Genetic variation in a human odorant receptor alters perception of sex steroid-derived odours

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Human olfactory perception differs enormously between individuals, with large reported perceptual variations in the intensity and pleasantness of a given odour. Androstenone (5α-androst-16-en-3-one), an odorous steroid derived from testosterone, is variously perceived by different individuals as offensive ("sweaty, urinous"), pleasant ("sweet, floral"), or odourless [1-3]. Up to 30% of humans have reduced sensitivity to androstenone, with 6% fitting the criteria of specific anosmia or "odour blindness" to androstenone, which may be a genetically determined trait [1,3,4]. The mechanistic basis of this phenomenon is unknown, but it has been hypothesized that genetic variation in odorant receptors [5-7] may account for interindividual variation in odour perception [1,4,8]. Here we show that a human odorant receptor, OR7D4, is selectively activated *in vitro* by androstenone and the related odorous steroid

androstadienone (androsta-4,16-dien-3-one) and does not respond to a panel of 64 other odours and two solvents. A common variant of this receptor (*OR7D4 WM*) contains two non-synonymous single nucleotide polymorphisms (SNPs), resulting in two amino acid substitutions (R88W, T133M) and severely impaired function *in vitro*. Human subjects with *RT/WM* or *WM/WM* genotypes are less sensitive to androstenone and androstadienone and find both odours less unpleasant than subjects with the functional *RT/RT* genotype. A second variant with reduced function *in vitro, OR7D4 P79L,* also results in reduced sensitivity to androstenone in human subjects. *OR7D4 S84N,* a variant with increased function *in vitro,* was found in several subjects who showed increased sensitivity to androstenone and androstadienone. We conclude that polymorphisms in *OR7D4* contribute to the variability in perception of these steroidal odours, which have been suggested to act as modulatory pheromones in humans [9-11]. Our results demonstrate the first link between the function of an odorant receptor *in vitro* and odour perception, establishing the basis for the unravelling of olfactory coding in humans.

We investigated the hypothesis that polymorphisms in odorant receptors contribute to the variability in human odour perception by a combining a cell-based assay technique to deorphanize odorant receptors [12] with an olfactory psychophysics study of a diverse population of human subjects in New York City [13]. A panel of 337 human odorant receptors was cloned and expressed in Hana3A cells, an HEK293T-derived cell line stably expressing accessory factors for odorant receptor expression[12], and screened for androstenone-mediated stimulation. A single odorant receptor, *OR7D4*, shows robust responses to this ligand (Fig. 1a).

A search for polymorphisms in *OR7D4* in existing SNP databases and our own sequencing efforts revealed 13 SNPs in this receptor (Table 1). Sequencing the coding region of *OR7D4* in 412 participants of the Rockefeller University Smell Study revealed that two polymorphisms (SNP5 and SNP7) occur at a reasonable frequency (p=0.154) and are in complete linkage disequilibrium in this population (r^2=1, D'=1). These non-synonymous substitutions lead to two amino acid changes (R88W and T133M), and thus we refer to the two most common alleles of this receptor as *OR7D4 RT* and *OR7D4 WM*. The allele frequency of these genotypes and the prevalence by racial group are detailed in Table 1 and Table S1. The *WM* allele is underrepresented in African-American subjects and overrepresented in Caucasian subjects relative to the *RT* allele (p=0.0008; Fisher's exact test)(Table S1).

We investigated the ligand specificity of both receptor variants *in vitro* against a panel of 66 odours and two solvents using a luciferase assay that converts odorant receptor activation to reporter gene activity. OR7D4 RT responds selectively to androstenone and the closely related odorous steroid, androstadienone, but shows no responses to any of the other 64 odours or two solvents (Fig. 1b, top). OR7D4 WM shows no responses to any compound at the concentrations tested here (Fig. 1b, bottom). Dose response curves with OR7D4 RT and OR7D4 WM suggest that the paired SNPs in the WM variant severely impair the function of this receptor (Fig. 1c). To investigate whether one or both of the variant residues causes this effect, we generated ORs with each one of the SNPs. OR7D4 R88W and OR7D4 T133M retain an intermediate level of function relative to OR7D4 RT,

suggesting that both R88 and T133 residues contribute to the function of OR7D4 (Figure 1c). *OR7D4* is situated on chromosome 19 in a cluster of 8 odorant receptor genes and one pseudogene, which is adjacent and 92% identical to *OR7D4*. In the chimpanzee genome, the orthologue of *OR7D4* and that of the linked pseudogene have intact open reading frames and the chimpanzee *OR7D4* orthologue exists as the *RT* allele. The closest homologue to *OR7D4* in the human genome is only 70% identical, suggesting that this receptor is not part of a larger subfamily of closely related receptors.

The non-synonymous substitutions in *OR7D4* affect amino acids distributed throughout the protein (Table 1 and Fig. 2a). We tested the function of the remaining 11 *OR7D4* variants *in vitro* and found five additional variants with reduced function (*D52G*, *S75C*, *P79L*, *M136I*, *L162P*, *A279D*, and *L292M*), two variants that have similar level of function to the reference sequence (*H131Q* and *C139Y*), and two variants that show increased function relative to *OR7D4 RT* (*S84N* and *C139R*) (Fig. 2b). Converse to the racial distribution of the *WM* allele, we find that *P79L* and *S84N* are overrepresented in African-American relative to Caucasian subjects when compared to the *RT* allele (p=0.0001 and p=0.03, respectively; Fisher's exact test)(Table S1). It is worth noting that the SNPs, *S75C*, *H131Q*, *M136I*, *C139R*, *C139Y*, and *L292M*, did not appear in a rather large cohort of ethnically diverse individuals and represent either rare or not naturally occurring SNPs.

To gain insight into the mechanism by which *OR7D4* polymorphisms affect receptor function, we examined the subcellular distribution, expression levels, and cell-surface distribution of the four major variants of *OR7D4* in Hana3A cells with an antibody that

recognizes the N-terminal epitope tag of the OR7D4 variants. Immunofluorescence staining of RT, WM, P79L, and S84N proteins in permeabilized cells reveals no obvious difference in subcellular distribution or expression level (Fig. 2c). Western blot analysis confirms that all are expressed at comparable levels (Fig. 2d). We therefore asked whether differences in cell surface expression could account for the functional differences between the variants. Flow cytometry analysis of live cells shows that RT, WM, and P79L have similar low levels of surface staining, while the S84N variant shows considerably more surface expression (Fig. 2e). We suggest that SNPs in the WM and P79L alleles may affect function by interfering with cell surface expression, ligand binding, signal transduction, or yet another mechanism, while the increased function of the S84N variant may stem from enhanced stability or cell surface trafficking.

We next asked whether variation in *OR7D4* correlates with variation in the perception of androstenone and androstadienone measured in the 412 participants of The Rockefeller University Smell Study. The common functional genotype (*OR7D4 RT/RT*) was found in 62% (N=255) of these subjects, the *OR7D4 WM/WM* genotype was found in 2% (N=10) of the subjects, and 24% (N=100) of the subjects have the heterozygous *OR7D4 RT/WM* genotype. The *OR7D4 RT/P79L* and RT/*S84N* genotypes were each found in ~2% of subjects (N=10 and N=7, respectively) and 2% (N=10) subjects had various combinations of these and rarer SNPs (Table 1 and Table S1). Extensive psychophysical data were collected on all 412 subjects over the course of a two-year study that involved three different tasks: subjects rated the perceived intensity and valence (pleasantness or unpleasantness) of 66 different odours at two concentrations; detection thresholds were

measured to androstenone and androstadienone in a subset of subjects and three control odours in all subjects [14,15]; subjects profiled five odours with 146 semantic labels [13,16] (see Supplemental Materials online for detailed information on the psychophysical methods). Psychophysical data were subsequently divided according to genotype and assessed for the influence of *OR7D4* genotype on perceptual phenotype. Rigorous statistical analysis, with Bonferroni correction for multiple comparisons, was imposed to establish the significance of our findings.

We first examined how the *OR7D4 WM* allele affects androstenone and androstadienone odour intensity perception. Strikingly, of the 66 odours and two solvents rated by all *OR7D4 RT/RT* and *RT/WM* subjects, only androstenone and androstadienone show a significant effect of genotype (Fig. 3a). These steroids are rated as less intense by *OR7D4 RT/WM* subjects (Fig. 3a). This phenotype is specific for these two compounds, as the perception of other similar steroidal and musky compounds such as pentadecalactone, ambrette, and galaxolide is not affected by *OR7D4* genotype (Fig. 3a-b). The reduction in androstenone and androstadienone odour intensity is even more marked in the few *OR7D4 WM/WM* subjects screened (Fig. 3b). Therefore, the reduced function of the WM variant measured *in vitro* (Fig. 1b) correlates with reduced perception of the OR7D4 ligands *in vivo*. Why do polymorphisms in *OR7D4* show a semi-dominant phenotype? It is likely that RT/WM heterozygous subjects have 50% fewer olfactory neurons expressing a functional OR7D4 variant, thus explaining their reduced sensitivity to androstenone and androstadienone.

Detection thresholds of a subset of these subjects were determined for both steroidal odours (Fig. 3c). As expected from the intensity ratings above, *OR7D4 RT/WM* subjects as a group have higher detection thresholds to both compounds (Fig. 3c) and a greater incidence of specific anosmia to androstenone than *RT/RT* subjects ($p<0.05$; chi-square test). We conclude that the WM allele affects human sensitivity to androstenone and androstadienone.

After establishing that the *OR7D4 WM* polymorphism affects sensitivity, we asked whether it also affects the perception of androstenone and androstadienone odour quality. *OR7D4 RT/WM* subjects rate both steroidal odours as more pleasant than *OR7D4 RT/RT* subjects (Fig. 4a). This change in odour quality perception is largest for the steroids although a less pronounced - but statistically significant - difference is found for vanillin and octyl acetate (Fig. 4b). *OR7D4 WM/WM* subjects as a group are not simply anosmic to both steroidal compounds as they rate these as more unpleasant than the solvent, propylene glycol (Fig. 4b). Subjects rated androstenone odour quality by profiling this odour with a standard set of 146 semantic labels [13,16]. We analyzed all descriptors used by more than 10% of subjects and compared the usage of individuals with differing genotypes. Of the 74 such descriptors used for androstenone, vanillin and the solvent, propylene glycol, only four differ significantly by genotype (see Supplementary Methods for details). *OR7D4 RT/WM* subjects are less likely to consider androstenone “sickening” and more likely to rate it as smelling like “vanilla” than *RT/RT* subjects (Fig. 4c). Interestingly, these same subjects show a comparable increase in their use of two descriptors for the odour vanillin (Fig. 4c).

