Optimization of insect odorant receptor trafficking and functional expression via transient transfection in HEK293 cells

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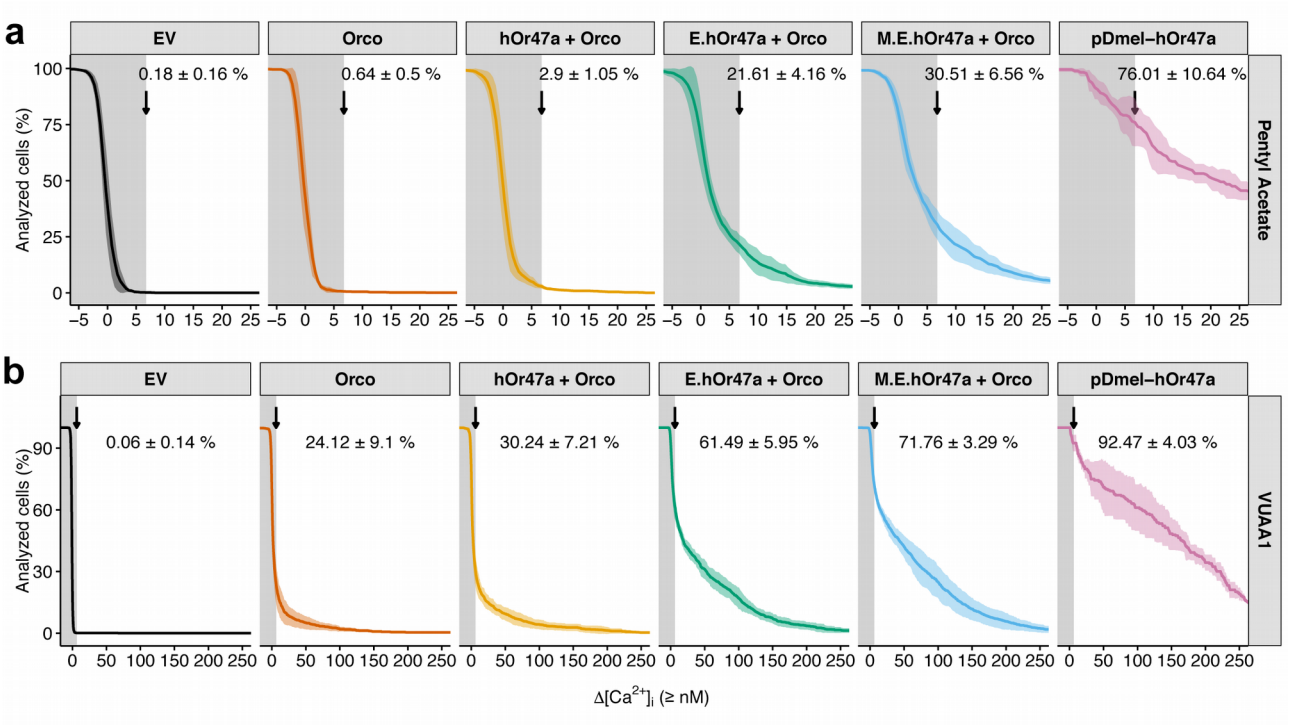
Supplementary Information

