# Chemo Sense

## ChemoSensory Science Noses Ahead

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**In the past decade** since the tumult and tragedy of 9/11, science has not stood still.

Chemosensory receptor technology which began in the 1990s is pressing ahead and new forms of database are needed to keep track of information growth. One of these is presented by Daniel Münch and Giovanni Galizia in this issue. Their freely accessible web-based information system can provide new insights into receptor-odorant functionality, through inter-linkage of information, obtained separately from any number of independent researchers. The database is a new tool for discovery. We pay tribute to the inclusive generosity of its inventors.

# DoOR: The Database of Odorant Responses

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**Both olfactory systems of animals,** or artificial noses, can increase the capacity of the system exponentially by combining many sensors with overlapping response profiles, thanks to the power of combinatorial coding. Studying animal systems helps to understand the underlying mechanism, and knowledge of odor-response profiles, in this pursuit, is of paramount importance. We present here a new approach towards this goal: DoOR, the database of odorant responses, including a freely available access to it on the Internet.

## THE COMBINATORIAL NATURE OF OLFACTORY CODING

The chemical environment provides the most complex sensory cues all living organisms on earth have to deal with, being composed of an uncountable number of different molecules. Many kinds of chemosensory systems have evolved and all organisms use some of them, but the most elaborate one is olfaction. This sense is amazingly efficient and enables organisms to orient and communicate within this incredibly complex chemical world.

Many technologies deal with detection or analysis of chemicals, but real olfactory systems

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Sweet treat

Eureka Moment

## ChemoSensory Science Noses Ahead

### continued

Australians lay claim to the discovery of, and national addiction to a white, sugary, fruit and cream-lashed tart, called Pavlova. The release of digitised back numbers of Sydney newspapers, spanning centuries, provides new resources on the historical voracity of such claims. While helping correct errors in the digitisation process, Robert Marks made interesting insights on the origin, recipe and name of this popular dessert. Whether in New Zealand or Australia, Pavlova performs gustatory ballet on your sensory perception.

ChemoSense congratulates Alan Mackay-Sim on his award of a Eureka Prize.

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# DoOR: The Database of Odorant Responses

### continued

still appear to be the most powerful.. We may think of a dog searching for drugs, dangerous substances or missing people, or of a winemaker or whisky distiller creating a special flavor. Indeed, even though it is much easier to describe a color or a tone as compared to a complex odor, and even though our vocabulary for odors quickly reaches its limits and we have to borrow words like "dense", "green" or "robust" from other contexts, we are still able to recognize and differentiate amazing numbers of complex odors..

Humans have around350 types of olfactory receptors (ORs), dogs over 1000. Even a rather simple olfactory system like that of the fruit fly *Drosophila melanogaster*, with about60 OR types, is able to detect and differentiate hundreds or thousands of different odors. Olfactory receptor neurons (ORNs) always express a single OR, so that 60 OR types in a species corresponds to 60 ORN types. The huge coding capability of countless different cues by only a few sensors arises from the combinatorial code created across ORN types. However, in order to decipher the code, it is necessary to know not only the best ligand of each ORN type (i.e. to "deorphan" it), but ideally its entire response spectrum across many substances: in other words, we need to know what is called the "olfactome".

### THE WAY TOWARDS THE OLFACTOME

It follows that "deorphaning" ORs (or ORNs) is a major challenge in olfactory research: very few ORs have had that honor yet. The animal species for which the best data is available is the fruit fly. Many labs around the world decided to use the fruit fly for olfactory experiments because these animals are easily modified genetically. Since the *Drosophila* OR

genes were published in 1999 (Clyne et al. 1999; Gao et al. 1999; Vosshall et al. 1999), responses of hundreds of ligand/OR combinations have been measured, with each data set contributing a small part to the *Drosophila* "olfactome" (e.g. de Bruyne et al. 2001; Hallem et al. 2006; Pelz et al. 2006). However, assembling these data remained a challenge.

