes a ligand acting as an agonist for said at least one chemoreceptor.

According to another embodiment of the method of the invention, when the level of signal determined in step b) in the biological sample after exposure to at least one test compound is higher than the level of signal determined in the same conditions with a negative control without exposure to a test compound, this indicates that said at least one test compound constitutes a ligand acting as an antagonist for said at least one chemoreceptor.

In the invention, a biological sample typically comprises isolated cells or cells within a tissue, wherein said cells express at least one chemoreceptor and wherein the gene encoding said chemoreceptor was naturally present in said cells or was introduced within said cells by genetic engineering or is present in said cells because said cells have been obtained from a transgenic non-human animal expressing at least one exogenous chemoreceptor gene as described herewith.

According to the invention, said chemoreceptor is selected among a G protein-coupled receptor protein (GPCR) or a non-GPCR protein.

In a particular embodiment of the invention, said chemoreceptor is a GPCR protein, more particularly a receptor selected from the group consisting of an olfactory receptor and a gustatory receptor.

Non-GPCR proteins useful in the invention include Trp channels, GCD/G, ENaC, PCKD channels, ionotropic 7TM ORs, 7TM GRs and ionotropic IRs.

In a particular embodiment, said chemoreceptor is an olfactory receptor.

Examples of human odorant receptors include those listed below, where the reference indicated in brackets provide access to their amino acid sequence and nucleic acid sequence available in public databases:

OR2W1 (ENSG00000229328), OR3A3 (ENSG00000159961), OR5P3 (ENSG00000182334), OR5AN1 (ENSG00000176495), OR11H4 (ENSG00000176198), OR10A3 (ENSG00000170683), OR52I2 (ENSG00000226288), OR7A5 (ENSG00000188269), OR6X1 (ENSG00000221931), OR52I1 (ENSG00000232268), OR4L1 (ENSG00000176246), OR5A2 (ENSG00000172324), OR52B2 (ENSG00000255307), OR4K17 (ENSG00000176230), OR8J1 (ENSG00000262796), OR5P2 (ENSG00000183303), OR56B4 (ENSG00000180919), OR5T3 (ENSG00000261897), OR51D1 (ENSG00000197428), OR6M1 (ENSG00000196099), OR2AG2 (ENSG00000188124), OR8K1 (ENSG00000263328), OR8J1 (ENSG00000172487), OR10K1 (ENSG00000173285), OR4N5 (ENSG00000184394), OR9G4 (ENSG00000262647), OR2H2 (ENSG00000229680), OR4C11 (ENSG00000172188), OR1J4 (ENSG00000239590), OR5T2 (ENSG00000262851), OR4C46 (ENSG00000185926), OR10R2 (ENSG00000198965), OR1N1 (ENSG00000171505), OR5T1 (ENSG00000262784), AC213223.1 (ENSG00000261958), OR1N2 (ENSG00000171501), OR1L8 (ENSG00000171496), OR4M2 (ENSG00000182974), OR5V1 (ENSG00000233046), OR12D2 (ENSG00000235966), OR2W1 (ENSG00000204704), OR4F4 (ENSG00000177693), OR6C75 (ENSG00000187857), OR10W1 (ENSG00000172772), OR2B3 (ENSG00000204703), OR2D3 (ENSG00000178358), OR51L1 (ENSG00000176798), OR8U1 (ENSG00000172199), OR8H2 (ENSG00000181767), OR1K1 (ENSG00000165204), OR7C2 (ENSG00000127529), OR7G3 (ENSG00000170920), OR2AE1 (ENSG00000244623), OR4P4 (ENSG00000181927), OR8K3 (ENSG00000262755), OR4S2 (ENSG00000174982), OR52A5 (ENSG00000171944), OR2Y1 (ENSG00000174339), OR4C6 (ENSG00000181903), OR2V1 (ENSG00000185372), OR8U8 (ENSG00000262315), OR2V2 (ENSG00000182613), OR1D5 (ENSG00000262628), OR2J3 (ENSG00000204701), OR1D2 (ENSG00000184166), OR8K3 (ENSG00000181689), OR4E2 (ENSG00000221977), OR52A1 (ENSG00000182070), OR7D2 (ENSG00000188000), OR13A1 (ENSG00000256574), OR2A42 (ENSG00000212807), OR2A7 (ENSG00000243896),

OR4A47 (ENSG00000237388), OR5A1 (ENSG00000172320), OR2J2 (ENSG00000204700), OR8B2 (ENSG00000204293), OR6Y1 (ENSG00000197532), OR6P1 (ENSG00000186440), OR8B3 (ENSG00000196661), OR14J1 (ENSG00000234195), OR10A6 (ENSG00000175393), OR2H1 (ENSG00000204688), OR2W1 (ENSG00000206525), OR8B4 (ENSG00000198657), OR8B8 (ENSG00000197125), OR4D6 (ENSG00000166884), OR8H3 (ENSG00000181761), OR2AG1 (ENSG00000170803), OR56A1 (ENSG00000180934), OR6A2 (ENSG00000184933), OR8J3 (ENSG00000167822), OR8D4 (ENSG00000181518), OR8K5 (ENSG00000181752), OR2A1 (ENSG00000221970), OR1E2 (ENSG00000127780), OR4D5 (ENSG00000171014), OR2F2 (ENSG00000221910), OR2B3 (ENSG00000225736), OR6T1 (ENSG00000181499), OR10S1 (ENSG00000196248), OR10G4 (ENSG00000254737), OR10G9 (ENSG00000236981), OR52J3 (ENSG00000205495), OR10G8 (ENSG00000234560), OR10G7 (ENSG00000182634), OR4K5 (ENSG00000176281), OR10X1 (ENSG00000186400), OR10Z1 (ENSG00000198967), OR5AP2 (ENSG00000172464), OR3A1 (ENSG00000180090), OR3A2 (ENSG00000221882), OR52E2 (ENSG00000176787), OR4K1 (ENSG00000155249), OR2B11 (ENSG00000177535), OR5K2 (ENSG00000231861), OR10C1 (ENSG00000204689), OR5AR1 (ENSG00000172459), OR5R1 (ENSG00000174942), OR10J5 (ENSG00000184155), OR51B6 (ENSG00000176239), OR8B12 (ENSG00000170953), OR8A1 (ENSG00000196119), OR8K1 (ENSG00000150261), OR52D1 (ENSG00000181609), OR1I1 (ENSG00000094661), OR2B3 (ENSG00000206524), OR2C3 (ENSG00000196242), OR14A2 (ENSG00000241128), OR13G1 (ENSG00000197437), OR10A5 (ENSG00000166363), OR6B2 (ENSG00000182083), OR2Z1 (ENSG00000181733), OR9A2 (ENSG00000179468), OR2J3 (ENSG00000206522), OR5M9 (ENSG00000150269), OR6V1 (ENSG00000225781), OR1G1 (ENSG00000183024), OR51B5 (ENSG00000242180), OR9G1 (ENSG00000174914), OR13F1 (ENSG00000186881), OR51Q1 (ENSG00000167360), OR13C4 (ENSG00000148136), OR13C3 (ENSG00000204246), OR6B3 (ENSG00000178586), OR4N4 (ENSG00000183706), OR13C8 (ENSG00000186943), OR51E1 (ENSG00000180785), OR6C65 (ENSG00000205328), OR4F3 (ENSG00000230178), OR7A10 (ENSG00000127515), OR5AC2 (ENSG00000196578), OR5H1

(ENSG00000231192), OR8H1 (ENSG00000262611), OR52N2 (ENSG00000180988), OR52N5 (ENSG00000181009), OR52K2 (ENSG00000181963), OR5B17 (ENSG00000197786), OR5M3 (ENSG00000174937), OR13C5 (ENSG00000277556), OR1F1 (ENSG00000168124), OR52W1 (ENSG00000175485), OR9K2 (ENSG00000170605), OR51M1 (ENSG00000184698), OR52E4 (ENSG00000180974), OR52B6 (ENSG00000187747), OR51B2 (ENSG00000184881), OR52E8 (ENSG00000183269), OR52E6 (ENSG00000205409), OR4F21 (ENSG00000176269), OR52N1 (ENSG00000181001), OR56B1 (ENSG00000181023), OR2F1 (ENSG00000213215), OR12D3 (ENSG00000112462), OR6C76 (ENSG00000185821), OR10C1 (ENSG00000206474), OR12D2 (ENSG00000168787), OR10G2 (ENSG00000255582), OR11H12 (ENSG00000257115), OR5V1 (ENSG00000243729), OR11G2 (ENSG00000196832), OR11A1 (ENSG00000204694), OR1M1 (ENSG00000170929), OR5H14 (ENSG00000236032), OR5J2 (ENSG00000174957), OR1Q1 (ENSG00000165202), OR1B1 (ENSG00000171484), OR7D4 (ENSG00000174667), OR11H1 (ENSG00000130538), OR10V1 (ENSG00000172289), OR52N4 (ENSG00000181074), OR6C70 (ENSG00000184954), OR6C2 (ENSG00000179695), OR1E1 (ENSG00000180016), OR2AP1 (ENSG00000179615), OR6C68 (ENSG00000205327), OR6C4 (ENSG00000179626), OR2J1 (ENSG00000226931), OR51A7 (ENSG00000176895), OR51A4 (ENSG00000205497), OR9G4 (ENSG00000172457), OR51F1 (ENSG00000188069), OR4B1 (ENSG00000175619), OR51G1 (ENSG00000176879), OR51G2 (ENSG00000176893), OR2H2 (ENSG00000204657), OR7A17 (ENSG00000185385), OR10A7 (ENSG00000179919), OR2H2 (ENSG00000206512), OR11H6 (ENSG00000176219), OR6J1 (ENSG00000255804), OR4C13 (ENSG00000258817), OR5AU1 (ENSG00000169327), OR4C12 (ENSG00000221954), OR2J2 (ENSG00000196231), OR51F2 (ENSG00000176925), OR1L1 (ENSG00000173679), OR1L3 (ENSG00000171481), OR51S1 (ENSG00000176922), OR51A2 (ENSG00000205496), OR52R1 (ENSG00000176937), OR8S1 (ENSG00000197376), OR6Q1 (ENSG00000172381), OR9I1 (ENSG00000172377), OR14J1 (ENSG00000237777), OR51H1P (ENSG00000176904), OR14J1 (ENSG00000234100), OR4D10 (ENSG00000254466), OR9Q1 (ENSG00000186509), OR51T1 (ENSG00000176900), OR9A4 (ENSG00000258083), OR4M1 (ENSG00000176299), OR4N2

(ENSG00000176294), OR4Q3 (ENSG00000182652), OR51E2 (ENSG00000167332), OR4A16 (ENSG00000181961), OR51I2 (ENSG00000187918), OR4A15 (ENSG00000181958), OR52H1 (ENSG00000181616), OR51I1 (ENSG00000167359), OR7E24 (ENSG00000237521), OR51V1 (ENSG00000176742), OR13C2 (ENSG00000276119), OR10A2 (ENSG00000170790), OR2J3 (ENSG00000229866), OR6C74 (ENSG00000197706), OR10A4 (ENSG00000170782), OR9Q2 (ENSG00000186513), OR13C9 (ENSG00000136839), OR52K1 (ENSG00000196778), OR4D11 (ENSG00000176200), OR1S2 (ENSG00000197887), OR4D1 (ENSG00000141194), OR5B3 (ENSG00000172769), OR5W2 (ENSG00000187612), OR6B1 (ENSG00000221813), OR5I1 (ENSG00000167825), OR2D2 (ENSG00000166368), OR1S1 (ENSG00000172774), OR4D2 (ENSG00000255713), OR4K15 (ENSG00000169488), OR2K2 (ENSG00000171133), OR2A5 (ENSG00000221836), OR7G2 (ENSG00000170923), OR6K2 (ENSG00000196171), OR2S2 (ENSG00000122718), OR4D9 (ENSG00000172742), OR5D13 (ENSG00000198877), OR5H15 (ENSG00000233412), OR52B4 (ENSG00000221996), OR7G1 (ENSG00000161807), OR10C1 (ENSG00000229412), OR1L4 (ENSG00000136939), OR12D3 (ENSG00000242022), OR10AG1 (ENSG00000174970), OR2A25 (ENSG00000221933), OR5B2 (ENSG00000172365), OR2J1 (ENSG00000204702), OR5K3 (ENSG00000206536), OR6K3 (ENSG00000203757), OR4K14 (ENSG00000169484), OR5H6 (ENSG00000230301), OR10T2 (ENSG00000186306), OR10K2 (ENSG00000180708), OR2AT4 (ENSG00000171561), OR4X2 (ENSG00000172208), OR5K4 (ENSG00000196098), OR5H2 (ENSG00000197938), OR5D14 (ENSG00000186113), OR52M1 (ENSG00000197790), OR12D2 (ENSG00000233481), OR6K6 (ENSG00000180433), OR10J1 (ENSG00000196184), OR4K13 (ENSG00000176253), OR13D1 (ENSG00000179055), OR5D18 (ENSG00000186119), OR4X1 (ENSG00000176567), OR4S1 (ENSG00000176555), OR4C3 (ENSG00000176547), OR4C5 (ENSG00000176540), OR6C6 (ENSG00000188324), OR1J1 (ENSG00000136834), OR4K2 (ENSG00000165762), OR1A2 (ENSG00000172150), OR4F29 (ENSG00000278566), OR2B2 (ENSG00000168131), OR6C1 (ENSG00000205330), OR2A12 (ENSG00000221858), OR2A4 (ENSG00000180658), OR6C3 (ENSG00000205329), OR5F1 (ENSG00000149133), OR1L6 (ENSG00000171459),

OR5AS1 (ENSG00000181785), OR5L2 (ENSG00000205030), OR5D16 (ENSG00000205029), OR5C1 (ENSG00000148215), OR56A3 (ENSG00000184478), OR1A1 (ENSG00000172146), OR13H1 (ENSG00000171054), OR2J2 (ENSG00000231676), OR52L1 (ENSG00000183313), OR4F17 (ENSG00000176695), OR2A2 (ENSG00000221989), OR5B12 (ENSG00000172362), OR6S1 (ENSG00000181803), OR56A4 (ENSG00000183389), OR5T2 (ENSG00000181718), OR5T3 (ENSG00000172489), OR5M11 (ENSG00000255223), OR10AD1 (ENSG00000172640), OR4F16 (ENSG00000273547), OR6F1 (ENSG00000169214), OR10D3 (ENSG00000197309), OR5T1 (ENSG00000181698), OR5M10 (ENSG00000254834), OR1C1 (ENSG00000221888), OR14A16 (ENSG00000196772), OR11L1 (ENSG00000197591), OR8H1 (ENSG00000181693), OR2C1 (ENSG00000168158), OR8I2 (ENSG00000172154), OR2W3 (ENSG00000238243), OR2T8 (ENSG00000177462), OR2AJ1 (ENSG00000177275), OR4F15 (ENSG00000182854), OR4F6 (ENSG00000184140), OR8D1 (ENSG00000196341), OR8D2 (ENSG00000197263), OR2L3 (ENSG00000198128), OR10P1 (ENSG00000175398), OR2L13 (ENSG00000196071), OR2L5 (ENSG00000197454), OR2AK2 (ENSG00000187080), OR2L8 (ENSG00000196936), OR1J2 (ENSG00000197233), OR2L2 (ENSG00000203663), OR2M5 (ENSG00000162727), OR2M2 (ENSG00000198601), OR2M3 (ENSG00000228198), OR2M4 (ENSG00000171180), OR2T33 (ENSG00000177212), OR10G3 (ENSG00000169208), OR5M1 (ENSG00000255012), OR2A14 (ENSG00000221938), OR5B21 (ENSG00000198283), OR2T12 (ENSG00000177201), OR4F5 (ENSG00000186092), OR2M7 (ENSG00000177186), OR14C36 (ENSG00000177174), OR6N2 (ENSG00000188340), OR5K1 (ENSG00000232382), OR2T4 (ENSG00000196944), OR2T6 (ENSG00000198104), OR2T1 (ENSG00000175143), OR2T7 (ENSG00000227152), OR2T2 (ENSG00000196240), OR2B6 (ENSG00000124657), OR7C1 (ENSG00000127530), OR2T3 (ENSG00000196539), OR2T5 (ENSG00000203661), OR2G6 (ENSG00000188558), OR2T29 (ENSG00000182783), OR2T34 (ENSG00000183310), OR2T10 (ENSG00000184022), OR2T35 (ENSG00000177151), OR2T27 (ENSG00000187701), OR14I1 (ENSG00000189181), OR4C15 (ENSG00000181939), OR4C16 (ENSG00000181935).