To extend our observation that *OR7D4* variation affects the perception of androstenone and androstadienone, we examined the severely impaired *OR7D4 P79L* variant and the variant with increased function, *OR7D4 S84N*. Dose-response analysis of OR7D4 P79L function *in vitro* shows severely impaired function at all concentrations of either steroidal odour tested (Fig. 5a). In contrast, OR7D4 S84N show remarkable sensitivity to both odours *in vitro*, exceeding the activity of the common functional RT variant at every concentration tested, with an EC50 value to androstadienone nearly 20 times lower than the RT variant (Fig. 5a; compare green and black curves). Psychophysical analysis of subjects carrying *RT/P79L* and *RT/S84N* genotypes is consistent with these *in vitro* results, although statistical analysis was constrained by the small number of such individuals in our study group. We found that *RT/P79L* subjects rated both androstenone and androstadienone as less intense and more pleasant than *RT/RT* controls (Fig. S1), and that these comparisons were significant for the androstadienone valence rating (Fig. S1b). Conversely, *RT/S84N* subjects rated both androstenone and androstadienone as more intense and less pleasant than *RT/RT* controls (Fig. S1). Detection thresholds of a subset of *RT/P79L* and *RT/S84N* subjects to both odorous steroids were also obtained (Fig. 5b-c). The detection threshold of *RT/P79L* subjects to both androstenone and androstadienone is lower than *RT/RT* subjects (Fig. 5b), as is the proportion of subjects anosmic to androstenone ($p=0.018$; chi-square analysis). The detection threshold of *RT/S84N* subjects to both steroids is higher than *RT/RT* controls, but the low number of such subjects precludes statistical analysis. Increased sensitivity to select musk compounds has been previously observed, suggesting that this type of specific hyperosmia may be a general phenomenon [17]. Taken together, we show that genetic

variation in *OR7D4* correlates with variation in the perception of two sex steroid-derived odours.

This study is the first to link specific polymorphisms in a single odorant receptor gene to altered perception of the ligands that activate this receptor. Sensory variation in bitter taste [18,19] and colour perception [20,21] have also been related to mutations in sensory receptors. Previous functional analysis of odorant receptors in olfactory neurons and in heterologous cells suggested a great deal of redundancy in the ligand specificity of odorant receptors [12,22-24]. Given this proposed combinatorial coding mechanism of odorant receptors [23,24] and the large number of odorant receptors in the human genome [6,7,25], it was not clear *a priori* that mutations in a single odorant receptor would be detectable as perceptual phenotypes. How does a single odorant receptor contribute to the complex odour quality of these sex steroid-derives odours? Based on our results, polymorphisms in the *OR7D4* protein-coding sequence alone do not account for specific anosmias to androstenone and androstadienone. Some *OR7D4 WM/WM* subjects can detect both odours and some *OR7D4 RT/RT* subjects cannot detect either odour. This suggests that a functional *OR7D4* gene is neither necessary nor sufficient to detect these compounds. Instead, our data show that *OR7D4* mutations with decreased function correlate strongly with decreased sensitivity and altered perception of these compounds. We suggest that *OR7D4* acts in parallel with one or more other odorant receptors to produce the “complete” sensation of androstenone and androstadienone. This would be consistent with the principle of combinatorial coding that has emerged from the *in vitro* analysis of odorant receptors [12,22-24]. The psychophysical data support a model in which *OR7D4 RT* activation contributes to the offensive odor quality of

androstenone, such that subjects lacking this receptor are less likely to find this odour unpleasant [26]. Further analysis of the ligand response properties of the entire repertoire of human odorant receptors will be required to resolve whether there are other receptors that together recognize androstenone and androstadienone.

The evidence that androstenone and androstadienone act as pheromones to modulate human behaviour is intriguing but controversial [9-11]. It will be interesting to ask whether humans with non-functional *OR7D4* alleles differ from humans with the *OR7D4 RT/RT* genotype in the neural and psychological effects induced by these odorous steroids. The combined functional genomic and psychophysical approach presented here may permit a comprehensive analysis of human odour coding.

METHODS

Heterologous expression of human odorant receptors. 423 human odorant receptors including 337 predicted functional receptors were cloned. Odorant receptors that contain the first 20 amino acid of human rhodopsin [27] in pCI (Promega) were expressed in the Hana3A cell line along with a short form of *mRTP1*, *RTP1S*, (M37 to the C-terminal end), which enhances functional expression of the odorant receptors (HZ and HM, manuscript in preparation). For immunocytochemistry, cells were fixed, permeabilized and incubated with monoclonal anti-rhodopsin antibody, 4D2 [28] , followed by Cy3-conjugated donkey anti-mouse IgG (Jackson Immunologicals). For FACS analysis, PE-conjugated donkey anti-mouse IgG (Jackson Immunologicals) was used. Western blot analysis was performed according to Mini-Protean 2 Cell (Bio-Rad) protocol. ECL (Amersham) was used for

detecting proteins on membranes. After the initial exposure, the membrane was incubated with stripping buffer (25mM Glycine-HCl [pH2], 1% SDS, 25mM Glycine, 0.036N HCl, 1% SDS) and incubated with rabbit anti-GFP (Invitrogen). Luciferase assays were performed as described[12]. All odours were supplied by Sigma-Aldrich at high purity, with these exceptions: androstadienone (a gift of Human Pheromone Sciences, Inc., Fremont, CA); banana (Bell Flavors and Fragrances); bourgeonal (Biomol); galaxolide (a gift of International Flavors and Fragrances); and r-carvone (Research Chemical Ltd.). The same batch and lot of each odour was used for both cell-based analysis and human olfactory psychophysics.

Human odorant receptor genotyping and sequencing. Venous blood was collected from all subjects and genomic DNA prepared with the Qiagen PAXgene blood DNA kit. Polymorphisms in *OR7D4* were assayed by sequencing and allele-specific polymerase chain reaction (PCR). For sequencing, human genomic DNAs were amplified, purified, and sequenced with a 3100 or 3730 Genetic Analyzer (ABI Biosystems) or by GeneWiz (North Brunswick, NJ). Detailed methods can be found in the Supplementary Materials.

Human olfactory psychophysics. All human subjects gave informed consent to participate in this study and were tested in a well-ventilated room of the Rockefeller University Hospital Outpatient Unit. All procedures involving human subjects were approved by the Rockefeller University Hospital Institutional Review Board. Normal human subjects (n=412; 218 female, 194 male; median age 34, age range 19-75) were pre-screened to exclude pregnant women and those with medical conditions causing general impairment of the sense of smell. All subjects completed two replicates of the test separated by at least 4 days. Odours were presented in amber vials as previously described [29] using bar-coded symbols to ensure that subjects were blind to the identity of all odours. The intensity and

valence of 66 odours at two concentrations ("high" and "low") and two solvents was rated using a 7-point scale. Thresholds were calculated using the single staircase method with seven reversals [14,15]. Threshold tests included both steroids as binary dilutions from 1:64 (binary dilution 6) to 1:134,217,728 (binary dilution 27). Subjects who could not reliably distinguish a 1:64 dilution of androstenone and androstadienone from solvent were operationally defined as anosmic to these odorous steroids, although we cannot exclude that these subjects could detect higher concentrations of these steroids [3]. Odour profiling used the method established by Dravnieks [16]. Detailed methods can be found in the Supplementary Material.

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Supplementary Information is linked to the online version of the paper at **www.nature.com/nature**.

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Author Contributions HZ and HM carried out the screen for androstenone and androstadienone receptors, identified polymorphisms, performed functional expression of receptor variants, and genotyped the human subjects. QC and HM carried out the cloning of human odorant receptors. QC assisted in the luciferase screening. AK and LBV devised and carried out the human olfactory psychophysics study, for which AK supervised the collection and performed the analysis of all the data and LBV prepared the human genomic DNA. The manuscript was co-written by AK, HZ, LBV, and HM.

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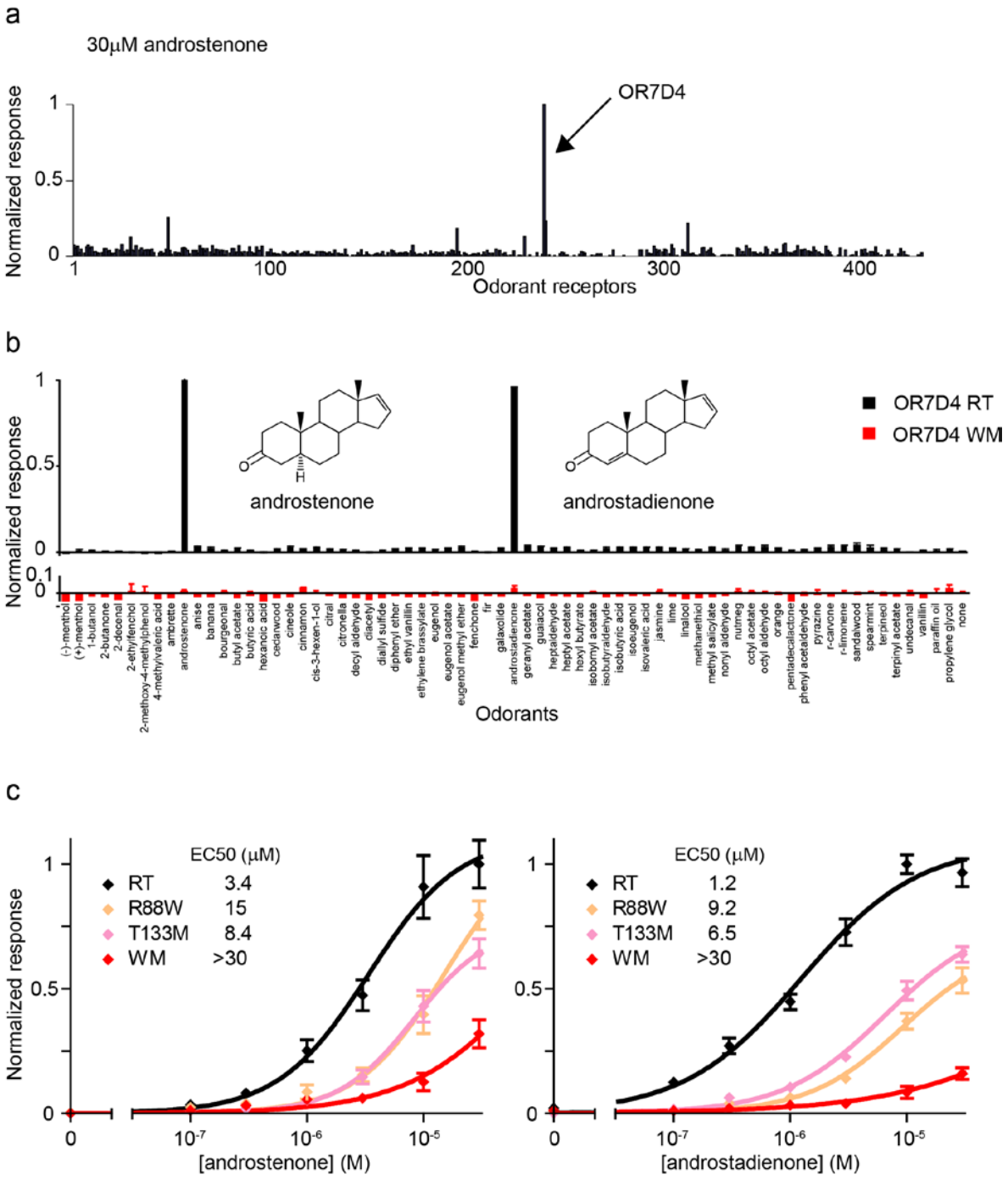

Figure 1 | *OR7D4* is a human odorant receptor selectively activated by androstenone and androstadienone.

a, Luciferase assays of a panel of 432 samples including 337 unique human odorant receptors, 45 odorant receptor pseudogenes, 27 variant pairs of the same genes, 14 duplicates, and 9 negative controls, all expressed in Hana3A cells stimulated with 30μM androstenone*. OR7D4* produces the most robust response. Y-axis denotes normalized response. **b**, Specificity of OR7D4 RT tested against a panel of 66 odours and 2 solvents presented at 30μM or 1/30,000 dilution. Only androstenone and androstadienone elicit a response. OR7D4 WM does not respond to any of the ligands tested. Y-axis denotes normalized response +SEM (n=4). **c**, Dose response curves of OR7D4 RT, WM, R88W, and T133M for androstenone and androstadienone. EC50 values in μM are provided. Y-axis denotes normalized response ±SEM (n=6).

