

## **Activity Patterns in the Rat Olfactory Bulb in Response to Straight-Chain Aliphatic Odorants Differing in Carbon Number**

### **Introduction**

The olfactory bulb is an organ in the brain that senses and processes odors. Even at very low concentrations, the olfactory system is able to distinguish among a virtually infinite number of chemical compounds. In order to understand how odorants with very similar molecular structures can be encoded in the rat brain, this project is testing the hypothesis that the location of the response in the glomerular layer of the olfactory bulb will vary in response to straight-chain aliphatic odorants differing in carbon number. Therefore, the nervous system activities in response to 1-octanol, 1-heptanol, 1-hexanol, and 1-pentanol will be compared with each other and with air as a control. Later, the results will also be compared to the patterns evoked by 2-octanol, 2-heptanol, 2-hexanol, and 2-pentanol. Response patterns will be studied by mapping the [<sup>14</sup>C]-2-deoxyglucose uptake in the glomerular layer of the bulb.

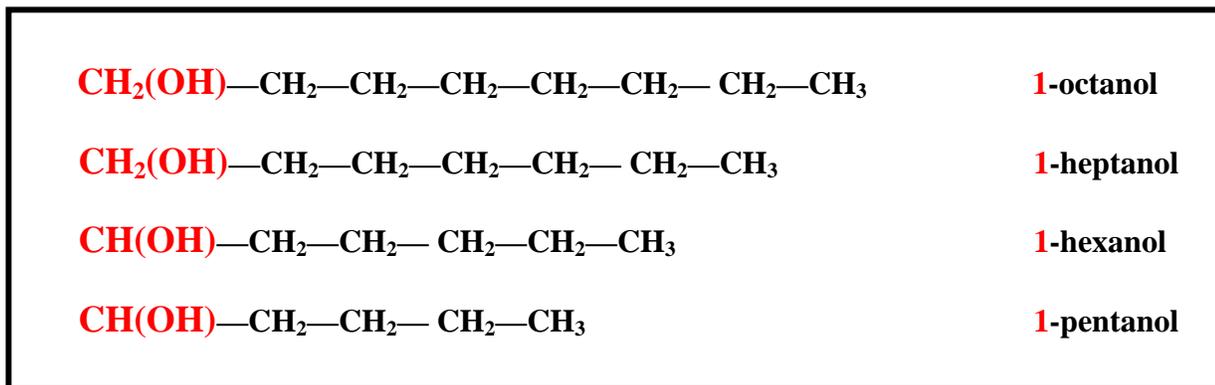
The general goal of this project is to understand the spatial coding of odorant chemistry in the rat olfactory bulb. The activity in the olfactory bulb in response to odorants can be analyzed by mapping [<sup>14</sup>C]-2-deoxyglucose uptake in the glomerular layer. Prior mapping experiments in response to various odorants in our lab have shown that the location of activity in the glomerular layer changes with different odorants. These results support the hypothesis that each odorant is recognized by a unique combination of olfactory receptors (Johnson et al., 1998). At the same time, however, the same odorants result in very similar activity patterns in different rats. This means that certain alcohols can easily be sensed by the rat olfactory system whereas other odorants with only minor differences in structure cannot be sensed at all.

### **Background Information**

The olfactory system is important for the sense of smell. In mammals, odors are initially detected by many different kinds of receptors on sensory neurons in the olfactory epithelium of the nose. These neurons project to specific glomeruli in the glomerular layer of the olfactory bulb, an organ that is located in the brain. Glomeruli are dense accumulations of synapses within which the axons or ends of receptor neurons come into contact with the dendrites or apices of mitral cells. The cell bodies of the mitral cells form a layer deeper within the bulb and send information to the olfactory cortex, which in turn projects to higher regions in the cerebral cortex, ultimately leading to the perception of odor.

Since the bulb is the first olfactory organ in the brain, transforming information about outside stimuli into codes that can be sent further to higher sensory centers of the brain, the glomerular layer itself should reveal information about the structure of the odorant molecule (Linster et al., 2001; Johnson et al., 2002). In fact, previous studies have shown that each stimulant is recognized by a unique combination of receptors and therefore produces a characteristic pattern of activity in the glomerular layer of the olfactory bulb (Johnson et al., 1998). It also has been suggested that different kinds of olfactory receptors respond to specific parts or functional groups of a molecule instead of to the whole molecule as an entity (Linster et al., 2001; Leon and Johnson, 2003). This is consistent with the fact that there are many more differentiable odorant molecules than there are receptors.