The sequences of the above-mentioned ORs can be retrieved from the uniprot.org or the ensembl.org (GRCh38 (GCA_000001405.15) assembly) websites.

In a particular embodiment, said olfactory receptor is a human odorant receptor, in particular a human odorant receptor selected from the group consisting of:

OR10A2, OR13C8, OR2AG2, OR2T8, OR4M2, OR52L1, OR5M3, OR7G2, OR10A3, OR13C9, OR2AJ1, OR2V1, OR4N2, OR52M1, OR5M8, OR7G3, OR10A4, OR13D1, OR2AK2, OR2V2, OR4N4, OR52N1, OR5M9, OR8A1, OR10A5, OR13F1, OR2AP1, OR2W1, OR4N5, OR52N2, OR5P2, OR8B12, OR10A6, OR13G1, OR2AT4, OR2W3, OR4P4, OR52N4, OR5P3, OR8B2, OR10A7, OR13H1, OR2B11, OR2Y1, OR4Q3, OR52N5, OR5R1, OR8B3, OR10AD1, OR13J1, OR2B2, OR2Z1, OR4S1, OR52R1, OR5T1, OR8B4, OR10AG1, OR14A16, OR2B3, OR3A1, OR4S2, OR52W1, OR5T2, OR8B8, OR10C1, OR14A2, OR2B6, OR3A2, OR4X1, OR56A1, OR5T3, OR8D1, OR10D3, OR14C36, OR2C1, OR3A3, OR4X2, OR56A3, OR5V1, OR8D2, OR10G2, OR14I1, OR2C3, OR4A15, OR51A2, OR56A4, OR5W2, OR8D4, OR10G3, OR14J1, OR2D2, OR4A16, OR51A4, OR56B1, OR6A2, OR8G1, OR10G4, OR14K1, OR2D3, OR4A47, OR51A7, OR56B3P, OR6B1, OR8G5, OR10G6, OR1A1, OR2F1, OR4A5, OR51B2, OR56B4, OR6B2, OR8H1, OR10G7, OR1A2, OR2F2, OR4B1, OR51B4, OR5A1, OR6B3, OR8H2, OR10G8, OR1B1, OR2G2, OR4C11, OR51B5, OR5A2, OR6C1, OR8H3, OR10G9, OR1C1, OR2G3, OR4C12, OR51B6, OR5AC2, OR6C2, OR8I2, OR10H1, OR1D2, OR2G6, OR4C13, OR51D1, OR5AK2, OR6C3, OR8J1, OR10H2, OR1D5, OR2H1, OR4C15, OR51E1, OR5AN1, OR6C4, OR8J3, OR10H3, OR1E1, OR2H2, OR4C16, OR51E2, OR5AP2, OR6C6, OR8K1, OR10H4, OR1E2, OR2J1, OR4C3, OR51F1, OR5AR1, OR6C65, OR8K3, OR10H5, OR1F1, OR2J2, OR4C46, OR51F2, OR5AS1, OR6C68, OR8K5, OR10J1, OR1G1, OR2J3, OR4C5, OR51G1, OR5AU1, OR6C70, OR8S1, OR10J3, OR1I1, OR2K2, OR4C6, OR51G2, OR5B12, OR6C74, OR8U1, OR10J5, OR1J1, OR2L13, OR4D1, OR51H1P, OR5B17, OR6C75, OR8U9, OR10K1, OR1J2, OR2L2, OR4D10, OR51I1, OR5B2, OR6C76, OR9A2, OR10K2, OR1J4, OR2L3, OR4D11, OR51I2, OR5B21, OR6F1, OR9A4, OR10P1, OR1K1, OR2L5, OR4D2, OR51L1, OR5B3, OR6J1, OR9G1, OR10Q1, OR1L1, OR2L8, OR4D5, OR51M1, OR5C1, OR6K2, OR9G4, OR10R2, OR1L3, OR2M2, OR4D6, OR51Q1, OR5D13, OR6K3, OR9G9, OR10S1, OR1L4, OR2M3, OR4D9, OR51S1, OR5D14, OR6K6, OR9I1, OR10T2, OR1L6, OR2M4, OR4E2,

OR51T1, OR5D16, OR6M1, OR9K2, OR10V1, OR1L8, OR2M5, OR4F15, OR51V1, OR5D18, OR6N1, OR9Q1, OR10W1, OR1M1, OR2M7, OR4F16, OR52A1, OR5F1, OR6N2, OR9Q2, OR10X1, OR1N1, OR2S2, OR4F17, OR52A5, OR5H1, OR6P1, OR10Z1, OR1N2, OR2T1, OR4F21, OR52B1P, OR5H14, OR6Q1, OR11A1, OR1Q1, OR2T10, OR4F29, OR52B2, OR5H15, OR6S1, OR11G2, OR1S1, OR2T11, OR4F3, OR52B4, OR5H2, OR6T1, OR11H1, OR1S2, OR2T12, OR4F4, OR52B6, OR5H6, OR6V1, OR11H12, OR2A1, OR2T2, OR4F5, OR52D1, OR5I1, OR6X1, OR11H4, OR2A12, OR2T27, OR4F6, OR52E2, OR5J2, OR6Y1, OR11H6, OR2A14, OR2T29, OR4K1, OR52E4, OR5K1, OR7A10, OR11L1, OR2A2, OR2T3, OR4K13, OR52E6, OR5K2, OR7A17, OR12D2, OR2A25, OR2T33, OR4K14, OR52E8, OR5K3, OR7A5, OR12D3, OR2A4, OR2T34, OR4K15, OR52H1, OR5K4, OR7C1, OR13A1, OR2A42, OR2T35, OR4K17, OR52I1, OR5L1, OR7C2, OR13C2, OR2A5, OR2T4, OR4K2, OR52I2, OR5L2, OR7D2, OR13C3, OR2A7, OR2T5, OR4K5, OR52J3, OR5M1, OR7D4, OR13C4, OR2AE1, OR2T6, OR4L1, OR52K1, OR5M10, OR7E24, OR13C5, OR2AG1, OR2T7, OR4M1, OR52K2, OR5M11, OR7G1.

In another particular embodiment, said olfactory receptor is a variant of a human odorant receptor, in particular a variant of any one of the human odorant receptors listed above, more particularly a variant having an amino acid sequence having at least 80% identity, for instance at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with the amino acid sequence of a human odorant receptor, in particular with any one of the human odorant receptors listed above.

In one embodiment, said GPCR is a non-OR GPCR.

In a particular embodiment, said chemoreceptor is a human non-OR chemoreceptor, in particular a human non-OR chemoreceptor selected from the group consisting of: a gustatory receptor, a Trp channel, a V1r or a V2r.

In another embodiment, said chemoreceptor is an invertebrate non-GPCR, in particular an insect chemosensor selected from the group consisting of ionotropic 7TM ORs, 7TM GRs and ionotropic IRs.

In a particular embodiment of the method of the invention, said biological sample and the gene encoding said chemoreceptor are from the same species, e.g. a biological sample from a mouse comprises cells expressing a gene encoding a mouse chemoreceptor.

In another particular embodiment, the identification of a mouse chemoreceptor as a receptor for a given ligand can be extended to the receptor or receptors of another species, in particular a human, because of the high sequence homology between the mouse receptor and the human receptor(s). Table 1 provides examples of human odorant receptors and murine odorant receptors which show at least 90% identity in their amino acid sequences.

Table 1

Mouse	Human	Percentage of Identity
Olfr727 (ENSMUSG00000059488)	OR4K15 (ENSG00000169488)	93.83
Olfr558 (ENSMUSG00000070423)	OR51E1 (ENSG00000180785)	93.67
Olfr713 (ENSMUSG00000073898)	OR10A5 (ENSG00000166363)	93.38
Olfr78 (ENSMUSG00000043366)	OR51E2 (ENSG00000167332)	93.10
Olfr1019 (ENSMUSG00000075208)	OR5AR1 (ENSG00000172459)	93.06
Olfr577 (ENSMUSG00000043354)	OR51G2 (ENSG00000176893)	93.01
Olfr449 (ENSMUSG00000049168)	OR6B1 (ENSG00000221813)	92.60
Olfr1032 (ENSMUSG00000042796)	OR5M3 (ENSG00000174937)	92.48
Olfr713 (ENSMUSG00000073898)	OR10A2 (ENSG00000170790)	92.38
Olfr734 (ENSMUSG00000045306)	OR4M1 (ENSG00000176299)	91.37
Olfr984 (ENSMUSG00000045812)	OR4D5 (ENSG00000171014)	90.45
Olfr152 (ENSMUSG00000068816)	OR5I1 (ENSG00000167825)	90.42
Olfr1510 (ENSMUSG00000063106)	OR10G2 (ENSG00000255582)	90.32
Olfr981 (ENSMUSG00000046678)	OR10G6 (ENSG00000198674)	90.28
Olfr554 (ENSMUSG00000073971)	OR52M1 (ENSG00000197790)	90.22
Olfr1420 (ENSMUSG00000060878)	OR10V1 (ENSG00000172289)	90.18
Olfr2 (ENSMUSG00000070417)	OR6A2 (ENSG00000184933)	90.18
Olfr1191-ps1 (ENSMUSG00000081948)	OR4S2 (ENSG00000174982)	90.03
Olfr1034 (ENSMUSG00000102091)	OR5M9 (ENSG00000150269)	90.00

In another embodiment, said biological sample and the gene encoding said chemoreceptor are from different species, e.g. a biological sample from a rodent comprises cells expressing a gene encoding a human chemoreceptor.

In one embodiment, the biological sample comprises a tissue comprising cells expressing at least one chemoreceptor as described above.

A tissue suitable for the invention typically comprises sensory neurons such as olfactory sensory neurons, gustatory sensory cells, or neurons from the trigeminal system.

Alternatively, a tissue suitable for the invention can comprise sensory cells from the sensillae from the antennae or from the palps in insects.

A tissue suitable for the invention can be selected from the group consisting of: main olfactory epithelium, vomeronasal organ, septal organ, Grueneberg ganglion, trigeminal tissue, sensory tissue from the oral cavity, for example.

In a particular embodiment, said tissue is the main olfactory epithelium.

In another particular embodiment, said tissue is the taste epithelium from the tongue.

In another particular embodiment, said tissue is the sensillae from the antennae or from the palps from an insect.

Said tissues can be obtained from biopsies of animals (including humans, non-human animals or transgenic non-human animals as described herewith), according to methods known in the art.

In another embodiment, the biological sample used in the invention comprises isolated cells expressing at least one chemoreceptor as described above.

The cells suitable for the invention are typically isolated from one of the above described tissues.

The cells suitable for the invention can also be cells from a cell line such as an olfactory sensory neuron cell line like Odora (*Murrel and Hunter, 1999, J. Neurosci., 19(19): 8260-70*).

In a particular embodiment, the cells suitable for the invention are sensory neurons, in particular olfactory sensory neurons isolated from the olfactory system comprising the main olfactory epithelium, vomeronasal organ, septal organ and/or Grueneberg ganglion of an animal or gustatory sensory cells isolated from the taste epithelium of an animal.

In another embodiment, said olfactory sensory neurons are isolated from the main olfactory epithelium.

In a further embodiment, said olfactory sensory neurons are isolated from the vomeronasal organ epithelium.

In a further embodiment, said olfactory sensory neurons are isolated from the Grueneberg ganglion.

In another embodiment, said gustatory sensory cells are isolated from the taste epithelium of the tongue of an animal.

In an alternative particular embodiment, the cells suitable for the invention are sensory cells isolated from the sensillae from the antennae or from the palps of an insect.

In a still further embodiment, said sensory neurons are isolated from a tissue from a transgenic non-human animal as described herewith.

In a particular embodiment, each cell suitable for the invention expresses one chemoreceptor gene as described herewith.

Methods to extract said olfactory sensory neurons are known in the art and include the method described by (*Bozza et al., 2002, J. Neuro, 22(8):3033-3043; Rivière et al, Nature 2009 May 28;459(7246):574-7).*

Methods to extract said gustatory sensory cells or neurons are known in the art and include the method described in *Huang et al., 2005, J. Neuro., 25(4) 843-847.*

The tissue or cells suitable for the invention can be obtained from an animal that can be a vertebrate or an invertebrate.

In one embodiment, the animal is a vertebrate such as a mammal.

In a particular embodiment, the animal is a mammal such as a human, a rodent, a pig or a cow.

In a particular embodiment, said animal is a non-human animal.

In a further particular embodiment, said animal is a rodent including mice, rats and rabbits.

In another embodiment, the animal is an invertebrate such as a tick, a fly or a mosquito.

In a still other particular embodiment, said non-human animal is a transgenic animal as described herewith.

In a particular embodiment, said non-human animal is a transgenic animal expressing at least 1, at least 2, at least 5, or at least 10, odorant receptor (OR) gene(s).

More particularly, said non-human animal is a transgenic animal expressing at least one, at least 2, at least 5, or at least 10, heterologous OR genes such as a human OR gene.

In a further particular embodiment, said non-human animal is a transgenic animal expressing at least one, at least 2, at least 5, or at least 10, gene(s) encoding a non-OR chemoreceptor.

More particularly, said non-human animal is a transgenic animal expressing at least one, at least 2, at least 5, or at least 10, heterologous non-OR chemoreceptor gene such as a human non-OR chemoreceptor gene.

According to the invention, the test compound to which the biological sample or the animal has been exposed can be of various natures including a peptide, a polypeptide, a

lipid, a carbohydrate, and a small organic or non-organic molecule including but not limited to an odorant, a fragrance compound, a palatable or non-palatable compound, a pheromone, a molecule from a synthetic or natural source, from a chemical or peptide library for instance.

In a particular embodiment, where the sensory neurons express at least one olfactory receptor, the test compound is selected from the group consisting of esters, linear terpenes, cyclic terpenes, aromatic, amines, alcohols, aldehydes, ketones, lactones, thiols, sulfated compounds, alkanes, gases.

In a particular embodiment, where the sensory neurons express at least one gustatory receptor, the test compound is selected from the group consisting of a sugar, an acid, or a fatty compound.

In another embodiment, more than one test compound can be tested in a mixture of several test compounds.

In a particular embodiment, the invention uses the following mixtures of test compounds:

- to mimick the odor of bad breath: volatile sulfur compounds including hydrogen sulfide, methanethiol (methyl mercaptan), dimethylsulfide;
- to mimick the odor of "coffee breath": 3-mercapto-methylbutylformate
- to mimick the odor of garlic breath: allyl methyl sulfide
- to mimick the odor caused by flatulence: sulfur containing compounds like hydrogen sulfide, methanethiol, dimethyl sulfide;
- to mimick the underarm odor: 3-methyl-2-hexenoic acid, 3-hydroxy-3-methylhexanoic acid, 3-methyl-3-sulfanylhexan-1-ol.
- to mimick the foot odor: methanethiol, propanoic acid, isovaleric acid.

The method of the invention is preceded by the exposure of the cells comprised in the biological sample to at least one test compound.

Said exposure can be carried out *ex vivo*, i.e. cells or tissues expressing at least one chemoreceptor are exposed to said test compound, or *in vivo*, i.e. the cells or tissues expressing at least one chemoreceptor are from an animal that has been exposed to said test compound.

In general, the method of the invention is preceded by the exposure of the biological sample or animal to the test compound, which lasted for about a few hours such as about 5 hours.

In one embodiment of the methods of the invention, the level of transcription of at least one gene encoding a chemoreceptor is determined. In particular, the level of transcription of at least 5, at least 10, at least 50, or at least 100 genes encoding a chemoreceptor is determined.

In another embodiment of the methods of the invention, the level of transcription of between about 10 and 2000 genes, for instance between about 20 and about 300, between about 20 and about 200 genes, encoding a chemoreceptor is determined.

The level of transcription of a gene can be determined according to standard methods in the field for quantifying said gene's transcript, including methods based on reverse transcription polymerase chain reaction (RT-PCR), mRNA sequencing. Examples of methods to determine the level of transcription of ORs include quantitative RT-PCR (herewith abbreviated "RT-qPCR") and high-throughput mRNA sequencing as illustrated in the example section.

