Author's response to reviews

**Title:** Expression of taste receptors in Solitary Chemosensory Cells of the rodent airways.

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**Version:** 3 **Date:** 6 December 2010

**Author's response to reviews:** see over
Dear Editor,

I’m glad I have a chance to resubmit our revised manuscript. We answered point by point to the concerns of the reviewers (in bold the answers). With the revised manuscript, I resubmitted 3 supplementary figures with the legends in the main manuscript. Again if you consider them to be more significant if integrated as part of the figure, I will be open to insert them in the manuscript. We hope to hear good news from you soon and happy to have choose a BMC journal again.

Regards,
Marco Tizzano

Reviewer's report
Title: Expression of taste receptors in Solitary Chemosensory Cells of the rodent airways.
Version: 2 Date: 8 November 2010
Reviewer: Joerg Fleischer
Major Compulsory Revisions:
1. A major flaw of the present manuscript are incorrect references to figures in the first and the fourth paragraph of the Results section (page 6 and 7). For example, it is stated that “numerous TrpM5 GFP+ SCCs are present throughout the length of the trachea approaching densities of 40-50 SCCs per 100 µm2 (Fig1B, 2C, 2E, 2G)”; in fact, TrpM5 GFP+ SCCs can be seen in Fig1B, 2B, 2D, 2F. Likewise, also in the Figure legend section (page 14), several errors can be found - in particular regarding figures 1 to 3 (for example, a Figure1G is mentioned which does not exist). Therefore, I strongly recommend that the authors carefully revise the references to the figures in these parts of the manuscript.

Fixed figure references in the text and figure legend 1 and 3.
Minor Essential Revisions:
1. In the Conclusions section of the Abstract (page 2), the authors use two different but synonymous abbreviations (Tas1R and T1R). For consistency, I would suggest that the authors only use the abbreviation Tas1R (as in the rest of the manuscript). Furthermore, the abbreviations Tas1R and Tas2R could be briefly explained in the Abstract (or at least in the list of abbreviations on page 9/10). Similarly, explanation of the abbreviations “OCT” (page 5, last paragraph), “RT” (page 6, first paragraph) and “TBs” (page 8, fifth paragraph) would be favorable.

Fixed abbreviations in the text.
2. Some extensive speculations in the Conclusions section of the Abstract (page 2) should be reduced (or at least phrased in a somewhat different way). Notably, it is critical to claim that Tas1Rs “are likely involved in detection of foreign foodstuffs which trigger protective reflexes in the airways” (page 2, last paragraph) as long as “there are no reports of any chemosensitivity mediated by Tas1R receptors in the airways or any experiment that show the natural ligands for the Tas1R+ SCCs in the respiratory tract” (page 8, fourth paragraph).
Abstract conclusions reduced in tone and phrased in a different way

3. As an important control, the authors have conducted RT-PCR experiments with primers for GAPDH. Unfortunately, they do not show the results of these approaches in figure 4 and 5. Anyway, it would be helpful if the authors mention at least in the legends of figure 4 and 5 that they have conducted these essential control experiments (so far, it is only mentioned in the Methods section where this important information can be easily overlooked by readers).

Added a supplementary figure with PCR for GAPDH and ‘minus RT’ amplification results. I have mentioned at page 7 paragraph 5 of the results section about the GAPDH amplification experiments using +RT or -RT templates (shown in the Supplementary Fig3).

4. Page 6, first paragraph of the Results section: It seems that the “SCCs in the nasal respiratory epithelium contacted repeatedly by“ should be “SCCs in the nasal respiratory epithelium are contacted repeatedly by“.

Fixed

5. Page 8, third paragraph: Could it be that there is a word missing in the sentence “by the presence of SCCs higher in the respiratory is unclear“ (after the word respiratory)?

Fixed

6. Page 8: It would be helpful if two references would be given: first, for the claim that „nasal SCCs co-express Tas1R3-WGA with T2R5 and T2R8“. And second, for the statement that “the SCCs/brush cells of the gut release peptides in a more paracrine fashion to modulate digestive activities”.

Added references for the 2 statements.