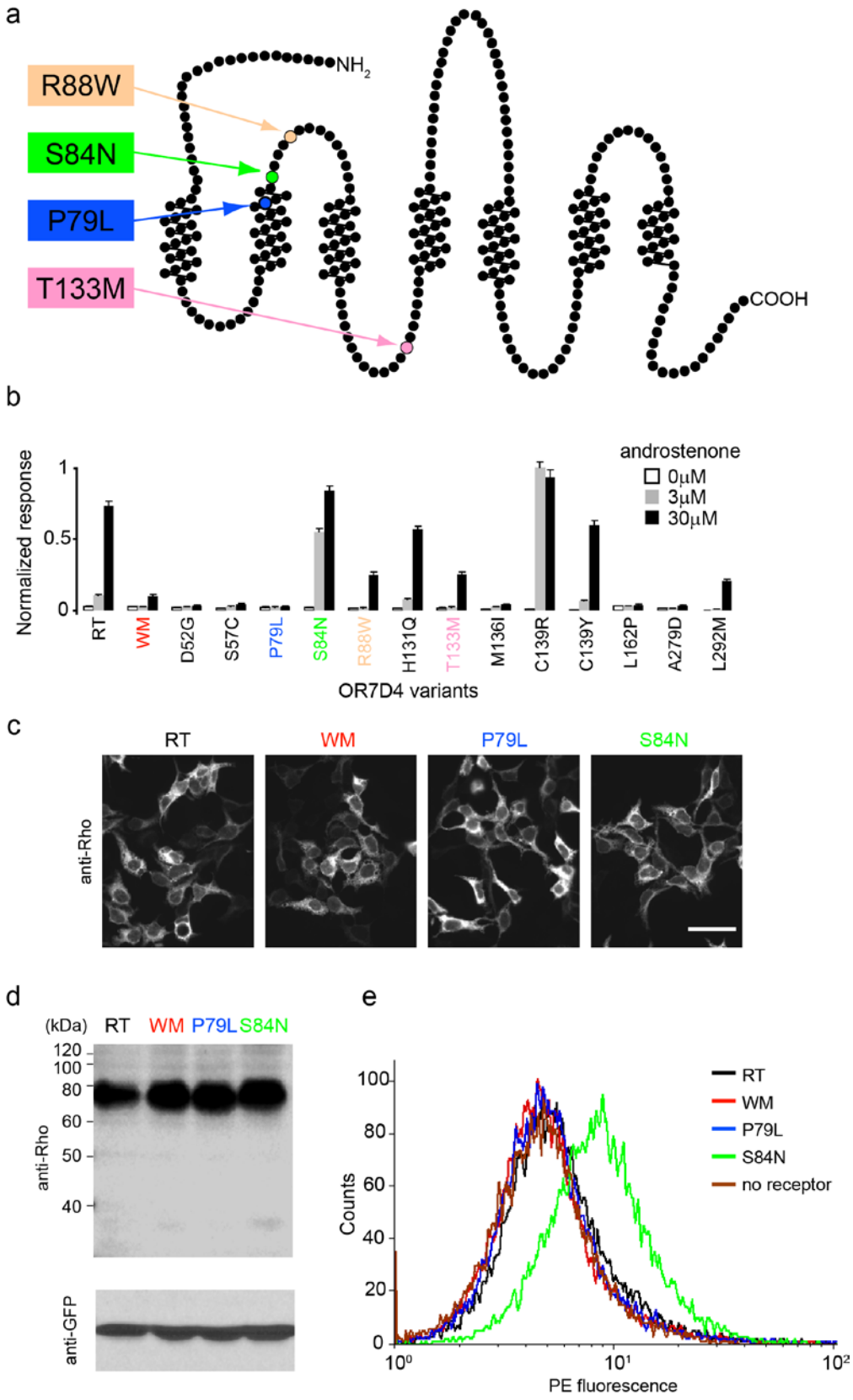
a
R88W
S84N
P79L
T133M
NH2
COOH
b
Normalized response
androstenone
0μM
3μM
30μM
RT
WM
D52G
S57C
P79L
S84N
R88W
H131Q
T133M
M136I
C139R
C139Y
L162P
A279D
L292M
OR7D4 variants
c
RT
WM
P79L
S84N
anti-Rho
d
(kDa)
RT
WM
P79L
S84N
anti-Rho
anti-GFP
e
RT
WM
P79L
S84N
no receptor
Counts
PE fluorescence

Figure 2 | Functional characterization of polymorphisms in *OR7D4*. **a**, Sequence of OR7D4 represented as a snake plot, with the major non-synonymous SNPs and their corresponding amino acid changes indicated as coloured circles. **b**, Characterization of receptor activity of 13 SNP variants in response to 3μM and 30μM androstenone in a luciferase assay. Y-axis denotes normalized response +SEM (n=4). **c**, Permeabilized cell immunofluorescence of Hana3A cells expressing OR7D4 RT, WM, P79L, and S84N. Cells were stained with anti-rhodopsin antibody. Scale bar = 50 μm. **d**, Western blot analysis of whole-cell lysates from HEK293T cells transfected with *OR7D4 RT*, *WM*, *P79L*, and *S84N* and *GFP* co-transfected as a control. **e**, Flow cytometry analysis of the cell-surface expression of OR7D4 RT, WM, P79L, and S84N, co-expressed with GFP. The intensity of phycoerythrin (PE) signal among the GFP-positive population was measured and plotted.

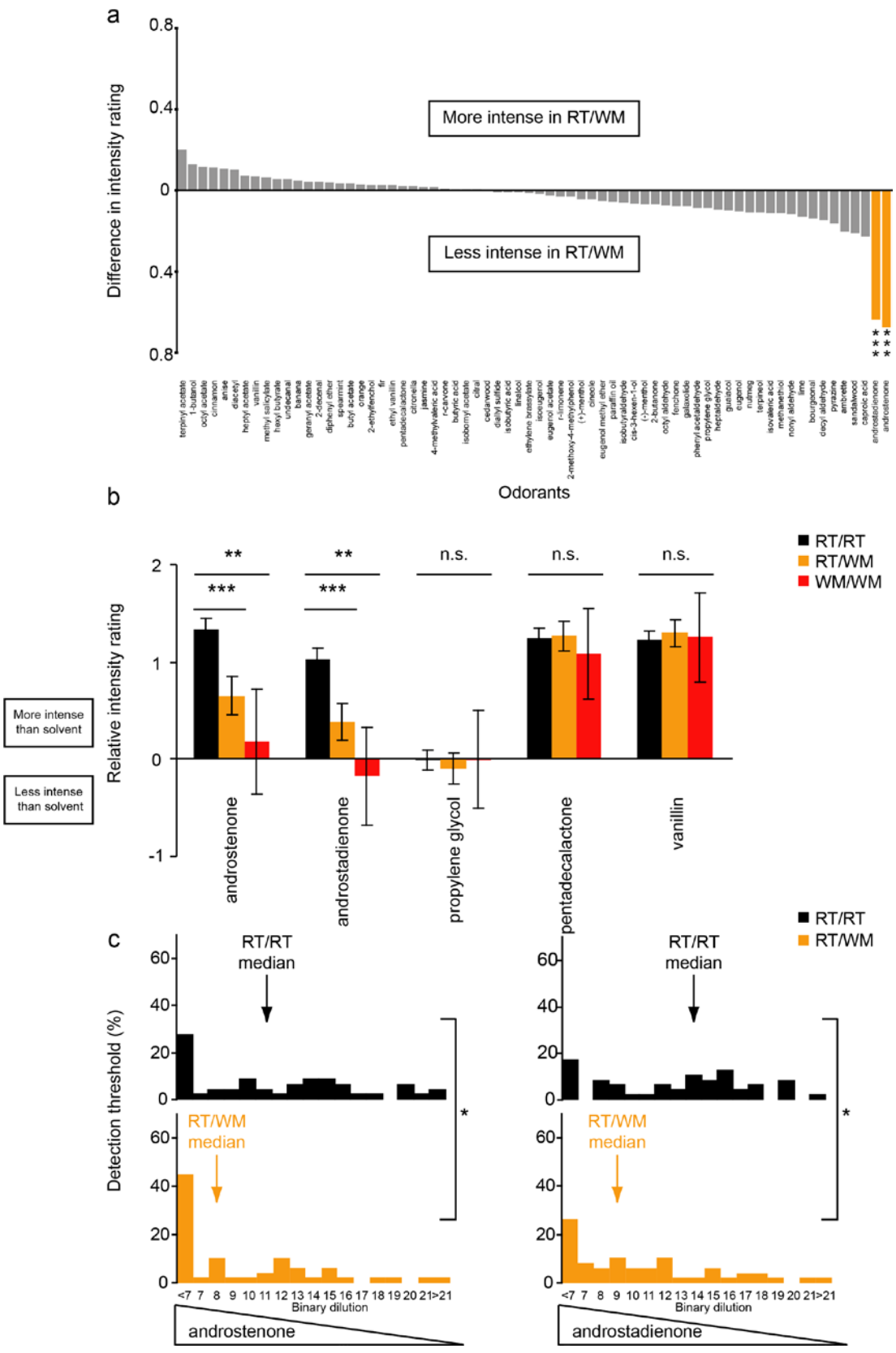
a
Difference in intensity rating
0.8
0.4
0
0.4
0.8
More intense in RT/WM
Less intense in RT/WM
Odorants
b
Relative intensity rating
RT/RT
RT/WM
WM/WM
More intense than solvent
Less intense than solvent
**
***
n.s.
androstenone
androstadienone
propylene glycol
pentadecalactone
vanillin
c
Detection threshold (%)
RT/RT median
RT/WM median
Binary dilution
androstenone
androstadienone

Figure 3 | *OR7D4 WM* correlates with reduced intensity perception of androstenone and androstadienone.

a, Mean differences in intensity rating for androstadienone and androstenone are significantly reduced in *OR7D4 RT/WM* relative to *OR7D4 RT/RT* subjects from a panel of 66 odours and 2 solvents. The data for the two different concentrations of each odour are pooled. **b**, Relative intensity ratings for five odours from **a** for *OR7D4 RT/RT*, *RT/WM*, and *WM/WM* subjects, compared to solvent (propylene glycol). Mean±S.E.M. **c**, Detection thresholds for androstenone plotted as per cent of subjects detecting this odour at a given binary dilution. **d**, Detection thresholds for androstadienone plotted as per cent of subjects detecting this odour at a given binary dilution. Significance in **a** and **b** was assessed with Student's t-test with a Bonferroni correction (**p<0.01; ***p<0.001). Significance in **c** and **d** was assessed with the Kolmogorov-Smirnov test (*p<0.05). Number of subjects tested in (a) and (b) was N=255 RT/RT, N=100 RT/WM, and N=10 WM/WM subjects and in (c) and (d) was N=47 RT/RT, N=49 RT/WM.

