Expression of an odorant receptor in the anhydrobiotic cell line Pv11 allows the recovery of odorant response after dry storage

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Abstract

The development of cell-based olfactory sensors is a growing field and odorant receptor-expressing cells have been shown to recognize various odors. However, cell cultures need controlled conditions and are therefore difficult to use outside a laboratory. In this study, odorant receptor DmOr47a, co-receptor DmOrco, and the calcium-sensing fluorescent protein GCaMP6f for recording the ligand responses were stably expressed in the desiccatable Pv11 cells (termed Pv11-00443-Or47a cell line). This cell line showed dose-dependent responses to the DmOr47a ligand pentyl acetate, at a similar level as that reported in other cell types. When Pv11-00443-Or47a cells were dried and stored for 14 days, they recovered significant response to pentyl acetate 12h after rehydration. Most importantly, the Pv11-00443-Or47a cells showed a response to the agonist of DmOrco just 1h after rehydration, as well as after inhibition of protein synthesis, demonstrating for the first time that a transmembrane protein can be dry-stored in an orthologous cell culture system.

We show that Pv11 cells can retain the function of an exogenous odorant receptor after dry storage and recovery. This constitutes a pivotal initial step towards the development of desiccatable sensing cells to be associated with portable biodevices.

Keywords

Odorant receptor, Pv11 cells, dry storage, odor sensor, GCaMP6f, pentyl acetate

1 Introduction

Olfactory systems across species identify odors with high selectivity and sensitivity. For example, dogs can recognize the scent of drugs and other contraband at airports, search for victims after disasters[1], and identify individuals with COVID-19 [2]. Physicians have long used odor to diagnose some diseases[3]. Recently even nematodes [4] and ants [5] have been shown to detect cancer.

Humans have been able to compensate for their relatively poor sense of smell by employing the noses of other animals, primarily dogs but also rodents[6], to aid in odor detection. However, such human-animal teams require considerable cost and time to train, they display individual variability, and they have low portability. Reproducibility can also be a concern, as is knowing the specific odor that leads to identification. There is thus a need to develop an artificial nose that reproduces the sense of smell for odor identification, and it should work in real-world environments, allow recording, and provide reproducibility. Therefore, odor sensing systems have been investigated for many years

as reviewed previously [7,8]. Various types of sensors have been used, including metal oxide (MO), conductive polymer, surface acoustic wave (SAW), membrane-type surface stress (MSS), electrochemical, and quartz crystal microbalances (QCM). However, there are still no general-purpose noses that span the natural chemical space and that can provide sufficient odor selectivity to rival that of an animal nose.

Attention has thus turned to utilizing olfactory receptors (ORs), which constitute the basis of olfaction in living organisms. Olfactory receptors are membrane proteins, which can be divided into mainly two types: the G protein-coupled receptor (GPCR) type found in vertebrates and the ligand-gated ion channel (LIC) type found in insects. When odorants bind to ORs in vertebrates, G proteins are activated, which in turn activate adenylate cyclase. The resulting cAMP opens ion channels, allowing cations such as Na^+ and Ca^{2+} to flow into cells, leading to an action potential [9,10]. In the case of insects, on the other hand, the receptors consist of a hetero-tetramer comprising an odorant-specific OR and an olfactory receptor co-receptor (Orco). This complex itself functions as a ligand-gated cation channel [11,12].

In order to utilize olfactory receptors, since they have limited stability alone[7], it is beneficial to express them in cells. Since no stable cell lines created from olfactory sensory neurons currently exist, there is interest in expressing ORs in heterologous cell lines. In the case of insects, it is possible to obtain a response to odorants in cells by simply expressing only two membrane proteins, an OR and Orco. Thus, this system can be used more easily than the more complex GPCR-type olfactory system. In fact, previous research has shown that it is possible to express insect olfactory receptors in various cell lines and evaluate their responsiveness to odorants [13-15].

However, a challenge to cell-based sensing has been the requirement of continuously keeping the cells alive in an aqueous culture medium and within a narrow temperature range, reducing device storability and portability. Although different cell shipping methods have been developed, cell viability at room temperature is usually limited from a few days to a week [16]. Cell-based sensors have thus been largely confined to the infrastructure of the laboratory.

One solution for solving this problem could be to use a cell line that can withstand long periods of stability outside laboratory settings. The Pv11 cells are a cultured cell line derived from the non-biting midge *Polypedilum vanderplanki*, an insect that lives in the semi-arid regions of Africa and that can withstand desiccation during its larval stage[17]. The ability of the midge to survive almost complete desiccation in an ametabolic state is called anhydrobiosis [18,19]. Like *P. vanderplanki* larvae, Pv11 cells also have the ability to tolerate desiccation and can be stored in the dry state at room temperature for over a year[20].

Desiccation tolerance is induced by immersing the cells in a highly concentrated trehalose solution for 48 hours [20]. Furthermore, an exogenous gene expression system has been established for Pv11 cells [21], and Pv11 cells in which luciferase was expressed as a foreign protein were successfully stored in a dry state at room temperature for over a year, while retaining the enzyme activity [22]. However, there has as yet been no proof that membrane proteins such as ORs can be preserved as functional in desiccated Pv11 cells, and to our knowledge no previous report has shown the dry preservation of functional membrane proteins in a cell culture orthologous expression system. Thus, we needed to verify if functional ORs could be dry-preserved on the surface of Pv11 cells.

Here, we show that Pv11 cells can be engineered to express an olfactory receptor, that they respond to their target odorant with a fluorescence response, and that they retain their desiccation tolerance. This is potentially a critical breakthrough for cell-based olfactory sensing. Specifically, we expressed olfactory receptors in Pv11 cells and investigated whether they remained responsive to their ligand even after dry storage at room temperature. We selected an insect OR derived from the fly *Drosophila melanogaster*, which is phylogenetically closely related to *P. vanderplanki* and which has already-characterized ligands. The fluorescent calcium sensitive protein GCaMP6f was employed as an evaluation system for olfactory receptor function, as has been done in previous studies [14,15]. The fluorescence signal is triggered by the influx of Ca^{2+} enabled by opening of the receptor upon ligand binding, the fluorescence intensity depending on the intracellular Ca^{2+} concentration. GCaMP6f, DmOrco and DmOr47a were knocked in at the genomic region downstream of the *Pv.00443* gene, controlled by the strongest promoter in Pv11 cell line [23], and a stable expression cell line was established. This stable cell line was dried and stored at room temperature. After rehydration the ligand response of the cells was evaluated. This work shows for the first time that membrane proteins (especially DmOrco) can be stored in the dry state while retaining their function, demonstrating an initial step towards room temperature dry storage of cell-based biosensors.

2 Results

2.1 Establishment of a Pv11 cell line stably expressing DmOr47a

Preliminary screening with transient expression confirmed that GCaMP6f, DmOrco and DmOr47a were functional in Pv11 cells (Fig. S1) and that DmOr47a protein was expressed at the surface of the cell membranes (Fig. S2). To generate a cell line stably

expressing DmOr47a, the genes selected above were knocked-in to the Pv11 cell genome using the CRISPR/Cas9 system and the precise integration of target chromosome (CRIS-PITCh) method, as described previously [24]. The genes were integrated downstream of the *Pv.00443* gene, whose expression is regulated by the strong 121 promoter, allowing the highest expression level in Pv11 cells [23]. Guide RNA was designed to target the 5'-flanking site of the stop codon of *Pv.00443*, and two donor vectors were constructed to produce polycistronic expression of three genes: the first *Pv.00443*, GCaMP6f, and DmOrco, and the second *Pv.00443*, zeocin resistance gene (Zeo^R), and DmOr47a (Fig. 1A). The Cas9 gRNA-expression vectors were transfected into Pv11 cells together with the donor vectors. After zeocin selection and single cell sorting for GCaMP6f fluorescence, a cell line expressing GCaMP6f, DmOrco, DmOr47a, and Zeo^R in a biallelic conformation was obtained (Fig. 1A). Genomic PCR from the obtained monoclonal cell line confirmed the presence of a 3562 bp band corresponding to "P2A-GCaMP6f-P2A-Orco" and a 2281 bp band corresponding to "P2A-Zeo^R-P2A-Or47a", whereas the primers generated only a 619 bp band corresponding to the 3'-region of *Pv.00443* gene in wildtype Pv11 cells (Fig. 1B, Fig. S3). The DmOr47a expressing monoclonal cell line was thus knocked-in as expected, and we named this cell line Pv11-00443-Or47a.

