Shared genetic requirements for ATF5 translation in the vomeronasal organ and main olfactory epithelium [version 1; referees: 3 approved with reservations]

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Abstract

Background: Both olfactory sensory neurons (OSNs) and vomeronasal sensory neurons (VSNs) require the transcription factor Atf5 for maturation and survival. In OSNs, ATF5 translation is controlled by olfactory receptor (OR) expression-mediated activation of the PERK branch of the unfolded protein response. This study evaluated whether OSNs and VSNs share genetic requirements for ATF5 translation.

Methods: ATF5 immunoreactivity was assayed in whole vomeronasal organs from a series of genetic mutant animals identified in studies of OR gene choice, OR feedback, and regulation and OSN development.

Results: ATF5 expression in VSNs required the histone demethylase Lsd1, which has been previously reported to be required for OR expression. ATF5 expression also required PERK-mediated phosphorylation of the translation initiation factor eIF2a. Finally, unlike previous observations in OSNs, ATF5 was found to be widespread in the mature VNO and co-expressed with mature VSN markers.

Conclusions: These data suggest that the initiation of ATF5 translation in VSNs and OSNs is under similar regulation, and that persistent/prolonged ATF5 translation in VSNs may serve VSN-specific gene regulatory programs. This study firmly establishes the unfolded protein response as a major controller of sensory neuronal maturation and diversification.
Introduction
Mice possess two olfactory organs: the main olfactory epithelium (MOE), which houses olfactory sensory neurons (OSNs), and the vomeronasal organ (VNO), which houses vomeronasal sensory neurons (VSNs). The MOE is thought largely to function in the detection of odors with no prior or innate behavioral importance, though there are notable exceptions. The VNO, on the other hand, detects pheromones, which drive important social and reproductive behaviors.

The VNO is a bilobal, crescent-shaped neuroepithelium. It is neurogenic, giving rise to new VSNs throughout the life of the animal. Immature VSNs are located at the tissue margins, or the tips of the crescents, while mature VSNs occupy more central areas. VSNs can be initially divided into two types, based on the expression of their primary signaling G proteins. Apical VSNs express the G protein Gna12, as well as type I vomeronasal receptors (V1Rs). Basal VSNs express the G protein Gnao and type II vomeronasal receptors (V2Rs). V1Rs are expressed monogenically and monoaesthetically. The situation is markedly more complicated for V2Rs. Type II VSNs express two V2Rs in non-random combinations: one from V2R family A, B, or D; and at least one from V2R family C. In addition, some basal VSNs also express at least one gene from the non-classical MHC H2-Mv gene family.

The VSNs express genes for basal type II VSNs) occur in non-random combinations, it is possible that the mechanism of ER trafficking in VSNs has yet to be established, resulting in global attenuation of mRNA translation initiation and a specific increase in translation of mRNA encoding the transcription factor Atf5. ATF5 is required for OSN maturation and expression of adenyl cyclase 3 (AC3). AC3 expression suppresses activity of a histone demethylase required for OR choice, LSD1. If OR choice fails to drive Adcy3 expression, as is the case with some OR pseudogenes as well as Atf5 and Adcy3 mutants, expression of the chosen OR is extinguished. This phenomenon, termed ‘gene switching’, appears to add a layer of quality control for ORs, and also indicates that OR choice is initially unstable. This lack of stability is probably due to the dual demethylase activities of LSD1, which presumably allow it to de-silence an OR allele, and then to re-silence the same allele. In this model, LSD1 downregulation by AC3 is required for stable transcription of the chosen OR. Together, these data support a model in which OR feedback acts to promote OSN maturation, to prevent further OR choice, and to stabilize expression of the chosen OR allele.