In a second aspect, the invention provides a method of identifying an agent able to modulate the action of a ligand on its chemoreceptor, comprising the steps of:

(a) Providing 3 sub-samples derived from the same biological sample comprising cells expressing at least one chemoreceptor;

where said sub-samples have been treated as follows:

(i) a first sub-sample has been exposed to a ligand of said chemoreceptor,

(ii) a second sub-sample has been exposed to the same ligand of said chemoreceptor as in (i) and to a test agent,

(iii) a third sub-sample has not been exposed to either a ligand of said chemoreceptor nor to a test agent, and constitutes a negative control,

(b) Measuring a signal that is proportional to the level of transcription of the gene encoding said chemoreceptor in each of said three sub-samples,

(c) Comparing the level of signal determined for each of said three sub-samples,

wherein:

(i) a level of signal determined in said second sub-sample that is lower than the level of signal determined in said first sub-sample, and a level of signal

determined in said first sub-sample that is lower than the level of signal determined in said third sub-sample, indicates that said agent enhances binding of said ligand to said chemoreceptor, and is an agonist of said chemoreceptor.

(ii) a level of signal determined in second sub-sample that is equal or comparable to the level of signal determined in said third sub-sample, and a level of signal determined in said second sub-sample and/or said third sub-sample that is higher than the level of signal determined in said first sub-sample, indicates that said agent inhibits the binding of said ligand to said chemoreceptor, and is an antagonist of said chemoreceptor, and

(iii) a level of signal determined in said first sub-sample that is equal or comparable to the level of signal determined in said second sub-sample, and a level of signal determined in said first sub-sample and/or said second sub-sample that is lower than the level of signal determined in said third sub-sample, indicates that said agent does not modulate the binding of said ligand to said chemoreceptor.

In a particular embodiment, said method of identifying an agent able to modulate the binding of a ligand to its chemoreceptor further comprises the steps of exposing said biological sample to a ligand for said chemoreceptor alone and in combination with a test agent.

In a more particular embodiment, it is provided a method of identifying an agent able to modulate the binding of a ligand for its chemoreceptor, comprising the steps of:

(a) Providing a biological sample comprising cells expressing at least one chemoreceptor;

(b) Dividing said biological sample into 3 groups,

(c) (i) Exposing a first group of said biological sample to a ligand of said chemoreceptor,

(ii) Measuring a signal that is proportional to the level of transcription of the gene encoding said chemoreceptor in said first group,

(d) (i) Exposing a second group of said biological sample to the same ligand of said chemoreceptor as in (c) (i) and to a test agent,

(ii) Measuring a signal that is proportional to the level of transcription of the gene encoding said chemoreceptor in said second group,

(e) (i) Keeping a third group of said biological sample as negative control with no exposition to either a ligand of said chemoreceptor nor to a test agent,

(ii) Measuring a signal that is proportional to the level of transcription of the gene encoding said chemoreceptor in said third group,

(f) Comparing the level of signal determined in steps (c), (d), and (e),

wherein:

(i) a level of signal determined in step (d) that is lower than the level of signal determined in step (c), and a level of signal determined in step (c) that is lower than the level of signal determined in step (e), indicates that said agent enhances binding of said ligand to said chemoreceptor, and is an agonist of said chemoreceptor.

(ii) a level of signal determined in step (d) that is equal or comparable to the level of signal determined in step (e), and a level of signal determined in step (d) and/or (e) that is higher than the level of signal determined in step (c), indicates that said agent inhibits the binding of said ligand to said chemoreceptor, and is an antagonist of said chemoreceptor, and

(iii) a level of signal determined in step (c) that is equal or comparable to the level of signal determined in step (d), and a level of signal determined in step (c) and/or (d) that is lower than the level of signal determined in step (e), indicates that said agent does not modulate the binding of said ligand to said chemoreceptor.

The same particular embodiments regarding the chemoreceptor, the biological sample, the tissue, the cells, the determination of the level of transcription of the gene encoding a chemoreceptor, as detailed above for the first aspect of the invention also apply to the method of the invention of this second aspect.

In a particular embodiment of the method of identifying an agent able to modulate the binding of a ligand for its chemoreceptor according to the invention, said biological sample comprises sensory neurons or a tissue comprising sensory neurons, in particular olfactory sensory neurons or a tissue comprising olfactory neurons.

In another particular embodiment of said method, said chemoreceptor is an olfactory receptor, more particularly a human olfactory receptor.

In a further particular embodiment of said method, said biological sample comprises olfactory sensory neurons or a tissue comprising olfactory sensory neurons, wherein said neurons express at least one, at least 5, or at least 10 human olfactory receptor genes.

In a particular embodiment, the duration of exposure to the test agent and/or ligand is of about few hours such as about 5 hours.

The agent to be tested in the methods of the invention can be of various natures including a peptide, a polypeptide, an antibody or antigen-binding fragment thereof, a lipid, a carbohydrate, a nucleic acid, a small organic or non-organic molecule including but not limited to an odorant, a fragrance compound and a pheromone, a molecule from a synthetic or natural source, from a chemical or peptide library for instance

Transgenic animals and use thereof

The invention also provides transgenic non-human animals expressing at least one exogenous chemoreceptor gene such as a human chemoreceptor gene, e.g. a human odorant receptor gene or a human non-odorant receptor gene, or a chemoreceptor gene not normally expressed in said non-human animals.

As will be understood by the skilled person, specifying that a transgenic animal expresses at least one exogenous chemoreceptor gene implies that the genome of said animal, or at least some cells of said animal, comprises said at least one exogenous chemoreceptor gene or its coding sequence.

In a particular aspect, the transgenic non-human animals according to the invention are vertebrates or invertebrates.

In a more particular aspect, the transgenic non-human animals according to the invention are vertebrates including mammals.

In a more particular aspect, the transgenic non-human animal according to the invention is a mammal such as a rodent, a pig or a cow.

In a more particular aspect, the transgenic non-human animal according to the invention is a rodent such as a mouse or a rat.

In a more particular embodiment, the transgenic non-human animal according to the invention is a mouse.

In another particular aspect, the transgenic non-human animals according to the invention are invertebrates, in particular insects, including flies and mosquitoes.

In a particular embodiment, the transgenic non-human animal according to the invention expresses at least one, in particular at least 2, at least 5, at least 10, at least 50, at least 100, for instance from 10 to 2000, more particularly from about 20 to about 300 or from about 20 to about 200, exogenous chemoreceptor genes.

In a more particular embodiment, the transgenic non-human animal according to the invention expresses at least 5 or at least 10 exogenous chemoreceptor genes.

In a particular embodiment, the transgenic non-human animal according to the invention expresses at least one, in particular at least 2, at least 5, at least 10, at least 50, at least 100, for instance from 10 to 384, human odorant receptor gene(s) selected from the group consisting of: OR10A2, OR13C8, OR2AG2, OR2T8, OR4M2, OR52L1, OR5M3, OR7G2, OR10A3, OR13C9, OR2AJ1, OR2V1, OR4N2, OR52M1, OR5M8, OR7G3, OR10A4, OR13D1, OR2AK2, OR2V2, OR4N4, OR52N1, OR5M9, OR8A1, OR10A5, OR13F1, OR2AP1, OR2W1, OR4N5, OR52N2, OR5P2, OR8B12, OR10A6, OR13G1, OR2AT4, OR2W3, OR4P4, OR52N4, OR5P3, OR8B2, OR10A7, OR13H1, OR2B11, OR2Y1, OR4Q3, OR52N5, OR5R1, OR8B3, OR10AD1, OR13J1, OR2B2, OR2Z1, OR4S1, OR52R1, OR5T1, OR8B4, OR10AG1, OR14A16, OR2B3, OR3A1, OR4S2, OR52W1, OR5T2, OR8B8, OR10C1, OR14A2, OR2B6, OR3A2, OR4X1, OR56A1, OR5T3, OR8D1, OR10D3, OR14C36, OR2C1, OR3A3, OR4X2, OR56A3, OR5V1, OR8D2, OR10G2, OR14I1, OR2C3, OR4A15, OR51A2, OR56A4, OR5W2, OR8D4, OR10G3, OR14J1, OR2D2, OR4A16, OR51A4, OR56B1, OR6A2, OR8G1, OR10G4, OR14K1, OR2D3, OR4A47, OR51A7, OR56B3P, OR6B1, OR8G5, OR10G6, OR1A1, OR2F1, OR4A5, OR51B2, OR56B4, OR6B2, OR8H1, OR10G7, OR1A2, OR2F2, OR4B1, OR51B4, OR5A1, OR6B3, OR8H2, OR10G8, OR1B1, OR2G2, OR4C11, OR51B5, OR5A2, OR6C1, OR8H3, OR10G9, OR1C1, OR2G3, OR4C12, OR51B6, OR5AC2, OR6C2, OR8I2, OR10H1, OR1D2, OR2G6, OR4C13, OR51D1, OR5AK2, OR6C3, OR8J1, OR10H2, OR1D5, OR2H1, OR4C15, OR51E1, OR5AN1, OR6C4, OR8J3, OR10H3, OR1E1, OR2H2, OR4C16, OR51E2, OR5AP2, OR6C6, OR8K1, OR10H4, OR1E2, OR2J1, OR4C3, OR51F1, OR5AR1, OR6C65, OR8K3, OR10H5, OR1F1, OR2J2, OR4C46, OR51F2, OR5AS1, OR6C68, OR8K5, OR10J1, OR1G1, OR2J3, OR4C5, OR51G1, OR5AU1, OR6C70, OR8S1, OR10J3, OR1I1, OR2K2, OR4C6, OR51G2, OR5B12, OR6C74, OR8U1, OR10J5, OR1J1, OR2L13, OR4D1, OR51H1P, OR5B17, OR6C75, OR8U9, OR10K1, OR1J2, OR2L2,

OR4D10, OR51I1, OR5B2, OR6C76, OR9A2, OR10K2, OR1J4, OR2L3, OR4D11, OR51I2, OR5B21, OR6F1, OR9A4, OR10P1, OR1K1, OR2L5, OR4D2, OR51L1, OR5B3, OR6J1, OR9G1, OR10Q1, OR1L1, OR2L8, OR4D5, OR51M1, OR5C1, OR6K2, OR9G4, OR10R2, OR1L3, OR2M2, OR4D6, OR51Q1, OR5D13, OR6K3, OR9G9, OR10S1, OR1L4, OR2M3, OR4D9, OR51S1, OR5D14, OR6K6, OR9I1, OR10T2, OR1L6, OR2M4, OR4E2, OR51T1, OR5D16, OR6M1, OR9K2, OR10V1, OR1L8, OR2M5, OR4F15, OR51V1, OR5D18, OR6N1, OR9Q1, OR10W1, OR1M1, OR2M7, OR4F16, OR52A1, OR5F1, OR6N2, OR9Q2, OR10X1, OR1N1, OR2S2, OR4F17, OR52A5, OR5H1, OR6P1, OR10Z1, OR1N2, OR2T1, OR4F21, OR52B1P, OR5H14, OR6Q1, OR11A1, OR1Q1, OR2T10, OR4F29, OR52B2, OR5H15, OR6S1, OR11G2, OR1S1, OR2T11, OR4F3, OR52B4, OR5H2, OR6T1, OR11H1, OR1S2, OR2T12, OR4F4, OR52B6, OR5H6, OR6V1, OR11H12, OR2A1, OR2T2, OR4F5, OR52D1, OR5I1, OR6X1, OR11H4, OR2A12, OR2T27, OR4F6, OR52E2, OR5J2, OR6Y1, OR11H6, OR2A14, OR2T29, OR4K1, OR52E4, OR5K1, OR7A10, OR11L1, OR2A2, OR2T3, OR4K13, OR52E6, OR5K2, OR7A17, OR12D2, OR2A25, OR2T33, OR4K14, OR52E8, OR5K3, OR7A5, OR12D3, OR2A4, OR2T34, OR4K15, OR52H1, OR5K4, OR7C1, OR13A1, OR2A42, OR2T35, OR4K17, OR52I1, OR5L1, OR7C2, OR13C2, OR2A5, OR2T4, OR4K2, OR52I2, OR5L2, OR7D2, OR13C3, OR2A7, OR2T5, OR4K5, OR52J3, OR5M1, OR7D4, OR13C4, OR2AE1, OR2T6, OR4L1, OR52K1, OR5M10, OR7E24, OR13C5, OR2AG1, OR2T7, OR4M1, OR52K2, OR5M11, OR7G1; and/or any variant thereof having at least 80% identity, for instance at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with any one of said human odorant receptor gene's sequences.

In a further particular embodiment, the transgenic non-human animal according to the invention expresses at least one, at least 2, at least 5, at least 10, at least 50, at least 100, for instance from 10 to 209, human odorant receptor gene(s) selected from the group consisting of: OR1I1, OR10H5, OR8H1, OR13G1, OR8G5, OR2S2, OR10AD1, OR5T1, OR6Y1, OR52B2, OR7C1, OR4D9, OR8H2, OR11L1, OR4D2, OR11H1, OR5B3, OR52A1, OR8G1, OR13C5, OR1J1, OR10W1, OR6B2, OR2T6, OR13A1, OR13C9, OR10K1, OR5P3, OR2L3, OR13C2, OR1L4, OR1L1, OR2V2, OR5B21, OR11H12, OR4D1, OR2Y1, OR4Q3, OR2M2, OR9A4, OR13C4, OR7D4, OR2T29, OR10Z1, OR11H2, OR5C1, OR5M3, OR4F15, OR2T5, OR5M9, OR10A6, OR4M2,

OR2L2, OR4K1, OR52W1, OR2T11, OR6K3, OR1Q1, OR11H4, OR51B4, OR13C3, OR1K1, OR11H6, OR5P2, OR8B2, OR4K2, OR4K17, OR52L1, OR2H2, OR10A5, OR4K13, OR56A4, OR11A1, OR2D2, OR4F21, OR2T10, OR14J1, OR51E2, OR5AN1, OR1D2, OR2J2, OR51I1, OR4F17, OR4N5, OR2J3, OR51Q1, OR51L1, OR56A3, OR6C1, OR8J3, OR51G2, OR51M1, OR52E6, OR2C1, OR2T33, OR51B2, OR5K3, OR12D2, OR2AJ1, OR6A2, OR2F1, OR13J1, OR2T8, OR6C70, OR6B1, OR10G3, OR2B11, OR4F16, OR2A5, OR6F1, OR4F4, OR2V1, OR3A2, OR5AU1, OR2D3, OR7A17, OR1C1, OR4K14, OR6B3, OR4F5, OR2F2, OR9K2, OR13D1, OR9Q1, OR2A25, OR10A3, OR9A2, OR9Q2, OR2A14, OR10A4, OR6C4, OR10H1, OR4E2, OR2AG1, OR6C2, OR2AK2, OR52B4, OR1M1, OR3A1, OR2T27, OR6V1, OR8B12, OR10Q1, OR52B6, OR52I2, OR2K2, OR10K2, OR51I2, OR2M3, OR2M4, OR51E1, OR7D2, OR4F3, OR1L6, OR56B4, OR2AG2, OR5K2, OR1L3, OR56A1, OR7A5, OR52I1, OR1B1, OR52E4, OR2G6, OR5K1, OR1N2 , OR52N2, OR2L13, OR5H15, OR1N1, OR52N1, OR2C3, OR4F29, OR2AT4, OR52N5, OR10S1, OR5H14, OR52A5, OR56B1, OR8D1, OR7E24, OR5A1, OR52N4, OR8B3, OR2W3, OR5A2, OR5AK2, OR14A16, OR1J4, OR5B12, OR6T1, OR52K1, OR5V1, OR5B2, OR8D4, OR2L8, OR2AE1, OR9I1, OR52D1, OR8B8, OR4D10, OR6Q1, OR52H1, OR1J2, OR10G4, OR9G4, OR8K3, OR6N1, OR5M10; and/or any variant thereof having at least 80% identity, for instance at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with any one of said human odorant receptor gene's sequences.

The above list of 209 genes corresponds to a group of ORs expressed in most humans.

In a further embodiment, said transgenic non-human animal according to the invention expresses at least one human non-OR chemoreceptor gene, such as at least one, at least 2, at least 5, at least 10, human TAAR receptor genes. More particularly, said transgenic non-human animal according to the invention expresses at least 5 human non-OR chemoreceptor genes.

In a still further embodiment, the transgenic animal according to the invention is a mouse expressing at least one, at least 5, at least 10, at least 50, at least 100, for instance from 10 to 209, human odorant receptor gene(s) selected from the group described above. In a more particular embodiment, said transgenic animal according to the

invention is a mouse expressing at least 5 human odorant receptor gene(s) selected from the group described above.