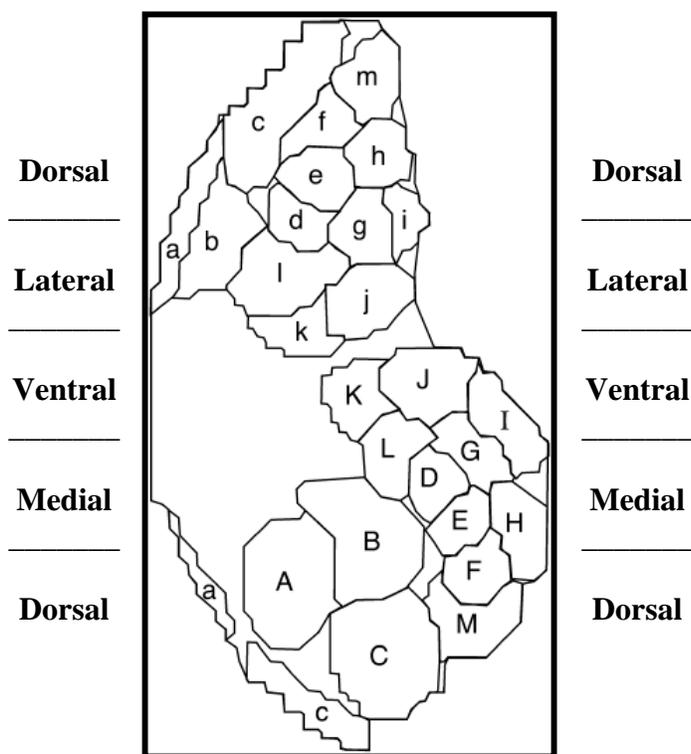
In order to study whether the carbon number in straight-chain aliphatic odorants affects the location of activity in the rat olfactory bulb, it is important to understand the structural differences between the odorants (Figure 1).



**Figure 1**

Straight chain aliphatic alcohols differing in carbon number. The carbon atom connected to the hydroxyl group is highlighted.

The hypothesis of this study is that each of these molecules, though very similar in structure, will be recognized by a different set of olfactory receptors, resulting in a unique pattern of activity in the glomerular layer of the olfactory bulb. To better analyze the relative positions of activity, neighboring glomeruli are considered units, or modules, and are assigned letters as shown in Figure 2.



**Figure 2**

Modular response model of the olfactory bulb. The same letters are used for lateral and medial modules that correspond to each other. A lower case letter describes the lateral section, and an upper case letter describes the medial section.

The glomerular activity can be measured by injecting [ $^{14}\text{C}$ ]-2-deoxyglucose (2-DG) into the rats before odorant exposure. This radioactive sugar is taken up by the animals but cannot be processed like regular glucose. In the olfactory bulb, it accumulates at regions of high activity, leaving those areas particularly radioactive. As a result, the activity in the entire glomerular layer can be quantified by mapping the 2-DG uptake in the bulb. If the glomerular activity patterns indeed vary significantly in response to the four heptanols, this would support the suggestion that olfactory receptors are sensitive not only to the actual functional group but also to the spatial position of the functional group within a molecule (Linster et al.,

2001; Leon and Johnson, 2003). This would mean that the receptors not only recognize the hydroxyl group but also differentiate between the different immediate environments surrounding it.