In contrast to the growing body of knowledge on OR gene regulation, comparatively little is known for VRs. However, a number of lines of evidence support a model in which both V1Rs and V2Rs employ a feedback signal similar to that used by ORs to prevent further VR gene activation. First, VSNs choosing a V1R pseudogene target axons widely across the accessory olfactory bulb, indicating that they have subsequently selected a second V1R gene. This finding suggests that V1R protein activates VR feedback. Second, VSNs that choose an OR gene knocked into a V1R gene locus do not express additional V1Rs. This result suggests both that canonical V1R signaling is unimportant—as ORs and V1Rs signal through different second messengers—and that ORs can activate VR feedback. Third, heterologous V2R expression activates the UPR, and both V1Rs and V2Rs, like ORs, fail to traffic from the ER when expressed heterologously. V2R trafficking appears to involve replacement of the ubiquitous chaperone Calreticulin with a VNO-specific homolog, Calreticulin 4. For V1Rs, the mechanism of ER trafficking in VSNs has yet to be established, but does not appear to involve either Calreticulin 4 or the OR transporters Rpt1p2. Finally, it was recently shown that Atf5 is required for maturation and survival of basal VSNs. This study also showed that while Atf5 mRNA expression is ubiquitous in VSNs, ATF5 protein is expressed in more limited patterns, suggesting that Atf5 mRNA is under translational control in the VNO. In sum, these data suggest that OR and VR feedback may employ a common framework, converging on PERK-driven translation of ATF5.

In order to begin to define the mechanistic outline of VR feedback, I have assayed ATF5 protein expression in a series of mouse mutants previously employed in studies of OR feedback. I have found that in Lsd1 mutant VNOs, ATF5 protein is absent, establishing a common genetic requirement for Lsd1 in ATF5 translation in both the VNO and the MOE. Appearance of ATF5 also required both the ER-resident kinase PERK and phosphorylation of the translation initiation factor eif2α, suggesting that ER stress drives ATF5 translation in basal VSNs. Finally, in adult animals, ATF5 is widespread and found in anatomical areas corresponding to both immature and mature VSNs, suggesting that mature VSNs experience continued or spurious ER stress events. Together, these results support a model in which V1Rs and V2Rs both employ ER stress-mediated feedback, potentially...
with different requirements for ATF5 and subsequently with different transcriptional outcomes.

**Results**

*Lsd1* is required for ATF5 expression

*Lsd1* has previously been deleted from the olfactory placode by crossing animals carrying *loxP*-surrounded *Lsd1* alleles to animals expressing Cre recombinase under the control of the FoxG1 promoter. These conditional mutants lose expression of most OR genes, resulting in a failure to translate ATF5 and a failure of OSNs to reach maturity. To test whether VSNs and OSNs share a genetic requirement for *Lsd1* in the ATF5 expression, ATF5 immunoreactivity was assayed in control (*FoxG1-Cre; Lsd1 fl/+*) and mutant (*FoxG1-Cre; Lsd1 fl/fl*) VNO at embryonic day 18.5 (E18.5). A later analysis was not possible due to the perinatal lethality of this combination of alleles. As can be seen in Figure 1A–B, control animals exhibited robust ATF5 immunoreactivity in the VNO. As previously described, ATF5 expression was found to be widespread and heterogeneous from cell to cell. In contrast, *Lsd1* mutants did not have observable ATF5 expression (Figure 1C–D). Consistent with previous findings showing that *Atf5* is required for VSN maturation and survival, the VNO of the mutant animals was greatly reduced in size. Despite its decrease in size, the VNO was still readily identifiable through the use of a number of structural features, including the surrounding bone and mesenchyme structure, bilateral symmetry, position relative to the MOE, and the presence of a lumen of stereotypical shape, adjacent to an epithelium with a single layer of apical sustentacular cells. Together, these data indicate that in the VNO *Lsd1* is required for ATF5 expression, and by extension for VSN maturation. On the basis of these data, I hypothesize that VR expression is under *Lsd1* control, and that VR expression drives ATF5 translation. This hypothesis will be addressed in further detail in the discussion section.