We also confirmed that Pv11-00443-Or47a did not lose its desiccation tolerance due to genome editing. One hour after the rehydration of dried cells, Pv11-00443-Or47a showed a viability of 26.9%, which was comparable to the viability of wild type Pv11 cells (Fig. 1C).

We next investigated the ligand response of Pv11-00443-Or47a. Exposure to ionomycin at 50 μM generated a maximal fluorescence response (average $\Delta F/F_0 = 0.84$), whereas the addition of the DmOrco agonist, VUAA1, at 500 μM induced an average response of $\Delta F/F_0 = 0.53$, confirming the sufficient expression of functional DmOrco (Fig. 1D). Finally, the ligand of Or47a, pentyl acetate, at a concentration of 1 mM elicited an average fluorescent response $\Delta F/F_0 = 0.19$, which was significantly higher than the response to the control solvent DMSO 0.5%, $\Delta F/F_0 = 0.05$. GCaMP6f fluorescence images showed that a majority of the Pv11-00443-Or47a cells responded to pentyl acetate at 1 mM (Fig. 1D). In conclusion, we succeeded in engineering a Pv11-00443-Or47a sensor cell line that recognizes the odorant pentyl acetate while retaining its desiccation tolerance.

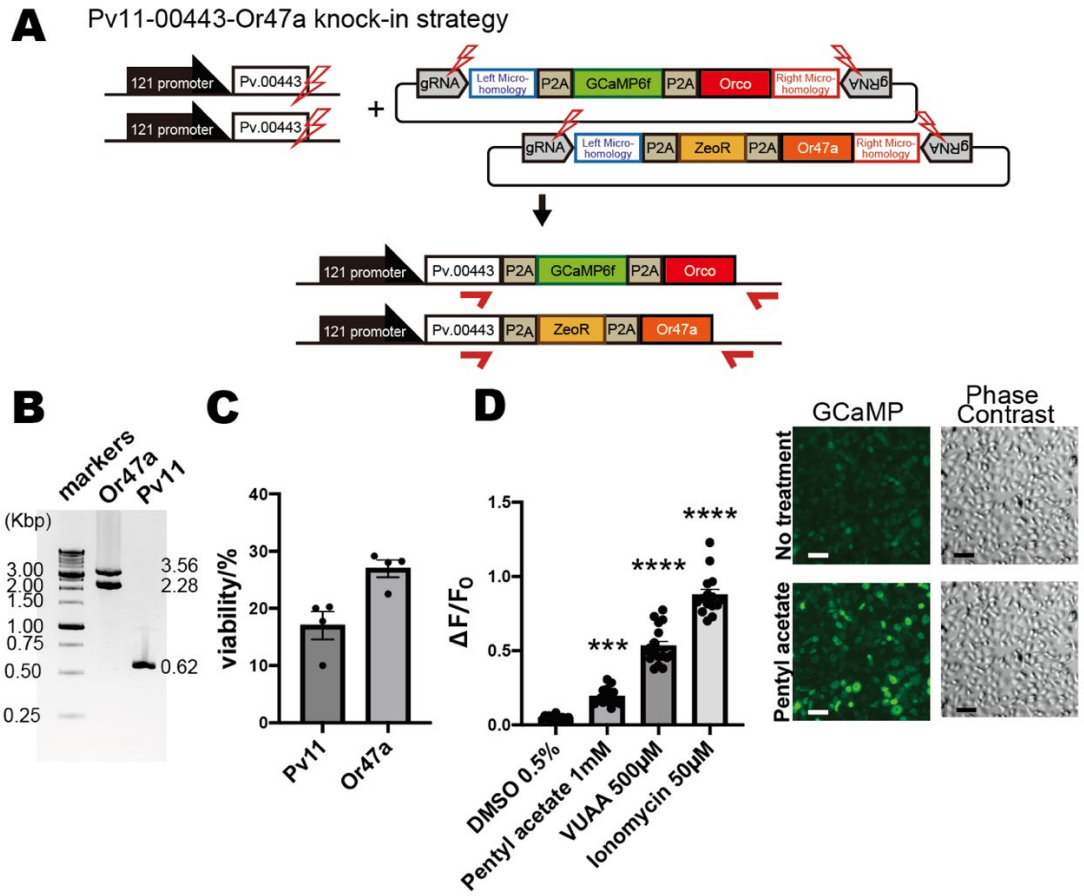

Figure 1: Generation of the Pv11-00443-Or47a stable cell line. **(A)** Strategy for knocking-in the genes of interest into the Pv11 genome. The CRISPR-Cas9 system was used to generate double strand breaks (red thunder marks) downstream of the stop codon of Pv.00443 gene, which is regulated by the endogenous strong 121 promoter (on the left) as well as on both sides of donor vectors containing either GCaMP6f and DmOrco genes or zeocin resistance and DmOr47a genes (on the right). As a result, GCaMP6f, DmOrco, zeocin resistance and DmOr47a genes were integrated into the Pv11 genome downstream of the Pv.00443 gene, in a polycistronic manner (on the bottom). Red arrowheads indicate the positions of primers for genomic PCR. **(B)** Genomic PCR of Pv11 control cells (Pv11) and Pv11-00443-Or47a cell line (Or47a) showing specific bands for the 3'-end of the Pv.00443 gene (619 bp), for the GCaMP6f-DmOrco insert (2,281 bp), and for the zeocin resistance-DmOr47a insert (3,562 bp). The original electrophoresis gel picture is available in Fig. S3. **(C)** Viability of the Pv11-00443-Or47a cell line compared to that of Pv11 control cells after dry storage, assessed 1 h after rehydration. **(D)** Ligand response expressed as change in fluorescence intensity ($\Delta F/F_0$) for Pv11-00443-Or47a cells in response to DMSO 0.5% as a control, pentyl acetate 1 mM, VUAA1 500 µM, and ionomycin 50 µM. Actual images of GCaMP fluorescence and phase contrast are shown on the right before and after exposure to pentyl acetate 1 mM. Scale bars represent 30 µm. Bar graphs show the mean of 4–14 replicates ± SD. Significant differences compared to DMSO 0.5% control (one-way ANOVA followed by Dunnett test) are expressed as *** (p-value < 0.001) and **** (p-value < 0.0001).

2.2 Evaluation of Pv11-00443-Or47a cell line dose-response to pentyl acetate

The stable Pv11-00443-Or47a cell line was exposed to pentyl acetate at concentrations between 0 and 1 mM. Monitoring GCaMP6f fluorescence after exposure to the ligand showed that Pv11-00443-Or47a cells responded to pentyl acetate in a dose-dependent manner (Fig. 2A). Exposure at 1 µM did not induce a significant fluorescent response, compared to the control solvent DMSO 0.5% (Fig. 2A, B). A pentyl acetate concentration of 10 µM, however, induced a significantly higher response than control, and the amplitude of the response was then stable up to 1 mM (Fig. 2B). The dose-response curve Pv11-00443-Or47a to pentyl acetate exhibited a baseline at $\Delta F/F_0 = 0.04$ and a plateau of maximal response at $\Delta F/F_0 = 0.24$ (Fig. 2B). The calculated EC_{50} for pentyl acetate was 3.4 µM (95% confidence interval (CI) 1.9 µM – 6.0 µM, n=6-13).