The transgenic non-human animal expressing at least one exogenous chemoreceptor gene according to the invention can be generated by standard procedures in the field including integration of said at least one chemoreceptor gene in said animal's genome following injection of at least one nucleic acid comprising said at least one chemoreceptor gene into oocyte pronuclei or delivery to the oocyte or germ cell by electroporation, viral vector, lipofection, or transfection.

A number of techniques may be used to introduce the transgene into an animal's genetic material, including, but not limited to, microinjection of the transgene into pronuclei of fertilized eggs and manipulation of embryonic stem cells (e.g. *Palmiter and Brinster, 1986, Ann. Rev. Genet, 20: 465-499*). Transgenic animals can carry the transgene in all their cells or can be genetically mosaic.

According to the method of conventional transgenesis, additional copies of normal or modified genes are injected into one of the pronuclei of the zygote and become integrated into the genomic DNA of the recipient animal. The transgene is transmitted in a Mendelian manner in established transgenic strains. Transgenes can be constitutively expressed or can be tissue specific or even responsive to an exogenous drug. A transgenic animal expressing one transgene or multiple transgenes can be crossed to a second transgenic animal expressing a second transgene or multiple transgenes such that their offspring will carry and express all transgenes.

According to one aspect, a transgenic animal of the invention is used as a tool to identify chemoreceptors responding to specific ligands according to a method of the invention.

Thus, in a further aspect, the invention provides a method for making a transgenic animal expressing at least one exogenous chemoreceptor comprising introducing into the genome of said animal a nucleic acid comprising at least one exogenous chemoreceptor gene or its coding sequence.

In a particular embodiment, said nucleic acids comprising said at least one exogenous chemoreceptor gene are bacterial artificial chromosomes (BACs) of typically 80-300 kb comprising said at least one chemoreceptor gene.

In a more particular embodiment, said nucleic acids comprising said at least one human odorant receptor gene are bacterial artificial chromosomes (BACs) of typically 80-300 kb comprising said at least one human odorant receptor gene.

In an alternative embodiment, said nucleic acids comprising said at least one exogenous chemoreceptor gene are short transgenes comprising said chemoreceptor gene's promoter followed by a 5'UTR, an intron, said chemoreceptor gene's coding sequence, and a polyA signal.

In a particular embodiment, said nucleic acids comprising said at least one human odorant receptor gene are short transgenes comprising an human odorant receptor gene's promoter followed by a 5'UTR, an intron, a human odorant receptor gene's coding sequence, and a polyA signal.

In a particular embodiment, the transgenic non-human animal expressing at least one, at least 5, at least 10, at least 50, at least 100, at least 200, human odorant receptor gene(s) according to the invention is generated by the integration, into said animal's genome, of at least one, at least 5, at least 10, at least 50, at least 90, bacterial artificial chromosomes selected from the group consisting of:

CTD-2184G2, RP11-160E10, RP11-378I20, RP11-635I20, RP11-81H21, RP11-1000I9, RP11-163E6, RP11-379F1, RP11-652F7, RP11-826F2, RP11-100F1, RP11-177D5, RP11-382A12, RP11-656I18, RP11-846G12, RP11-1029E16, RP11-203G14, RP11-384C21, RP11-659M23, RP11-910P5, RP11-1040N14, RP11-206D24, RP11-409C1, RP11-65A10, RP11-918H8, RP11-1042J13, RP11-21N2, RP11-429J13, RP11-661M13, RP11-947H5, RP11-1044H15, RP11-236L12, RP11-42G15, RP11-663K1, RP11-950A2, RP11-105B16, RP11-23F9, RP11-430I15, RP11-681D10, RP11-952I8, RP11-1069J21, RP11-242C5, RP11-432E18, RP11-696P18, RP11-960L8, RP11-1105A4, RP11-243N6, RP11-438H8, RP11-69E17, RP11-98N22, RP11-1107C18, RP11-24N17, RP11-452G22, RP11-69N15, RP11-98P19, RP11-110A12, RP11-252I5, RP11-454O22, RP11-720H19, RP4-669L17, RP11-1115M8, RP11-259N2, RP11-456D1, RP11-74B15, RP11-1144E12, RP11-27N2, RP11-462C5, RP11-759P17, RP11-1150B23, RP11-297D12, RP11-466F22, RP11-75J4, RP11-115H4, RP11-299I2, RP11-585F1, RP11-76K18, RP11-1205H24, RP11-30H21, RP11-599O3, RP11-777K22, RP11-126P23, RP11-320A14, RP11-62C23, RP11-79N3, RP11-144C16, RP11-

345A24, RP11-630D14, RP11-806H4, RP11-146C10, RP11-361I19, RP11-632E19, RP11-806P5.

The sequence of the above-mentioned BACs can be retrieved from public databases at www.ncbi.nlm.nih.gov/clone/.

In a further particular embodiment, the transgenic non-human animal according to the invention that expresses the 209 human odorant receptor genes listed above is generated by the integration, into said animal's genome, of the 94 bacterial artificial chromosomes listed above.

A further aspect of the invention provides a cell isolated from said transgenic non-human animal, in particular a sensory neuron (such as an olfactory sensory neuron isolated from said transgenic non-human animal), as well as a tissue sample extracted from said transgenic non-human animal, in particular a tissue sample extracted from the olfactory system of said transgenic animal.

Said cells and tissues can be isolated as described in the previous sections.

Another aspect of the invention provides the use of the transgenic non-human animal as described herewith, or a cell or a tissue thereof, in the method of identifying at least one chemoreceptor for at least one ligand according to the invention and/or in the method of identifying an agent able to modulate the binding of a ligand for its chemoreceptor according to the invention.

Compositions useful in the invention

In another aspect, the invention provides a ligand binding to a chemoreceptor, in particular an olfactory receptor, as well as agents modulating the binding of a ligand to its chemoreceptor, which can be identified by the methods of the invention.

Ligands of at least one chemoreceptor and agents modulating the binding of a ligand to its chemoreceptor, as those identified by the methods according to the invention, are useful for controlling perceived scents and/or tastes. For instance, undesired scents can be blocked, covered or altered by using antagonists of an olfactory receptor and desired scents can be enhanced by using a ligand and/or an agonist of an olfactory receptor.

Ligands of at least one olfactory receptor and agents modulating the binding of a ligand to its olfactory receptor, as those identified by the methods according to the invention, are useful in methods of treatment and/or prevention of disorders involving a chemoreceptor.

Thus, the invention provides compositions comprising at least one ligand of at least one chemoreceptor and/or at least one agent modulating the binding of a ligand to its chemoreceptor.

In a particular embodiment, the invention provides a composition mimicking or reducing the perception of a scent and/or of a taste in a subject, comprising (a) at least one ligand for at least one first chemoreceptor and/or at least one agent modulating the binding of a ligand to a first chemoreceptor

Uses of ligands and agents according to the invention

The invention provides a method for modulating the perception of at least one scent and/or at least one taste in a subject comprising the use of at least one ligand of at least one chemoreceptor involved in the perception of said scent and/or taste and/or at least one agent modulating the binding of a ligand to said chemoreceptor.

In a particular embodiment, the invention provides a method for modulating the perception of at least one scent in a subject comprising the use of at least one ligand of at least one olfactory receptor involved in the perception of said scent and/or at least one agent modulating the binding of a ligand to said olfactory receptor.

In a more particular embodiment, the invention provides a method for enhancing the perception of at least one scent in a subject comprising the use of at least one ligand of at least one olfactory receptor involved in the perception of said scent and/or at least one agonist of said olfactory receptor.

In another particular embodiment, the invention provides a method for reducing the perception of at least one scent in a subject comprising the use of at least one antagonist of at least one olfactory receptor involved in the perception of said scent.

In the method for modulating the perception of at least one scent and/or at least one taste in a subject according to the invention, various means for exposing said subject to said ligand and/or antagonist can be used including pulverization of said components in the form of an aerosol or incorporating said components in a solid or liquid form in the food or beverage of said subject.

As used herewith, a subject encompasses any animal including a human, a rodent, a cow, a pig, or an insect.

In a particular embodiment, said ligand and/or said agonist and/or antagonist have been identified with the methods of the invention described herewith.

References cited herein are hereby incorporated by reference in their entirety. The present invention is not to be limited in scope by the specific embodiments described herein, which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

The invention having been described, the following examples are presented by way of illustration, and not limitation.

EXAMPLES

Animals

Male C57BL/6 mice were ordered at 7 weeks of age from Charles River Laboratories (www.criver.com). Upon arrival they were stored in double filter-equipped disposable plastic cages (Innovive) in groups of 5-6 for 5 days. They were kept in an overpressurized "experimental odorant-free" room. After 5 days mice were isolated into identical cages until they were needed for an experiment (from 1 to 7 days).

Drosophila (W1118) were collected between 0 and 14 hours after hatching. Males and females were exposed separately. Gender did not affect their responses, and the data for both sexes were thus pooled.

Odorant exposures

In vivo exposure to odorant: Starting 48 h before experiments, mice were habituated to pure DMSO on a cotton ball. 200 µl were pipetted directly onto the cotton and placed in the cage, replacing any cotton that was already in the cage for nest-making purposes. The DMSO cotton ball was then changed daily until the experiment began.

On the day of the experiment, mice receiving odorants were transferred to a different room, where they were exposed to a cotton ball on which 200 µl of odorant diluted in pure DMSO were deposited. Exposures started in the morning between 8 and 10 am (that is two hours after lights were on) and mice were killed and dissected between 1 and 3 pm. Mice did not react differently to cotton balls containing odorants or only DMSO. If a number of different odorants were tested in the same experiment, a

different overpressurized room would always be used for each odorant to avoid odor cross-contamination.

In vitro exposure to odorant: The olfactory sensory mucosa of a live or recently dead vertebrate is collected and maintained alive *in vitro*. Sensory neurons are either left untouched or are dissociated. The exposure to the odorants is then performed *in vitro*, in a tube, in the liquid phase. The explants/neurons are kept alive in a physiological medium (such as 145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose). The procedure is then comparable to the one used for in vivo exposure, that is to divide the collected sensory neurons in two separate populations, and activate one of these populations (for 2-5 hours but this can be variable) while the other one is used as a negative control. The potential modulations of mRNA levels between the two populations are then evaluated, by qPCR (Figure 2), RNAseq or other techniques, to identify the receptors that have been activated.

RNA extractions

After odorant exposure, mice were killed by cervical dislocation and immediately decapitated. The main olfactory epithelia (MOE), were harvested in their entirety and transferred to a tube containing 600 µl of lysis buffer and β-mercaptoethanol (RNAeasy kit, Qiagen, following the manufacturer protocol) and a 0.5 cm diameter steel ball, and then put on ice. Samples were then homogenized using a FastPrep®-24 instrument (MP Biomedicals) at 6 m/s for 30 s and left on ice. RNA extraction was performed on all samples in parallel using the Qiagen RNeasy Mini® kit following the manufacturer's instructions. Two DNase treatments were performed on all samples: first using the Qiagen RNase-Free DNase Set® and then using the Life Technologies Ambion DNase I® kit. RNA samples were then aliquoted and stored at -80°C.

For flies, after exposure, vials containing the animals were placed upside down in a -80°C freezer for five minutes, then kept in a -20°C freezer until dissection. Whole heads were removed by hand and placed in a screw-cap tube containing 1ml of Trizol (Ambion) and a 0.5 cm diameter steel ball, and kept on ice. Samples were homogenized using four runs of 15 s at 6 m/s on a FastPrep®-24 instrument (MP Biomedicals). Samples were cooled on ice between each run. RNA extraction followed the Trizol protocol according to the manufacturer instructions, and using 5 µg of RNase-free glycogen as carrier for RNA precipitation. The RNA pellet was resuspended in 20 µl of

RNase-free water and a DNAse treatment (Life Technologies Ambion DNase I®) was performed.

qPCR

RNA concentrations were determined using an Amersham Ultrospec® 3100 Pro spectrophotometer, and RNA quality was evaluated with an Agilent Technologies 2100 Bioanalyzer®. Each reverse transcription was performed with 0.5 µg RNA using the Takara Primescript® kit, in a final volume of 10 µl. Primers for reverse transcription were equal mixtures of poly-T nucleotides and random hexamers. Negative controls (omitting the reverse transcriptase enzyme) were performed for each sample. 1/14th of the cDNA preparation was used for each triplicated qPCR reaction. The forward and reverse primers for carrying out the qPCR reactions were as presented in Table 2 below.

Table 2

Gene name	ENSMUST Accession Number	SEQ ID NO. forward primer	SEQ ID NO. reverse primer
Olfr160	00000104875	1	2
Olfr556	00000098219	3	4
Olfr1377	00000075177	5	6
Olfr983	00000050996	7	8
Olfr1079	00000111582	9	10
Olfr609	00000055787	11	12
Olfr611	00000078108	13	14
Olfr2	00000094109	15	16
Olfr168	00000078554	17	18
Olfr167	00000054606	19	20
Olfr109	00000031086	21	22
Olfr15	00000080917	23	24
Olfr16	00000038432	25	26
Olfr73	00000099838	27	28
Olfr171	00000079891	29	30
Olfr1019	00000102634	31	32
Omp	00000098281	33	34
Adcy3	00000020984	35	36
b Actin	00000100497	37	38

RT-qPCR was performed on a 7900HT SDS® thermocycler from Applied Biosystems. Every reaction was systematically run in triplicate. Conditions were the following: 50°C 2 min, 95°C 10 min, 40x (95°C 15 sec, 60°C 1 min), using SYBR Green® dye.

Raw data were analyzed using the SDS® 2.2.1 software. Detection threshold was set at $\Delta Rn = 0.3$, with this limit always within the 2n exponential amplification phase of

genes. Dissociation curves were checked for aspecific products and samples were discarded if such products were detected.

qPCR Ct values were analyzed using an in-house developed Microsoft Excel® macro file. Triplicate values were compared and outliers (values with a difference higher than 0.5 Ct to their nearest neighbor) were discarded. The number of conditions with an outlying value was always less than 10% of the total number of conditions. Ct values were converted to quantities relative to the maximum for each given gene. Selected reference genes were then analyzed for variance using the geNorm algorithm. All values were normalized with the olfactory tissue-specific genes Omp and Adcy3. β-Actin was used as a non-tissue-specific reference gene to control for differences in dissection between samples.

RNAseq

Animals were exposed to 5% acetophenone for 5 hours before RNA extraction (as previously described). cDNA libraries were generated with the Truseq RNA and DNA sample preparation kits after selection of polyA-containing mRNAs. Adapters for RNAseq multiplexing were added to the cDNAs. The cDNA libraries were sequenced with a HiSeq®2500 Sequencing system. 100 bp reads were mapped using Bowtie2 with a 28 bp seed. The differential gene expression analyses were performed with DESeq2. Because of the high sequence similarity between OR gene paralogs, some reads are mapped to multiple OR genes. These reads were removed from the analysis.

Example 1: Method of the invention for deorphanizing odorant receptors in mice, with *in vivo* exposure to odorant

Adult C57BL/6 mice were exposed to 5% ethyl isobutyrate for 5 hours, and the level of transcripts of odorant receptors' genes were measured by RT-qPCR on extracts of olfactory epithelia from these mice, as described in the materials and methods section.

In the sensory neuroepithelium of adult C57BL/6 mice, the mRNA concentration of *Olfr171 and Olfr167*, was significantly decreased when exposed to 5% ethyl isobutyrate for 5 hours (ethylisobutyrate was previously shown to be an agonist for *Olfr171* and *Olfr67*) (74% and 47% relative to unexposed controls respectively, n=7 mice) (Figure 1a, f). The concentration of none of the control odorant receptor genes (8 genes) was significantly affected by ethyl isobutyrate exposure (a margin of ± 20% relative to

control mice was considered not significant for the *in vivo* exposure approach, grey zone on Figure 1).

To test if this activation-induced transcript downregulation was specific to ethyl isobutyrate-responsive olfactory sensory neurons, or was a general characteristic shared by activated olfactory neurons, the transcriptional responses of 7 additional odorant receptor genes were evaluated.