Primer name	Sequence (5'→3')
E.hOr47a_fwd	GCCCTCTAGAGCCGCCACCATGGATGGAAAACCCATCCCAAATCCTCTTC
hOr47a_fwd	GCCCTCTAGAGCCGCCACCATGGATGGAAAACCCATCCCAAATCCTCTTCTGGGGCTTGATAGCACC-GACTCTTTCCTCCAAGTGCAGAAATC
(E.)hOr47a_rev	GCCGCTCGAGTCAAGAAAAGCTG
pCMVTNT_fwd	CGGTTATCCAGATCGCCCTTCCCAACAG
pCMVTNT_rev	CCTGATAAATATAAATGTACATATTATGATATAGATACAACGTATGC
pBI-CMV1_fwd	GTACATTTATATTTATCAGGGTTATTGTCTCATG
pBI-CMV1_rev	AAGGGCGATCTGGATAACCGTATTACCG
hOrcoExon1_fwd	TTAGTGAACCGTCAGATCCGCTAGGGATCCGCCACCATGGAACAGAAACTGATCTCTGAAGAAGACCTG-GCTAGCACAACTAGCATGCAAC
hOrcoExon1_rev	CCTTGATACTTACCTGGGCCAGAGCATAGCC
chimeric_intron_fwd	TGCTCTGGCCCAGGTAAGTATCAAGGTTACAAGACAGG
chimeric_intron_rev	TGCAAAAGTGAAACACCTGTGGAGAGAAAGGCAAAG
hOrcoExon2_fwd	TTTCTCTCCACAGGTGTTTCACTTTTGCATTTTC
hOrcoExon2_rev	TGATCCTCTGGAGATATCGTCGACAAGCTTTCACTTCAGTTGGACCAG
BI-R.E.Or47a_fwd	AACCGTCAGATCGCCTGGAGGCCGCCACCATGGATCAA
BI-R.E.Or47a_rev	CCCGCGGCATATGACCGGTGTCAAGAAAAGCTGCGCAGC
hOr56a_fwd	GTTTAGTCTGTTCAAGGTAAAGGACTTGTTG
hOr56a_rev	CCCGCGGCATATGACCGGTGTCAGTACAGATGAGAACTCCTC
hOr56aTag_fwd	AACCGTCAGATCGCCTGGAGGCCACCATGGATCAAGTC
hOr56aTag_rev	CTTTACCTTGAACAGACTAAACTTGTTAACGGTG
pBI-CAG_for	GGGGTCATTAGTTCATAGCCCATAT
pBI-CAG_rev	CAAAACCGCATCACCATGGTAATAG
pBI-Orco_for	GTGTACGGTGGGAGGTCTATATAAG
pBI-Orco_rev	GTGGTATGGCTGATTATGATCCTCT
pBI-OrX_for	CATTTTATGTTTCAGGTTCAGGGGG
pBI-OrX_rev	GGCCTATATAAGCAGAGCTCGTTT

Supplementary Table 1. Primers used in this study.

