Identification of Molecular Properties Coding Areas in Rat's Olfactory Bulb by Rank Products

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Abstract: Neural coding of chemical information is still under strong debate. It is clear that, in vertebrates, neural representation in the olfactory bulb is a key for understanding a putative odour code. To explore this code, in this work we have studied a public dataset of radio images of 2-Deoxyglucose uptake (2-DG) in the olfactory bulb of rats in response to diverse odorants using univariate pixel selection algorithms: rank-products and Mann-Whitney U (MWU) test. Initial results indicate that some chemical properties of odorants preferentially activate certain areas of the rat olfactory bulb. While non-parametric test (MWU) has difficulties to detect these regions, rank-product provides a higher power of detection.

1 INTRODUCTION

Olfaction is the main chemical sense and it is key for basic animal survival since it determines food intake, sexual mating among others basic functions. It is known that humans are able to differentiate thousands of low molecular mass, typically organic compounds. This sense is the least studied and, it is not known how olfaction encodes chemical information about the odorants yet. While due to the advances in genomics, we know today the family of G-coupled protein receptors in sequenced species, the affinity those receptors have for the huge number of putative ligands is only barely known (Hallem and Carlson, 2006); (Mori et al., 2006). However, it is well known that chemical information is coded in a combinatorial way and that OR are only partially selective. That is, a single odorant may excite many diverse OR and an OR responds to a set of ligands with diverse affinity levels (Malnic et al., 1999).

The current dogma of olfaction is that olfactory sensory neurons (OSN) express a single type of olfactory receptor (OR) and that sensory neurons expressing the same receptor converge to the same glomerolous. The system is characterized by a large degree of convergence where millions of OSN converge to few thousands of glomeruli. In the rat about 1200 OR are supposed to be active for a total number of glomeruli of about 2400 (Johnson and Leon, 2007); (Meister and Bonhoeffer, 2001).

Since each glomerulus only receives inputs from OSN featuring the same OR receptor, it has been argued that glomerular activity maps could be a convenient way to explore how chemical information is encoded in the olfactory bulb. In this sense, Leon and Johnson (LJ), in a long and persistent effort, have acquired 2-DG uptake radio images for a large number of diverse odorants (Leon and Johnson, 1999). In this work, we attempt to determine if certain molecular properties excite particular areas or modules within the olfactory bulb. This hipothesis has been formulated by Leon and Johnson, but without backing statistical analysis of the available data. This Leon and Johnson dataset has been previously analyzed by the authors for clustering (Falasconi et al., 2012), coding capacity (Fonollosa et al., 2012) and properties coding (Auffarth et al., 2011). In this work, we go deeper in this last point using a non-parametric hypothesis testing and newer state of the art feature selection methods originally proposed for Microarray data analysis.

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2 MATERIALS AND METHODS

2.1 Activity Maps and Molecular Features Datasets

In order to carry out this study we employed the olfactory bulb (OB) activity dataset obtained by the group of Leon & Johnson at the University of California in Irvine. They captured OB activity in response to a large set of odorants with diverse chemical structures. This activity was measured crossways the complete glomerular layer of the rat OB and mapped using uptake of [14C]-radio labeled 2-deoxyglucose (2DG) (Leon and Johnson, 2003). A remarkable advantage of this technique is that it allows observation of the complete olfactory bulb, but the main drawback is that one can examine the image for just one odor at one concentration per experimental animal (Johnson and Leon, 2007).

Examples of glomerular activity maps are shown in figure 1 for two selected odorants. It is important to realize that these glomerular maps are not the result of direct imaging of the olfactory bulb, but synthetic images built from a series of autoradiographies of sections of the OB. The exact imaging procedure is described in detail by Johnson. (Johnson et al., 1999). For the sake of completeness here we present a short summary of the process to build these images. For more details, please refer to the original publication.

The OB was cut perpendicularly to the long axis and every sixth 20µm section was used to autoradiography. The original autoradiographies were digitized at 108 pixels/mm achieving glomerular resolution.

A main objective of the image formation process is to align the images in order to standardize the anatomical differences from animal to animal. On the one hand, three anatomical landmarks were used to standardize rostral–caudal distances between bulbs: the first cresyl violet–stained section that possessed an external plexiform layer, the first section that contained an accessory olfactory bulb and the last section that contained a mitral cell layer on its medial aspect. Using a total of 78 animals they found that the average bulb measures 3.0 mm (25 sections) from the first external plexiform layer to the first accessory bulb and 2.28 mm (19 sections) from here to the last mitral cell section. These 44 sections correspond to the columns of the image. On the other hand, they created a standard grid for y axis of the image with 80 pixels by row. The image has 80 active pixels in the section with the largest glomerular layer. For the other sections, the number of active pixels is reduced in such a way that each pixel corresponds to the mean activity in circular areas of about 120µm of diameter. Due to these image formation procedures, the resulting maps are aligned to anatomical landmarks and do not require further alignment.