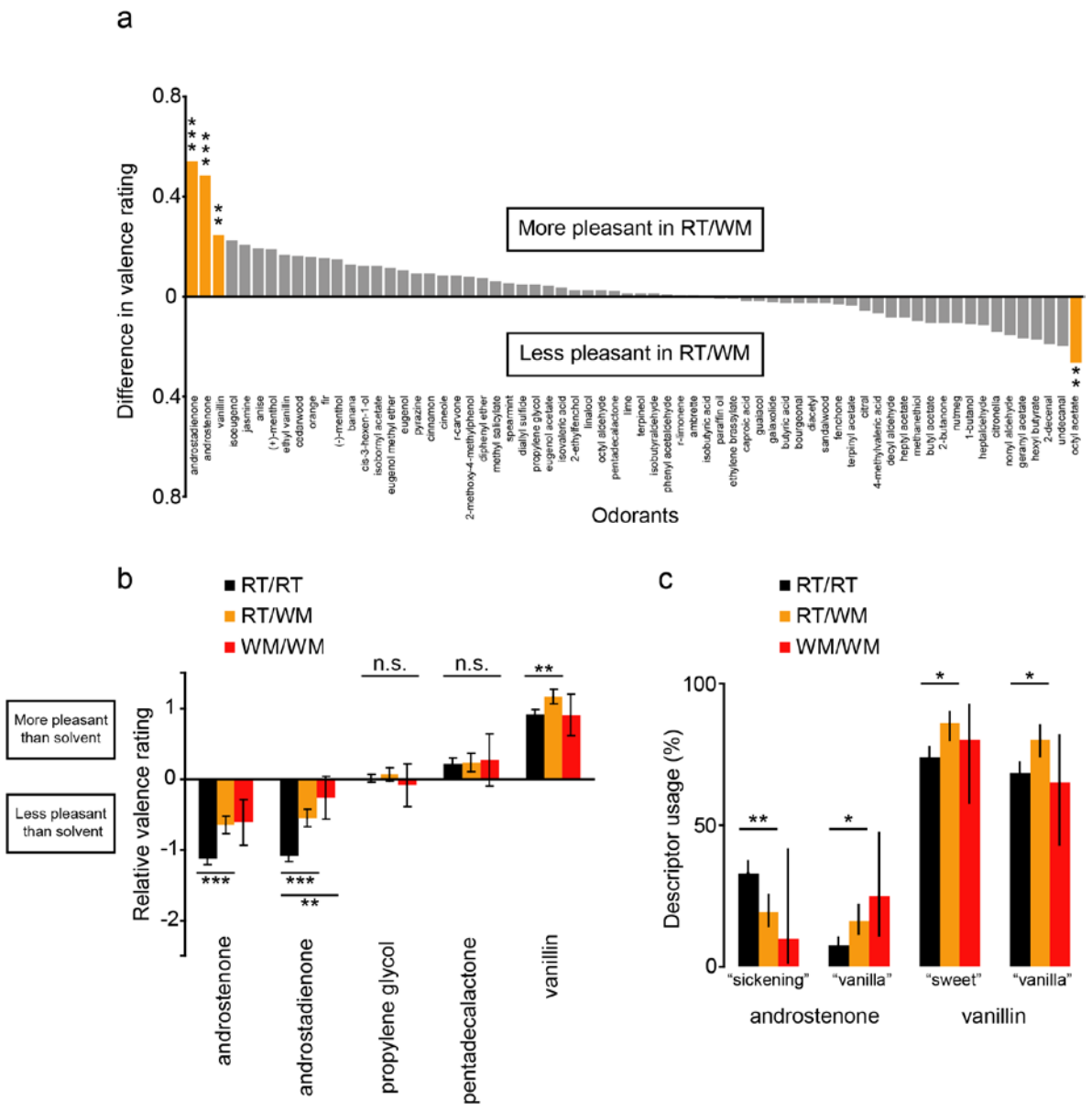

Figure 4 | *OR7D4 WM* correlates with changes in quality perception of androstenone and androstadienone.

a, Mean differences in valence rating for androstadienone and androstenone are significantly increased in *OR7D4 RT/WM* relative to *OR7D4 RT/RT* subjects from a panel of 66 odours and 2 solvents. The data for the two different concentrations of each odour are pooled. **b**, Relative valence ratings for five odours from **a** for

OR7D4 RT/RT, *RT/WM*, and *WM/WM* subjects compared to solvent (propylene glycol). Ratings that are less pleasant than the solvent are negative in this figure. Mean±S.E.M. **c**, Odour profiling of androstenone, vanillin, pentadecalactone, and solvent (propylene glycol) by *OR7D4 RT/RT*, *RT/WM*, and *WM/WM* subjects show that *RT/WM* subjects differ in their usage of 2/146 descriptors for androstenone and vanillin. Data are plotted as % of individual sessions in which a given descriptor was used for an odour, with 95% confidence intervals represented as black lines. Significance in **a** and **b** was assessed with Student's t-test with a Bonferroni correction (*p<0.05; **p<0.01; ***p<0.001). Significance in **c** was assessed with a chi-square test with Bonferroni correction (*p<0.05). Number of subjects tested was N=255 RT/RT, N=100 RT/WM, and N=10 WM/WM subjects.

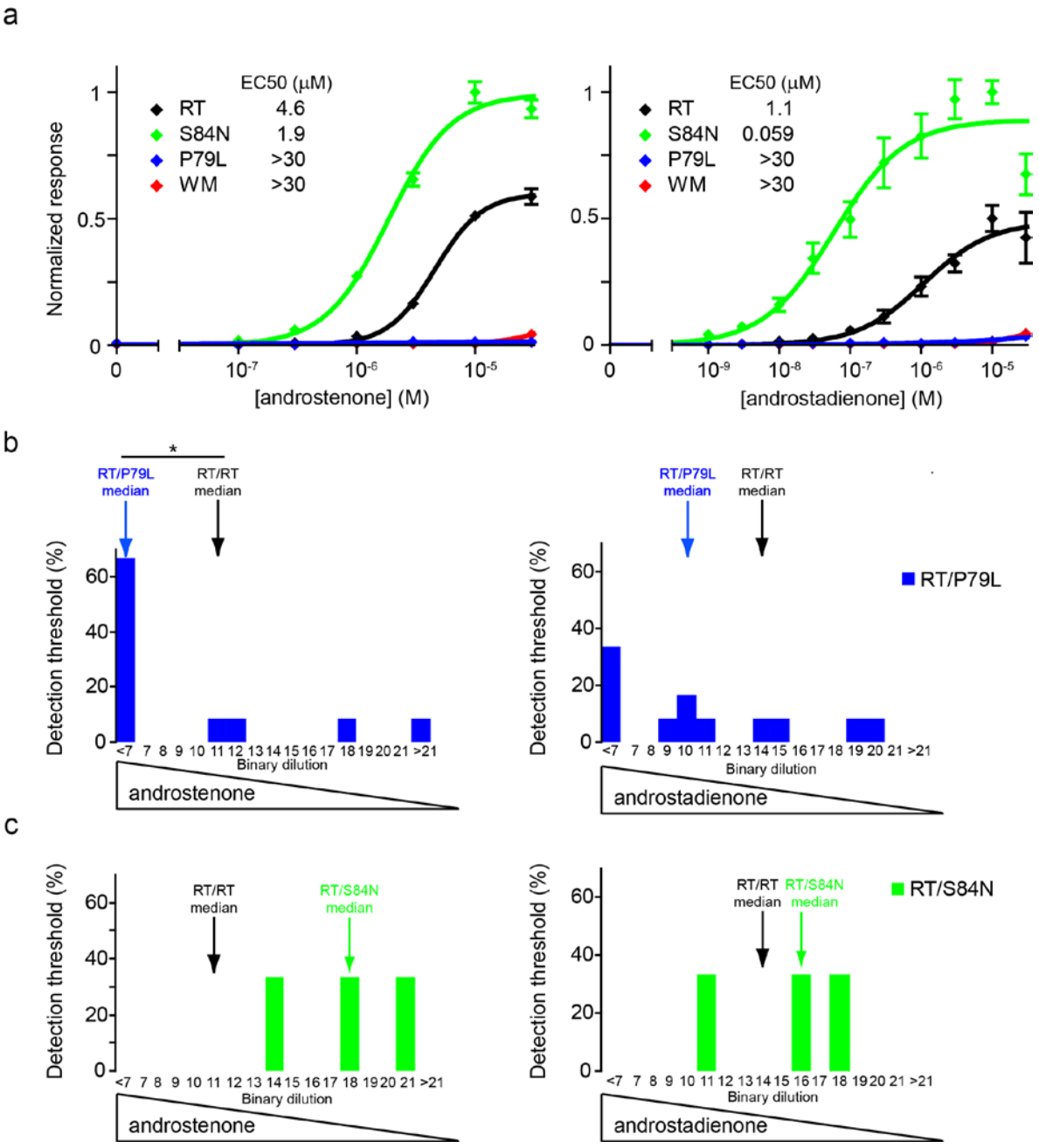

Figure 5 | *OR7D4 P79L* and *S84N* polymorphisms affect androstenone and androstadienone activity *in vitro* and perception *in vivo*.

a, Dose response curves of *OR7D4 RT*, *WM*, *P79L*, and *S84N* for androstenone and androstadienone. EC50 values in μM are provided. Y-axis denotes normalized

response ±SEM (n=6 for androstenone and n=4 for androstadienone). **b**, Detection thresholds for androstenone and androstadienone in *OR7D4 RT/P79L* subjects plotted as per cent of subjects detecting these odours at a given binary dilution. Significance in was assessed with the Kolmogorov-Smirnov test (*p<0.05) N=12 subjects. **c**, Detection thresholds for androstenone and androstadienone in *OR7D4 RT/S84N* subjects plotted as per cent of subjects detecting these odours at a given binary dilution. N=3 subjects.

Table 1 | Single nucleotide polymorphisms in *OR7D4*

SNP	dbSNP ID	Allele	AA change	Codon	Protein region	Allele frequency**
refseq						0.788
1		T/C	D52G	2	IC1	0.002
2	rs5020281	G/C	S75C	2	TM2	0
3		G/A	P79L	2	TM2	0.042
4	rs5020280	C/T	S84N	2	EC1	0.012
5		G/A	R88W	1	EC1	0.154
6	rs5020279	G/C	H131Q	3	IC2	0
7	rs5020278	G/A	T133M	2	IC2	0.154
8	rs5020277	C/T	M136I	3	IC2	0
9	rs5020276	A/G	C139R	1	IC2	0
10	rs5020275	C/T	C139Y	2	IC2	0
11		A/G	L162P	2	TM4	0.001
12		G/T	A279D	2	TM7	0*
13	rs4564704	G/T	L292M	1	TM7	0

*one individual with this SNP was found but not screened in the psychophysical study
**allele frequency is calculated based on the 412 subjects in this study

Supplementary Materials for "Genetic variation in a human odorant receptor…"

Supplementary Methods

Cloning of human odorant receptors

423 human odorant receptors were cloned with sequence information from The Olfactory Receptor Database (http://senselab.med.yale.edu/senselab/ORDB/default.asp). Of these, 337 were predicted to encode functional receptors, 45 were predicted to encode pseudogenes, 27 were variant pairs of the same genes, and 14 were duplicates. We adopted the nomenclature proposed by the D. Lancet group [1]. SNPs in OR7D4 were identified from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP) or through genotyping. OR7D4 single nucleotide variants were generated by overlap extension PCR.