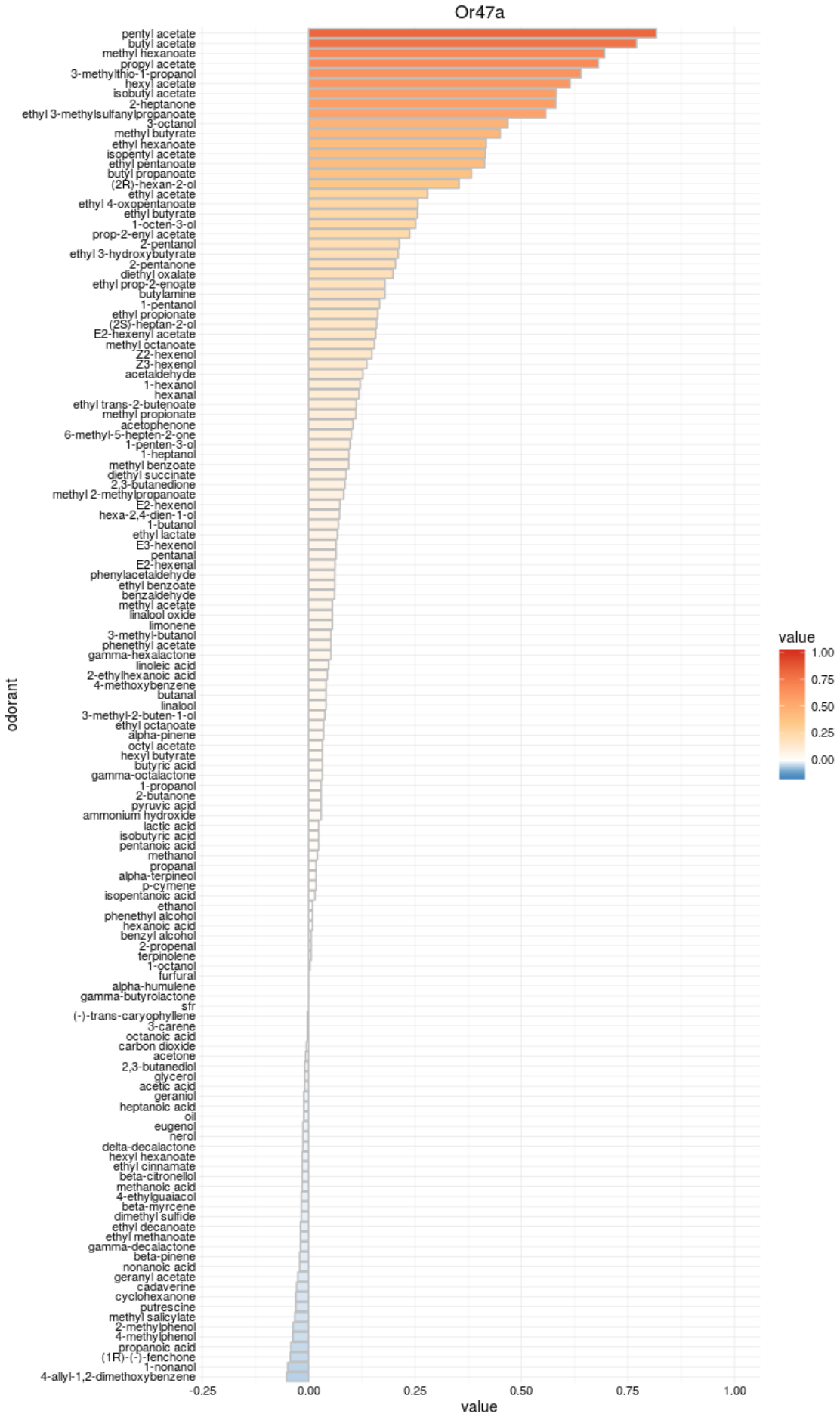
Platform	Plugin (Pl) or package (Pk) name	Citation
ImageJ	Image Stabilizer (Pl)	K. Li, "The image stabilizer plugin for ImageJ," http://www.cs.cmu.edu/~kangli/code/Image_Stabilizer.html, February, 2008.
R		R core team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
R	ggplot2 (Pk)	H. Wickham. ggplot2: Elegant Graphics for Data Analysis (Wickham, 2016)
R	gridExtra (Pk)	(Auguie, 2017) gridExtra: Miscellaneous Functions for "Grid" Graphics. R package version 2.3. https://CRAN.R-project.org/package=gridExtra
R	ggthemes (Pk)	(Arnold, 2017) ggthemes: Extra Themes, Scales and Geoms for 'ggplot2'. R package version 3.4.0. https://CRAN.R-project.org/package=ggthemes
R	scales (Pk)	(Wickham, 2017) scales: Scale Functions for Visualization. R package version 0.5.0. https://CRAN.R-project.org/package=scales
R	multcomp (Pk)	Torsten Hothorn, Frank Bretz and Peter Westfall (2008). Simultaneous Inference in General Parametric Models. (Hothorn et al., 2008)
R	extrafont (Pk)	(Chang, 2014) extrafont: Tools for using fonts. R package version 0.17. https://CRAN.R-project.org/package=extrafont
R	drc (Pk)	Ritz, C., Baty, F., Streibig, J. C., Gerhard, D. (2015) Dose-Response Analysis Using R (Ritz et al., 2015)
R	DescTools	Andri Signorell. 2019. DescTools: Tools for Descriptive Statistics. Version 0.99.28 https://cran.r-project.org/web/packages/DescTools/index.html
R	FSA (Pk)	Ogle, D.H. 2017. FSA: Fisheries Stock Analysis. R package version 0.8.17.
RStudio		RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

Supplementary Table 2. Software plugin and packages used for data analysis.



Supplementary Figure 1. Distribution of calcium responses in transfected HEK293 cells

Distribution of the $\Delta[Ca^{2+}]_i$ in transfected HEK293 cells following a stimulation with a 100µl of 100 µM pentyl acetate (a) or 100 µM VUAA1 (b). (a) The $\Delta[Ca^{2+}]_i$ values were calculated for each cell 50 s after stimulation (Time = 100 s in Figure 1c and Figure 2c). Cells were identified as "responding" to the stimulus if a stimulation with pentyl acetate induced a $\Delta[Ca^{2+}]_i \geq 6.75$ nM (non shaded area of the graphs). This threshold value is defined as the mean + 2 × SD response intensity ($\Delta[Ca^{2+}]_i$, in nM) value of the top (most responsive) 0.5 percentile of the cumulative distribution of analyzed cells in the control (Empty Vector) group. (b) The $\Delta[Ca^{2+}]_i$ values were calculated for each cell 20 s after stimulation (Time = 380 s in Figure 1c and Figure 2c). Cells were identified as "responding" to the stimulus if a stimulation with VUAA1 induced a $\Delta[Ca^{2+}]_i \geq 6.25$ nM (non shaded area of the graphs). This threshold was defines in the same was as for (a). The percentage of responding cells is reported for each panel as mean ± SD. Graphs represent mean ± SD. $3 \leq n \leq 5$ for each graph, each distribution (n = 1) is constituted by a number of cells x, with $56 \leq x \leq 355$.