The products of these genes were previously shown to have as agonists (with an EC50 of less than 50 μM), acetophenone (to which *Olfr1377*, *Olfr556*, and *Olfr983* are responsive when expressed in HEK cells, and to which *Olfr160* is responsive in dissociated neurons), heptanal (to which *Olfr2* responds in HEK cells), lyral (to which *Olfr16* responds in dissociated neurons), and vanillic acid (to which *Olfr609* is responsive in HEK cells). Mice (n=21) were individually exposed for 5 hours to 5% of each of the 4 compounds. The concentration of their corresponding receptor mRNAs was evaluated by RT-qPCR immediately after exposure. For the 7 receptor-ligand pairs, a decrease in mRNA levels relative to unexposed controls was observed (Figure 1b-e). In none of the control receptor genes (whose products were previously shown *in vitro* not to respond to the specific ligands) did we observe such decrease of odorant receptor transcript levels (6-9 genes per condition, Figure 1).

The potential response to agonist exposure of TAAR genes, that encode the second class of olfactory receptor present in the mammalian olfactory mucosa, was then tested using the same method as the one used for ORs, except that ligand exposure lasted 48 hours.

Transcript levels of olfactory receptor gene were evaluated following olfactory stimulation during 48 hours by beta-phenylethylamine. Olfactory receptor gene transcript levels were evaluated by RT-qPCR for each receptor gene, and the ratios between the values obtained in exposed versus control mice are shown in Figure 6. A decrease in the number of transcripts encoding TAAR3 and TAAR4 was observed (two TAAR genes previously shown to respond to this ligand) (*Zhang et al, J. Neurosc. 2013, 33(7), 3228-3239*). The negative controls TAAR6 and Olfr556 were not affected by beta-phenylethylamine (Figure 6).

These results establish a remarkable correlation between *in vitro*-identified receptor/ligand pairs, and a specific *in vivo* transcriptional effect each of these ligands

had on neurons expressing the corresponding receptor gene. Therefore, this demonstrates that a transcript-based receptor deorphanization approach can be used to identify, *in vivo*, novel receptor/ligand pairs and that the present technique applies to a very large variety of chemoreceptors across receptor types.

Example 2: Method of the invention for deorphanizing odorant receptors in mice, with *ex vivo* exposure to odorant

Mouse olfactory epithelia were collected and kept *in vitro* in ACSF medium where they were exposed for 2 hours to 50µM ethyl isobutyrate. The number of transcripts for *Olfr* genes was then evaluated by qPCR, and those of *Olfr167* and *Olfr171* were both repressed after agonist exposure. Ethyl isobutyrate was previously shown to be an agonist for *Olfr167* and *Olfr171*.

Example 3: Large scale odorant receptor deorphanization according to the method of the invention

Examples 1 and 2 demonstrate a solid correlation between olfactory neuron activation and olfactory receptor mRNA downregulation. Current example aimed at demonstrating the feasibility of the method of the invention as a large scale method for receptor deorphanization following olfactory stimulation. An RNAseq approach was chosen to evaluate potential modulations of olfactory receptor gene transcripts.

Acetophenone was tested, which downregulates the messenger levels of *Olfr1377*, *Olfr556*, *Olfr983* and *Olfr160* (Figure 1).

Mice (n=6) were exposed to 5% acetophenone or to a "neutral" control condition for 5 hours. An olfactory cDNA library corresponding to each animal was deep sequenced and the ratios of odorant receptor mRNA concentrations relative to controls were plotted (Figure 3). Odorant receptor genes that exhibited a transcriptional reduction consistently larger than 20% were considered as downregulated. Based on transcriptional downregulation levels observed by RNAseq following acetophenone exposure, a list of 26 candidate responsive ORs was determined (Figure 3), that reached 74% decrease (Olfr145) or even over 80% (Olfr169) relative to controls. [Among the identified receptor genes, the four corresponding to the previously known and most sensitive acetophenone receptors (*Olfr1377*, *Olfr556*, *Olfr983* and *Olfr160*) were all downregulated, fitting well with the data obtained by RT-qPCR in Example 1 (Figure

1b). The transcript downregulation of 21 out of the 26 olfactory receptor candidates obtained by RNAseq was confirmed by qPCR. The remaining 5 candidates were false RNAseq positive candidates, namely OR candidates for which in fact mRNA was not downregulated following acetophenone exposure (Table 3).

In order to independently validate the identity of an acetophenone receptor obtained with the RNAseq approach, the potential colocalization following acetophenone exposure of a neuronal activity marker (the phosphorylated ribosomal protein S6 (pS6) (*Knight et al., 2012, Cell, 151, 1126-1137*) and specific olfactory receptors were evaluated. To this aim, in-situ hybridizations were performed on sections of olfactory tissue with probes targeting receptor mRNAs followed by immunolabeling of pS6. The probes used were directed against the transcripts of most of the ORs determined as responsive including Olfr983 transcripts (encoding a known acetophenone receptor), *Olfr171* transcripts (encoding a known ethyl isobutyrate receptor), and *Olfr145* transcripts, encoding a newly identified receptor by RNAseq. Before analysis, mice were exposed to either 5% acetophenone, 5% ethyl isobutyrate, or a negative control condition for 60 minutes. The results indicate that ethyl isobutyrate exposure led to a large fraction of Olfr171-expressing neurons to exhibit signal for pS6 (67%), while neurons expressing *Olfr983* and *Olfr145* showed pS6 colabeling in only 12% and 28% of the cases respectively (Figure 4). 66% (*Olfr983*) and 70% (*Olfr145*) of the neurons were positive for pS6 after acetophenone exposure, while only 11% of the neurons expressing *Olfr171* were positive for pS6 after exposure to this compound. The list of OSN populations (i.e. OSNs expressing the same odorant receptor gene) that exhibited neuronal activation (i.e. pS6 signal in a fraction of neuron exceeding 15% relative to controls) following odorant stimulation is shown in Table 3. A perfect correspondence between olfactory mRNA decrease and pS6 positive signals was observed for all 15 tested OSN populations, and no mRNA modulation and no pS6 signals for all 5 tested OSN populations.

Thus, taken together, the deorphanization approach according to the invention faithfully identifies the neurons that respond as agonists, *in vivo*, to volatile chemicals.

Therefore, the method according to the invention represents a large scale method for receptor deorphanization following olfactory stimulation.

Table 3

	candidate ORs (RNAseq)	confirmed by qPCR	ps6 positive
Acetophenone	olfr145	y	y
	olfr736	y	na
	olfr901	y	y
	olfr983	y	y
	olfr556	y	y
	olfr476	y	na
	olfr1501	y	na
	olfr1448	y	na
	olfr478	y	y
	olfr922	y	y
	olfr229	y	y
	olfr19	y	y
	olfr160	y	y
	olfr935	y	y
	olfr745	y	y
	olfr47	y	na
	olfr109	y	y
	olfr1377	y	y
	olfr143	y	y
	olfr1054	y	na
	olfr30	y	y
	olfr378	n	n
	olfr750	n	n
	olfr490	n	n
	olfr1135	n	n
	olfr1350	n	n

Example 4: Production of transgenic mice expressing at least 10 human odorant receptor genes

Transgenic mice expressing at least 10 human odorant receptor genes are generated. Human odorant receptor genes (384 genes as cited in paragraph extending from page 26 to 28) can be used. In particular, 209 human odorant receptor genes (cited in full paragraph at page 28) expressed in over 90% of humans with an OR gene repertoire of at least 250 genes are targeted.

To this aim, two separate and independent approaches have been taken:

1) Bacterial Artificial Chromosomes (BACs) of 80-300 kb and containing the human ORs are integrated into the mouse genome following standard procedures.

The nucleic acids are injected into oocyte pronuclei (they can alternatively be delivered to the cells by electroporation, viral delivery, lipofection, or transfection of oocytes or germ cells). The recipient species is not necessarily mice and could be any vertebrate species, in particular rats. The transgene often integrated as it organizes head to tail. These multimeric insertions contain between 1 and a few hundreds transgenes that are heterogenous as different transgenes are co-inserted in the genome. The targets to be used can be insertions containing 10-200 transgenes (a situation known to occur in about 1 in 15 founders). All F0 founders are tested for multiple integrations and the identity of the integrants is defined. Complementary founders are crossed to generate a single mouse expressing most human OR genes.

To cover the 209 odorant receptor genes of interest, 94 BACs are co-injected.

2) Conventional transgenes are also generated, each containing an OR promoter followed by a 5'UTR (such as in *Vassalli et al., 2002, Neuron, 35(4):681-96*), an intron, a human OR coding sequence (one of the 209 OR genes) and a polyA signal. The promoters can be chimeric, and include enhancers/stabilization/choice elements such as in *Vassalli et al., 2011, Mol. Cel. Neurosci., 46:381-96.* These transgenes are co-injected into mouse oocytes as described above in order to obtain mice expressing most of the human OR genes.

Example 5: Method of the invention for identifying antagonists of odorant receptors in mice

Adult C57BL/6 mice were exposed to 5% acetophenone for 5 hours, and the level of transcripts of odorant receptors' genes were measured by RT-qPCR on extracts of olfactory epithelia from these mice, as described in the materials and methods section. The mRNA concentration of *Olfr1178 and Olfr730* was significantly increased when exposed to 5% acetophenone (fold changes of 1.5 and 1.4 were observed respectively).

Example 6: Method of the invention for deorphanizing chemoreceptors in Drosophila

24 hours-old Drosphila melanogaster flies were kept in plastic tubes and exposed to either 5% ethyl lactate diluted in DMSO or to 5% geranyl acetate diluted in DMSO. The odorants were spotted on papers that were introduced into the plastic tubes. 10 flies

were used per condition (8 independent extracts per condition). 5 hours after exposure heads were collected and RNA extracted.

The transcript levels for Or67c (a known receptor for ethyl lactate) and Or82a (a known receptor for geranyl acetate) were evaluated by RT-qPCR. Or67c mRNA levels were downregulated after ethyl lactate exposure (0.7x) relative to control ORs, and Or82a mRNA levels were downregulated after geranyl acetate exposure (0.6x) relative to control ORs (Figure 5).

OR67c and OR82a were previously shown to respond to ethyl lactate and geranyl acetate respectively (*Hallem and Carlson, 2006, Cell, 125(1):143-60*) and of the three negative control olfactory receptors analyzed, Or2a was previously shown to be weakly responsive only to three esters, Or49b was responsive to some aromatic compounds and Or67a was strongly responsive to several odorants (acids, aldehydes, ketones, aromatics, alcohols and esters) (*Hallem and Carlson, 2006, supra*).

These insect ORs are non-GPCR olfactory receptors, therefore, those data indicate that the present technique applies to a very large variety of chemoreceptors across species, including invertebrates.

SEQUENCE LISTING

SEQ ID NO.	Forward primer
1	GAGGGCTAACTAACAGGCCA
3	ATGCACAGTGGAAGGCTTTG
5	TGAAGATACCATCTGCCCGC
7	ACCTGCAGCTCTCACATGAT
9	CCTGATCATCCTTGGCTCCT
11	CGCTTCTAAGACTGAACGCC
13	ATGGCCTTCGATCGGTATGT
15	AAGGAACCACACTGGGAGAG
17	CTATCCTTACCCCCATGCTCA
19	ATTCTAGGGCGGGGAAGAAG
21	CAACCTTCTCTCGAGGCGTA
23	AGGAGACAGATGCAAAGCCT
25	TTTTTGTCACCTTGGCCACC
27	TTGGGATCCTATGCTTGGGG
29	GAGTGCCTTCTCTTGGCAGT
31	ACCTGCGGGTCTCATCTTAC
33	CGCCATGGATTGGAATGAGG
35	GGTGCCTTCCAAGTACTCCA
37	CTAAGGCCAACCGTGAAAAGAT
SEQ ID NO.	Reverse primer
2	TCAAGGTGATCATGCCCAGA
4	CCTAGCCAGGCCACATAGAT
6	GAAGGACGCATGTAGACACC
8	ATCCACTGACCCAACAGGAG
10	CAGAAACCACGGTCAGATGG
12	CACTTGCCAAATCGGTGGAT
14	CTCAAGAGGATAGGGGCAGG
16	CACGTAGGCCAACAGAGAAA
18	TTCATGGAAGAGAATGTCCCAAG
20	AGGTGTAAGCAAATGGTGCG
22	GCCTCCGTACTTCCCAGAAA
24	CTTGCTGGTCCCTTTTGCAT
26	ACCAACAAGGCACACATTCC
28	GACAGATGTGTCAGAGCGTG
30	GAGCCTGCAGCCAGGAGCCC
32	TGTAGAACACAGAGGCCCAC
34	TCGCCAAAGGTGATGAGGAA
36	AGTGTTCGGGCCAGTTTTTC
38	CACAGCCTGGATGGCTACGT

Claims

1. A method of identifying at least one chemoreceptor responding at least one ligand comprising the steps of:

a) providing a biological sample comprising cells expressing at least one chemoreceptor, wherein said biological sample (i) has been exposed to at least one test compound or (ii) was obtained from an animal that has been exposed to at least one test compound;

b) measuring a signal that is proportional to the level of transcription of a gene encoding at least one chemoreceptor in said biological sample,

c) comparing the level of signal determined in step b) to the level of signal determined in the same conditions with a negative control where the biological sample or animal has not been exposed to said at least one test compound;

wherein a difference between the level of signal determined in step b) and the level of signal determined in the same conditions with a negative control indicates that said at least one test compound constitutes a ligand for said at least one chemoreceptor and is able to bind and modulate the activity of said at least one chemoreceptor.

2. The method according to claim 1, wherein said chemoreceptor is an olfactory receptor.

3. The method according to any one of claims 1 or 2, wherein said chemoreceptor is a G protein-coupled receptor (GPCR).

4. The method according to any one of claims 1 or 2, wherein said chemoreceptor is a trace amine receptor (TAAR).

5. The method according to any one of claims 1 or 2, wherein said chemoreceptor is a non-GPCR.

6. The method according to any one of claims1 to 3, wherein said chemoreceptor is an odorant receptor.

7. The method according to claim 6, wherein said odorant receptor is a human odorant receptor selected from the group consisting of: OR10A2, OR13C8, OR2AG2, OR2T8, OR4M2, OR52L1, OR5M3, OR7G2, OR10A3, OR13C9, OR2AJ1, OR2V1, OR4N2, OR52M1, OR5M8, OR7G3, OR10A4, OR13D1, OR2AK2, OR2V2, OR4N4, OR52N1, OR5M9, OR8A1, OR10A5, OR13F1, OR2AP1, OR2W1, OR4N5, OR52N2, OR5P2, OR8B12, OR10A6, OR13G1, OR2AT4, OR2W3, OR4P4, OR52N4, OR5P3, OR8B2, OR10A7, OR13H1, OR2B11, OR2Y1, OR4Q3, OR52N5, OR5R1, OR8B3, OR10AD1, OR13J1, OR2B2, OR2Z1, OR4S1, OR52R1, OR5T1, OR8B4, OR10AG1, OR14A16, OR2B3, OR3A1, OR4S2, OR52W1, OR5T2, OR8B8, OR10C1, OR14A2, OR2B6, OR3A2, OR4X1, OR56A1, OR5T3, OR8D1, OR10D3, OR14C36, OR2C1, OR3A3, OR4X2, OR56A3, OR5V1, OR8D2, OR10G2, OR14I1, OR2C3, OR4A15, OR51A2, OR56A4, OR5W2, OR8D4, OR10G3, OR14J1, OR2D2, OR4A16, OR51A4, OR56B1, OR6A2, OR8G1, OR10G4, OR14K1, OR2D3, OR4A47, OR51A7, OR56B3P, OR6B1, OR8G5, OR10G6, OR1A1, OR2F1, OR4A5, OR51B2, OR56B4, OR6B2, OR8H1, OR10G7, OR1A2, OR2F2, OR4B1, OR51B4, OR5A1, OR6B3, OR8H2, OR10G8, OR1B1, OR2G2, OR4C11, OR51B5, OR5A2, OR6C1, OR8H3, OR10G9, OR1C1, OR2G3, OR4C12, OR51B6, OR5AC2, OR6C2, OR8I2, OR10H1, OR1D2, OR2G6, OR4C13, OR51D1, OR5AK2, OR6C3, OR8J1, OR10H2, OR1D5, OR2H1, OR4C15, OR51E1, OR5AN1, OR6C4, OR8J3, OR10H3, OR1E1, OR2H2, OR4C16, OR51E2, OR5AP2, OR6C6, OR8K1, OR10H4, OR1E2, OR2J1, OR4C3, OR51F1, OR5AR1, OR6C65, OR8K3, OR10H5, OR1F1, OR2J2, OR4C46, OR51F2, OR5AS1, OR6C68, OR8K5, OR10J1, OR1G1, OR2J3, OR4C5, OR51G1, OR5AU1, OR6C70, OR8S1, OR10J3, OR1I1, OR2K2, OR4C6, OR51G2, OR5B12, OR6C74, OR8U1, OR10J5, OR1J1, OR2L13, OR4D1, OR51H1P, OR5B17, OR6C75, OR8U9, OR10K1, OR1J2, OR2L2, OR4D10, OR51I1, OR5B2, OR6C76, OR9A2, OR10K2, OR1J4, OR2L3, OR4D11, OR51I2, OR5B21, OR6F1, OR9A4, OR10P1, OR1K1, OR2L5, OR4D2, OR51L1, OR5B3, OR6J1, OR9G1, OR10Q1, OR1L1, OR2L8, OR4D5, OR51M1, OR5C1, OR6K2, OR9G4, OR10R2, OR1L3, OR2M2, OR4D6, OR51Q1, OR5D13, OR6K3, OR9G9, OR10S1, OR1L4, OR2M3, OR4D9, OR51S1, OR5D14, OR6K6, OR9I1, OR10T2, OR1L6, OR2M4, OR4E2, OR51T1, OR5D16, OR6M1, OR9K2, OR10V1, OR1L8, OR2M5, OR4F15, OR51V1, OR5D18, OR6N1, OR9Q1, OR10W1, OR1M1, OR2M7, OR4F16, OR52A1, OR5F1, OR6N2, OR9Q2, OR10X1, OR1N1,