Cell culture, immunocytochemistry, and flow cytometry

Odorant receptors that contain the first 20 amino acid of human rhodopsin tag [2] in pCI (Promega) were expressed in the Hana3A cell line along with a short form of mRTP1, RTP1S, (M37 to the C-terminal end), which enhances functional expression of the odorant receptors (HZ and HM, manuscript in preparation). For immunocytochemistry, cells were seeded in a 35mm dish (Falcon) containing a piece of cover glass coated with poly-D-lysine (Sigma) 24 hrs prior to transfection in M10. Lipofectamine2000 (Invitrogen) was used for transfection of plasmid DNA. Blue fluorescent protein (BFP) was cotransfected as a control for transfection efficiency. For live cell-surface staining, typically 24 hrs post-transfection, cells were incubated in M10 containing mouse monoclonal anti-rhodopsin antibody, 4D2 [3], 15mM NaN_3, and 10mM HEPES at 4°C for 45 min. Cells were then washed in Hank's Balanced Buffer Solution (Gibco) containing

15mM NaN_3 and 10mM HEPES, followed by incubation with Cy3-conjugated donkey anti-mouse IgG (Jackson Immunologicals) at 4°C for 30 min and then fixation in 1% PFA at 4°C and mounting in Mowiol. For permeabilized staining, 24 hrs post-transfection, cells were fixed in 4% PFA for 15 min and permeabilized with methanol at 4°C. Cells were blocked in 5% skim milk diluted in PBS, incubated in 5% skim milk/PBS containing mouse monoclonal anti-rhodopsin antibody, 4D2, at room temperature for 45 min. Cells were then washed in PBS followed by incubation with Cy3-conjugated donkey anti-mouse IgG (Jackson Immunologicals) at RT for 30 min. For FACS analysis, Hana3A cells were seeded in 35mm dishes. At the time of transfection, green fluorescent protein (GFP) expression vector was co-transfected as a control for transfection efficiency. 24 hrs post-transfection, cells were incubated with 4D2 and then washed and incubated with PE-conjugated donkey anti-mouse IgG (Jackson Immunologicals). 7-amino-actinomycin D (Calbiochem) was added before flow cytometry to eliminate dead cells from analysis. The intensity of PE signal among the GFP-positive population was measured and plotted.

Western blot analysis

Hana3A cells in 35mm dishes were transfected with Rho-tagged receptor variants and RTP1S using Lipofectamine2000 (Invitrogen). GFP expression vector was cotransfected as a control. 24 hrs post-transfection, cells were lysed with sample loading buffer (20mM Tris [pH 7.5], 2mM EDTA, 5% SDS, 20% glycerol, 0.002% BPB, 0.25M DTT) and sonicated. SDS-PAGE and Western blot analysis were performed according to Mini-Protean 2 Cell (Bio-Rad) protocol. Membranes were incubated with 4D2 and

subsequently with donkey anti-mouse HRP (Jackson Immunologicals). The membrane was then incubated with stripping buffer (25mM Glycine-HCl [pH2], 1% SDS, 25mM Glycine, 0.036N HCl, 1% SDS) for 30 min at room temperature and then with rabbit anti-GFP and subsequently with donkey anti-rabbit HRP. ECL (Amersham) was used for detecting proteins on membranes.

Luciferase assay and data analysis

Dual-Glo™ Luciferase Assay System (Promega) was used for luciferase assay as previously described [4]. CRE-luciferase (Stratagene) was used to measure receptor activation. Renilla luciferase driven by a constitutively active SV40 promoter (pRL-SV40; Promega) was used as an internal control for cell viability and transfection efficiency. Hana3A cells were plated on poly-D-lysine-coated 96-well plates (BioCoat; Becton Dickinson). Plasmid DNA of the receptor variants and RTP1S was transfected using Lipofectamine2000. ~24 hrs post-transfection, the medium was replaced with CD293 chemically defined medium (Gibco) and then incubated for 30 min at 37°C. The medium was then replaced with 25uL of odorant solution diluted in CD293 and incubated for 4 hrs at 37°C. We followed manufacturer’s protocols for measuring luciferase and Renilla luciferase activities. Luminescence was measured using Wallac Victor 1420 (Perkin-Elmer). Normalized luciferase activity was calculated by the formula [luc(N)–luc(lowest)]/luc(highest)/RL(N) where luc(N) = luminescence of luciferase of a certain well; luc(lowest) = lowest luminescence of luciferase of a plate or a set of plates; luc(highest) = highest luminescence of luciferase of a plate or a set of plates; RL(N) =

luminescence of Renilla luciferase of a certain well. Data was analyzed with Microsoft Excel and GraphPad Prism 4.

Human odorant receptor genotyping and sequencing

Venous blood was collected from all subjects and genomic DNA prepared with the Qiagen PAXgene blood DNA kit. Polymorphisms in OR7D4 were assayed by both sequencing and allele-specific PCR. In allele-specific PCR, an OR7D4 RT and an OR7D4 WM PCR were performed on each genomic DNA sample, each using a pair of internal primers containing the residues of interest. The RT forward primer is specific for R88 (5’-CTAGTGAGCATCCAGGCAC-3’) and reverse primer is specific for T133 (5’-CAGGGGTTCATGATGACCG-3’). The WM forward primer contains W88 (5’-CTAGTGAGCATCCAGGCAT-3’) and the reverse primer contains M133 (5’-CAGGGGTTCATGATGACCA-3’). The PCR was done using HotStar Taq (Qiagen). Cycling protocol was: 95°C, 15 min; 30 cycles of 95°C, 15 sec; 66°C, 30s; 72°C, 1 min; and then 72°C, 10 min. Fifty percent of each reaction was analyzed on a 1% agarose gel (Research Products International Corp.). For sequencing, human genomic DNAs were PCRed with HotStar Taq (Qiagen) with primers upstream (5’-AAGTGATGACAAGCTGAGCTGC-3’) and downstream (5’-CCACAACATTTGCCTTAGGGGTA-3’) of the OR7D4 open reading frame. The PCR products were then either gel-purified using MiniElute Gel Extraction Kit (Qiagen) or Sephadex™-purified (GE HealthCare Biosciences AB; Uppsala, Sweden) and sequenced with 3100 or 3730 Genetic Analyzer (ABI Biosystems) or by GeneWiz (New Brunswick, NJ). All samples were sequenced in addition to allele-specific PCR.

Human subjects

Subjects for this study were recruited from the greater New York City area. To control for inter-test variation, all subjects completed the same protocol on two different visits that were four or more days apart. Exclusion criteria for subjects were: allergies to odours or fragrances, a history of nasal illness, upper respiratory infection, seasonal allergy, prior endoscopic surgery on the nose, pre-existing medical condition that has caused a reduced sense of smell such as head injury, cancer therapy, radiation to head and neck, or alcoholism, and pregnancy. All experiments were approved by the Institutional Review Board of the Rockefeller University and subjects gave their informed consent to participate and were financially compensated for their time and effort. We collected data on the demographics, habits, and usage of the subjects in a computer-administered questionnaire. Demographic questions were largely based on standard US Census questions. The intensity and valence ratings and the assignment of descriptors to odours shown in Figures 3, 4, and 5 were measured in sessions between March 2005 and May 2006. At this time the genotype of the subjects was unknown. The thresholds shown in Figures 4 and 5 were measured between August and December 2006. At this time the genotypes of the subjects were known, but the subjects and the test administrators were blind to subject genotype information. We invited 100/255 subjects with the RT/RT genotype and all subjects with other genotypes back for thresholding to androstenone and androstadienone. Not all invited subjects participated in androstenone and androstadienone thresholding. All subjects participating in this thresholding previously participated in the intensity and valence ratings and the assigning of descriptors to odours. We obtained evaluable data from 412 subjects, who had to meet the minimum

criteria of qualifying for the study, completing two study sessions, and providing a blood sample of adequate size for DNA isolation. Subjects whose blood sample subsequently failed to yield adequate quantities of DNA were excluded. Our yield of evaluable subjects represents about 77% of all subjects who enrolled in the study, with most subjects being excluded for failing to complete two visits.

The numbers of subjects with different *OR7D4* genotypes are:

OR7D4 genotype	# Subjects
RT/RT	255
RT/WM	100
RT/P79L	30
WM/WM	10
RT/S84N	7
WM/P79L	4
RT/D52G	2
WM/S84N	2
WM/L162P	1
S84N/P79L	1
	412

Stimuli for olfactory psychophysics

All odours were presented as one ml of an odour dilution in either propylene glycol or paraffin oil in 20 ml amber glass vials. The concentrations used in the intensity and valence rating are shown in Supplementary Table 2. All of these compounds were used in the intensity and valence rating portion of the smell test.

For the descriptors task, the following odours and concentrations were used:

- propylene glycol: pure
- pentadecalactone: 1/500
- vanillin: 1/200
- androstenone: 1/10,000

The following odours were used for thresholding:

- adrostenone
- androstadienone

These were tested at an initial concentration of binary dilution 23 (1/8,388,608) in propylene glycol and moved from there in binary steps. The range of dilutions tested was from binary dilution 27 (1:134,217,728) to binary dilution 6 (1:64).

Odour vials used for intensity and valence ratings and the assigning of descriptors to odours were used for 40 sessions and then replaced by a new set. Master stocks of each odour were established at the beginning of the study to avoid intertrial variability in odour concentrations.

Procedures for olfactory psychophysics

All testing was performed in a well-ventilated room in the Rockefeller University Hospital. On the first visit, basic vital signs were collected from each subject and an 8.5cc venous blood sample was collected. All women of child-bearing age took a urine pregnancy test to confirm that they were not pregnant. Smell tests were self-administered

and computerized using custom-written applications in FilemakerPro and Microsoft Access. The average subject took 2.5 hours for the sessions that included the rating and descriptor data shown here and 30 minutes for the sessions in which the thresholds were determined. A screenshot from the computerized intensity and valence rating is shown in Figure S2. This application as well as the application in which odour descriptors are assigned to odours was written in Filemaker Pro. To prevent odour sampling errors, subjects must scan the vial containing the stimulus before being able to rate the stimulus. They are only allowed to proceed with the experiment if the correct vial was scanned. The application for the thresholding was written in Microsoft Access. The computer application for the intensity and valence rating had a built-in mandatory 15 seconds inter-stimulus interval. However, it took most subjects much longer to move from one stimulus to the next so that it was rarely enforced.

Olfactory ratings

The intensity and valence of 66 odours were rated at two different concentrations (high and low) and the intensity and valence of the two solvents (paraffin oil and propylene glycol) was rated three times. Prior to these ratings, six stimuli that represented the spectrum of intensity and valence of the stimuli used in the study were presented to allow the subjects to calibrate the usage of the scale. The subjects were unaware that the six first stimuli served that purpose. After the rating of the 66 odours at two concentrations and the rating of the solvents, 15 stimuli that were presented earlier in the experiment were repeated to test for the effect of adaptation and olfactory fatigue on the ratings. The subjects were not aware that the last 15 stimuli served this purpose. These 15 stimuli and

the six first stimuli were not included in the analysis presented here. The stimuli were presented in the same order in all sessions to not introduce a bias caused by adaptation and olfactory fatigue and to make the measurements between different sessions as comparable as possible.