The data across five rats exposed to the same stimulus was averaged to obtain the two-dimensional (80x44 pixels) activity maps reducing biological variability. Possibly, if authors only had used one image per rat, the resolution of the images could be glomerular (the magnification is 108 pixels/mm).

The complete dataset has 472 group-averaged maps in response to 339 diverse odorants, some of them at different concentrations. Furthermore, as a result of the experimental process, most of the activity maps include missing values, generally dispersed in the ventro-caudal and dorsal parts and on the border of the activity map (Falasconi et al., 2012). We restricted our analysis to the 1778 pixels that were represented in all the maps; these pixels cover almost the entire OB, except its borders.

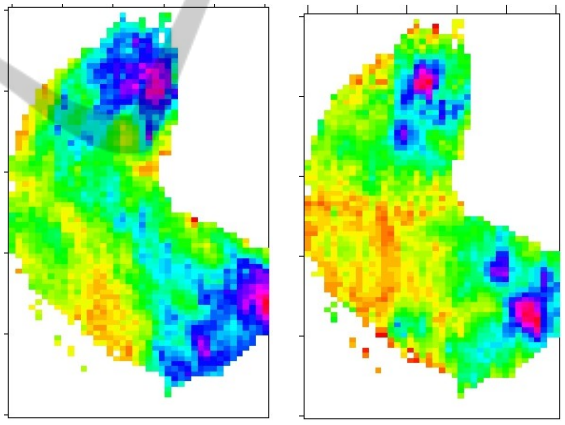

Figure 1: The activity maps of 4-tert-butylpyridine (left), and 2-acetylpyridine (right).

Besides the activity dataset, we have a molecular descriptor dataset for some of odorant stimuli. It contains a list of odorants, identified with their CAS number, and with 67 molecular properties that describes them (Leon and Johnson, 2003). From the initial 339 individual odorants, for this work we performed the analysis with 155 odorants and a set of binary molecular properties. Table 1 lists the selected binary properties that refer to different functional groups and different cyclization structures.

2.2 Data Analysis

For each molecular descriptor, we statistically tested whether a pixel was differentially activated in the target group vs. the control group. For this analysis we have used, as baseline technique, a non-parametric hypothesis test: the Mann-Whitney U test. However, in the last decade permutation tests have been proposed to improve test power. Among the different options for permutation tests, we have chosen rank-products.

2.2.1 Rank Products

Rank Products is a rather new technique mostly used to identify differentially expressed genes in microarray experiments. This procedure is derived from biological analysis and provides us a simple way to establish the significance level of each element analyzed calculating rank products (RP) from replicate experiments (Breitling et al., 2004). In our case we will use it in order to detect differentially activated pixels in a neuroimage. This test can be used in other application domains: for the analysis of diverse –omics data and general feature selection (Smit et al., 2007). As far as we know, it has not been previously used for the analysis of brain activity.

The underlying assumptions for this technique are fairly weak thanks to its non-parametric nature and, additionally, the results also are consistent in highly noisy data or when a small amount of replicates are obtainable, and it is very robust against outliers. For this analysis we used the RankProd Package for R. The used function permits to control the estimated percentage of false predictions (pfp) (Hong et al., 2006). We performed 1000 permutations and we selected the pixels that have a pfp < 10^{-5}.

2.2.2 Mann-Whitney U Test

The Wilcoxon rank-sum test, also called Mann-Whitney U test (MWU), is a non-parametric statistical hypothesis test that determines if one distribution is stochastically greater than the other.

When we use conventional hypothesis testing in this context, we must take into account the multiple comparison problems. The p-values have to be adjusted to control the probability of any pixel hypothesis. This is formally known as *family-wise* Type I error rate. To carry out this test we use multtest package of R (Pollard et al., 2005). To cope with the multiple comparison problems we have used the Benjamini and Yekutieli correction (Yekutieli and Benjamini, 1999).We selected the pixels with a corrected p-value < 0.05.

3 RESULTS

In order to obtain the significant pixels of each molecular feature analyzed, we compute both statistics. For the MWU, more demanding p-values resulted in few selected pixels. Even at this significance level ($p<0.05$) there are some molecular properties that do not show significant pixels. Table 1 shows the number of significant pixels obtained for the selected molecular features, by Rank Products and by MWU Test. It can be observed that even at very demanding pfp rank-products selects more pixels than MWU.