Or47a
pentyl acetate
butyl acetate
methyl hexanoate
propyl acetate
3-methylthio-1-propanol
hexyl acetate
isobutyl acetate
2-heptanone
ethyl 3-methylsulfanylpropanoate
3-octanol
methyl butyrate
ethyl hexanoate
isopentyl acetate
ethyl pentanoate
butyl propanoate
(2R)-hexan-2-ol
ethyl acetate
ethyl 4-oxopentanoate
ethyl butyrate
1-octen-3-ol
prop-2-enyl acetate
2-pentanol
ethyl 3-hydroxybutyrate
2-pentanone
diethyl oxalate
ethyl prop-2-enoate
butylamine
1-pentanol
ethyl propionate
(2S)-heptan-2-ol
E2-hexenyl acetate
methyl octanoate
Z2-hexenol
Z3-hexenol
acetaldehyde
1-hexanol
hexanal
ethyl trans-2-butenoate
methyl propionate
acetophenone
6-methyl-5-hepten-2-one
1-penten-3-ol
1-heptanol
methyl benzoate
diethyl succinate
2,3-butanedione
methyl 2-methylpropanoate
E2-hexenol
hexa-2,4-dien-1-ol
1-butanol
ethyl lactate
E3-hexenol
pentanal
E2-hexenal
phenylacetaldehyde
ethyl benzoate
benzaldehyde
methyl acetate
linalool oxide
limonene
3-methyl-butanol
phenethyl acetate
gamma-hexalactone
linoleic acid
2-ethylhexanoic acid
4-methoxybenzene
butanal
linalool
3-methyl-2-buten-1-ol
ethyl octanoate
alpha-pinene
octyl acetate
hexyl butyrate
butyric acid
gamma-octalactone
1-propanol
2-butanone
pyruvic acid
ammonium hydroxide
lactic acid
isobutyric acid
pentanoic acid
methanol
propanal
alpha-terpineol
p-cymene
isopentanoic acid
ethanol
phenethyl alcohol
hexanoic acid
benzyl alcohol
2-propenal
terpinolene
1-octanol
furfural
alpha-humulene
gamma-butyrolactone
sfr
(-)-trans-caryophyllene
3-carene
octanoic acid
carbon dioxide
acetone
2,3-butanediol
glycerol
acetic acid
geraniol
heptanoic acid
oil
eugenol
nerol
delta-decalactone
hexyl hexanoate
ethyl cinnamate
beta-citronellol
methanoic acid
4-ethylguaiacol
beta-myrcene
dimethyl sulfide
ethyl decanoate
ethyl methanoate
gamma-decalactone
beta-pinene
nonanoic acid
geranyl acetate
cadaverine
cyclohexanone
putrescine
methyl salicylate
2-methylphenol
4-methylphenol
propanoic acid
(1R)-(-)-fenchone
1-nonanol
4-allyl-1,2-dimethoxybenzene
odorant
-0.25
0.00
0.25
0.50
0.75
1.00
value
value
1.00
0.75
0.50
0.25
0.00

Supplementary Figure 2. Expected odor tuning properties of *D. melanogaster* Or47a

Expected response profile for *D. melanogaster* Or47a according to the DoOR 2.0 database. Query retrieved on 24 April 2019 at http://neuro.uni-konstanz.de/DoOR/default.html.

Supplementary Code. ImageJ and R files used for data analysis

Code used in this study is available on GitHub at the following URL:
https://github.com/fmiazzi/ORs_HEK293.git

Fura2_ImageJ.js: JavaScript code used to calculate $[Ca^{2+}]_i$ and perform cell segmentation for Figure 1-2.

Figure_1-2.R: R analysis for data shown in Figure 1-2 and Supplementary Figure 1.

Figure3.R: R analysis for data shown in Figure 3.

References

Arnold, J.B. 2017. ggthemes: Extra Themes, Scales and Geoms for "ggplot2".

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