### OUR APPROACH TOWARDS ASSEMBLING THE OLFACTOME

With so many data sets of olfactory responses in *Drosophila*, a notable part of the olfactome is known, and one would think that we are already close to the full picture. But far from it: these data are very heterogeneous and cannot be compared easily. Different labs use different techniques, based on slightly different research questions: ORN responses can be recorded *in situ* using electrophysiology (de Bruyne et al. 2001), or in transgenic animals, where the OR of interest is expressed ectopically (Hallem et al. 2006). ORN responses can also be recorded *in situ* as changes in intracellular calcium (Pelz et al. 2006). ORs can be expressed in cell culture, and their responses to odors measured using either electrophysiology or calcium imaging (Kiely et al. 2007). Additional heterogeneity comes from different approaches: some labs record odor responses to single concentrations, other to full dose response curves, odor pulse length and/or temporal dynamics vary across labs, etc. Nevertheless, one rule should always be fulfilled (give or take experimental variability): a better ligand gives a stronger response, irrespective of the technique or the lab involved.

Using this notion, we decided to develop a technique that allows merging all available data into a consensus database,

# DoOR: The Database of Odorant Responses

**continued**

the Database of Olfactory Responses – DoOR (Galizia et al. 2010).

## MERGING HETEROGENEOUS DATA

Merging data is performed stepwise, whereby in each step we integrate two data sets from different studies of the same OR into a new merged data set, and iteratively repeat the procedure with the data from the next study until all existing responses for a given OR are used. We use the following strategy. First, the odorant responses (e.g. spike counts, fluorescence change, half-optimal concentrations) are scaled from zero to one within each study. Then, they are plotted against each other (Fig. 1). Five monotonic functions (linear, exponential, sigmoid, asymptotic, and asymptotic with an offset) are fitted to the overlapping data points (i.e. the odorants measured in both studies). Next, we select the function with the best fit. As the function describes the relationship of the two studies only within the range of overlapping measurements, extrapolation beyond that range follows a linear identity relationship. All measurements are then projected orthogonally onto the function, producing a new, merged data set containing more odors than each of the parent data sets. This data set is again scaled from zero to one according to the position of the responses along the curve, and the merging now continues iteratively until all data sets are used. This way, in the end all the data sets describing a given OR/ORN end up in one consensus data set, scaled from 1 (best ligand) to 0 (either no response, or corresponding to an antagonistic, inhibitory response).

Merging of two data sets is only performed when they overlap at least by four odors and the fit performance is good. The sequence of merging data set

pairs affects the outcome: suitable methods were developed to select the best sequence (Galizia et al. 2010). As a result we obtain a database that combines all currently available information about odor responses in the fruit fly. This database is open access, and can be used interactively or as a downloaded package. In the following, we present how to use the database, and show a few study cases where such a tool can be put to good use. Most importantly, such a database can be evaluated from two points of view that are orthogonal to each other: for a given receptor, it is possible to obtain all available responses as an odor-response profile, or for a given odor it is possible to obtain the combinatorial odor-response pattern across all receptors.



Figure 1: *Merging process for data sets of receptor Or22a. Best fitting function is given above each plot, black circles are odorants measured in both data sets, their number is indicated as n, MD = mean distance, blue and yellow lines are projections of odors present in only one study.*

## USING DoOR: HOMEPAGE

The interactive mode is the fastest: any particular OR response profile or odor-response pattern is available as the newest DoOR data in form of tables and figures on the DoOR homepage

(http://neuro.uni-konstanz.de/DoOR). In addition, we list chemical information on the odor molecules, data on each given receptor, and all the studies used that deal with a given receptor or odorant together with links to corresponding external resources from PubChem (http://pubchem.ncbi.nlm.nih.gov/) or FlyBase (http://www.flybase.org).