2.3 Dried Pv11-00443-Or47a cells fully recover their function 24 h after rehydration

To investigate whether the Pv11-00443-Or47a cell line would retain its sensor function through desiccation and dry storage, the cells were desiccated and stored for 14 days in the dry state prior to rehydration. Ligand response was observed 24 h after rehydration. Cells were exposed to pentyl acetate at concentrations between 0 and 1 mM. As for Pv11-00443-Or47a cells investigated prior to desiccation, the cells showed a dose-dependent response to pentyl acetate (Fig. 2C), with cells responding to pentyl acetate 1 µM (Fig. 2C), the exposure of rehydrated cells to pentyl acetate 1 µM did not induce a significant fluorescent response ($\Delta F/F_0 = 0.05$) in average, compared to the control solvent DMSO 0.5% (Fig. 2D). Pentyl acetate concentrations of 5 µM, 10 µM, 100 µM, and 1 mM induced significant changes in GCaMP6f fluorescence ($\Delta F/F_0 = 0.16$, $\Delta F/F_0 = 0.13$, $\Delta F/F_0 = 0.27$ and $\Delta F/F_0 = 0.21$, respectively), compared to the solvent control (Fig. 2D). The dose-response curve of rehydrated Pv11-00443-Or47a cells to pentyl acetate exhibited a baseline at $\Delta F/F_0 = 0.04$ and a plateau of maximal response at $\Delta F/F_0 = 0.24$ (Fig. 2D). The calculated EC_{50} for pentyl acetate was 8.1 µM (95% 4.6 µM – 14.2 µM, n=7-16). These values were similar to those of the dose-response curve to pentyl acetate prior to desiccation (compare Fig. 2B), except for a slightly higher EC_{50}.

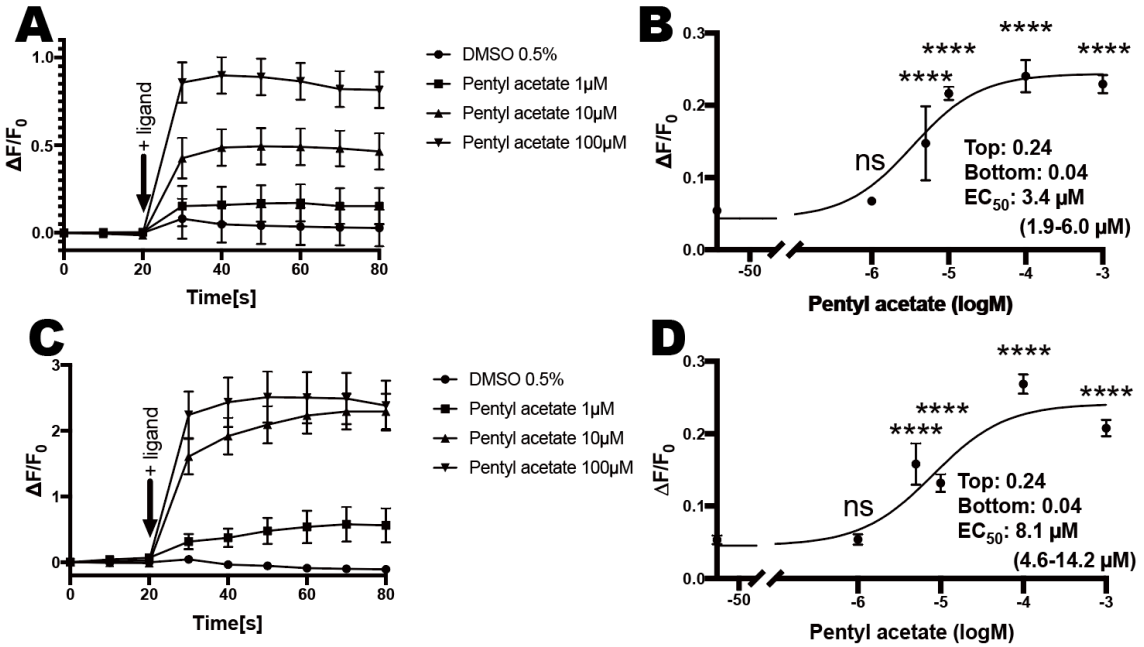

Figure 2: Response of the stable cell line Pv11-00443-Or47a to pentyl acetate before dry storage (A, B) and 24 h after rehydration (C, D). Ligand responses are expressed as a change in fluorescence ($\Delta F/F_0$) in response to the DMSO 0.5% control and to the ligand pentyl acetate at different concentrations from 1 µM to 1 mM. **(A)** Traces of individual cells responses to DMSO 0.5% control and pentyl acetate 1 µM, 10 µM, 100 µM. The arrow shows the timing of ligand addition. **(B)** Dose-response curve fitted to a nonlinear regression curve. **(C)** Traces of individual cells responses to DMSO 0.5% control and pentyl acetate 1 µM, 10 µM, 100 µM, 24 h after rehydration. The arrow shows the timing of ligand addition. **(D)** Dose-response curve 24 h after rehydration fitted to a nonlinear regression curve. Response traces represent the mean of 15 individual cells randomly selected ± SEM (A, C). On the dose-response curves (B, D), significant differences compared to DMSO 0.5% control (one-way ANOVA followed by Dunnett test) are expressed as **** (p-value < 0.0001) and ns (not significant). Top and bottom responses are expressed as $\Delta F/F_0$ and the EC_{50} is shown with a 95% CI.

2.4 DmOrco and GCaMP6f partially retain their function after dry storage

The response of Pv11-00443-Or47a cells was first investigated just 1 h after rehydration with culture medium. Rehydrated cells had a significant response to 50 µM ionomycin (Fig. 3A), showing that GCaMP6f was functional, although the amplitude of the fluorescent response was 5-fold weaker than the response observed for cells prior to desiccation (Figs. 1D, 3A). Similarly, rehydrated Pv11-00443-Or47a had a significant response to 500 µM VUAA1, showing that DmOrco was functional, although the fluorescent response was 3.3-fold weaker than prior to desiccation (Figs. 1D, 3A). The cells did not show any significant response to 1 mM pentyl acetate, having apparently lost

DmOr47a function during dehydration and rehydration (Fig. 3A). To verify whether the responses of rehydrated Pv11-00443-Or47a were due to dry-preserved DmOrco and GCaMP6f proteins or alternatively to *de-novo* synthesized proteins, desiccated Pv11-00443-Or47a cells were rehydrated with culture medium containing the translation inhibitor cycloheximide (CHX). One hour after rehydration Pv11-00443-Or47a cells still showed significant responses to 50 µM ionomycin and 500 µM VUAA1, with a fluorescence change amplitude not significantly different from that of cells rehydrated without CHX, $\Delta F/F_0 = 0.17$ and $\Delta F/F_0 = 0.14$, respectively (Fig. 3A, B). Cells rehydrated with CHX again did not show any significant response to 1 mM pentyl acetate (Fig. 3B).