7. Page 8, second last paragraph: It is mentioned that “SCCs expressing elements of the taste transduction cascade are prevalent in both the gastrointestinal tract [12, 56] as well as the respiratory tree [10, 12, 13, 16, 17, 25, 57, 58]“. To my (limited) knowledge, (putative) chemosensory cells in the gastrointestinal tract with certain similarities to SCCs are usually not designated as SCCs; they are most often called brush cells. Accordingly, the abovementioned sentence should be “Chemosensory cells expressing elements of the taste transduction cascade are prevalent in both the gastrointestinal tract [12, 56] as well as the respiratory tree [10, 12, 13, 16, 17, 25, 57, 58]“. In this context, the authors themselves emphasize that “diverse chemoresponsive cells including SCCs and brush cells, are different in terms of function and therefore should not be considered to be a single cell type”. Consequently, the statement “the SCCs/brush cells of the gut release peptides in a more paracrine fashion” (page 8, second last paragraph) should be converted to “the brush cells of the gut release peptides in a more paracrine fashion”.

Replaced cell type definitions with more appropriate ones.

Discretionary Revisions:

1. While the authors thoroughly investigate the expression of Tas1Rs (at least subtype Tas1R3) in different regions of the airways (at least in mice), they rely on a few PCR experiments regarding the expression of Tas2R subtypes in these tissues (moreover, they only use cDNA from rats). This is a pity since the results of these PCR experiments indicate some interesting findings: for instance, in
contrast to other Tas2R subtypes, Tas2R126 seems to be strongly expressed in
the lungs (figure 5). Consequently, using in situ hybridization, it would have been
interesting to assess potential differential expression patterns of diverse Tas2Rs
in SCCs along the airways which might suggest interesting functional
implications (similar to the differential expression pattern of Tas1R3). In addition,
double-staining in situ hybridization experiments with probes for Tas2Rs and
TRPM5 would have confirmed that Tas2Rs are indeed expressed in SCCs of the
trachea, the bronchi and the lung [and not in other cell types of these tissues
such as the ciliated epithelial airway cells which have been recently reported to
express Tas2Rs (Shah et al. 2009)]. Finally, using double-staining in situ
hybridization experiments with probes for Tas2Rs and Tas1R3, it might have
been worthwhile to investigate (for example) whether SCCs in the trachea
coop-express Tas1Rs and Tas2Rs [similar to nasal SCCs (Ohmoto et al. 2008)].
Maybe, the authors could consider these aspects for future studies.

Thank you so much for the suggestions about future studies. We are in the
process to perform the in situ experiments, but it will require more time.
For the purpose of the manuscript, these experiments show the presence
of SCCs in the entire respiratory tract and that subpopulation of them
express at least one of the taste receptors. I agree that co-localization and
double in situ hybridization experiments would represent in full the
promising results shown in this manuscript.

2. I would be interested in knowing why Dig-RNA probes were explicitly dilute,
denatured and cooled in the dark (page 6).

Because Digoxigenin is photo sensible to light and I noted less
background doing most of the probe production and in situ steps in the
dark.

3. Page 7, second paragraph: Actually, it is not entirely correct to claim that “all
Tas1Rs were present in taste tissue and airway (Fig5)” since figure 5 only shows
results for Tas1R1 and Tas1R2; the relevant results for Tas1R3 are depicted in
other figures.

Fixed

4. Legend of figure 1 (page 14): It is mentioned that SCCs “are intimately
innervated by peptidergic nerve fibers of the trigeminal nerve which express both
PGP9.5 (B) and CGRP (C)“. Is it really true that these fibers are generally
positive for both PGP9.5 and CGRP? In my opinion, this is not shown in the
manuscript. If it has been demonstrated previously, the corresponding reference
would be helpful. If not, it is critical to call PGP9.5-positive fibers peptidergic
since PGP9.5 is not a peptide but a hydrolase (as also mentioned by the authors
in the present manuscript). In the latter case, I recommend omission of the words
“peptidergic” and “both”.

Fixed
**Reviewer's report**

**Title:** Expression of taste receptors in Solitary Chemosensory Cells of the rodent airways.

**Version:** 2  **Date:** 12 November 2010  
**Reviewer:** Wolfgang Meyerhof

**Major Compulsory Revisions**

1) The paper does not contain control experiments. In fact, the authors declare in the method section that they have performed these experiments and that the outcomes were as expected. However, in my opinion such data are essential to make a strong case and should be included. I am referring to RT-PCR data which lack the ‘minus RT’ lanes for all amplification reactions (and I think they all are required) and to appropriate tissue specimens of wild type animals demonstrating the absence of any fluorescence. It is well-known that certain cells display greenish autofluorescence (perhaps because they are aminergic) that cannot easily be distinguished from GFP fluorescence using false color. Having sense probe controls for the in situ hybridization protocols would also be helpful at least for the epiglottis sections.