The example in Fig. 2a shows the response to the odor 2-heptanone (a substance that smells somewhat like glue; the structure is shown in figure 2c). The response is shown as a *virtual* activity pattern across olfactory glomeruli in the antennal lobe of the fruit fly (the antennal lobe forms a spatial mapping of all olfactory receptors in the fly brain, akin to what happens in the human olfactory bulb), with the four frames
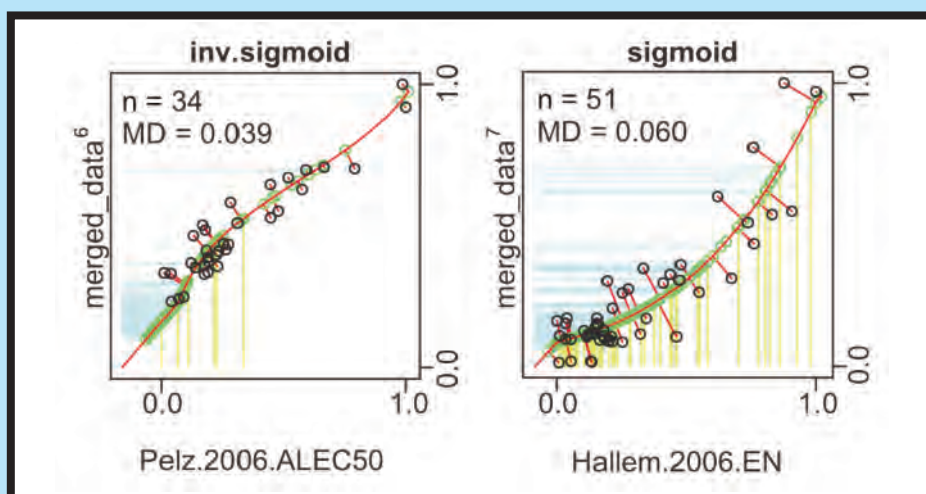
showing four sections going deeper into the brain. The odor elicits strong activity in some glomeruli (red), an inhibition in others (blue) and no activity in the rest (white). The responses for some glomeruli are as yet not known (gray),

# DoOR: The Database of Odorant Responses

and for some glomeruli not even the receptor innervation is known (black). Receptor responses can be shown as a histogram (Fig. 2b), with glomeruli/receptors sorted so that the strongest response is in the center, and weak or inhibitory responses to the sides. Such histograms are useful to describe overall response properties, e.g. odors that activate only a few receptors (with a pointed shape) or those that activate many receptors (with a flat shape). In this case, the histogram is moderately broad, with two inhibited glomeruli. Because the antennal lobe is a three-dimensional structure in the brain, we also mapped the responses onto a 3-D model (Fig. 3). On the homepage, this model is interactive, and can be rotated by the user in order to see the response from whatever angle is needed.
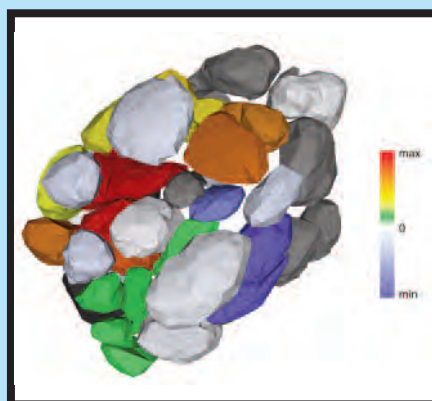


Figure 3: *Responses to 2-heptanone mapped onto 3-D model of the antennal lobe.*

## DoOR-PACKAGE

If you are interested in more detailed data and tools for further calculations you can download the complete Database of Odorant Responses

(http://neuro.uni-konstanz.de/DoOR). DoOR is written in Gnu-R (R Development Core Team 2011), a statistical programming language which is available for free from http://www.R-project.org. The DoOR download contains all available data, and all programs needed to compute the consensus database. Furthermore, several tools for plotting data, performing various calculations on it, and tools for adding data sets are included.

The DoOR package consists of two parts, one containing all the functions and one containing all the data. The advantage of this separation is that updating the data is independent from updating the functions. Importantly, with each update, all previous versions of the database (both functions and data tables) remain as downloadable links, and therefore stay available as fully referable snapshots.