2.5 The responses to pentyl acetate after rehydration depend on *de novo* protein synthesis

Waiting 24h after rehydration, the cells showed significant responses not only to VUAA1 500µM and ionomycin 50µM, but also to pentyl acetate 1 mM, compared to the control DMSO, with average fluorescence change responses of $\Delta F/F_0 = 0.81$, $\Delta F/F_0 = 1.03$ and $\Delta F/F_0 = 0.21$, respectively (Fig. 3C). These ligand responses were similar to or even stronger than those from cells prior to desiccation (Fig. 1D). Specifically, the responses to control DMSO 0.5% and pentyl acetate 1 mM recorded 24 h after rehydration were not significantly different from the responses observed prior to desiccation (Fig. 1D). However, the responses to VUAA1 500 µM and ionomycin 50µM were significantly higher 24 h after rehydration (Fig. 3C) than prior to desiccation (Fig. 1D), with *p*-values of 0.000002 and 0.002211, respectively. These results show that not only DmOr47a, but also GCaMP6f and DmOrco fully recovered their function 24 h after rehydration. Pv11-00443-Or47a cells rehydrated with the translation inhibitor CHX were also tested for ligand response 24 h after rehydration. The results showed significant responses to ionomycin 50 µM ($\Delta F/F_0 = 0.16$) and VUAA1 500µM ($\Delta F/F_0 = 0.12$), but no significant response to pentyl acetate 1 mM ($\Delta F/F_0 = 0.10$) (Fig. 3D). This suggests that the complete recovery of the pentyl acetate response of Pv11-00443-Or47a cells 24 h after rehydration (Figs. 2 & 3C) was principally due to *de novo* synthesis of GCaMP6f, DmOrco, and DmOr47a proteins.

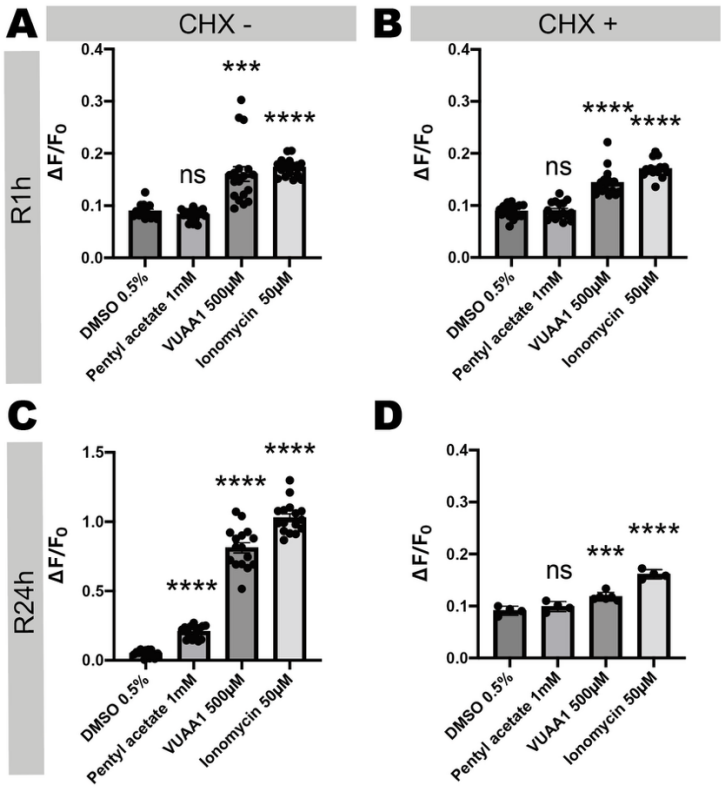

Figure 3: Ligand response expressed as change in fluorescence intensity ($\Delta F/F_0$) for desiccated and then rehydrated Pv11-00443-Or47a cells. **(A)** Ligand response one hour after rehydration in culture medium (CHX -). **(B)** Ligand response one hour after the rehydration in medium containing the translation inhibitor cycloheximide at 0.35 mM (CHX +). **(C)** Ligand response 24 h after rehydration in culture medium. **(D)** Ligand response 24 h after the rehydration in medium containing the translation inhibitor cycloheximide (0.35 mM). Cells were exposed to DMSO 0.5% as a control, pentyl acetate 1 mM, VUAA1 500 µM, or ionomycin 50 µM. Bars represent the mean of 14–18 replicates ± SD. Significant differences compared to DMSO 0.5% control (one-way ANOVA followed by Dunnett test) are expressed as **** (p-value < 0.0001); *** (p-value < 0.001); ns (not significant).

In conclusion, Pv11-00443-Or47a cells in the dry state can at least partly preserve the function of intracellular GCaMP6f and DmOrco on the surface of their membranes. However, the function of DmOr47a was either not preserved or was too weak for detection just after rehydration, needing time for *de novo* protein synthesis and for the full recovery of its ligand detection.

2.6 Ligand responses and cell growth after rehydration

The response of Pv11-00443-Or47a cells to pentyl acetate was recovered 24 h after the rehydration of dry-stored cells. To verify when Pv11-00443-Or47a cells recovered significant response to pentyl acetate, cell responses were monitored during the 48 h following rehydration. As observed above, Pv11-00443-Or47a cells did not show significant response to pentyl acetate 1mM just 1 h after rehydration and no significant difference was observed at 6 h after rehydration neither (Fig. 4A). In contrast, Pv11-00443-Or47a cells showed a significant response to pentyl acetate 1 mM from 12 h until 48 h after rehydration (Fig. 4A). It is worth to note that the response observed here to pentyl acetate was low (average $\Delta F/F_0$ around 0.11), compared to the average response $\Delta F/F_0$ around 0.24 observed in previous experiments at the same timing of 24 h after rehydration (Fig. 2D). This difference is probably due to a lower cell viability observed in this experiment after rehydration (13% of live cells), compared to other experiments (Fig. 1C).

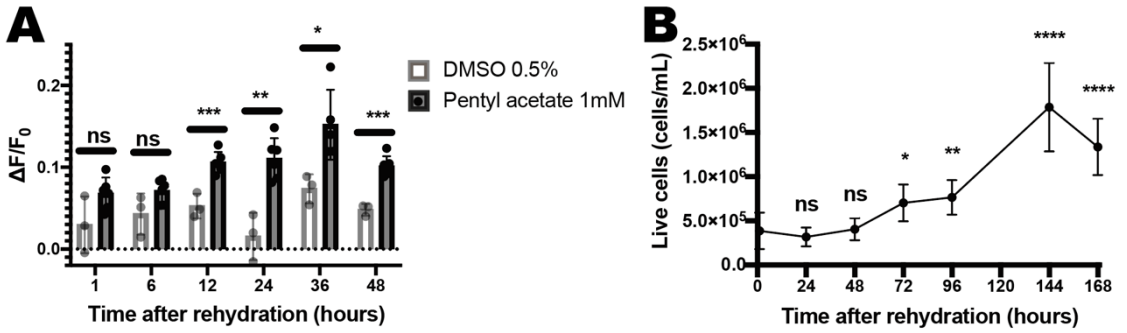

Figure 4: Monitoring ligand responses and cell growth after the rehydration of dry Pv11-00443-Or47a cells. **(A)** Rehydrated Pv11-00443-Or47a cells were exposed to the solvent DMSO 0.5% (white bars) as a control or to a solution of 1 mM pentyl acetate (grey bars). Ligand responses were expressed as a change in fluorescence intensity ($\Delta F/F_0$). Bars represent the mean of 3–6 replicates ± SD. Significant differences were expressed in comparison to the DMSO 0.5% control (*t*-tests) **(B)** Cell growth monitored for one week (1-168 h) after rehydration. The concentration of live cells is expressed as a mean ± SD (n=12). Significant differences compared to the cell concentration just after rehydration (1 h) are shown over the growth curve (one-way ANOVA followed by Dunnett test). The significant differences are expressed as **** (*p*-value < 0.0001); *** (*p*-value < 0.001); ** (*p*-value < 0.01); * (*p*-value < 0.05); ns (not significant).