OR2S2, OR4F17, OR52A5, OR5H1, OR6P1, OR10Z1, OR1N2, OR2T1, OR4F21, OR52B1P, OR5H14, OR6Q1, OR11A1, OR1Q1, OR2T10, OR4F29, OR52B2, OR5H15, OR6S1, OR11G2, OR1S1, OR2T11, OR4F3, OR52B4, OR5H2, OR6T1, OR11H1, OR1S2, OR2T12, OR4F4, OR52B6, OR5H6, OR6V1, OR11H12, OR2A1, OR2T2, OR4F5, OR52D1, OR5I1, OR6X1, OR11H4, OR2A12, OR2T27, OR4F6, OR52E2, OR5J2, OR6Y1, OR11H6, OR2A14, OR2T29, OR4K1, OR52E4, OR5K1, OR7A10, OR11L1, OR2A2, OR2T3, OR4K13, OR52E6, OR5K2, OR7A17, OR12D2, OR2A25, OR2T33, OR4K14, OR52E8, OR5K3, OR7A5, OR12D3, OR2A4, OR2T34, OR4K15, OR52H1, OR5K4, OR7C1, OR13A1, OR2A42, OR2T35, OR4K17, OR52I1, OR5L1, OR7C2, OR13C2, OR2A5, OR2T4, OR4K2, OR52I2, OR5L2, OR7D2, OR13C3, OR2A7, OR2T5, OR4K5, OR52J3, OR5M1, OR7D4, OR13C4, OR2AE1, OR2T6, OR4L1, OR52K1, OR5M10, OR7E24, OR13C5, OR2AG1, OR2T7, OR4M1, OR52K2, OR5M11, OR7G1; and/or any variant thereof having an amino acid sequence having at least 80% identity with the amino acid sequence of one of said human odorant receptors.

8. The method according to any one of claim 1 to 6, wherein said biological sample comprises sensory neurons expressing at least one chemoreceptor.

9. The method according to any one of claim 1 to 8, wherein said biological sample comprises olfactory sensory neurons expressing at least one chemoreceptor.

10. The method according to any one of claim 1 to 9, wherein said biological sample comprises a tissue from the olfactory system.

11. The method according to any one of claim 1 to 10, wherein said biological sample comprises cells or tissues from a transgenic non-human animal expressing at least one exogenous chemoreceptor gene.

12. The method according to any one of claims 1 to 11, wherein a level of signal determined in step b) in the biological sample after exposure to at least one test compound that is lower than the level of signal determined in the same conditions with a negative control without exposure to a test compound, indicates that said at least one

test compound constitutes a ligand acting as an agonist for said at least one chemoreceptor.

13. The method according to any one of claims 1 to 11, wherein a level of signal determined in step b) in the biological sample after exposure to at least one test compound that is higher than the level of signal determined in the same conditions with a negative control without exposure to a test compound, indicates that said at least one test compound constitutes a ligand acting as an antagonist for said at least one chemoreceptor.

14. The method according to any one of claims 1 to 13, wherein the level of transcription of at least 5 genes, or at least 10 genes encoding a chemoreceptor is determined.

15. A transgenic non-human animal expressing at least 5 exogenous chemoreceptor genes.

16. The transgenic animal according to claim 15, wherein said animal expresses at least 5 or at least 10 human odorant receptor genes.

17. The transgenic animal according to any one of claims 15 to 16, wherein said animal expresses at least 5 or at least 10 human odorant receptor genes selected from the group consisting of: OR10A2, OR13C8, OR2AG2, OR2T8, OR4M2, OR52L1, OR5M3, OR7G2, OR10A3, OR13C9, OR2AJ1, OR2V1, OR4N2, OR52M1, OR5M8, OR7G3, OR10A4, OR13D1, OR2AK2, OR2V2, OR4N4, OR52N1, OR5M9, OR8A1, OR10A5, OR13F1, OR2AP1, OR2W1, OR4N5, OR52N2, OR5P2, OR8B12, OR10A6, OR13G1, OR2AT4, OR2W3, OR4P4, OR52N4, OR5P3, OR8B2, OR10A7, OR13H1, OR2B11, OR2Y1, OR4Q3, OR52N5, OR5R1, OR8B3, OR10AD1, OR13J1, OR2B2, OR2Z1, OR4S1, OR52R1, OR5T1, OR8B4, OR10AG1, OR14A16, OR2B3, OR3A1, OR4S2, OR52W1, OR5T2, OR8B8, OR10C1, OR14A2, OR2B6, OR3A2, OR4X1, OR56A1, OR5T3, OR8D1, OR10D3, OR14C36, OR2C1, OR3A3, OR4X2, OR56A3, OR5V1, OR8D2, OR10G2, OR14I1, OR2C3, OR4A15, OR51A2, OR56A4, OR5W2, OR8D4, OR10G3, OR14J1, OR2D2, OR4A16, OR51A4, OR56B1, OR6A2, OR8G1, OR10G4, OR14K1, OR2D3, OR4A47, OR51A7, OR56B3P, OR6B1, OR8G5, OR10G6, OR1A1,

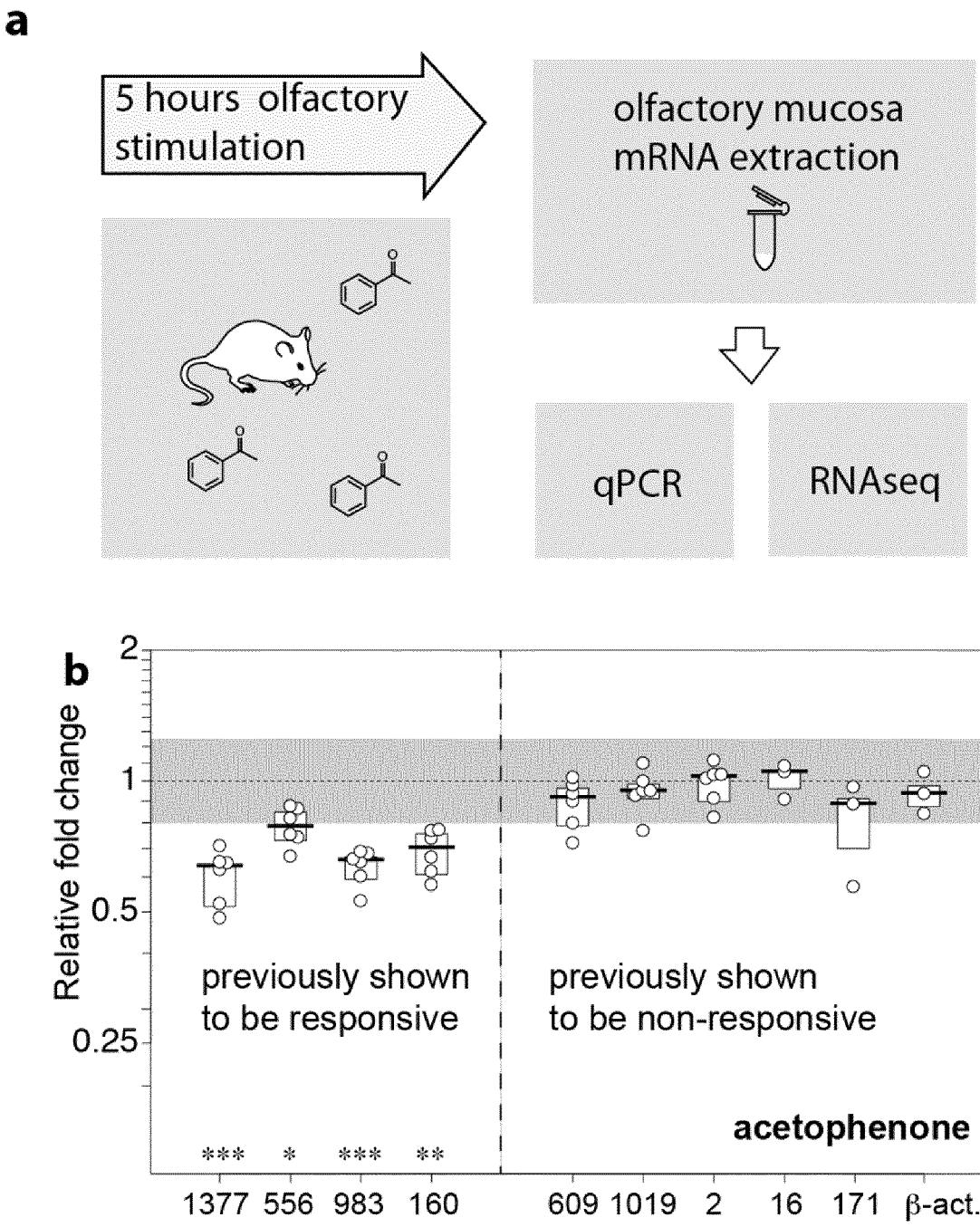
OR2F1, OR4A5, OR51B2, OR56B4, OR6B2, OR8H1, OR10G7, OR1A2, OR2F2, OR4B1, OR51B4, OR5A1, OR6B3, OR8H2, OR10G8, OR1B1, OR2G2, OR4C11, OR51B5, OR5A2, OR6C1, OR8H3, OR10G9, OR1C1, OR2G3, OR4C12, OR51B6, OR5AC2, OR6C2, OR8I2, OR10H1, OR1D2, OR2G6, OR4C13, OR51D1, OR5AK2, OR6C3, OR8J1, OR10H2, OR1D5, OR2H1, OR4C15, OR51E1, OR5AN1, OR6C4, OR8J3, OR10H3, OR1E1, OR2H2, OR4C16, OR51E2, OR5AP2, OR6C6, OR8K1, OR10H4, OR1E2, OR2J1, OR4C3, OR51F1, OR5AR1, OR6C65, OR8K3, OR10H5, OR1F1, OR2J2, OR4C46, OR51F2, OR5AS1, OR6C68, OR8K5, OR10J1, OR1G1, OR2J3, OR4C5, OR51G1, OR5AU1, OR6C70, OR8S1, OR10J3, OR1I1, OR2K2, OR4C6, OR51G2, OR5B12, OR6C74, OR8U1, OR10J5, OR1J1, OR2L13, OR4D1, OR51H1P, OR5B17, OR6C75, OR8U9, OR10K1, OR1J2, OR2L2, OR4D10, OR51I1, OR5B2, OR6C76, OR9A2, OR10K2, OR1J4, OR2L3, OR4D11, OR51I2, OR5B21, OR6F1, OR9A4, OR10P1, OR1K1, OR2L5, OR4D2, OR51L1, OR5B3, OR6J1, OR9G1, OR10Q1, OR1L1, OR2L8, OR4D5, OR51M1, OR5C1, OR6K2, OR9G4, OR10R2, OR1L3, OR2M2, OR4D6, OR51Q1, OR5D13, OR6K3, OR9G9, OR10S1, OR1L4, OR2M3, OR4D9, OR51S1, OR5D14, OR6K6, OR9I1, OR10T2, OR1L6, OR2M4, OR4E2, OR51T1, OR5D16, OR6M1, OR9K2, OR10V1, OR1L8, OR2M5, OR4F15, OR51V1, OR5D18, OR6N1, OR9Q1, OR10W1, OR1M1, OR2M7, OR4F16, OR52A1, OR5F1, OR6N2, OR9Q2, OR10X1, OR1N1, OR2S2, OR4F17, OR52A5, OR5H1, OR6P1, OR10Z1, OR1N2, OR2T1, OR4F21, OR52B1P, OR5H14, OR6Q1, OR11A1, OR1Q1, OR2T10, OR4F29, OR52B2, OR5H15, OR6S1, OR11G2, OR1S1, OR2T11, OR4F3, OR52B4, OR5H2, OR6T1, OR11H1, OR1S2, OR2T12, OR4F4, OR52B6, OR5H6, OR6V1, OR11H12, OR2A1, OR2T2, OR4F5, OR52D1, OR5I1, OR6X1, OR11H4, OR2A12, OR2T27, OR4F6, OR52E2, OR5J2, OR6Y1, OR11H6, OR2A14, OR2T29, OR4K1, OR52E4, OR5K1, OR7A10, OR11L1, OR2A2, OR2T3, OR4K13, OR52E6, OR5K2, OR7A17, OR12D2, OR2A25, OR2T33, OR4K14, OR52E8, OR5K3, OR7A5, OR12D3, OR2A4, OR2T34, OR4K15, OR52H1, OR5K4, OR7C1, OR13A1, OR2A42, OR2T35, OR4K17, OR52I1, OR5L1, OR7C2, OR13C2, OR2A5, OR2T4, OR4K2, OR52I2, OR5L2, OR7D2, OR13C3, OR2A7, OR2T5, OR4K5, OR52J3, OR5M1, OR7D4, OR13C4, OR2AE1, OR2T6, OR4L1, OR52K1, OR5M10, OR7E24, OR13C5, OR2AG1, OR2T7, OR4M1, OR52K2, OR5M11,

OR7G1 ; and/or any variant thereof having at least 80% identity with any one of said human odorant receptor gene's sequences.

18. An olfactory sensory neuron isolated from the transgenic animal according to any one of claims 16 to 17.

19. A tissue sample extracted from the olfactory system of the transgenic animal according to any one of claims 16 to 17.

20. A method according to any one of claims 1 to 15 wherein the biological sample is a biological sample isolated from a transgenic animal according to any one of claims 16 to 17.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5 hours olfactory stimulation
olfactory mucosa mRNA extraction
qPCR
RNAseq
b
Relative fold change
2
1
0.5
0.25
previously shown to be responsive
previously shown to be non-responsive
acetophenone
***
*
***
**
1377
556
983
160
609
1019
2
16
171
β-act.