## USING THE DoOR

### Accounting for negative responses

A feature of ORNs is that they do not only respond with excitation but that some ligand/receptor combinations lead to an inhibition of that cell. The DoOR data consists of values scaled from 0 to 1 after merging, not yet accounting for negative responses. Therefore, during the merging process, we treat the spontaneous firing rate of a receptor (SFR, the firing rate in absence of any stimulus) as a normal odorant. We then set the SFR to zero, so that all the values lower than SFR become negative. The range SFR – maximum is rescaled from zero to one and the inhibitory responses are as large as dictated by the linear remapping.

### Global scaling

The most important feature of an olfactory response to an odorant is the
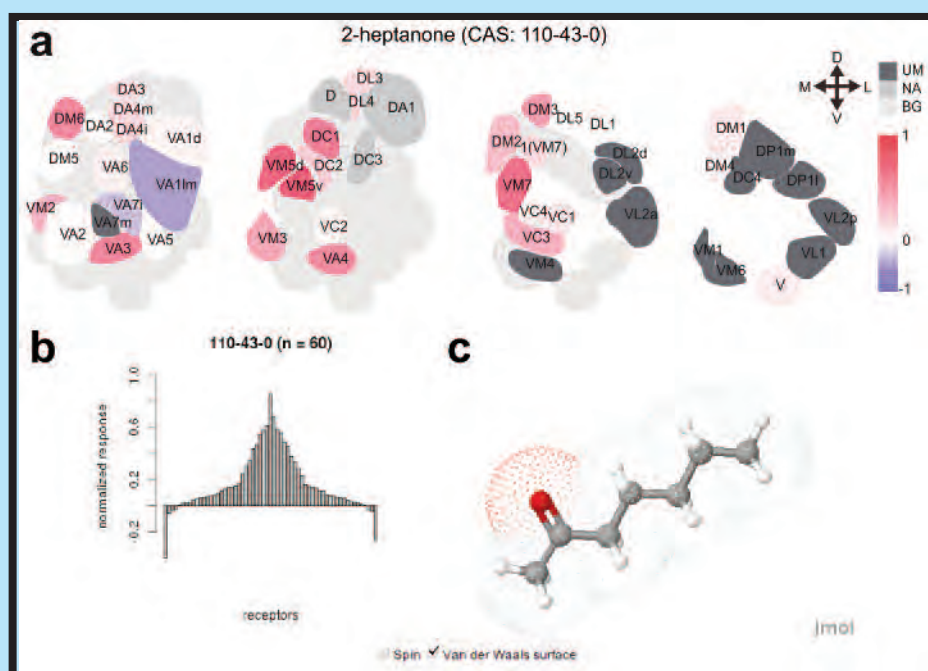


Figure 2: *Odorant profile of 2-heptanone as found on the DoOR homepage. a: The DoOR data for 2-heptanone visualized as a false color coded representation of the antennal lobe. UM: OR for these glomeruli is still unknown; NA: no data available for 2-heptanone in this glomerulus; BG: background, glomeruli from deeper layers. b: Odorant response histogram for 2-heptanone. c: 3-D model of 2-heptanone.*

# DoOR: The Database of Odorant Responses
**continued**

combinatorial code represented by the responses across all receptors. In order to reveal the combinatorial features of the DoOR, the data sets that have been computed for each receptor have to be rescaled relative to each other. Thus, we recalculate the responses using inter-receptor information gained from studies that measured more than one receptor. This information is important for a correct interpretation of the data. For example, in Fig. 4 (top), the odor-responses of the two receptors Or65a (left) and Or67a (right) are shown as histograms (again in center-lateral order, with best responses at the center, and increasingly weak responses to the sides). Or65a has a pointed shape (few good ligands) with many inhibitory responses (the two downwards pointing tails), while Or67a has a broad response profile (many intermediate ligands with varying affinity) and no negative responses. However, when scaling is corrected for differences across receptors (Fig. 4, bottom), it is apparent that the picture for Or65a changes: even the best known ligand is a very weak ligand, eliciting a weak response. Similarly, the inhibitory responses are weak. This result has two possible interpretations: either
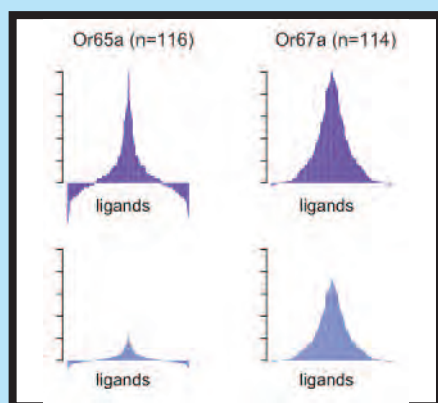