The average GCaMP6f raw fluorescence of individual cells was also plotted in the baseline and at the highest response to pentyl acetate 1 mM before desiccation and until

48h after rehydration (Fig. S4). Results showed that GCaMP6f fluorescence decreased by about 20% at 1–6 h after rehydration, compared to cells prior to desiccation. Twelve hours after rehydration and later, fluorescence recovered a level similar to that observed in cells prior to desiccation (Fig. S4). This suggests that not only DmOr47a, but also GCaMP6f require *de novo* protein synthesis to recover their original functional level at 12 h after rehydration and later.

One hour after rehydration, the concentration of live Pv11-00443-Or47a cells was 3.8×10^5 cells/mL and this concentration did not differ significantly during the first 48 h following rehydration (Fig. 4B). Significant cell growth was observed from 72 h after rehydration and reached a plateau at 1.8×10^6 cells/mL after 144 h following rehydration without changing culture medium (Fig. 4B). According to these data, we conclude that Pv11-00443-Or47a cells can detect significantly pentyl acetate from 12 h to 48 h after rehydration, but this response to pentyl acetate is likely to be influenced by cell growth after 72 h following rehydration.

3 Discussion

In the present work, we succeeded in creating a Pv11 cell line stably expressing functional odorant receptor DmOr47a and in detecting pentyl acetate in a dose-dependent manner within an estimated range from 5 µM to 100 µM, while retaining desiccation tolerance and thus storability in the dry form at room temperature. We have demonstrated that 1) in our Pv11 cell system, DmOr47a showed an EC_{50} of 1.9 µM to 6.0 µM for its ligand pentyl acetate, within the range reported in other expression systems, 2) DmOr47a function was lost just after the rehydration of dry-stored cells, but recovered upon *de novo* protein synthesis 12–48 h after rehydration, and 3) the co-receptor protein DmOrco was partly preserved in the dry state, while retaining its agonist response even only 1 h after rehydration, independently from de novo protein synthesis. To our knowledge, this is the first report of dry preservation of a functional membrane protein in an orthologous biological expression system.

3.1 Pv11-00443-Or47a cell line shows ligand responses within the range observed in other cultured cell expression systems

We genetically engineered the Pv11-00443-Or47a cell line that stably expresses the fluorescent calcium-sensing protein GCaMP6f, DmOrco and DmOr47a. The three proteins were functional and the latter two responded to their respective ligand (Fig. 1D).

DmOr47a responded to its ligand pentyl acetate in a dose-dependent manner with a calculated EC_{50} of 3.4 µM (Fig. 2B). This response of DmOr47a in Pv11 cells was comparable to or better than the response reported in other cell lines. For example, DmOr47a expressed in HEK293 showed an EC_{50} of 2.2 µM [15], which is similar to our data in Pv11 cells prior to desiccation. Another system of expression in HEK293 cells had an apparent EC_{50} for pentyl acetate of approximately 50 µM [25]. When expressed in *Xenopus* oocytes, a dose-dependent response of DmOr47a to pentyl acetate was observed from 50 µM to 300 µM [11] (suggesting a higher EC_{50}). Such a high apparent EC_{50} was also reported for pentyl acetate in transgenic *D. melanogaster* expressing GCaMP6m and DmOr47a [26]. After rehydration, Pv11-00443-Or47a cell line showed a calculated EC_{50} of 8.1 µM (Fig. 2D), similar to the EC_{50} of 14µM reported for the co-expression of DmOr47a with DmOrco [27]. As listed above, the responses of DmOr47a to pentyl acetate are varying between expression systems and these differences can be explained for example by the amount of Or47a expressed at the surface of the cells, the proportion of co-receptor Orco (supplementary Fig. S2) or its origin [27], the expression level and sensitivity of GCaMP protein, or even the cell membrane environment.

3.2 After rehydration, DmOr47a recovers its function upon *de novo* protein synthesis

One hour after the rehydration of dry-preserved Pv11-00443-Or47a cells, no significant response to pentyl acetate was detected (Fig. 3A) The response to pentyl acetate recovered to a level similar to that observed prior to desiccation 24 h after rehydration (Figs. 3C and 1D), but this recovery was not detected in the presence of a protein synthesis inhibitor (Fig. 3D). This suggests that DmOr47a is not enough protected in dry cells to observe significant ligand response just after rehydration and that DmOr47a function is recovered upon *de novo* protein synthesis. Newly synthesized DmOr47a (and probably DmOrco and GCaMP6f) allow Pv11-00443-Or47a cells to recover their function at a significant level from 12 h after rehydration (Fig. 4A). Exceeding 48 h after rehydration, cell growth resumed (Fig. 4B) and the resulting effect on the stability of pentyl acetate detection by the cells remains to be investigated. Concerning the stability of exogenous proteins expressed in Pv11 cells, our previous work reported that GFP expression was stable through multiple passages for 1 year[28], but the stability of exogenous proteins expressed in the cytosol following rehydration was only assessed for luciferase and suggested no significant change of activity 1 h after rehydration [22]. Here, our data suggest that cytosolic GCaMP6f was not fully preserved in Pv11-00443-Or47a cells just at 1–6 h after rehydration (Fig. S4). However, remaining GCaMP6f was enough

to reveal significant response of DmOrco to VUAA1 500 µM even 1 h after rehydration (Fig. 3). In comparison, the loss of function of DmOr47a 1 h after rehydration suggests also at least partial loss of functional DmOr47a. However, since DmOr47a function is directly dependent on the presence of functional co-receptor DmOrco and GCaMP6f, de novo synthesis of these 3 proteins is required to recover Pv11-00443-Or47a cells response to pentyl acetate after 12 h of rehydration.

3.3 Co-receptor DmOrco was partially preserved in dry Pv11-00443-Or47a cells

One important finding here is that DmOrco protein retained its function in Pv11 cells at least partially after dry storage and immediately after rehydration (Fig. 3). Our previous work showed that the enzyme luciferase can be preserved in the cytosol of desiccated Pv11 cells, while retaining enzymatic activity immediately after rehydration [22]. Here, using the same translation inhibitor experiment, we showed that at least one fraction of DmOrco protein was preserved in the dry state and functional at the Pv11 cell surface immediately after rehydration. To our knowledge, this is the first report of dry preservation of a membrane protein in a cell culture orthologous expression system (for further discussion, see supplementary material section 3.1). From previous reports, we expect that the protective role of trehalose [29] and chaperone proteins such as Late Embryogenesis Abundant (LEA) proteins [30,31] contributed to the preservation of functional DmOrco in dry Pv11-00443-Or47a cells. The reason why DmOrco was preserved during dry storage and DmOr47a not is unclear, but we hypothesize that the expression of DmOr47a prior to desiccation was too low to allow detectable ligand response just after rehydration with only partial protection, as observed for DmOrco and GCaMP6f.