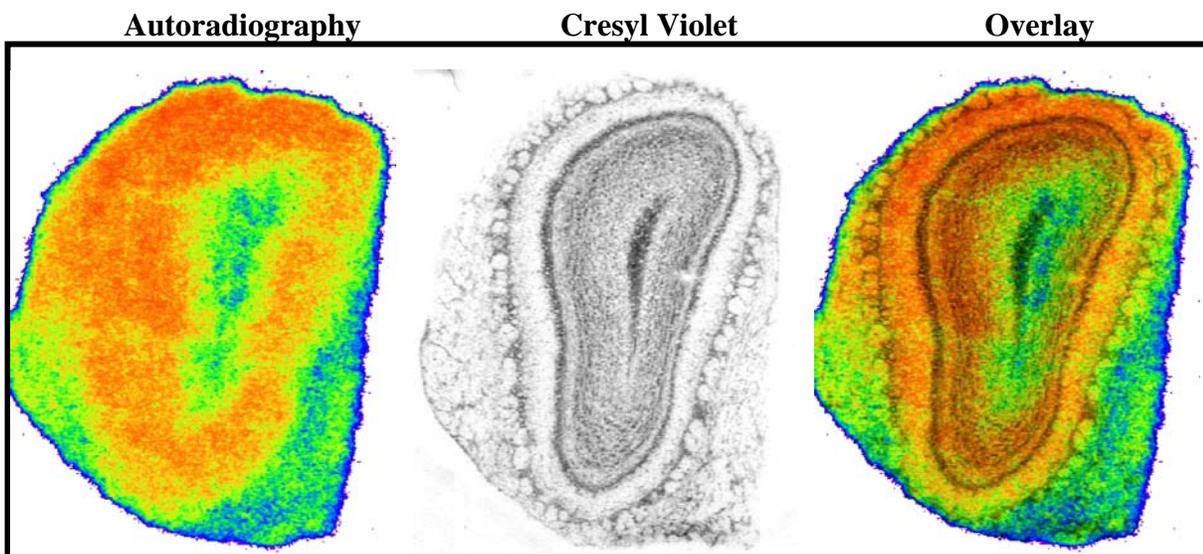
## Materials and Methods

### *Subjects and Exposures*

A total of 20 male rats (19-22 days of age) from four litters will be used for this project, four rats for each odorant and four for exposure to air as a standard. Within a litter, no two rats will be exposed to the same odorant. The vapor-phase concentration will be approximately the same for all of the odorants. The order of the odorants given to the rats within one litter is never repeated for a second litter to avoid any systematically repeated contamination between odorants. Before odorant exposure, 0.19 mCi/kg [ $^{14}\text{C}$ ]-2-DG will be injected into the rats subcutaneously. After 45 min of exposure, the rats will be decapitated, the olfactory bulbs removed and immediately frozen to  $-45\text{ }^{\circ}\text{C}$  in isopentane.

### *Glomerular Activity Quantification*

Using a cryostat, the olfactory bulbs are sectioned coronally at  $20\text{ }\mu\text{m}$ . Every sixth section will be exposed to autoradiographic film for ten days, and each adjacent section was stained with cresyl violet. The autoradiography was performed with  $^{14}\text{C}$  standards adjusted to tissue equivalents (nCi/g) of isotope (Johnson et al, 1999) and shows the radioactivity in the bulb by indicating the 2-DG uptake across the glomerular layer. The cresyl violet staining will be used to detect the glomerular layer and the anatomical landmarks of the bulb. Overlays will then be created to map the radioactivity distribution by tracing the glomerular layer on the autoradiographic images. The measurements are anatomically standardized and then converted into nCi/g units of radioactivity relative to the standards used. The arrays of animals exposed to air are subtracted from the arrays of the animals exposed to the odorants, and the 2-DG uptake will be averaged for each animal and transformed into units of z-scores relative to the mean and standard deviation of uptake across the glomerular layer. Finally, the data of all the rats exposed to the same odorant will be averaged.



**Figure 3**

Sections taken to map the radioactivity in the glomerular layer of the olfactory bulb. *Autoradiography*: Every sixth section of  $20\text{ }\mu\text{m}$  thickness is exposed to autoradiographic film for ten days together with  $^{14}\text{C}$  standards adjusted to tissue equivalents (nCi/g) of isotope. The image displays the [ $^{14}\text{C}$ ]-2-DG uptake in the bulb. *Cresyl Violet*: Sections adjacent to the ones taken for autoradiography are stained with cresyl violet to detect the glomerular layer and the anatomical landmarks of the bulb. *Overlays*: Images of the cresyl violet-stained sections are superimposed on images of the adjacent autoradiographic images to map the radioactivity distribution by tracing the glomerular layer on the autoradiographic images.

### **Project Design, Feasibility, and Student Familiarity**

My role will be to take histological sections of the olfactory bulbs of rats stimulated with the various alcohols, identify the EPL, AOB, and MCL layers of the olfactory bulbs first, then save images of the histological sections, develop autoradiographic films, map the [<sup>14</sup>C]-2-deoxyglucose uptake on autoradiograms, and finally analyze all of the data obtained.

Each alcohol is expected to produce a characteristic spatial pattern of response in the glomerular layer; one that is similar in different rats exposed to the same alcohol. For each odorant (including air as a control), I will be analyzing the brains of four rats in order to obtain statistically correct averages of the results.

This project is very feasible since I am not asking whether or not there will be a response but rather *where* in the glomerular layer the response will be. During my past year in the lab and my SURP research experience, I have been trained extensively to identify the significant structures of the olfactory bulb, use the autoradiogram, map the uptake in the glomerular layer, and analyze the results.

### **General Timeline of the Project**

I have already started this project, which is an interesting variation to the one that I did during SURP over the summer. I am planning to complete the experiment and all of the analysis by the end of December. I have already identified the important structures of the olfactory bulb, saved images of the relevant histological sections, and used the autoradiogram to obtain the radioactivity distribution. For the remaining time until the end of December, I will map the uptake of [<sup>14</sup>C]-2-deoxyglucose in the glomerular layer of the rats stimulated with the different alcohols, analyze all of the data obtained and compare the location of activity resulting from different odorants to identify a pattern in the nervous system response.

### **References and Works Cited**

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- Leon M and Johnson BA. Olfactory Coding in the Mammalian Olfactory Bulb. 2003. Brain Research Brain Research Review 42(1): 23-32.

**Proposed Budget**

<b>Quantity</b>	<b>Item Description</b>	<b>Unit Price</b>	<b>Total Price</b>
1	10pk 22x22mm coverglass	109.00	109.00
1	10pk 60x24mm coverglass	100.00	100.00
5	5ft corrugated FEP tubing 3/8" id	75.00	375.00
5	5ft corrugated FEP tubing 1/4" id	58.00	290.00
5	10 KYNAR T connectors 1/4" id	18.50	92.50
		<b>TOTAL:</b>	<b>966.50</b>