ATF5 expression requires PERK-mediated eif2α phosphorylation

I next asked whether ATF5 translation in the VNO is under the same regulatory control as in the MOE. The *Atf5* mRNA contains an inhibitory upstream open reading frame (iuORF) that under basal conditions suppresses its translation. However, upon phosphorylation of the translation initiation factor eIF2α at Serine-51, ribosomes bypass this iuORF to translate the *Atf5* mRNA coding sequence. OR expression in the MOE promotes this phosphorylation event and ATF5 translation by activating the ER-resident kinase PERK. OR-driven ATF5 translation can be blocked either through PERK deletion or through mutation of the serine phosphorylation site on eIF2α to alanine. I therefore asked whether ATF5 was lost in the VNO of PERK mutants and

![Figure 1](https://example.com/figure1.png)

**Figure 1. Lsd1 is required for ATF5 expression.** (A–B) Representative coronal section of embryonic day 18.5 (E18.5) VNO from a *FoxG1-Cre; Lsd1 fl/+* animal. (C–D) Representative coronal section from a *FoxG1-Cre; Lsd1 fl/fl* VNO, also stained for ATF5 and DAPI. For all images, ATF5 immunoreactivity is shown in red and DAPI nuclear counterstain in blue.
eIF2α phosphor-mutants. While P0 Perk+/– VNO exhibited robust ATF5 immunoreactivity (Figure 2A–B), ATF5 was completely absent in littermate Perk−/− animals (Figure 2C–D). Similarly, ATF5 was completely absent in eIF2αS51A/S51A animals, in which PERK is still present but cannot exert translational control through eIF2α phosphorylation (Figure 2E–F). These data indicate that, as has been observed in the MOE and elsewhere, Atf5 mRNA in the VNO is under translational regulation via PERK-dependent phosphorylation of eIF2α.

ATF5 expression is widespread in adult animals
In the MOE, ATF5 expression is restricted to immature OSNs24. This expression pattern is intriguing, as both Atf5 and OR mRNA continue to be expressed in mature OSNs. It has been proposed that this context-dependence for ATF5 translation is due to increased expression of OR transporters such as RTP1/2 in mature OSNs, which could compete ORs away from PERK or simply relieve the ER burden imposed by ORs. In the VNO, a previous report demonstrated that at P0, Atf5 mRNA expression is essentially

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Figure 2. Translational control of ATF5. (A–B) Coronal section of postnatal day 0 (P0) VNO from Perk+/– animal. (C–D) Coronal section of P0 VNO from a Perk−/− littermate. (E–F) Coronal section from a P0 eIF2α S51A/S51A animal. For all images, ATF5 immunoreactivity is shown in red and DAPI nuclear counterstain in blue.
homogenous across the neuronal area, but that ATF5 protein is more heterogeneous. However, this report did not assay ATF5 expression in adult animals. While in young animals the VNO and MOE are dominated by immature neurons, in older animals the immature and mature neuronal compartments separate and resolve. In the adult VNO, a number of reports, have shown that immature VSNs are restricted to the VNO margins (i.e. the ‘tips’ of the VNO crescents)\textsuperscript{38,39}. Surprisingly, ATF5 was not restricted to the tissue margins in the adult VNO. Instead, it was widespread, heterogeneous, and found in areas corresponding to both immature and mature VSNs (Figure 3A–B). Furthermore, co-staining sections from this animal with antibody against olfactory marker protein (OMP) to label mature VSNs revealed that some ATF5-labeled cells co-express OMP. While most OMP+ cells were either ATF5-negative, or displayed barely-detectable ATF5, other OMP+ cells displayed saturating levels of ATF5. These observations suggest that, unlike in the MOE, in VSNs ATF5 continues to be expressed after

**Figure 3.** ATF5 expression in the adult VNO. (A–B) Coronal section of a postnatal day 35 VNO. ATF5 immunoreactivity is in red and DAPI in blue. (C–F) A close-up section from the same VNO as shown in A–B. Olfactory marker protein (OMP) immunoreactivity is shown in green, ATF5 in red, and DAPI in blue.
maturation. Given the nature of ATF5 translational control and the consequences of persistent UPR activation, this raises a number of interesting questions regarding PERK activation dynamics and the transcriptional output of ATF5 in VSNs.