3.4 Potential of dry storage at room temperature for shipping and long-term preservation

Concerning the stability of dry-stored Pv11-00443-Or47a cell line, most experiments in the present study were performed with a 14-days desiccation and dry storage period. From previous studies, we know that Pv11 cells are almost completely dried 36h after transfer into a desiccator box [20]. Thus, the 14-day dry storage period ensured complete desiccation of the cells prior to rehydration. Under these conditions, Pv11-00443-Or47a cells showed 27% viability one hour after rehydration, confirming that desiccation tolerance was not altered in this monoclonal cell line compared to the original Pv11 cells. Longer dry storage periods yielded lower viability in previous studies. For example, dry

storage of Pv11 cells for 9 months led to a drop in cell viability to only 7% [20]. Similarly, luciferase-expressing Pv11 cells stored for more than one year showed less than 3% viability after rehydration; however surviving cells still showed luciferase activity [22]. In the present study, Pv11-00443-Or47a cells were desiccated in NARO, Japan and sent to the University of Maryland, where the cells were preserved at room temperature for over 3 years (from July 2019 to September 2022) prior to successful rehydration. This demonstrates that OR-expressing Pv11 cells can be handled and preserved at room temperature, significantly lowering shipping cost, while remaining functional after rehydration and culture (Fig. S5). The cell line also retained some sensitivity to pentyl acetate and VUAA1 through multiple desiccation-rehydration cycles and culture over a period of 4 years (from September 2020 through October 2024) (Fig. S6). Recording individual cells responses to 10 successive exposures to pentyl acetate 1 mM in perfusion assay showed that, even after multiple dry storage and culture passages, Pv11-00443-Or47a cells are functional and not limited to a single use (Fig. S7)

3.5 Limitations of this work and future perspectives

Although we showed that Pv11-00443-Or47a cells can be stored in the dry state, shipped at room temperature and stored for long periods while recovering their function after rehydration and subsequent passages, this work has limitations, and some problems need to be solved before practical application of these cells as odor sensors. First, the fluorescence signals from GCaMP6f are very faint and need long exposure times (1–3 s) to be detected. This problem affects the stability and reproducibility of quantitative results. Furthermore, the viability rate of the cells after rehydration (i.e. the number of cells responding to the ligands) and their physiological state (i.e. the proportion of OR expressed on the cell membranes) are subjected to variation and thus affect the stability of the responses to pentyl acetate with a sensible shift of EC_{50} compared to the responses observed prior to desiccation (Fig. 2A and 2B).

To solve these problems, improvement of the expression system could include an increase in GCaMP6f, DmOrco and DmOr47a protein levels, that would allow brighter and more stable fluorescent responses to pentyl acetate prior to desiccation. Higher DmOr47a protein levels prior to desiccation are expected to lead to a significant response to pentyl acetate just after rehydration. Actually, DmOr47a was expressed here in the Pv11-00443-Or47a cell line in a polycistronic system, producing a large protein including the Pv.00443 gene product, zeocin resistance protein, and DmOr47a (Fig. 1A). Subsequent cleavage of P2A peptide generated 3 independent proteins. However,

polycistronic expression systems with P2A peptide can show lower expression levels, especially for the protein in the third position [32]. Recent work on Pv11 cells allowed efficient protein expression from genes directly under the control of the strong 121 promoter and inserted in newly identified genomic safe harbors [23,28]. We anticipate that this alternative expression system will help to improve DmOr47a expression in Pv11 cells and allow the detection of a response to its ligand in the early hours following rehydration and also repeatable calcium detection measurements closer to those observed before desiccation. The effect of cell viability on the stability of the responses to pentyl acetate could be overcome by correction through an internal fluorescent marker or relative to the maximal response to VUAA1 or ionomycin. Individual cells recording could also attenuate the variability of the responses to pentyl acetate after rehydration.

3.6 Conclusion

To summarize, DmOr47a, DmOrco, and GCaMP6f were expressed in the desiccation-tolerant cell line Pv11 and showed a dose-dependent response to the DmOr47a ligand pentyl acetate prior to desiccation and also following dry storage and 24h after rehydration. Our data also suggest for the first time that a transmembrane protein, DmOrco, can be functionally preserved in the dry state in Pv11 cells. Future improvements of the expression system in Pv11 cells will allow stronger and more stable ligand responses and also increase the panel of ORs that can be functionally expressed in Pv11 cells. This work is the first step toward the development of cell-based odorant sensors storable at room temperature and practically usable outside of the laboratory. The use of Pv11 cells has several advantages for cell-based sensing. Since Pv11 cells are insect cells, they can be maintained in culture at room temperature without carbon dioxide gas. Secondly, Pv11 cells are non-adherent, floating in the medium, so the culture can be rapidly scaled up.

Our final aim is to associate dry-stored sensor Pv11 cells with miniaturized detector devices based on the detection of fluorescence [33,34] or electrical signals [35-37].

4 Methods

4.1 Cell culture

Pv11 cells were cultured as described previously [20,24]. Briefly, Pv11 cells were cultured in IPL-41 medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 2.6 g/L tryptose phosphate broth (Becton, Dickinson and Company,

Franklin Lakes, NJ, USA), 10% (v/v) fetal bovine serum, and 0.05% (v/v) of an antibiotic and antimycotic mixture (AA mix; penicillin, amphotericin B, and streptomycin; Millipore Sigma, Burlington, MA, USA). Cells were passaged at a concentration of 3 × 10^5 cells/mL weekly and incubated at 25 ºC.

4.2 Expression vectors for gene knock-in

Total RNA was extracted from dissected heads of *Drosophila melanogaster* (Canton-S) flies with ReliaPrep™ RNA Tissue Miniprep System (Promega, Madison, WI). RNA was then reverse transcribed to cDNA with PrimeScript II 1st strand cDNA Synthesis Kit (Takara, Kusatsu, Japan) and this cDNA was used as a template to clone DmOrco and DmOr47a coding regions. The GCaMP6f coding sequence was cloned from the plasmid vector pGP-CMV-GCaMP6f (Addgene #40755). The expression vectors constructed for transient expression (Supplementary methods 2.1.), namely pPv121-Orco (Supplementary data 8), pPv121-Or47a (Supplementary data 11), pPv121-GCaMP6f (Suplementary data 12), and an expression vector including zeocin resistance gene, pCR4-Zeocin-P2A (supplementary data 13), were used as templates and amplified by PCR with primers so that the encoding nucleotide sequence for the P2A peptide (GSGATNFSLLKQAGDVEENPGP) was added to the 5'-end of the sequences encoding each gene. PCR products were used as inserts and pCR4-Pv.00443#1µH-P2A-BbsI [24] was used as a vector, which contains the guide RNA (gRNA) target and microhomology, P2A, and BbsI sequences. The vector was treated with the restriction enzyme BbsI (New England Biolabs) and PCR product inserts corresponding to P2A-GCaMP6f and P2A-Orco were assembled using NEBuilder HiFi Assembly kit (New England Biolabs), generating the donor vector pCR4-Pv.00443#1µH-P2A-GCaMP6f-P2A-Orco (supplementary data 15). Alternatively, pCR4-Pv.00443#1µH-P2A-MCS (supplementary data 14) was used as a backbone vector containing the guide RNA (gRNA) target and microhomology, P2A, and multiple cloning site sequences. pCR4-Pv.00443#1µH-P2A-MCS and the PCR products corresponding to Zeocin-P2A and Or47a were treated with the restriction enzymes BamHI, HindIII and XhoI before performing a ligation that generated the donor vector pCR4-Pv.00443#1µH-P2A-Zeocin^R-P2A-Or47a (supplementary data 16). The primer oligonucleotides used to build these expression vectors are listed in table S2.