Figure 4: *Receptor tuning plots before (top) and after (bottom) global normalization for the two ORs Or65a (left) and Or67a (right).*

the best ligand for this receptor is not known (meaning that more odor-response screening is needed), or this is indeed a very weak receptor (which would have far-reaching implication for how the brain extracts information from combinatorial response patterns).

## Back projection

When working with a particular technique, 0-1 information in the DoOR database is useful, but abstract and different from the values in the physiological data (e.g. spikes, $Ca^{2+}$ increase). For this reason, we included a tool for back projection of the consensus data onto the value-space of any of the input data types. This tool uses the same algorithms that are used for merging the single data sets, but calculates in reverse, transforming the 0-1 data to the unit of interest.

## Identifying ORs from an orphan response profile

Another tool is useful when the mapping of OR and ORN is not yet known. In these cases you may have an orphan response profile and want to know to which OR/ORN it belongs. One example is receptor Or13a which we were measuring using $Ca^{2+}$ imaging. The $Ca^{2+}$ reporter was expressed under control of the Or13a promoter, but the corresponding ORN was not known by then.

In *Drosophila*, each OR gene (e.g. Or22a, a name related to its position in the genome) is expressed in a particular population of receptor neurons (e.g. ab3A, for "cell **A** in the **a**ntennal **b**asiconic sensillum number **3**"), and these receptor neurons send their axons to a particular glomerulus (e.g. DM2 for dorso-medial glomerulus number 2). This isomorphism is also the reason why

activity patterns across receptor types can be shown as patterns across glomeruli (see above). The specific mapping of gene-receptor-glomerulus (e.g. Or22a -> ab3A -> DM2) is not yet known in all cases. Therefore where data sets are available for a particular neuron type (e.g. ab6A), they have been included as such when the corresponding OR is not known.

The program performs fits between the orphan data set and all the data in DoOR and thereby finds the best matching partner. In our case Or13a had the best match to ab6A, a receptor neuron that had been characterized before but for which the expressed receptor was not yet known (de Bruyne et al. 2001; Galizia et al. 2010). We confirmed this result by mapping another data set from a recent study measuring Or13a (Kreher et al. 2008) (Figure 5).

## OUTLOOK

The DoOR project is a first step towards the complete olfactome, and is intended as a service to the broader scientific community, not only Drosophila researchers.. Clearly, the database does yet completely solve the "olfactome" problem: information about odor-concentration is not yet included, because too few reported data sets contain sufficiently detailed information. Similarly, odor mixtures are not covered, and natural odors (e.g. plant extracts) are also missing. DoOR is, however, the most complete and most comprehensive step towards the olfactome currently available.

We will keep DoOR up to date and upload new versions as soon as new olfactory data is published. Any additional data set is welcome and will be added: the more data is fed into the database, the more reliable and complete
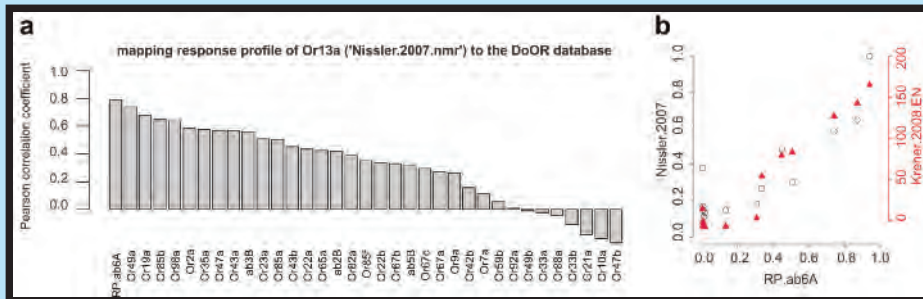
# DoOR: The Database of Odorant Responses