Dataset 1. Raw images of immunofluorescence presented in figures

http://dx.doi.org/10.5256/f1000research.13659.d190561

Discussion

Receptor-driven feedback programs endow developing olfactory neurons with a means by which to establish distinct, unambiguous cell fates. These programs are therefore essential in the construction of the basic architecture of the olfactory system. Furthermore, because these feedback programs allow the appearance of a single protein to establish cell fate, they also act as an engine in neuronal diversification, forging a direct relationship between the number of chemoreceptor genes and the number of chemosensory cell fates.

My previous work uncovered that in OSNs, OR feedback is executed by co-option of the PERK branch of the unfolded protein response. An obvious follow-up question to this work was whether this feedback mechanism was employed in other tissues in which sensory cells express single or small numbers of sensory receptors. In the present work, I demonstrate that expression of ATF5, which is required for OR feedback and for the maturation and survival of basal VSNs, has the same genetic requirements in the VNO and the MOE. This work therefore strongly suggests that ORs and VRs have a shared mechanism of feedback, converging on activation of the PERK branch of the UPR. This work was undertaken at the tissue level, and therefore more detailed analyses will likely be required in order to determine the specific requirements of VSN subtypes. Below I discuss some of the caveats of this work, as well as interesting questions for future work.

First, I hypothesized above that VR expression is under Lsd1 control and is required for ATF5 expression. Several pieces of data prompted this hypothesis. Among them are the shared elements in VR and OR feedback discussed in the introduction such as their activation of the UPR in cell lines, as well as the requirement of Lsd1 for ATF5 expression in the VNO demonstrated herein. However, it has yet to be directly demonstrated whether and how Lsd1 influences VR expression, or whether VR expression in VSNs drives ATF5 translation. A number of experimental considerations make these analyses difficult. Among them are the prenatal lethality of Lsd1 mutants and the requirement of Atf5 for VSN survival, which together result in exceedingly small amounts of tissue for analysis of VR expression or of the epigenetic landscape of the VR gene family. Additional genetic models would likely be useful in determining the role of Lsd1 in VR choice, and a combination of biochemical and genetic approaches would be powerful in the determination of the mechanisms by which chemoreceptors influence PERK activity.

Second, it has been shown that a key element of OR feedback is AC3-driven downregulation of LSD1. In the MOE, LSD1 downregulation both prevents further OR choice and acts to stabilize expression of the chosen OR. LSD1 downregulation therefore must be exquisitely timed. No analogous situation has yet been demonstrated for VSNs. It is worth noting that the requirements for VSNs are likely different than for OSNs. In particular, VSNs choosing VR pseudogenes continue to express them while also selecting another VR from a different VR gene cluster. This finding indicates that VR choice may involve the permanent engagement of a single or limiting element in cis to a given VR cluster. VR feedback may therefore act to prevent further choice, but not to stabilize VR expression. Thus, if VSNs employ a mechanism similar to that of AC3 in OSNs, it may only act to terminate further VR choice, but not to stabilize VR choice. The mechanistic basis of this difference is a fascinating area for future study.

Third, the convergence on ATF5 in OSNs and VSNs prompts a number of questions on the role and transcriptional output of ATF5. For example, how could ATF5 control OR feedback in OSNs and VR feedback in VSNs? It seems likely, given that ATF5 is a bZIP-family transcription factor, that ATF5 has different binding partners in different tissues. This model would allow for co-factors to tune the transcriptional specificity of ATF5, but would prevent their engagement until ATF5 has been translated. For example, in OSNs this may allow ORs to promote expression of RTP1/2 such that they can subsequently be targeted to the plasma membrane. In contrast, given that basal VSNs express non-random combinations of receptors and that the expression of these receptors is sequential, expression of one VR may drive ATF5 expression to aid in selection of a second VR (or an H2-Mv). The identity of these potential binding partners is a fascinating outstanding question and is likely to greatly aid in our understanding of chemoreceptor feedback programs.