4.3 Knock-in into Pv11 genome and monoclonal cell selection

Integration of the target sequences into the Pv11 genome downstream to Pv.00443

gene was performed using the CRIS-PITCh genome editing method [38] as described previously [24]. In detail, a mixture of 0.3 μg of each donor vector obtained above, 5 μg of gRNA expression vector pPvU6b-DmtRNA-Pv.00443#1 [24], and 5 μg of Cas9 expression vector pPv121-SpCas9 [24] was transfected into Pv11 cells, following the electroporation protocol described previously[21] (for details, see Supplementary methods 3.2.). Transfected cells were cultured in 2 mL of supplemented IPL-41 medium for 5 days before performing zeocin selection treatment by inseminating 10^5 cells/mL in supplemented IPL-41 medium containing zeocin at a final concentration of 400 μg/mL and incubating the cells for 1 week under zeocin selection. Then, half the medium was replaced by fresh supplemented IPL-41 medium containing zeocin (400μg/mL), and the cells were again selected over a second week. After these 2 weeks of zeocin selection, cells were transferred into fresh supplemented IPL-41 medium without zeocin and cultured for one more week to let the selected cells grow. After this week of recovery, the cells were subjected to single cell sorting in order to obtain a monoclonal cell line. Single cell sorting was performed with a MoFlo Astrios cell-sorter (Beckman Coulter, Brea, CA) equipped with 355, 488, and 640 nm lasers, as described previously [24]. One thousand wild-type Pv11 cells were seeded as feeder cells in each well of a 96-well plate prior to sorting. The cells were stained with DAPI (Dojindo, Kumamoto, Japan) and then DAPI and GCaMP6f were excited with 355 nm and 488 nm lasers, respectively. DAPI- and GCaMP6f-positive cells were selected for single cell sorting, and the obtained single cells were grown for 2 weeks with feeder cells. Then, zeocin selection was performed as above to eliminate the feeder cells. The obtained monoclonal cell line was named Pv11-00443-Or47a. The Pv11-00443-Or47a cell line was passaged and cultured at 25 ºC for 8 days in a 25 cm^2 flask (BD Falcon, 353018) prior to a ligand binding assay.

4.4 Genomic PCR

Genomic DNA was extracted from the obtained monoclonal cells with FavorPrep Blood Genomic DNA Extraction Mini Kit (Favorgen, Taiwan). To confirm precise knock-in, the knock-in target region of the *Pv.00443* gene was amplified by PCR using KOD One PCR Master Mix-Blue (Toyobo, Osaka, Japan) with the following primer set: 5′-GCCAAAGCGAGCCAATTCAA-3′ and 5′-GGGTGTTATTGCTACTTTAATGCGT-3′. The presence of the knock-in band was verified by electrophoresis of the PCR product. After gel purification of the PCR products, sequencing was carried out with BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Tokyo, Japan) and precise integration of the target genes into Pv11

genome was verified.

4.5 Glass coating for immobilizing cells

Experiments were performed in 96-well plates (EZview culture plate LB glass bottom; AGC Techno Glass, Shizuoka, Japan). The coating protocol was described previously [39]. In detail, Cellmatrix type I-C (3 mg/mL; Nitta Gelatin Inc., Osaka, Japan) was diluted 10-fold in HCl (1 mM; pH 3), spread on the glass bottom of each well of the 96-well plate, and incubated for 60 min at room temperature for collagen coating. The wells were then washed twice with milliQ water and left to dry. Subsequently, BAM powder (Sunbright OE-040-CS; NOF corporation, Tokyo, Japan) was dissolved in dimethyl sulfoxide (DMSO) (Fujifilm Wako, Osaka, Japan) to obtain a 10 mM solution. This concentrated BAM solution was diluted with phosphate buffered saline (PBS(-): Fujifilm Wako) to a concentration of 100 µM in 1% DMSO. The BAM solution was added to each well of the collagen-coated 96-well plate and incubated for 60 min at 37 ºC. The 96-well plate was then washed once with PBS(-) and twice with MilliQ water before drying.

4.6 Ligand binding assay

OR-expressing cells were diluted into modified artificial cerebrospinal fluid buffer (aCSF: NaCl 125 mM; KCl 2.5 mM; Na_2HPO_4 1.25 mM; HEPES 10 mM; $CaCl_2$ 4.5 mM; pH 7.4) at a concentration of $1x10^7$ cells/mL and applied to each well of the BAM-coated 96-well plate as described above (4.5) for 60 min at room temperature to achieve cell attachment. Once cells adhered to the bottom of the wells, aCSF buffer with remaining floating cells was removed and replaced by fresh aCSF buffer for the ligand binding assay.

Ionomycin (> 95% purity; Fujifilm Wako, Osaka, Japan) was used as an ionophore to artificially increase intracellular calcium concentration and evaluate the GCaMP6f fluorescence response. VUAA1 (>98% purity; Sigma-Aldrich, Tokyo, Japan) was used as the DmOrco co-receptor agonist. The ligand pentyl acetate (>97% purity; Fujifilm Wako) was used to induce the responses of DmOr47a. These chemicals were first dissolved in dimethyl sulfoxide (DMSO; Fujifilm Wako) and then diluted in modified aCSF buffer at the following final concentrations: DMSO 1%, ionomycin 100 µM, VUAA1 1mM, and pentyl acetate 2 mM. To evaluate the Or47a ligand dose-response, the pentyl acetate was diluted to final concentrations of 2 µM, 20 µM, 200 µM and 2 mM in modified aCSF buffer, 1% DMSO. Fifty µL of these ligand solutions were added to the 50 µL aCSF buffer covering the attached cells and mixed gently so that the cells were

exposed to a final ligand concentration diluted by one half.

Image acquisition was performed with an Axio Observer 7 fluorescence inverted microscope (Carl Zeiss, Oberkochen, Germany) equipped with a 10x objective (EC PlnN 10x/0.3 PhI DICI, Carl Zeiss) and a high-resolution camera (Axiocam 506 mono, Carl Zeiss), using Zen 3.2 (Zen pro) software. GCaMP6f fluorescence excitation was obtained with an LED at 475nm through 38 HE Green filter set (EX BP 470/40, BS FT 495, EM BP 525/50). Nine frames, one taken every 10 s, were acquired at an exposure of 400 ms for a Region of Interest (ROI) of 2048 x 2048 pixels with resolution binning of 2x2. The first 3 frames corresponded to the baseline and the remaining 6 frames were acquired after addition of the ligand. These time lapse pictures were analyzed with Fiji (ImageJ v.2.1.0) software [40], measuring the fluorescence intensities of the whole ROI or alternatively of individual cells for Figs. 2A, 2B and S7. The time series of fluorescence intensity change ($\Delta F/F_0$) was calculated as follows: $\Delta F/F_0 = (F_{max}-F_0)/F_0$, where F_0 is the fluorescence intensity of the frame preceding ligand addition and F_{max} is the maximal fluorescence intensity measured during the 6 frames following ligand addition.

4.7 Evaluation of cell viability after desiccation, dry storage, and rehydration

Pv11 cells and Pv11-00443-Or47a cells were desiccated following the protocol described previously [20,28]. Cells were first treated with a trehalose mixture (9 volumes of 600 mM trehalose for 1 volume of supplemented IPL-41 culture medium) at a concentration of $2x10^7$ cells/mL and incubated for 48 h at 25 ºC. After trehalose treatment, cells were centrifuged at 300 g for 5 min and recovered into fresh trehalose mixture at a concentration of $1x10^8$ cells/mL. A drop of 40 µL of these cells in fresh trehalose mixture was deposited at the center of a tissue culture dish (BD Falcon, 353001). Twenty of these dishes, covered with their lids but unsealed, were transferred to a plastic desiccator (250 x 250 x 250 mm, AsOne, UD-1) containing 1 kg of silica gel (Toyota Kako Co., Ltd., Toyota Silica Gel) to reach a relative humidity <10% at 25 ºC. Cells were almost completely desiccated after 36 h. The cells were kept in the desiccator for 14 days.

Cells were rehydrated by adding 1 mL of supplemented IPL-41 medium to the tissue culture dish with dried cells and incubating at 25 ºC. One hour after rehydration, cells were double-stained with Hoechst 33342 (Hoechst; Dojindo, Kumamoto, Japan) at a final concentration of 2 µg/mL and with propidium iodide (PI; Dojindo, Kumamoto, Japan) at a final concentration of 0.75 µg/mL in supplemented ILP-41 culture medium.