Figure 5: *Mapping response profiles to ORs. a: Pearson's correlation coefficient of the Or13a response profile to the consensus responses of all ORs existing in DoOR, the best match was found for RP.ab6A. b: Plotting the data from the known receptor gene (left ordinate: $Ca^{2+}$ imaging; right ordinate: electrophysiological recording) against the measured response profile of receptor neuron ab6A (abscissa).*

the overall result. While the current data only covers the fruit fly, algorithms and the program package can be immediately ported to any other species (or even to a set of artificial odor sensors). Whenever enough information is available, a new DoOR to other olfactomes can be opened ∎

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# Pavlova

*by Robert Marks*

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**Whence the pavlova?** There has long been disagreement between Aussies and Kiwis about the origin of the sweet meringue cake, called the pav after the Russian danseuse, Anna Pavlova, who visited the Antipodes in 1926 and again in 1929. In 1931 she died of pleurisy, aged 49, in Amsterdam.

Recently, the National Library of Australia, in its Trove archive, has been scanning and digitising Australian newspapers from 1803 to 1955 (after which copyright is retained by the newspaper companies). In an inspired move, the NLA allows the converted output, often garbled thanks to dodgy printing, to be corrected by anyone on-line. It occurred to me to seek the earliest reference to the pavlova cake in their newspaper archive. Although this research is unlikely to resolve the controversy, it might shed light on the evolution of the meringue cake into the pavlova.

Can we agree that a pavlova is an open meringue cake, in which the meringue in the centre is not brittle (by the use of cornflour), and which is decorated with cream and fruit? We could further ask when the passionfruit had become associated with such meringue cakes, and when the name "pavlova" first appeared for these desserts.

There are several early recipes for meringue cakes that are recognisable antecedents of today's pav. The earliest I found was a recipe for "Lemon Meringue Pudding" of 29 July 1893 in the Rockhampton *Capricornian*, and there were a handful of recipes for cakes topped with meringue before 1934.

The *Australian Women's Weekly* of Saturday, 25 August 1934, page 33, published an feature entitled, "Meringue Dishes," by Margaret Shepherd, which includes seven recipes, but nary a mention of the word, "pavlova," which suggests that the name was not in wide use for meringue dishes in mid-1934 Australia.

Among the seven recipes is one that is significant. It is for an open "Meringue Cake," and introduces passionfruit to the meringue, as well as cornflour (and coffee essence!), but not vinegar (although the feature notes that "About three drops of lemon juice or vinegar whitens the [egg white] mixture, and improves the flavour"):

> Six tablespoons castor sugar, 2 egg whites, 1 tablespoon cornflour, coffee essence. Mix castor sugar and cornflour together. Add to the stiffly beaten egg whites gradually, beating all the time, add one teaspoon coffee essence. Beat well. Put into a shallow, well-buttered cake tin, cook 60 minutes in a slow oven. Lift on to a plate and cover just before serving with whipped cream and passionfruit mixed together, or whipped cream flavored with rum.

Three years later the first reference to a "Pavlova Cake Sweet" (were there savoury pavlovas?) appears, in the *Australian Women Weekly* of Saturday, 19 July 1937, page 39S. This recipe is an open cake, and includes both cornflour and vinegar, a modern pavlova:

> Whites of 4 eggs, 8 oz. castor sugar, 1 dessertspoon cornflour, 2 teaspoons vinegar, strawberries, or fruit in season, whipped cream, pistachio nuts or chopped walnuts. Stiffly beat whites of eggs. Add sugar gradually and beat well, or until stiff and frothy. Fold sifted cornflour in lightly and add the vinegar. Place mixture into an 8 in. sandwich tin which has been well greased and lightly dredged with cornflour. Bake in slow oven for 1¼ hours. Decorate with whipped cream, strawberries and nuts. Serve as cold summer sweet.