**Added a supplementary figure with PCR for GAPDH and ‘minus RT’ amplification results.** I have mentioned at page 7 paragraph 5 of the results section about the GAPDH amplification experiments using +RT or -RT templates (shown in the Supplementary Fig3).

In Supplementary Fig1 it is shown that wild type mice miss green autofluorescence and the same sections are stained with gustducin antibody to show location of the SCCs in respiratory and tracheal epitheliums.

Finally Supplementary Fig2 show the sense probe controls for the epiglottis (dotted lines and blue arrows show the location of laryngeal taste buds).

2) A problem is that the authors performed only RT-PCR experiments for Tas2r bitter receptor mRNAs but no in situ hybridization experiments. Thus, no cellular localization is provided and therefore, the authors cannot conclude that the SCCs are those cells that express Tas2Rs. I admit it may be likely that they do. On the other hand, based on recent numerous reports on Tas2r
expression in extragustatory cells it is impossible to exclude other cells such as contaminating blood cells as the site of Tas2r expression. Moreover, Tas2rs have been examined in rats only, not in mice. Either the authors add ISH data or must tone down their conclusions. In the latter case numerous places of the ms are to be edited (including the abstract).

**Manuscript have been edited and toned down.**

**Minor Essential Revisions**

1) There appears to be confusion in labeling/describing the panels of fig. 1 (text, page 6 versus images.

**Fixed figure references in the text and figure legend 1.**

2) Why are the data regarding the nasal respiratory epithelium included? I think they are published and it is superfluous to show them here again.

**Nasal respiratory epithelium (RE) is only included in figure 1 to show how the innervations contact the SCCs in the larynx (very similar to the RE) and how the SCCs in the trachea are normally not innervated. Furthermore, it has never been reported if T1R3+ SCCs are innervated, either in RE, larynx or trachea.**

3) Have the authors observed GFP fluorescence or ISH signal in laryngeal taste buds and, if so, why have they not been mentioned and, if not, is there an explanation?

**Yes, taste bubs are observed in the larynx of the GFP mice and in the ISH experiments. Text has been fixed and an explanation of taste buds presence in the laryngeal tissue has been added.**

4) From my copy of fig.4 it appears that ISH signals for both gustducin and Tas1r3 in the epiglottis are clustered raising the question as to whether the positive cells are truly SCCs or taste bud cells.

**Text and figure 4 have been fixed regardless taste buds presence in the laryngeal.**

5) In the introduction (page 3, para5) the authors cite refs 33-36 for the claim that bitter substances are detected by GPCRs of the numerous TAS2R family. Neither ref 33, 35 nor 36 contains such evidence. It would be fair to cite work of the Meyerhof group here as they contributed more than others to the alignment of Tas2rs with their cognate bitter compounds (best suited paper would be Meyerhof et al 2010). I should also say that not the Tas2r family is numerous but their members.

**Added reference**

6) Quite recently a paper appeared in Nature Med describing airway smooth muscle Tas2Rs raising the question if the authors observed taste signaling compounds in these cells as well. Nevertheless, some comment on this report appears required in my opinion.

**Although our manuscript was submitted before the Nature Med paper was online, we also think that a comment on this report is required. Text modified in the discussion paragraph.**

7) The claim that nasal SCCs coexpress Tas1R3 and Tas2R5 and-8 is not referenced (p8, para5).
Added reference
8) Fig 1 B,C show cells that either express TRPM5-GFP or Tas1r3-GFP not both (legend fig 1)

Figure 1 legend fixed

Discretionary Revisions
1) The cells in images fig 1 D / E appear to have very different shapes. Do the authors wish to comment on this?

Although the shape of the SCCs in the nasal respiratory epithelium is clearly different than the tracheal SCCs, some ultrastructural experiments are more suitable to address this question. Future studies will focus on this aspect and on ISH experiments for the members of the T2R family, not only in rat.