Fourth, as demonstrated herein, ATF5 continues to be expressed in mature VSNs, unlike findings in OSNs. In addition, cell-to-cell levels of ATF5 appeared to be extremely variable, with signal nearly undetectable in most cells, but reaching saturation levels in other cells. This is a fascinating observation, as it would indicate that mature VSNs continue to experience ER stress events. Atf5 is ubiquitous in VSNs and the UPR-driven mRNA translation program is rapidly induced but brief. I therefore hypothesize that the ATF5 expression patterns I observed reflect transient ER stress events experienced by many or all VSNs. However, it is impossible to rule out an alternate scenario in which some cells (or even VSN sub-types) experience continuing ER stress while others do not experience ER stress at all. An additional implication of the prolonged ATF5 expression pattern in VSNs could be that VRs and ORs have different mechanisms of PERK activation, for example direct versus indirect. A number of studies support an indirect model of PERK activation by ORs, in which ORs activate PERK only in the absence of RTP1/2, but this question is unaddressed for VRs. In addition, it is intriguing that ATF5 could be continuously expressed in mature VSNs, as it would beg the question of
how VSNs can differentiate between bona fide ER stress and this developmental signal. Whether ATF5 has direct anti-apoptotic functions in VSNs as has been observed in other cell types has yet to be determined.

Finally, these findings firmly establish that a pathway canonically thought to be involved in the detection and resolution of cellular stress responses is fundamental in the designation of cellular identity and in cell maturation. This not only begs a reassessment of the role of PERK signaling, but also suggests specific additional studies. Given that activation of PERK provides such a powerful means by which to coordinate receptor appearance to the cellular gene expression program, and given that a multitude of cell types are defined by their expression of one or a handful of receptors, it would be surprising if PERK were not involved in other receptor-driven feedback programs. Excellent candidates include somatosensory neurons expressing Mas-related GPR family members, taste receptor cells, and photoreceptor cells. Specific chaperone or transporter requirements for these different receptors would provide a simple and generalizable mode for receptors to activate PERK in order to drive global gene expression programs, whose outputs can then be tuned by the use of tissue or cell type-specific co-factors.

Methods

Mice and strains used

All mice were housed in standard conditions with a 12-hour light/dark cycle and access to food and water in a UCSF barrier facility. All mouse experiments as well as euthanasia were approved by and were in accordance with University of California, San Francisco Institutional Animal Care and Use Committee (IACUC) protocol as described previously. Animals used in this study were under protocols held by the Lomvardas laboratory. Details on standard procedures including euthanasia can be found at the UCSF IACUC website (http://iacuc.ucsf.edu/Policies/awStandardProcedures.asp). Because all animals described in this study were only used for tissue collection, the relevant UCSF IACUC sections are those that deal with proper euthanasia. For all animals used in this study, animals were single or pair-bred (for animals harvested during pregnancy) or were group housed (for animals harvested as adults). For prenatal experiments, pregnancies were timed such that pregnant females and perinatal pups, and then immediately dissected. Main olfactory neurons expressing Mas-related GPR family cell type-specific co-factors.

Immunofluorescence

Immunofluorescence (IF) was performed as previously described. All animals were dissected immediately following euthanasia by CO2 exposure. Briefly, tissue was directly dissected into optimal cutting temperature compound (OCT, Tissue-Tek #4583). 14µm sections were air-dried on glass slides (VWR #48311-6703) for 10 minutes, fixed in 4% paraformaldehyde (PFA, Sigma Sigma #158127) in phosphate buffer solution (PBS) for 10 minutes, washed 3x5 minutes in PBS + .1% Triton-X (PBST), blocked for 1 hour in 4% donkey serum in PBST, then incubated with primary antibodies diluted in PBST and under coverslips (VWR # 470019-008) overnight at 4C. The following day, slides were washed 3x15 minutes in PBST and then incubated with secondary antibodies and DAPI in PBST at concentrations of 1:1000 under cover slips. Slides were then washed 3x15 minutes in PBST and mounted with vectashield (Vector Laboratories # H-1000) for imaging. Imaging was performed on Leica 700-series laser scanning confocal microscopes. The following antibodies were used: goat anti-Atf5 (Santa Cruz Biotechnology, SC-46934, dilution 1:250), rabbit anti-OMP (Abcam ab93127, dilution 1:250). For each panel, at least one mouse per genotype was sectioned. Differences were not noted between males and females. Mice were genotyped in-house using genotyping protocols suggested by the original generators of the mouse line. Genotyping protocols included positive and negative controls reactions. Full MOE or VNO were sectioned. To minimize variability between slides, control and experimental genotypes were sectioned onto the same slide. Slides with strong antibody signal and low background were selected for analysis. For each section, microscope settings were optimized for signal:noise. All image analysis was done in Fiji (version 2.0.0-rc-39/1.50b, build 8dcf1e65a6) and consisted only of changing brightness and contrast for each channel.