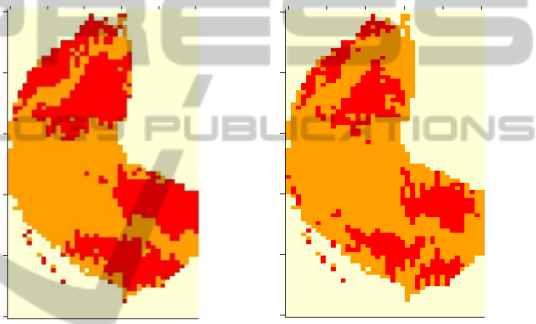

Figure 2: Active regions in olfactory bulb for aromatic descriptor obtained by RP (left), and MWU Test (right).

We can locate the significant pixels obtained in the olfactory bulb to visualize the active areas. In figure 2 we can observe the results of both tests for the aromatic feature. As we can observe both tests show approximately the same active regions, but the Rank-products seems less noisy.

At figure 3 we can notice that there are different actives areas for each molecular feature. For some properties (aromatic, alcohol or ketone) the technique identifies clear regions that show the well-known image symmetry of the activation in the OB. Instead, other properties show scattered pixels and the interpretation is not so clear.

Table 1: Number of significant pixels for each method.

Molecular feature	Rank Products (pfp < 10^{-5})	Wilcoxon Test (p-value < 0.05)
Aromatic	796	530
Alcohol	512	39
Alicyclic	127	0
Heterocyclic	126	0
Ester	311	0
Ketone	303	62

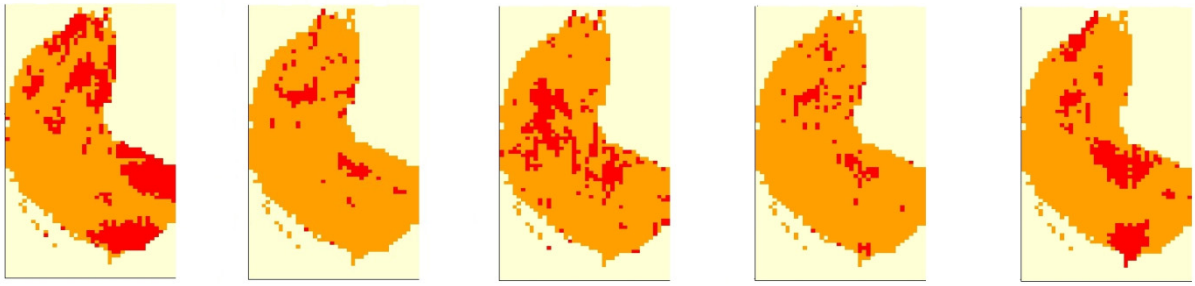

Figure 3: From left to right alcohol, alicyclic, ester, heterocyclic and ketone significant regions obtained by Rank Products with pfp=10^{-5}.

4 DISCUSSION

A first outcome of this analysis is the clear difference in hypothesis test power from rank-products to the MWU test. This was somewhat expected, but the results clearly show that MWU fails completely to identify differentially active areas for some properties.

Concerning the biological interpretation of the present results, we have to consider that the existence of a chemotopical organization at the level of the OB is controversial. While few groups consider this proven (Johnson and Leon, 2007); (Mori et al., 2006), recent results seem to prove the contrary (Soucy et al., 2009).

The chemotopic organization in the OB has been supported observing that odorants containing similar functional groups have similar responses of the activation in the olfactory bulb (Mori et al., 2006); (Takahashi et al., 2004). Eventually, this could allow the prediction of the neural activation pattern from an odorant and vice versa. This is not proved yet.

The identification of coding areas for different chemical properties is hindered by the high dimensionality of chemical information, that is, one odorant can be described by hundreds (or thousands) of chemical properties. When the target group shares one property, all the rest may change introducing a high level of noise.

While for some properties our results are very clear (aromatic or ketones), many recent studies claim that the real code is spatio-temporal, and that 2-DG images fully neglect the temporal dimension of the code. While this could be true, these results seem to indicate that the spatial part of the code can convey important information at least for selected properties.

The authors are aware of the limitations of the LJ dataset, and consider the present analysis and discussion limited to the analyzed data. Further elaboration on this topic will come from the analysis of newer imaging techniques with glomerular resolution that additionally are able to see the activation dynamics in response to the odorant pulse.

5 CONCLUSIONS

Reported results indicate rank-products as a convenient pixel selection technique to locate differentially active areas in response to particular stimulus (in this case odorants sharing certain molecular descriptors). This technique succeeds for properties where conventional non-parametric testing catastrophically fails.

Previously discussions on the existence of chemotopy in the OB lacked a supporting statistical analysis of the available data. This results support the argument that at least for selected chemical properties, there are differentially active areas that are topologically connected (Takahashi et al., 2004). This goes against recent studies that report that particular chemical properties are encoded in scattered glomeruli in the OB (Meister and Bonhoeffer, 2001); (Ma et al., 2012).

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