A year later the first "Pavlova cake" recipe with passionfruit (but without cornflour) appears, in the *Perth West Australian* of 11 November 1938. So perhaps the *passionfruit* pavlova comes from Perth after all.

# 13th Scientific Meeting of the Australasian Association for ChemoSensory Science (AACSS)

## 7-9 December 2011
### Ascension Wine Estate, Matakana, New Zealand
(*one hour's drive north of Auckland*)

## Book Now!

The 13th meeting of the AACSS will be held north of Auckland in wonderful Matakana close to wineries and beautiful beaches. The program will cover all aspects of chemosensory science in both invertebrate and mammalian systems.  Please lock in those dates.

Please watch the AACSS website for more information including the programme,  invited speakers, registration and abstract submission dates and details of accommodation:    http://www.aacss.org/

Any queries regarding the meeting can be directed to Richard Newcomb,
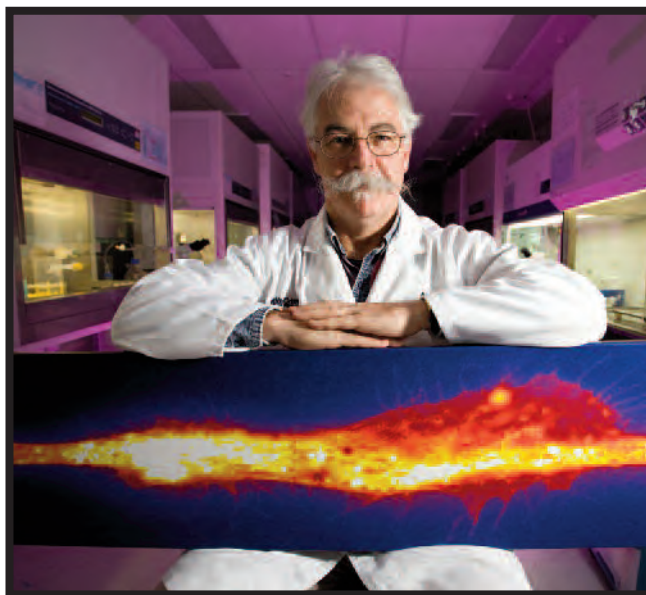Richard.Newcomb@plantandfood.co.nz

# NEWS

# Eureka Moment for
# Olfactory Cell Researcher

**The promise of a new therapy** to treat spinal cord injury, using olfactory cells, won the most online votes and a share of the $240,000 Eureka Prize for Alan Mackay-Simm of Griffith University's Eskitis Institute for Cell and Molecular Therapies. The award was made at a gala dinner in Sydney on September 6.

Alan's research involves taking an individual's own cells from the nose, growing them in the lab and transplanting them into the injured spinal cord. The nose is the source of cells which continuously regenerate from a pool of adult "stem cells". This means that the person provides his own cells to do the repair work and neither another adult, nor an embryo has to donate cells.

Ethical issues that have bedevilled embryonic stem cell research and risk of transplant rejection are neatly circumvented by Alan's approach. It has appeal to both the public and members of the scientific community who voted in greatest numbers for Alan to win the prestigious Australian Museum's Eureka People's Choice Award ■



Imagination, excitement and discovery: Alan Mackay-Simm wins a 2011 Eureka Prize

# Upcoming Events

| | |
|---|---|
| 12-16 November 2011 | **Neuroscience 2011**<br>Washington DC, USA<br>www.sfn.org/am2011/ |
| 7-9 December 2011 | **Australasian Association for ChemoSensory Science (AACSS)**<br>Annual Scientific Meeting<br>Matakana, Auckland, New Zealand<br>www.aacss.org |
| 29 January – 1 February 2012 | **Australian Neuroscience Society (ANS)**<br>Annual Conference<br>Gold Coast, Queensland |
| 7-9 March 2012 | **EcoForum**<br>Conference and Exhibition<br>Australian Technology Park, Sydney<br>www.ecoforum.net.au |

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