Amelioration of animal suffering

All efforts were made to ameliorate the suffering of animals used in this study. Animals received regular care from the author and from the animal facility personnel, as well as monitoring for health and injury. Unhealthy or injured animals were either treated or, in severe cases, humanely euthanized. In accordance with UCSF IACUC guidelines (see above), all animals were euthanized by exposure to CO2 at a rate deemed to minimize stress and suffering. Animals were not bred unnecessarily, and when possible multiple types of tissue were dissected from each animal and saved for potential future use.

Data availability

Dataset 1: Raw images of immunofluorescence presented in figures 10.5256/f1000research.13659.d19056

Competing interests

No competing interests were disclosed.

Grant information

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Open Peer Review

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Version 1

Referee Report 27 March 2018

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Pelin Cayirlioglu Volkan, Scott Barish
Department of Biology, Duke University, Durham, NC, USA

This study from Dalton aims to understand whether the same mechanisms that control olfactory receptor (OR) gene choice also govern the selection of vomeronasal receptors (VRs). The paper presents several immunohistological assays demonstrating that the expression of ATF5 is regulated by similar genes to those observed in the olfactory epithelium. Finally, the author shows that the expression pattern of ATF5 in the vomeronasal organ is distinct from the observed pattern in the olfactory epithelium.

Overall, this work is well presented and clearly described. A few points deserve revision.

Major points:
The author acknowledges that there is significant cell death caused by mutation of Lsd1. An additional control demonstrating that the expression of a housekeeping gene would be useful to demonstrate that gene expression is not globally altered in the surviving cells.

Minor points:
The statement of a hypothesis in the Results section should be deleted. This hypothesis is adequately addressed in the Discussion section.

Lines delineating regions that contain immature vs mature VSNs could be drawn in Figure 3 to aid readers who are less familiar with the structure of the VNO.

The second to last paragraph of the introduction lacks several references. The authors should review this paragraph and add citations.

The sentence beginning "A number of studies support..." In the second to last paragraph of the discussion lacks a citation.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes
If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

**Gustav F. Jirikowski** 1, **Andrea Rodewald** 2
1 Institute of Anatomy II, Jena University Hospital, Jena, Germany
2 Institute of Anatomy II, Jena University Hospital, Jena, Germany

This is a morphological study on histochemical similarities between vomeronasal and MOE sensory neurons in mouse during pre- and postnatal development with regard to the distribution of transcription factor *Atf5*. The expression of ATF5 depends on the histone demethylase Lsd1. ATF5 staining of samples from Lsd1-mutants revealed negative results. The expression of ATF5 also seems to require PERK. There was no ATF5 staining seen in *Perk* -/- mutants. The observations are original, interesting to a wide audience and deserve publication. However a few issues need to be addressed prior to final acceptance of this paper:

Photomicrographs need to be presented at higher magnifications and in higher quality. The overall morphology of the mouse VNO is most likely known to the majority of readers. So one low power image may be enough to give an overview. All other pictures should be at higher magnifications in order to reveal the details described in the results section. Micrographs showing ATF5 staining alone can be omitted since sections counterstained with DAPI provide the same information.

It seems that ATF5 is confined to the sensory epithelium only in newborn mice. In both E 18,5 and PNE 35 animals ATF5 seems to occur also in cells of the non sensory epithelium. This observation needs to be mentioned in results and discussion.

This is mere histochemical study. Neither physiological nor molecular data are presented. Therefore these aspects need to be discussed more cautiously.