Figure 1

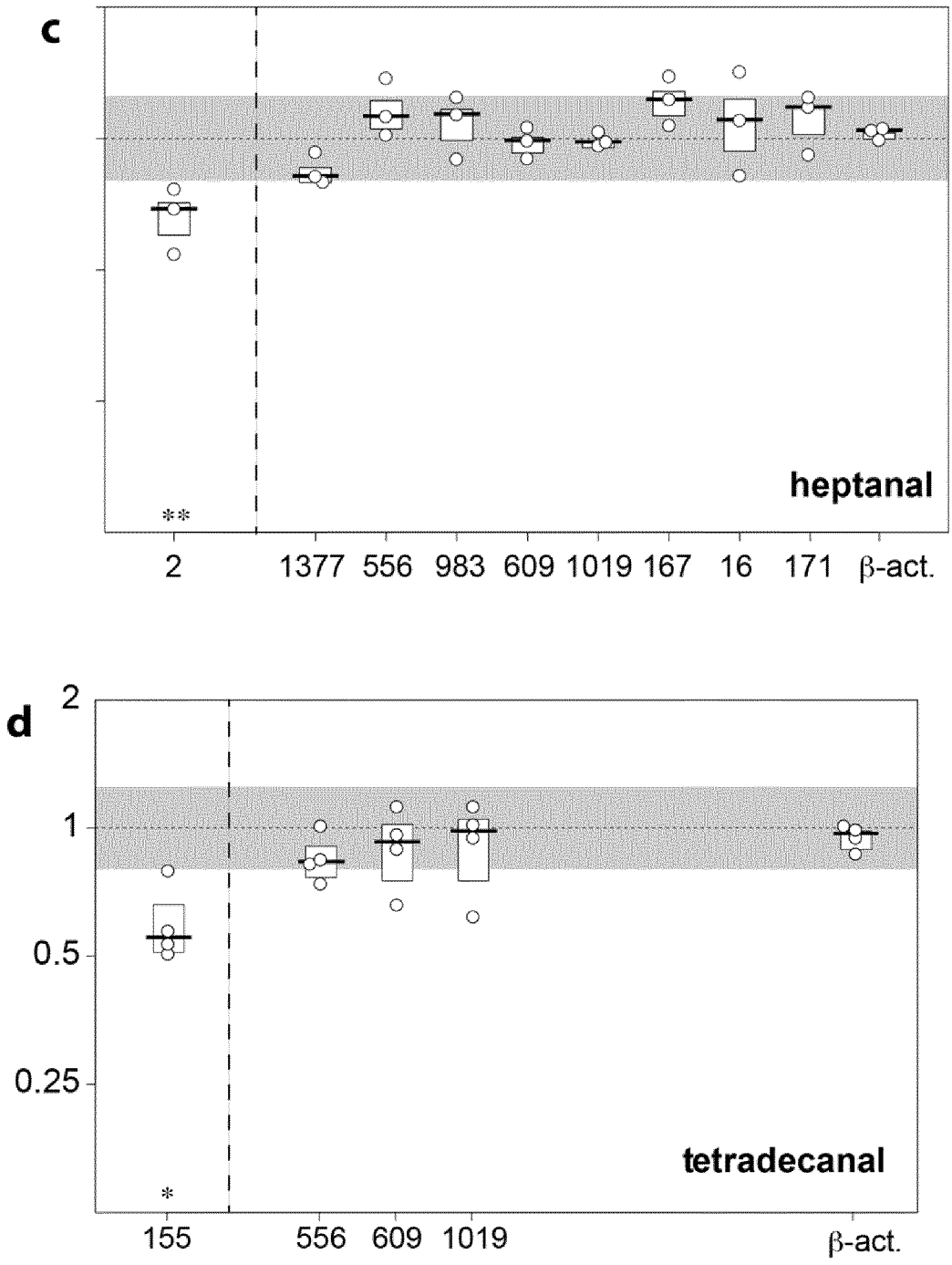


Figure 1 (continued)

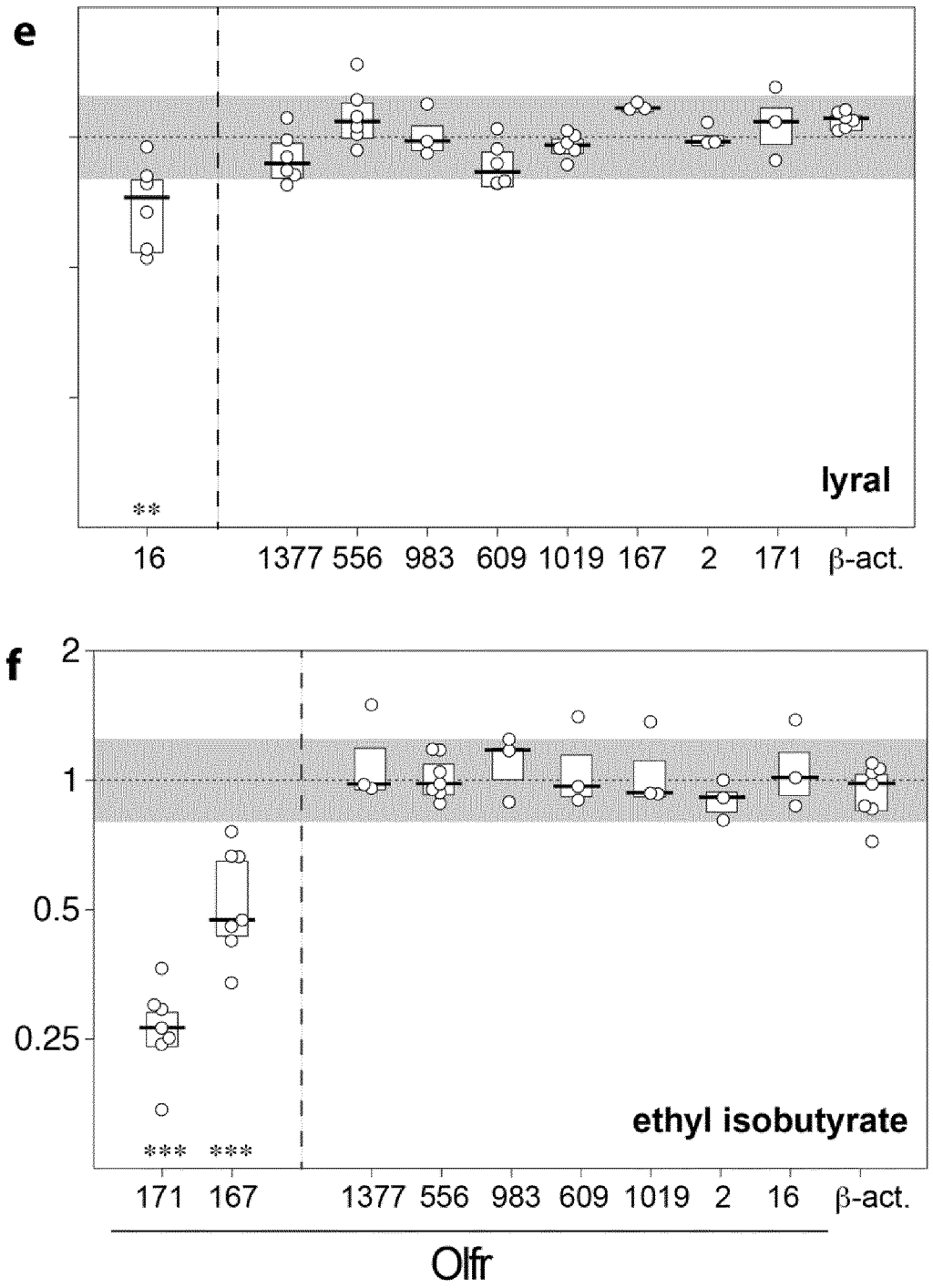


Figure 1 (continued)

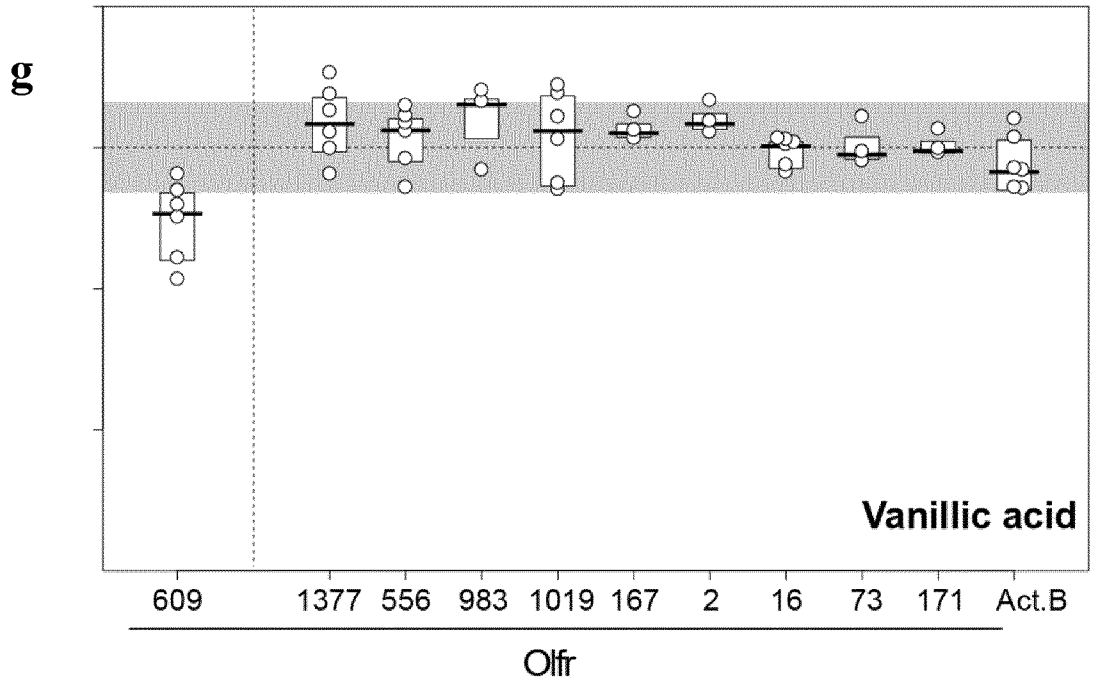


Figure 1 (continued)

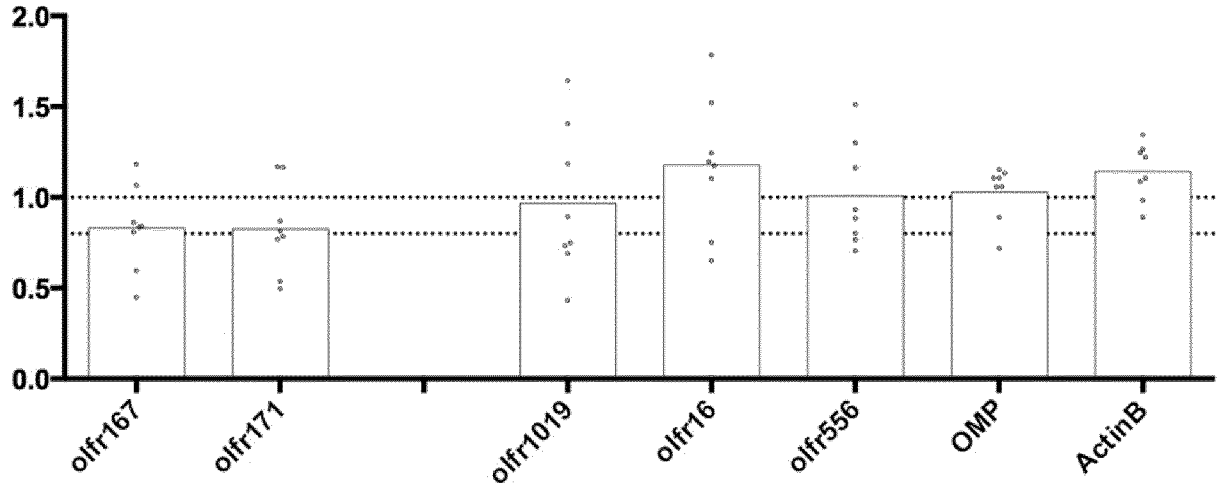
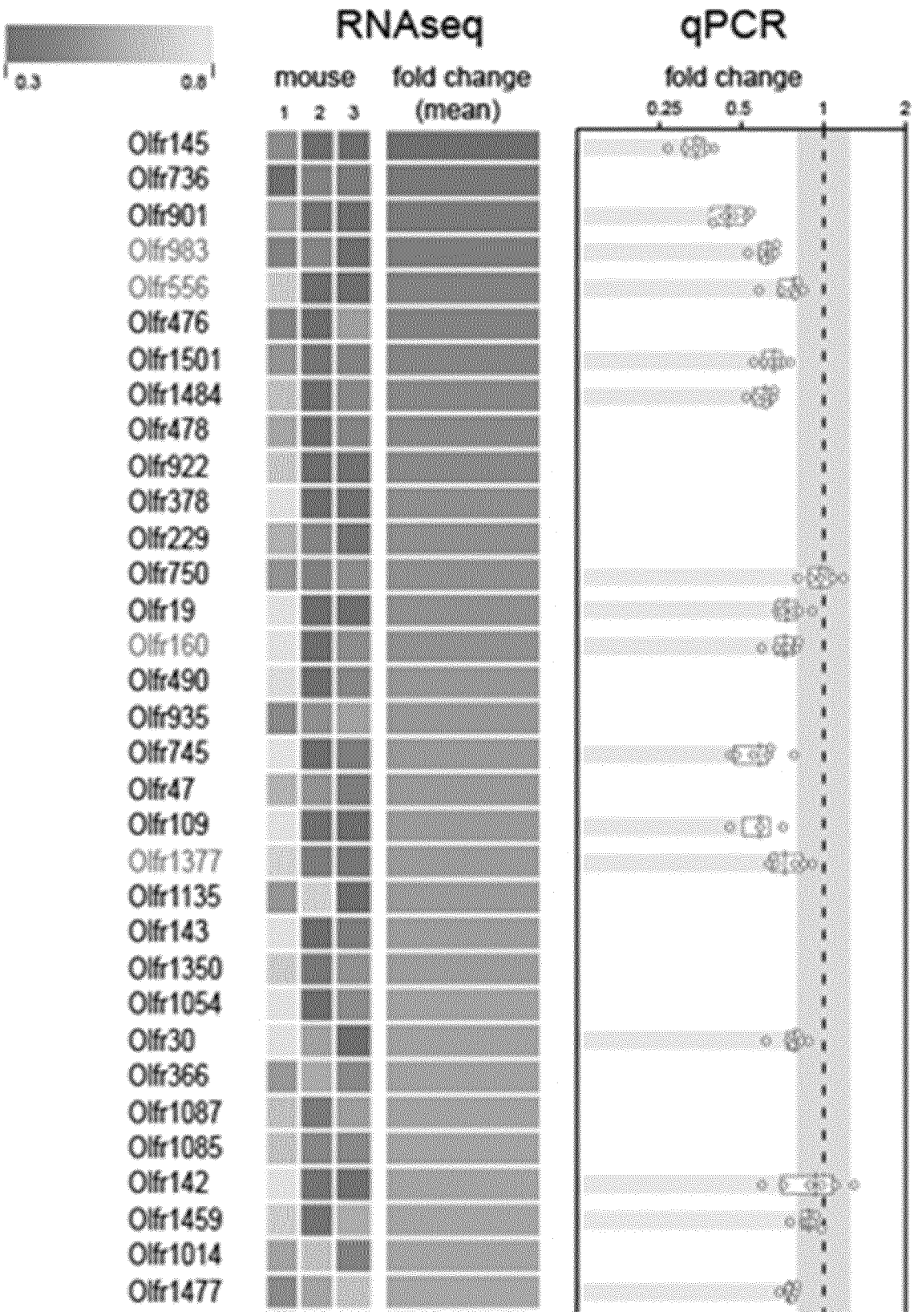


Figure 2


0.3
0.8
RNAseq
mouse
1 2 3
fold change (mean)
qPCR
fold change
0.25 0.5 1 2
Olfr145
Olfr736
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Olfr983
Olfr556
Olfr476
Olfr1501
Olfr1484
Olfr478
Olfr922
Olfr378
Olfr229
Olfr750
Olfr19
Olfr160
Olfr490
Olfr935
Olfr745
Olfr47
Olfr109
Olfr1377
Olfr1135
Olfr143
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Olfr1085
Olfr142
Olfr1459
Olfr1014
Olfr1477