The 15 control stimuli and their concentrations were as follows:

1. guaiacol (high)
2. octyl acetate (high)
3. undecanal (high)
4. paraffin oil
5. heptyl acetate (low)
6. hexyl butyrate (low)
7. butyric acid (high)
8. hexyl butyrate (high)
9. decyl aldehyde (high)
10. 2-decenal (low)
11. cis-3-hexenal (low)
12. nonyl aldehyde (high)
13. 2-methoxy-4-methylphenol (low)
14. decyl aldehyde (low)
15. propylene glycol

Although there was variability between the first and second presentation of these stimuli, there was no indication for a systematic difference between the intensity rating at the beginning and end of the session. Eight of the 15 stimuli were rated on average as more intense at the end of the session, whereas seven were rated as less intense.

A seven point scale was used to rate intensity and valence with these choices:

INTENSITY RATING

- Extremely Weak
- Very Weak
- Slightly Weak
- Neither Weak nor Strong
- Slightly Strong
- Very Strong
- Extremely Strong

VALENCE RATING

- Extremely Unpleasant
- Very Unpleasant
- Slightly Unpleasant
- Neither Unpleasant nor Pleasant
- Slightly Pleasant
- Very Pleasant
- Extremely Pleasant

In addition there was a button on the screen labeled "I can't smell anything" and a button labeled "Don't Know". If the "Don't Know" button was pressed, no rating was recorded. If the "I can't smell anything" button was pressed, a 0 was recorded for the intensity rating and no rating was recorded for the valence rating. The other ratings were transformed according to the following scheme:

INTENSITY RATING

- Extremely Weak =1
- Very Weak =2
- Slightly Weak =3
- Neither Weak nor Strong =4
- Slightly Strong =5
- Very Strong =6
- Extremely Strong =7

VALENCE RATING

- Extremely Unpleasant =1
- Very Unpleasant =2
- Slightly Unpleasant =3
- Neither Unpleasant nor Pleasant =4
- Slightly Pleasant =5
- Very Pleasant =6
- Extremely Pleasant =7

Averages for the ratings of the different genotypes were calculated.

Prior to the study (in November and December 2004) the concentrations used for each odorant were determined in intensity matching experiments in which control subjects rated the intensity of stimuli. Odours were considered "low" intensity when the intensity rating was within one standard deviation of the rating for a 1:10,000 dilution of 1-butanol. Odours were considered "high" intensity when the intensity rating was within one standard deviation of a 1:1,000 dilution of 1-butanol. For ethylene brassylate, eugenol methyl ether, (-)-menthol, (+)-menthol, and vanillin the pure odour or the saturated dilution was rated less intense than the criteria for "high" intensity and these odours were therefore presented at the highest possible concentration. Androstenone and androstadienone could not be intensity matched in any meaningful way because of the high variability in the responses across subjects. The intensity matching protocol was approved by the Rockefeller University Institutional Review Board. All subjects gave their informed consent to participate and were financially compensated for their time and effort. Ten subjects participated in the intensity matching and six visits were necessary to match all stimuli. The subjects were aware of the purpose of the intensity matching and were instructed to focus on the intensity of the stimulus and disregard the valence. The stimuli used for the intensity and valence rating are shown in Table S2.

Assigning descriptors to odours

Subjects assessed the quality of six odours using an odour profiling method that has shown to produce stable profiles of odorants. Subjects rated 146 odour descriptors (for

example: fishy, fruity, tar) on a scale from 0 to 5 with 0 being the default. Odour profiling typically took five minutes per odorant and was performed as a computer-controlled self-test in which the subject’s responses are directly recorded. This has the benefit that each subject could work at his or her own pace. We provided large posters listing all 146 odour descriptors so that subjects could study these before beginning this part of the test. Of the six odours the first (spearmint) was meant to allow the subject to become familiar with the procedure and the descriptors and was not included in the analysis. The subjects were not aware that the first odour served this purpose. For a second odour (methanethiol), we failed to produce reproducible intensities of the odour due to the high volatility of methanethiol. These data was therefore also not evaluated. The descriptors used to describe the other four odours (vanillin, pentadecalactone, androstenone, and propylene glycol) were evaluated for this paper. In Figure 4c the four descriptors that are used in more than 10% of the sessions and that show statistically significant differences between the genotypes are shown. 19/146 descriptors were used in more than 10% of the sessions to describe pentadecalactone, 11/146 for propylene glycol, 23/146 for vanillin, and 21/146 for androstenone (sweet, fragrant, aromatic, musky, bitter, stale, sweaty, light, heavy, rotten fruit, sickening, rancid, putrid foul, vanilla, dirty linen, urine, sharp pungent, ammonia, chemical, cleaning fluid, musty). Of these 74 descriptors, only the four shown in Figure 4c showed statistically significant differences between the genotypes. In Figure 4c the percentage with which a given descriptor was used by subjects of a given genotype is plotted.

Thresholding

Detection thresholds (Fig. 3c and Fig. 5 b-c) were determined using the “Single Staircase Threshold Detection Method” [5,6]. This method produces very accurate data on the threshold concentration of a given odour and is easy to administer and for the subjects to perform. We tested the thresholds for each subject on two occasions at least four days apart. This helped to control for inter-trial variability in olfactory performance. The average difference between the thresholds determined on the two days was three binary dilution steps for androstenone and five for androstadienone.

A custom computer-controlled thresholding test was administered as a self-test to each subject. Briefly, subjects are instructed to sniff two vials, one containing the solvent, the other a dilution of the odorant. Both vials have barcode labels and the procedure is done at a computer equipped with a scanner. Subjects are asked to scan the vial with the stronger odour. Depending on their answer, the procedure will be repeated at an adjusted concentration. The total time to determine the threshold varies between subjects but is typically between 15 and 25 minutes per odorant. We started the thresholding procedure at binary dilution 23 for the conventional odours and binary dilution 27 for the steroidal odours. If the subject failed to identify the right vial, the computer prompted the subject to move to a higher concentration in binary dilution steps. This continued until the subject chose the correct vial at one concentration five times in a row. Then the direction of the change in concentration was reversed and a lower concentration was tested. After this first reversal the direction of the change in concentration was reversed whenever on the way down a mistake is made or, on the way up, two right choices were made at one

concentration. The experiment continued until the seventh reversal. The thresholds reported in this paper are the average of the last four reversals. The data in Figures 3 and 5 shows the distribution of the thresholds for different genotypes in histograms. Each subject's threshold is the average of two replicates of the experiments on two days four or more days apart. An example of a threshold procedure is shown in Figure S3.

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2. Krautwurst, D., Yau, K. W. & Reed, R. R. Identification of ligands for olfactory receptors by functional expression of a receptor library. *Cell* **95**, 917-26 (1998).
3. Laird, D. W. & Molday, R. S. Evidence against the role of rhodopsin in rod outer segment binding to RPE cells. *Invest Ophthalmol Vis Sci* **29**, 419-28 (1988).
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5. Doty, R. L., McKeown, D. A., Lee, W. W. & Shaman, P. A study of the test-retest reliability of ten olfactory tests. *Chem Senses* **20**, 645-56 (1995).
6. Doty, R. L. & Laing, D. G. in *Handbook of Olfaction and Gustation* (ed. Doty, R. L.) pp. 203-228 (Marcel Dekker, Inc., New York, 2003).

Supplementary Data

Supplementary Table 1| Single nucleotide polymorphisms in OR7D4 and their distribution among 412 subjects

SNP	dbSNP ID	Chromosomal Position	AA change	Allele frequency		Racial self-identification					
						African-American	Asian	Caucasian	Do not wish to specify	Native American	Other**
refseq				649/824	0.788	25%	8%	44%	3%	1%	19%
1		9186359	D52G	2/824	0.002	100%	0%	0%	0%	0%	0%
2	rs5020281	9186290	S75C	0/824	0						
3		9186278	P79L	35/824	0.042	80%	0%	0%	6%	0%	14%
4	rs5020280	9186263	S84N	10/824	0.012	50%	0%	10%	0%	0%	40%
5		9186252	R88W	127/824	0.154	14%	9%	61%	5%	1%	10%
6	rs5020279	9186121	H131Q	0/824	0						
7	rs5020278	9186116	T133M	127/824	0.154	14%	9%	61%	5%	1%	10%
8	rs5020277	9186106	M136I	0/824	0						
9	rs5020276	9186099	C139R	0/824	0						
10	rs5020275	9186098	C139Y	0/824	0						
11		9186029	L162P	1/824	0.001	0%	0%	100%	0%	0%	0%
12		9185678	A279D	0/824	0*						
13	rs4564704	9185640	L292M	0/824	0						

*one individual with this SNP was found but was not used for psychophysical analysis.
**Of 73 subjects who chose "Other" as a race category, 60% self-identified as Hispanic, 16% as mixed race, and 10% as African.