Minor points:

- p.2, line 1: There are more than 2 olfactory organs in rodents: In addition to the MOE and the VNO there are Grüneberg ganglion and, Septal organ.
- p.2, line 3-4: The introduction also need to mention the non-sensory epithelium of the VNO
- p.2, line 12: A reference is necessary here.
- p.2, line 36: The definition of the abbreviation OR is missing.
- p.7, methods: How many mice were examined?
- There are still a few typos throughout the text.
Lettering of figures (small letters) should match lettering in captions (capital letters).

**Is the work clearly and accurately presented and does it cite the current literature?**
Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Not applicable

**Are all the source data underlying the results available to ensure full reproducibility?**
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**Are the conclusions drawn adequately supported by the results?**
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**Paolo E. Forni**  
Department of Biological Sciences, University at Albany, Albany, NY, USA

The research presented by Dalton aims to investigate mechanisms that control the expression of vomeronasal receptors in mice. The paper is very clear, well written and analyzes a relevant question in the field. Moreover, the author nicely discusses how his findings could be relevant for the study of different types of neurons.

Selective expression of olfactory neuron receptors (OR), in the main olfactory epithelium, relies on a complex mechanism that include epigenetic DNA modification and interaction of different transcriptional regulators.

Olfactory receptor expression activates the ER-resident kinase PERK, which controls phosphorylation of the translation initiation factor eif2α, and increase in expression of the transcription factor ATF5. ATF5 expression controls Adenylate cyclase 3 expression. In the main olfactory epithelium, it has been proposed that the OR selective choice depends on removal of silencing chromatin modifications by the demethylase Lsd1 and that the adenylate cyclase 3 expression blocks LSD1 activity.

Previous work (Nakano et al 2015) has shown that ATF5 is important for maturation and survival of basal vomeronasal sensory neurons. Based on these premises Dalton proposes that vomeronasal receptors share a similar expression feedback mechanism, to the one described in the MOE, based on ER-resident
kinase PERK activation, EIF2α phosphorylation and ATF5 translation. To understand if a similar regulatory network, to the one described in olfactory neurons, exist in the VNO Dalton analyzed ATF5 expression in Foxg1Cre;Lsd1<sup>flox/flox</sup>, Foxg1Cre;PERK<sup>flox/flox</sup> conditional mutants and in mice carrying a mutated form of elf2α (elf2α<sub>S51A/S51A</sub>) that prevents its phosphorylation and therefore AFT5 expression.

**Major points:**

- In line with the hypothesis ATF5 expression is lost in the vomeronasal epithelium of Lsd1, PERK and elf2α<sub>S51A/S51A</sub> mutants suggesting that ATF5 is under control of PERK, Lsd1 and EIF2a. However, while the PERK mutants appear to have a relatively normal vomeronasal epithelium in Lsd1 and elf2α mutants the VNO appears to be almost vestigial suggesting that these genetic manipulations affect much more than ATF5 expression alone. The phenotypes of Lsd1 and elf2α mutants do not overlap with the one described in the ATF5 conventional KO (see Nakano et al. 2015<sup>1</sup>). In fact in the ATF5 KOs a reduction has been described in basal vomeronasal neurons only, in the Lsd1<sup>flox/flox</sup> and elf2α<sub>S51A/S51A</sub> mutants all the vomeronasal neurons seem to be affected. Are there vomeronasal genes that are not affected after Lsd1 and elf2α<sub>S51A/S51A</sub> mutations? Is proliferation altered? Are the neurons dying? All mutants should be presented including at least GAP43 and OMP, Ki67 and activated Caspase-3 immunostaining. Though the lack of ATF5 expression is in line with the main hypothesis more internal controls are needed to support the conclusion.

- Dalton nicely shows that ATF5 is expressed in mature vomeronasal neurons. Is ATF expression still controlled by Lsd1, PERK and elf2α in mature (OMP+) vomeronasal sensory neurons? Characterizing ATF5 expression after OMPCre drive deletion of Lsd1 and PERK would strengthen the quality of the paper and its relevance.

- The number of analyzed animals is not clearly indicated.

- Heterozygous Foxg1-cre mice can have developmental phenotypes (Eagleson et al. 2007<sup>2</sup>) ATF5 expression should be also analyzed in FoxG1Cre<sup>+/−</sup> controls.

**References**


**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

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Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes
If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

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