Figure 3

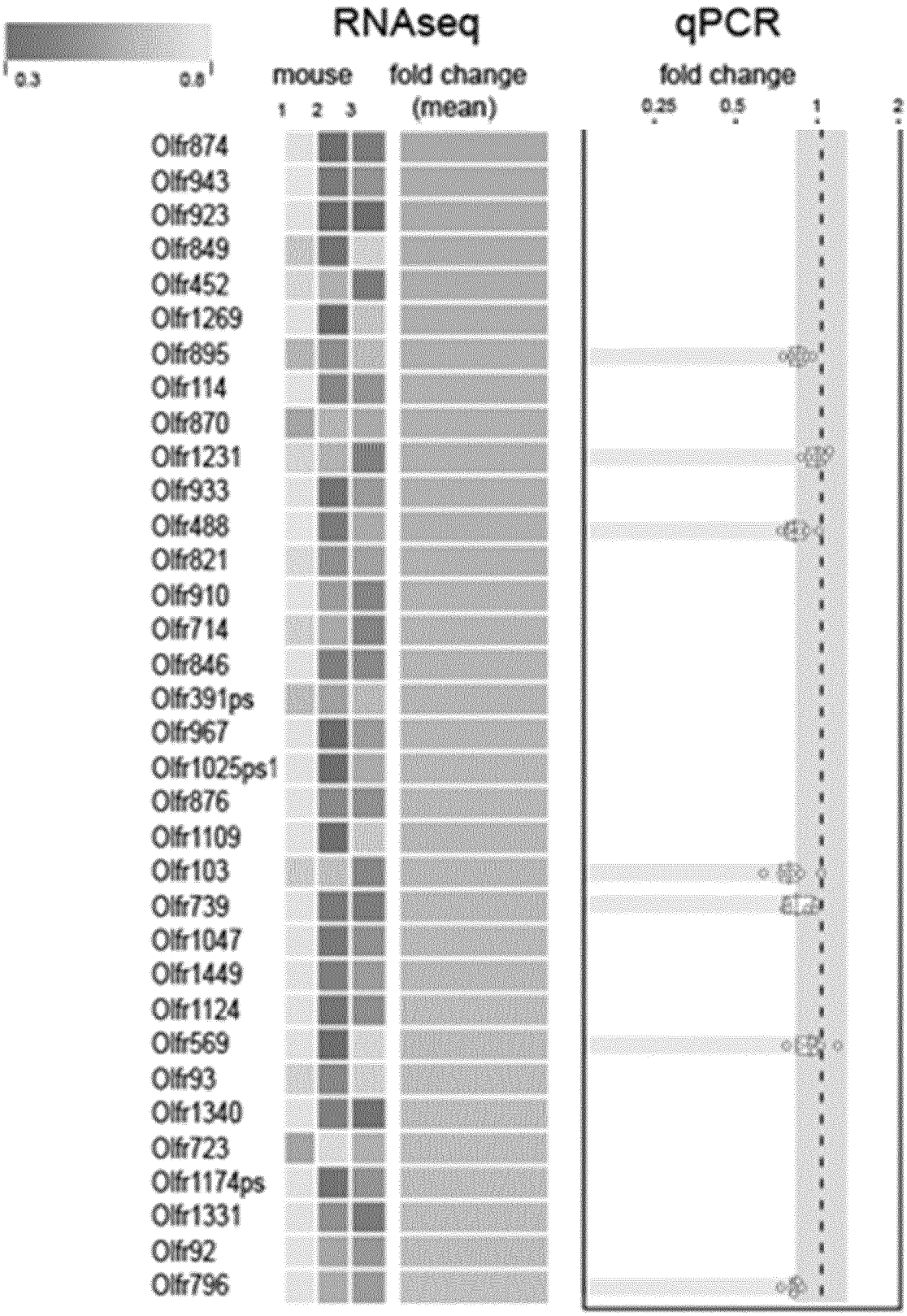

Figure 3 (continued)

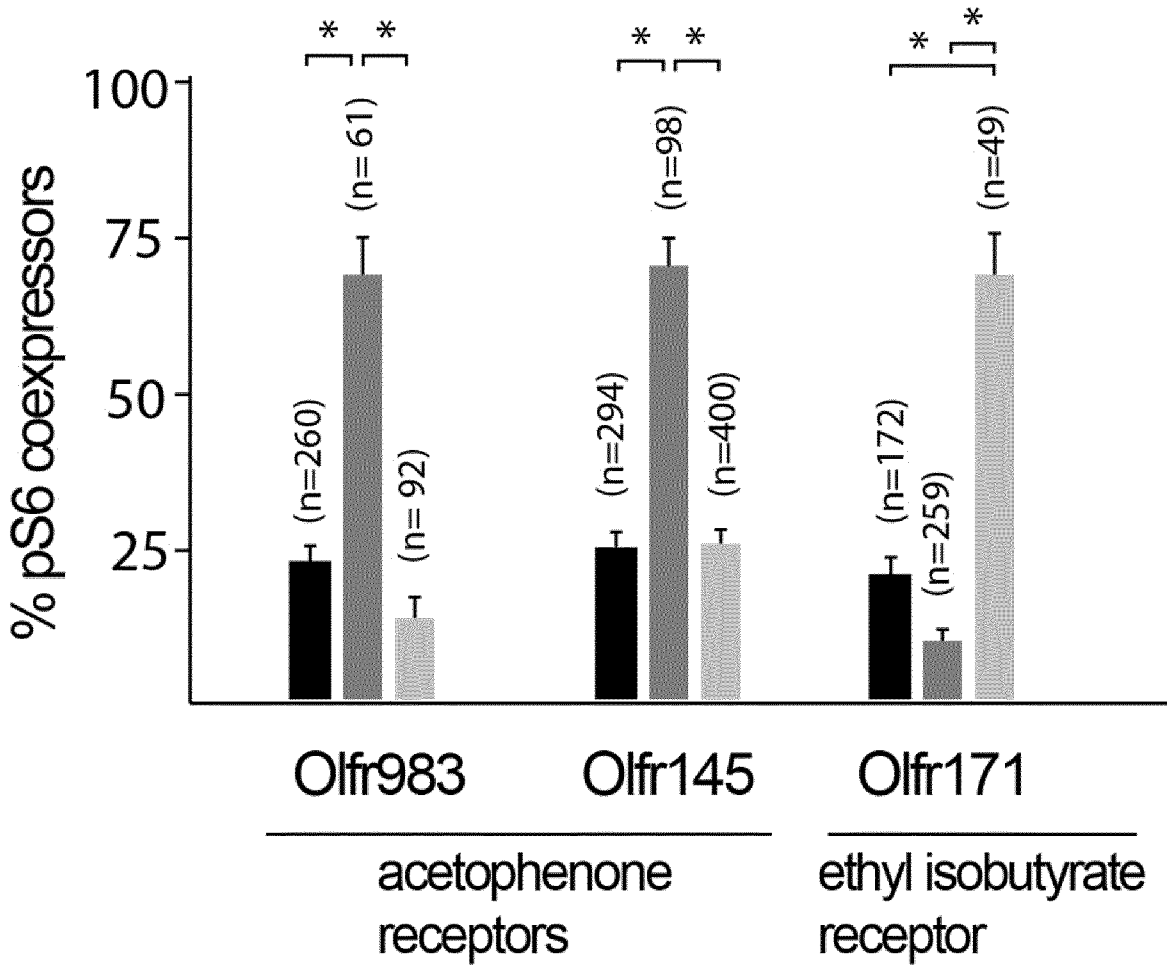
% pS6 coexpressors
100
75
50
25
(n=260)
(n= 61)
(n= 92)
(n=294)
(n=98)
(n=400)
(n=172)
(n=259)
(n=49)
*
*
*
*
*
*
Olfr983
Olfr145
Olfr171
acetophenone receptors
ethyl isobutyrate receptor

Figure 4

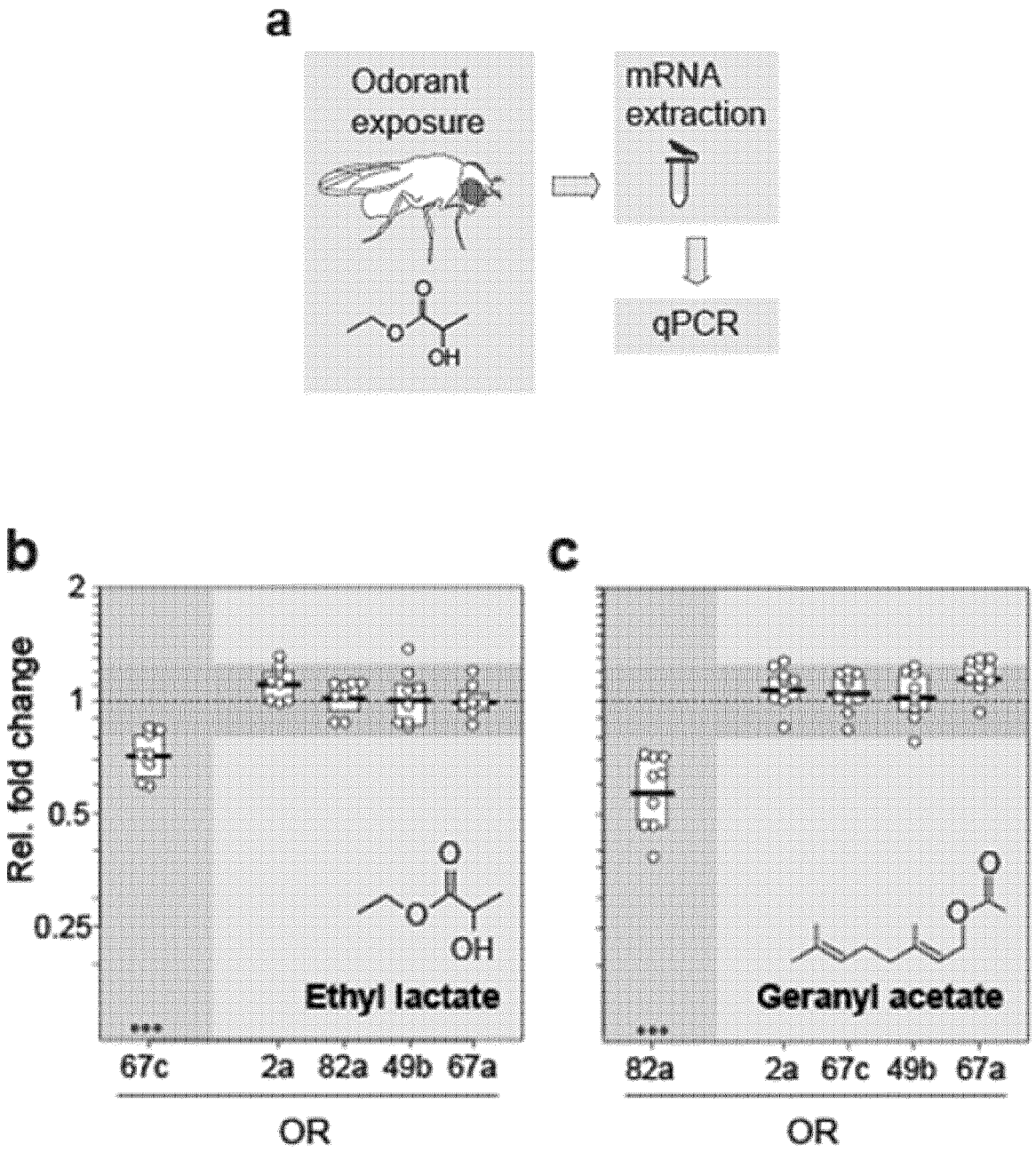
a
Odorant exposure
mRNA extraction
qPCR
OH
b
Rel. fold change
2
1
0.5
0.25
Ethyl lactate
***
67c
2a
82a
49b
67a
OR
c
Geranyl acetate
***
82a
2a
67c
49b
67a
OR

Figure 5

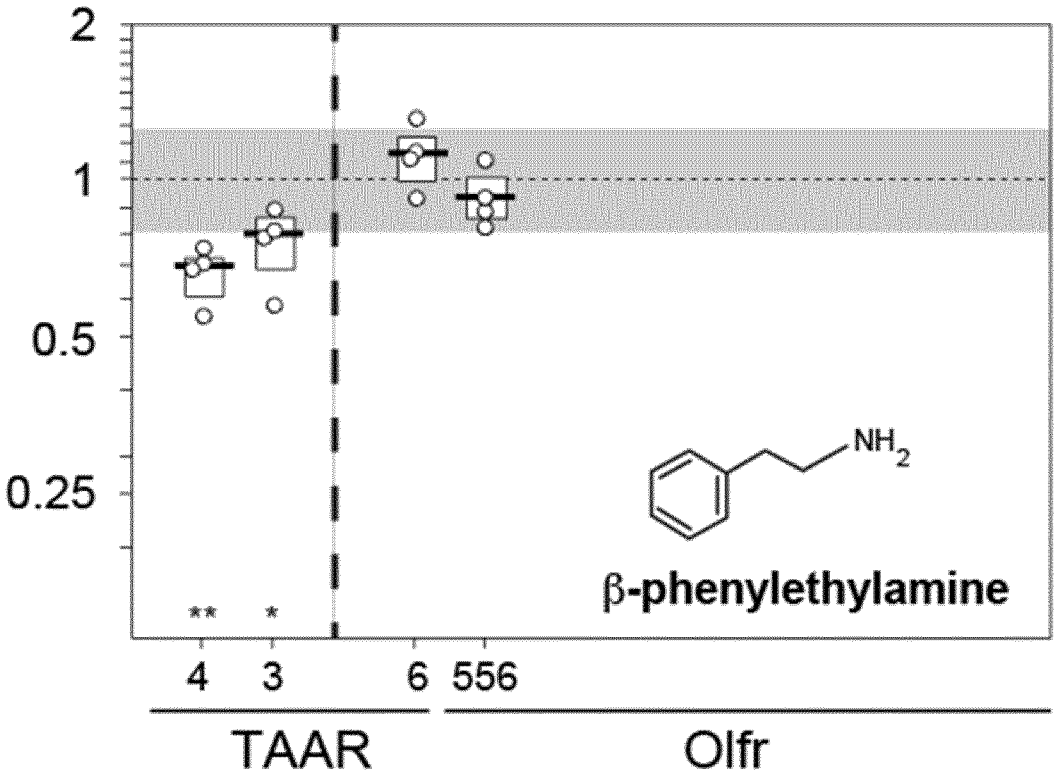

Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2015/069454

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

1-20(partially)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/069454

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/68 A01K67/027 G01N33/566
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CASEY TRIMMER ET AL: "High-throughput Analysis of Mammalian Olfactory Receptors: Measurement of Receptor Activation via Luciferase Activity", JOURNAL OF VISUALIZED EXPERIMENTS, no. 88, 2 June 2014 (2014-06-02), XP55225547, DOI: 10.3791/51640 the whole document In particular: Title; Abstract; Protocol section; Figures 1 and 2. ----- -/--	1-20

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 February 2016	18/02/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	C.F. Angioni

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DAVID C. RINKER ET AL: "Novel high-throughput screens of Anopheles gambiae odorant receptors reveal candidate behaviour-modifying chemicals for mosquitoes", PHYSIOLOGICAL ENTOMOLOGY, vol. 37, no. 1, 23 February 2012 (2012-02-23), pages 33-41, XP55225549, GB ISSN: 0307-6962, DOI: 10.1111/j.1365-3032.2011.00821.x	1-14,20
A	the whole document In particular: Title; Abstract; Introduction, last section with title "High throughput approach to insect control by modifying behaviour"; Materials and methods section; Figures 1-4	15-19
X	WO 03/020913 A2 (SENTIGEN CORP [US]; LEE KEVIN J [US]; ONG JANE [US]; NGUYEN THUY-AI T) 13 March 2003 (2003-03-13)	1-14,20
Y	the whole document In particular: Table 2, 5th item; Page 13, line 10-22; Claims 75-118.	15-19
X	P. K. RICHGELS ET AL: "Genetic Variation in Odorant Receptors Contributes to Variation in Olfactory Behavior in a Natural Population of Drosophila melanogaster", CHEMICAL SENSES., vol. 37, no. 3, 29 October 2011 (2011-10-29), pages 229-240, XP55225245, GB ISSN: 0379-864X, DOI: 10.1093/chemse/bjr097	1-14,20
A	the whole document In particular: Abstract; Materials and methods section; Figure 5d + page 235-236, bridging paragraph.	15-19
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 01/68805 A2 (SENOMYX INC [US]) 20 September 2001 (2001-09-20) the whole document In particular: p.1, field of invention; p. 19-21, detailed description of invention; p. 74-75, bridging paragraph; p. 170, AOLFR207 (SEQ ID NOs: 385 and 386); Claims 85-124. -----	1-14,20 15-19
Y	EP 1 391 508 A1 (HOFFMANN LA ROCHE [CH]; GIVAUDAN SA [CH]) 25 February 2004 (2004-02-25) the whole document In particular: Claims 1-28. -----	1-20
Y	WO 00/35274 A1 (UNIV JOHNS HOPKINS MED [US]; REED RANDALL R [US]; KRAUTWURST DIETMAR [] 22 June 2000 (2000-06-22) the whole document In particular: Claims 29-40. -----	1-20

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/069454

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-20(partially)

A screening method as defined in claim 1, wherein the receptor to be tested is OR10a.

2-386. claims: 1-20(partially)

A screening method as defined in claim 1, wherein the receptor to be tested is one of the remaining genes listed in claim 7.