Supplementary Table 2: Odours used in this study

ODOR	LOW CONCENTRATION	HIGH CONCENTRATION	SOLVENT	CAS #
(-)-menthol	1/400	1/40	propylene glycol	2216-51-5
(+)-menthol	1/400	1/40	propylene glycol	15356-60-2
1-butanol	1/10.000	1/1.000	paraffin oil	71-36-3
2-butanone	1/10.000	1/5.000	paraffin oil	78-93-3
2-decenal	1/10.000	1/1.000	paraffin oil	3913-71-1
2-ethylfenchol	1/100.000	1/5.000	paraffin oil	18368-91-7
2-methoxy-4-methylpheno	1/1.000.000	1/100.000	paraffin oil	93-51-6
4-methylvaleric acid	1/5.000.000	1/10.000	paraffin oil	646-07-1
ambrette	1/1.000.000	1/1.000	paraffin oil	8015-62-1
androstadienone	1/100.000	1/1.000	propylene glycol	794-58-9
androstenone	1/100.000	1/1.000	propylene glycol	18339-16-7
anise	1/50.000	1/5.000	paraffin oil	8007-70-3
banana	1/250.000	1/10.000	paraffin oil	
bourgeonal	1/2.000	1/200	paraffin oil	18127-01-0
butyl acetate	1/1.000.000	1/1.000	paraffin oil	123-86-4
butyric acid	1/1.000.000	1/250.000	paraffin oil	107-92-6
caproic acid	1/1.000.000	1/250.000	paraffin oil	142-62-1
cedarwood	1/5.000	1/2.000	paraffin oil	68990-83-0
cineole	1/100.000	1/1.000	paraffin oil	470-82-6
cinnamon	1/50.000	1/10.000	paraffin oil	8015-91-6
cis-3-hexen-1-ol	1/250.000	1/100.000	paraffin oil	928-96-1
citral	1/50.000	1/5.000	paraffin oil	5392-40-5
citronella	1/250.000	1/10.000	paraffin oil	8000-29-1
decyl aldehyde	1/25.000	1/5.000	paraffin oil	112-31-2
diacetyl	1/10.000.000	1/10.000	paraffin oil	431-03-8
diallyl sulfide	1/2.000.000	1/100.000	paraffin oil	592-88-1
diphenyl ether	1/500	1/200	paraffin oil	101-84-8
ethyl vanillin	1/1.000	1/200	propylene glycol	121-32-4
ethylene brassylate	1/500	1/100	paraffin oil	105-95-3
eugenol	1/25.000	1/1.000	paraffin oil	97-53-0
eugenol acetate	1/1.000.000	1/100	paraffin oil	93-28-7
eugenol methyl ether	1/500	1/10	paraffin oil	93-15-2
fenchone	1/25.000	1/1.000	paraffin oil	7787-20-4
fir	1/100.000	1/10.000	paraffin oil	8002-09-3
galaxolide	1/10	1/1.000	paraffin oil	1222-05-5
geranyl acetate	1/10.000	1/200	paraffin oil	105-87-3
guaiacol	1/50.000.000	1/1.000.000	paraffin oil	90-05-1
heptaldehyde	1/10.000.000	1/25.000	paraffin oil	111-71-7
heptyl acetate	1/25.000	1/2.500	paraffin oil	112-06-1
hexyl butyrate	1/1.000	1/100	paraffin oil	2639-63-6
isobornyl acetate	1/2.000.000	1/100	paraffin oil	125-12-2
isobutyraldehyde	1/100.000	1/1.000	paraffin oil	78-84-2
isobutyric acid	1/10.000	1/1.000	paraffin oil	79-31-2
isoeugenol	1/25.000	1/2.000	paraffin oil	97-54-1
isovaleric acid	1/2.000.000	1/20.000	paraffin oil	503-74-2
jasmine	1/200.000	1/1.000	paraffin oil	8022-96-6
lime	1/2.000.000	1/5.000	paraffin oil	8008-26-2
linalool	1/100.000	1/100	paraffin oil	78-70-6
methanethiol	1/50.000.000	1/10.000.000	water	5188-07-8
methyl salicylate	1/25.000	1/1.000	paraffin oil	119-36-8
nonyl aldehyde	1/100.000	1/5.000	paraffin oil	124-19-6
nutmeg	1/25.000	1/1.000	paraffin oil	8008-45-5
octyl acetate	1/100	1/200	paraffin oil	112-14-1
octyl aldehyde	1/250.000	1/25.000	paraffin oil	124-13-0
orange	1/2.500	1/100	paraffin oil	8008-57-9
pentadecalactone	1/2.000	1/500	propylene glycol	106-02-5
phenyl acetaldehyde	1/8.000.000	1/2.000.000	paraffin oil	122-78-1
pyrazine	1/500	1/10	propylene glycol	290-37-9
r-carvone	1/100.000	1/1.000	paraffin oil	6485-40-1
r-limonene	1/250	1/10	paraffin oil	5989-27-5
sandalwood	1/10.000	1/1.000	paraffin oil	8006-87-9
spearmint	1/250.000	1/5.000	paraffin oil	8008-79-5
terpineol	1/10.000	1/100	paraffin oil	98-55-5
terpinyl acetate	1/1.000	1/500	paraffin oil	80-26-2
undecanal	1/10.000	1/1.000	paraffin oil	112-44-7
vanillin	1/1.000	1/200	propylene glycol	121-33-5
paraffin oil				8012-95-1
propylene glycol				57-55-6

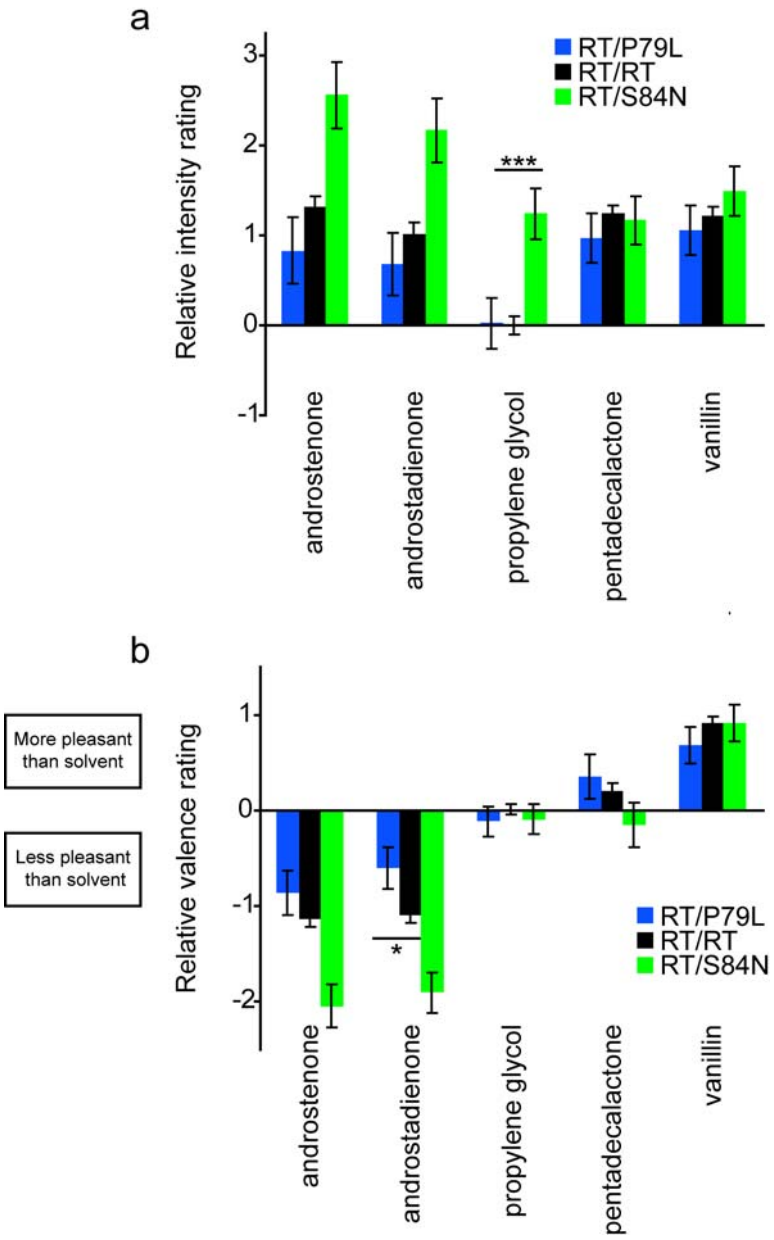

Figure S1: RT/P79L and RT/S84N intensity and valence rating

a, Intensity rating of RT/P79L (N=30) and RT/S84N (N=7) subjects compared to RT/RT subjects (N=255). **b**, Valence rating of RT/P79L (N=30) and RT/S84N (N=7) subjects in comparison to RT/RT subjects (N=256). Mean±S.E.M. is plotted. There is a trend for RT/P79L subjects to perceive androstenone and androstadienone as less intense and more

pleasant than the RT/RT subjects and for RT/S84N to perceive androstenone and androstadienone as more intense and less pleasant than the RT/RT subjects. This is consistent with the *in vitro* findings and the thresholds, but the difference between the genotypes is only statistically significant in one case (androstadienone, RT/RT vs. RT/P79L). Significance in was assessed with Student’s t-test with a Bonferroni correction (*$p<0.05$; ***$p<0.001$).

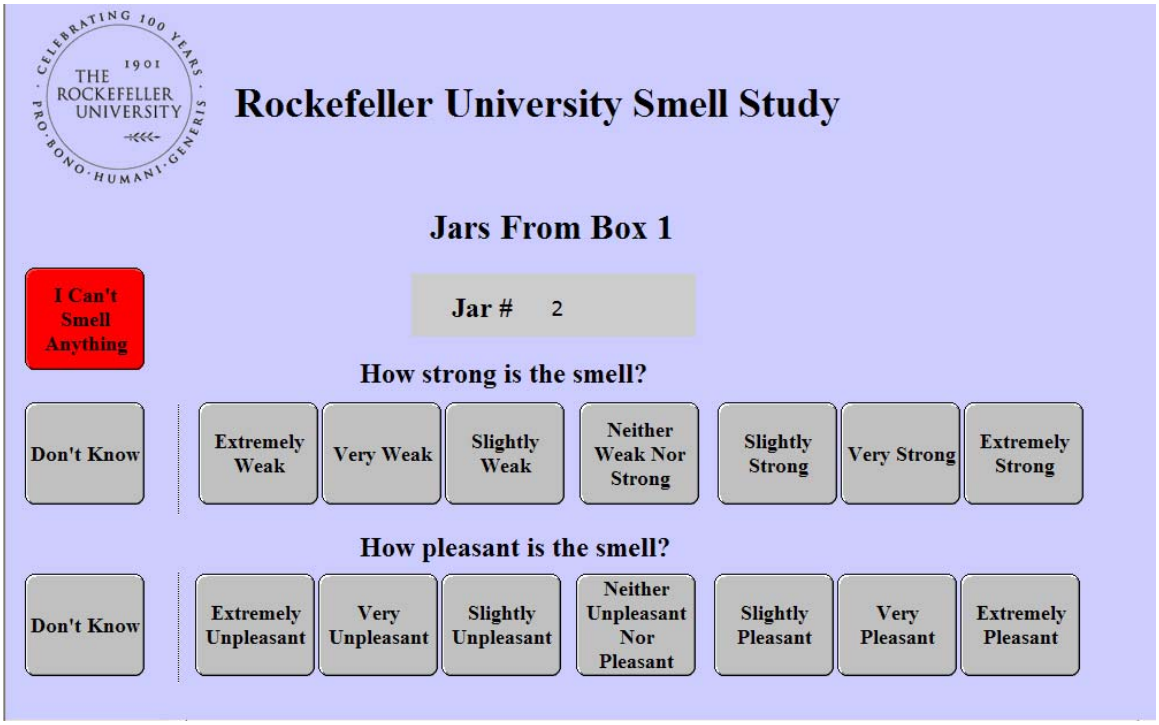

Figure S2: Screenshot from the computerized intensity and valence rating

This is the screen the subjects see after scanning in vial #2 in the intensity and valence rating portion of the smell test. Subjects are instructed to click the "I Can't Smell Anything" button if they can't perceive any odour. If subjects are able to perceive the odour, they must rate the strength and valence of the odour. After both selections are made, subjects are prompted to scan vial #3 on the next screen.