Image acquisition was performed with an all-in-one fluorescence microscope (BZ-X710, Keyence). The excitation/emission wavelengths were 544 nm / 605 nm and 405

nm / 460 nm for PI and Hoechst, respectively. The number of dead cells (PI-positive cells) was subtracted from the total number of cells (Hoechst-positive cells) to obtain the number of live cells. Cell viability was expressed as a percentage of live cells, relative to the total number. For the ligand binding assay of rehydrated cells, the cells taken 1 h or 24 h after rehydration were collected by centrifugation at 300g for 5 min and recovered into aCSF buffer. Cell attachment and ligand binding assays were performed as described above.

4.8 Assessment of protein dry preservation with a translation inhibitor

To examine if the ligand response observed after rehydration was due to dry-preserved proteins, i.e. proteins produced before desiccation that were preserved during the drying and rehydration process, or to *de novo* synthesized proteins, we used cycloheximide (CHX, Sigma-Aldrich, St Louis, MO), a translation inhibitor for eukaryotic cells that interferes with elongation in protein synthesis. Dried Pv11-00443-Or47a cells were rehydrated with 1 mL of complete ILP-41 medium containing CHX at 0.35 mM, an optimal concentration determined previously [22]. Cells were collected 1 h or 24 h after rehydration and subjected to the ligand binding assay as described above, except that cells were recovered into aCSF buffer containing CHX 0.35 mM for cell attachment.

4.9 Statistics

GraphPad Prism 8 software (GraphPad, San Diego, CA) was used for statistical analyses. Differences between ligand samples and solvent control (DMSO 0.5%) were examined for statistical significance by one-way ANOVA corrected with post-hoc Dunnett test. In paired experiments (Fig. 4A), statistical differences were calculated with Student's *t* test. Dose-response fit curves were obtained by nonlinear regression with the equation log(agonist) vs. response (three parameters) to determine bottom and top responses and EC_{50}.

Data availability statement

The DNA sequences generated during the current study are available in the NCBI repository with accession numbers PV368676, PV368677, PV368678, PV368679, PV368680, PV368681, PV368682, PV368683, and PV368684.

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Author contributions

The authors confirm contribution to the paper as follows: study conception and design: RC, TK, HF, YM, ST; data collection: HF, RC, SS, RA, RH, ES; analysis and interpretation of results: HF, RC, RA, RCA, ES; draft manuscript preparation: HF, RC, ES, PA, RCA. Author. All authors reviewed the results and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Figure Legends

Figure 1: Generation of the Pv11-00443-Or47a stable cell line. **(A)** Strategy for knocking-in the genes of interest into the Pv11 genome. The CRISPR-Cas9 system was

used to generate double strand breaks (red thunder marks) downstream of the stop codon of Pv.00443 gene, which is regulated by the endogenous strong 121 promoter (on the left) as well as on both sides of donor vectors containing either GCaMP6f and DmOrco genes or zeocin resistance and DmOr47a genes (on the right). As a result, GCaMP6f, DmOrco, zeocin resistance and DmOr47a genes were integrated into the Pv11 genome downstream of the Pv.00443 gene, in a polycistronic manner (on the bottom). Red arrowheads indicate the positions of primers for genomic PCR. **(B)** Genomic PCR of Pv11 control cells (Pv11) and Pv11-00443-Or47a cell line (Or47a) showing specific bands for the 3'-end of the Pv.00443 gene (619 bp), for the GCaMP6f-DmOrco insert (2,281 bp), and for the zeocin resistance-DmOr47a insert (3,562 bp). The original electrophoresis gel picture is available in Fig. S3. **(C)** Viability of the Pv11-00443-Or47a cell line compared to that of Pv11 control cells after dry storage, assessed 1h after rehydration. **(D)** Ligand response expressed as change in fluorescence intensity ($\Delta F/F_0$) for Pv11-00443-Or47a cells in response to DMSO 0.5% as a control, pentyl acetate 1 mM, VUAA1 500 µM, and ionomycin 50 µM. Actual images of GCaMP6f fluorescence and phase contrast are shown on the right before and after exposure to pentyl acetate 1 mM. Scale bars represent 30 µm. Bar graphs show the mean of 4–14 replicates ± SD. Significant differences compared to DMSO 0.5% control (one-way ANOVA followed by Dunnett test) are expressed as *** (p-value < 0.001) and **** (p-value < 0.0001).

Figure 2: Response of the stable cell line Pv11-00443-Or47a to pentyl acetate before dry storage (A, B) and 24 h after rehydration (C, D). Ligand responses are expressed as a change in fluorescence ($\Delta F/F_0$) in response to the DMSO 0.5% control and to the ligand pentyl acetate at different concentrations from 1 µM to 1 mM. **(A)** Traces of individual cells responses to DMSO 0.5% control and pentyl acetate 1 µM, 10 µM, 100 µM. The arrow shows the timing of ligand addition. **(B)** Dose-response curve fitted to a nonlinear regression curve. **(C)** Traces of individual cells responses to DMSO 0.5% control and pentyl acetate 1 µM, 10 µM, 100 µM, 24 h after rehydration. The arrow shows the timing of ligand addition. **(D)** Dose-response curve 24 h after rehydration fitted to a nonlinear regression curve. Response traces represent the mean of 15 individual cells randomly selected ± SEM (A, C). On the dose-response curves (B, D), significant differences compared to DMSO 0.5% control (one-way ANOVA followed by Dunnett test) are expressed as **** (p-value < 0.0001) and ns (not significant). Top and bottom responses are expressed as $\Delta F/F_0$ and the EC_{50} is shown with a 95% CI.

Figure 3: Ligand responses expressed as a change in fluorescence intensity ($\Delta F/F_0$) for dry-preserved and rehydrated Pv11-00443-Or47a cells. (A) Ligand responses 1 h after rehydration in culture medium (CHX -). (B) Ligand responses 1 h after the rehydration in culture medium containing the translation inhibitor cycloheximide at 0.35 mM (CHX +). (C) Ligand response 24 h after rehydration in culture medium. (D) Ligand response 24 h after the rehydration in medium containing the translation inhibitor cycloheximide (0.35 mM). Cells were exposed to DMSO 0.5% as a control, pentyl acetate 1 mM, VUAA1 500 µM, or ionomycin 50 µM. Bars represent the mean of 4–16 replicates ± SD. Significant differences compared to DMSO 0.5% control (one-way ANOVA followed by Dunnett test) are expressed as **** (p-value < 0.0001), *** (p-value < 0.001) or ns (not significant).

Figure 4: Monitoring ligand responses and cell growth after the rehydration of dry Pv11-00443-Or47a cells. **(A)** Rehydrated Pv11-00443-Or47a cells were exposed to the solvent DMSO 0.5% (white bars) as a control or to a solution of 1 mM pentyl acetate (grey bars). Ligand responses were expressed as a change in fluorescence intensity ($\Delta F/F_0$). Bars represent the mean of 3–6 replicates ± SD. Significant differences were expressed in comparison to the DMSO 0.5% control (*t*-tests) **(B)** Cell growth monitored for one week (1-168 h) after rehydration. The concentration of live cells is expressed as a mean ± SD (n=12). Significant differences compared to the cell concentration just after rehydration (1 h) are shown over the growth curve (one-way ANOVA followed by Dunnett test). The significant differences are expressed as **** (*p*-value < 0.0001); *** (*p*-value < 0.001); ** (*p*-value < 0.01); * (*p*-value < 0.05); ns (not significant).