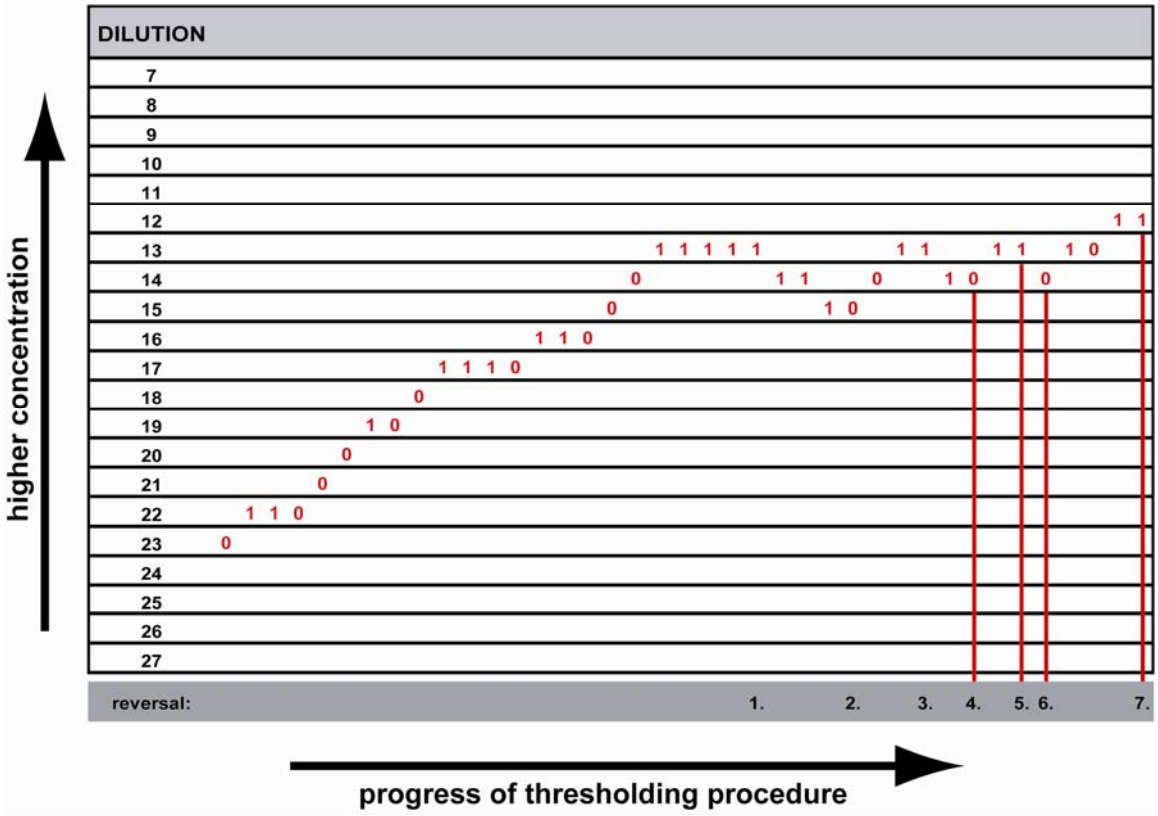

Figure S3: Example of the threshold detection procedure

Detection thresholds were determined using the “Single Staircase Threshold Detection Method” with a computer-controlled thresholding algorithm. Subjects were instructed to sniff two vials, one containing the solvent, the other a dilution of the odorant. All vials are marked with a bar code on the side of the vial and a number on the top of the vial, but contain no other identifying marks. Subjects scan the bar code of the vial with the stronger odour. If subjects choose the odour vial correctly, they are next prompted to test the next lower concentration of the odour. If subjects choose the solvent vial, they are next prompted with a higher concentration of the odour. As an example of how this algorithm works, we present representative data from a single subject above. In the representation above, when the subject scans the vial containing the odour, this is denoted

with a red "1". When the subject scans the vial containing the solvent this is denoted with a red "0". The thresholding procedure starts at dilution 23. Whenever the subject chose the solvent vial, the next higher concentration was tested until dilution 13, where the subject chose the odour vial five times in a row. This is considered the first reversal. Lower concentrations were then tested and the subject was prompted to choose among more dilute odours until two correct odour vial choices were made for one concentration. Note that this subject made an error at dilution 15 and the computer then moved to a higher concentration. This is the second reversal. After this the direction of the change in concentration is reversed. The experiment continues till the seventh reversal at dilution 12. The thresholds reported in the paper are the average of the last four reversals. In this example the last four reversals are at dilutions 12, 14, 13, and 14, the threshold for this session is therefore computed to be 13.25.

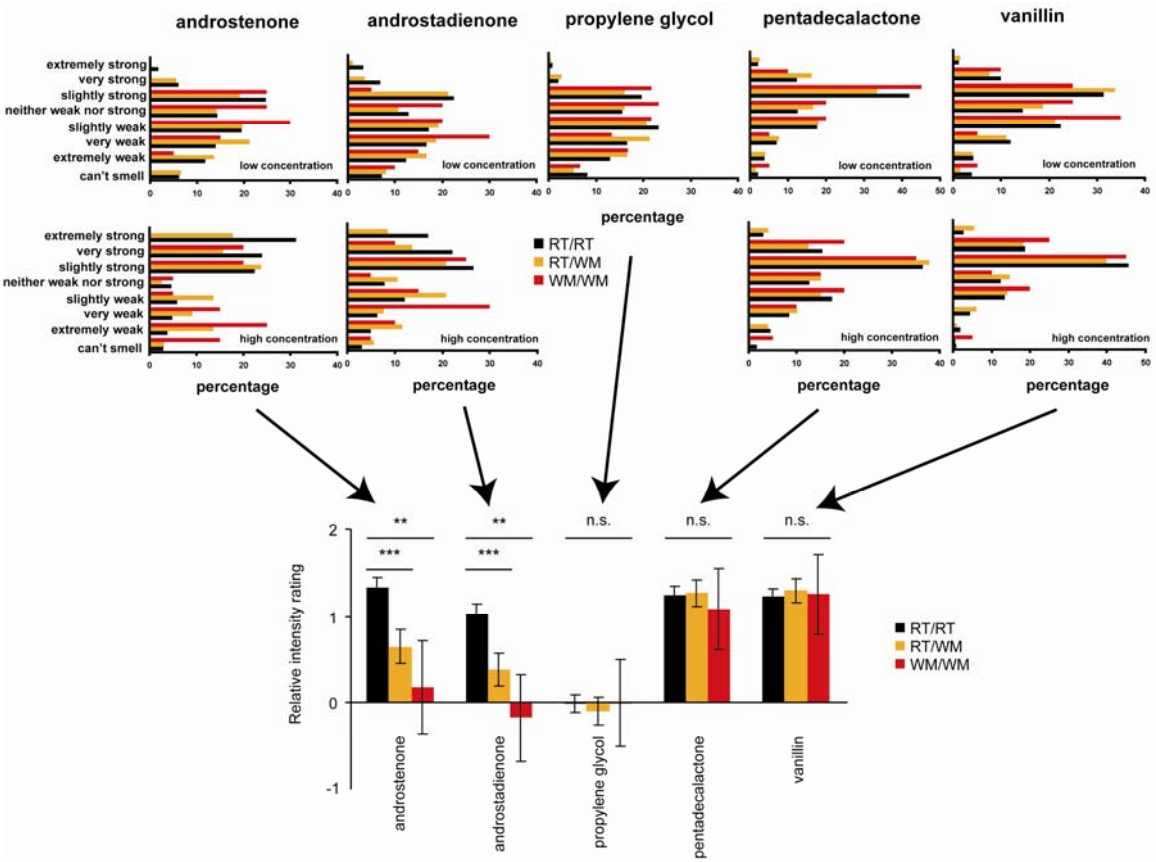

Figure S4: RT/RT, RT/WM, WM/WM intensity rating

The data used to calculate the values shown in Figure 3b are shown. The upper row shows the histograms for the low concentrations and the solvent. The lower row shows the histograms for the high concentrations. These histograms represent all the raw data used to calculate Figure 3b. N=255 for RT/RT, N=100 for RT/WM, and N=10 for WM/WM. Since each subject was tested twice, the number of data points represented in the histograms is twice the number of the subjects. In Figure 3b and in the other figures showing results from ratings (Fig. 3a, Fig. 4a-4b, and Fig. S1), the ratings for the two concentrations of an odour are pooled. Note that the high concentration of androstenone

(left in lower row) is rated "extremely weak" by less than five percent of the RT/RT subjects, but by a much higher percentage of RT/WM, and WM/WM subjects. For "extremely strong", this situation is reversed.

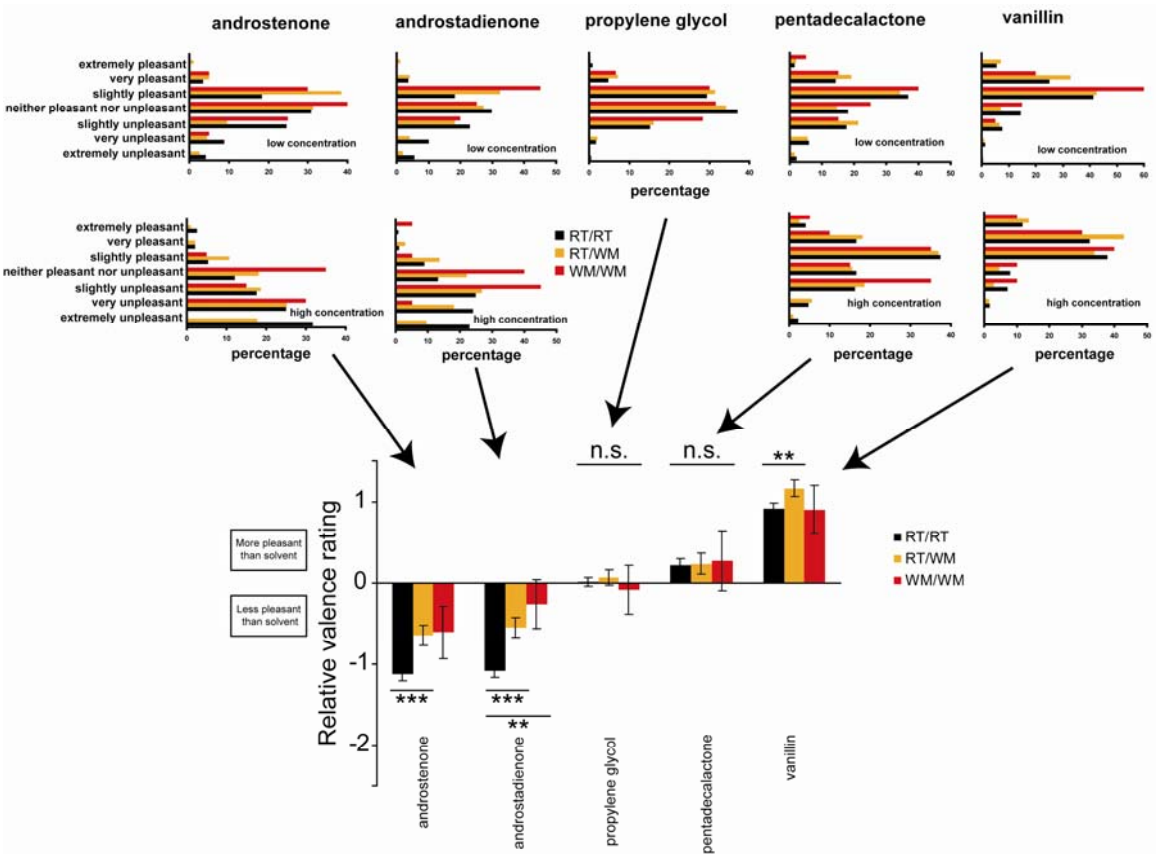

Figure S5: RT/RT, RT/WM, WM/WM valence rating

The data used to calculate the values shown in Figure 4b are shown. The upper row shows the histograms for the low concentrations and the solvent. The lower row shows the histograms for the high concentrations. These histograms represent all the raw data used to calculate Figure 4b. N=255 for RT/RT, N=100 for RT/WM, and N=10 for WM/WM. Since each subject was tested twice, the number of data points represented in the histograms is twice the number of the subjects. The ratings for the two concentrations of an odour are pooled. Note that at both concentrations of androstenone and androstadienone "extremely unpleasant" is used more frequently by RT/RT subjects than by RT/WM subjects. None of the WM/WM subjects rated any of these four stimuli as "extremely unpleasant".