12-1972

The Frequency Coding Theory of Olfaction

William B. Stewart

Western Michigan University

Follow this and additional works at: https://scholarworks.wmich.edu/masters_theses

Part of the Experimental Analysis of Behavior Commons

Recommended Citation
https://scholarworks.wmich.edu/masters_theses/2798

This Masters Thesis-Open Access is brought to you for free and open access by the Graduate College at ScholarWorks at WMU. It has been accepted for inclusion in Master's Theses by an authorized administrator of ScholarWorks at WMU. For more information, please contact maira.bundza@wmich.edu.
THE FREQUENCY CODING THEORY OF Olfactory

by

William B. Stewart

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
December 1972

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
ACKNOWLEDGEMENTS

I would like to thank Professors J. Lawrence and B. Huitema for their encouragement of this study. I would also like to express special thanks to Dr. F.F. Gault in whose laboratory this study was performed.

William B. Stewart
MASTERS THESIS

STEWART, William Bennett
THE FREQUENCY CODING THEORY OF OLFACITON.

Western Michigan University, M.A., 1972
Psychology, experimental

University Microfilms, A XEROX Company, Ann Arbor, Michigan
PLEASE NOTE:

Some pages may have
indistinct print.
Filmed as received.

University Microfilms, A Xerox Education Company
## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>SECTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
</tr>
<tr>
<td>III</td>
<td>7</td>
</tr>
<tr>
<td>IV</td>
<td>11</td>
</tr>
<tr>
<td>V</td>
<td>14</td>
</tr>
<tr>
<td>VI</td>
<td>15</td>
</tr>
<tr>
<td>VII</td>
<td>25</td>
</tr>
</tbody>
</table>

- INTERROGATION
- METHODS
  - Subjects
  - Surgery
  - Procedure
  - Data Analysis
- RESULTS
- DISCUSSION
- SUMMARY
- FIGURES
- BIBLIOGRAPHY

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
INTRODUCTION

Recordings of gross electrical activity from the mammalian olfactory bulb have been numerous (Adrian, 1942; Adrian, 1950; Hernandez-Peon et al, 1960 and Mozell and Pfaffmann, 1954), as have been single unit studies (Shepherd, 1963 and Yamamoto et al, 1962). There has, however, been little progress towards discovery of the mechanism for the neural coding of olfactory stimuli.

Olfactory researchers have encountered several major problems. The stimulus is difficult to quantify with ordinary laboratory methods. At best, its concentration is expressed relative to saturation in an air stream. Traces of impurities in the odorant may obscure the response. Pure biologic odors are difficult to obtain and define, so common laboratory chemicals must often serve as stimuli. It is difficult to ensure that the subject is constantly and uniformly exposed to the odor and that it sniffs at a constant rate and breathes through its nose. Atrophic rhinitis often necessitates the use of young animals. The stimuli for the chemical senses do not vary along relatively simple dimensions as do light and sound.

The use of anesthetics alters the responsiveness of the olfactory system to odors by manipulating the
state of arousal (Adrian, 1942) or by influencing the electrical activity of the bulb by other means (Orr, 1972).

Intracellular recordings in the bulb have been difficult to obtain (Yamamoto et al, 1962). Extracellular single and multiple unit recordings have provided some information (Macrides, 1972), but the results are difficult to reproduce.

Gross recordings, on the other hand, have been more easily reproduced but hard to interpret.

Similar gross electrical activity has been observed across several species of mammals. Inspiration is accompanied by high voltage, low frequency (30-70cps) electrical activity, called induced activity and expiration by high frequency (70-100cps), low voltage electrical activity, called intrinsic or background activity. This pattern, called bursting, occurs with pure oxygen and nitrogen as stimuli, suggesting that a mechanoreceptor in the mucosa may be responsible for some of the induced activity, though it is most easily demonstrated with odorous stimuli (Domino and Ueki, 1960 and Domino and Ueki, 1961).

Bursting will not occur without nasal airflow (Gault and Leaton, 1963) and occurs synchronously with a slow potential believed to be indicative of the electroolfactogram (EOG) (Ottoson, 1959) (Fig. 1).
The amplitude of the bursting is directly related to the volume of the nasal airflow and can be modulated by stimulation in the reticular formation (Pagano, 1966).

It has been suggested that the induced activity recorded in the bulb is a result of cyclical mitral cell activation modulated by dendrodendritic reciprocal synapses in the external plexiform layer with granule cells (Nicoll, 1969).

Hughes and Hendrix (1967) have examined the electrical activity of the bulb for evidence of frequency coding. The frequency component theory of olfaction states that odor quality is encoded as action potential frequency in the olfactory bulb. Their data, recorded from rabbit and human olfactory bulbs, gave some support for the theory. A series of odors had responses with different frequency characteristics. Small molecule compounds induced lower frequency activity than larger molecules. In most cases the odorant was presented to a restrained animal or a human undergoing concurrent surgery by holding a soaked cotton swab in front of the nostrils. Only sample data is presented and little information on variability is available.

The purpose of this experiment was to examine the frequency component theory of olfaction under more rigorous conditions, with a controlled sniff rate and volume,
with a more discrete stimulus and with as little complicating effect of anesthesia as possible.
METHODS

Subjects

Subjects were 190-350 gm. male rats procured from The Upjohn Company, Kalamazoo, Michigan.

Surgery

Subjects were anesthetized by intraperitoneal and supplemental subcutaneous injections of a short-acting barbiturate, Brevital. A tracheotomy was performed, two tubes being inserted, one caudally to provide an airway for respiration and one rostrally to provide nasal insufflation. The subjects were immobilized by a spinal cord section at C-1 or administration of Flaxedil and placed in a stereotaxic instrument. A craniotomy was performed, exposing both olfactory bulbs. All wound edges and pressure points were infiltrated with Procaine. Respiration was maintained with a small animal respiration pump operating at one cycle per second and heart rate was monitored. Another pump operating at two cycles per second, with a variable volume control provided nasal insufflation. The subjects were then left for at least one hour to allow the effects of the anesthesia to dissipate. Control experiments had shown that the olfactory bulb EEG returned to normal in this period.
Procedure

The olfactory stimuli were delivered through a flow-dilution olfactometer (Braun et al., 1967), in which air zero was purified by flowing through wash bottles containing silica gel and charcoal and moisturized by bubbling through water, to a tube placed over the subject's nostrils. To provide camphor stimulation, a saturated solution of camphor (C\textsubscript{10}H\textsubscript{16}O) in water was placed in the air line. The nasal insufflation pump mimicked a natural sniff, allowing the subject to have a near physiologic exposure to the stimulus.

Electrodes, made from 00 insect pins insulated with formvar and bared 0.5 mm from the tip, were placed on or below the surface of the bulb until a bursting pattern to pure air could be elicited. A typical recording session consisted of several recordings each of background activity, with no nasal airflow, and of activity elicited by pure air and camphor.

The electrical activity was simultaneously displayed on a Grass Model VII polygraph and stored on a Sanborn seven track FM tape recorder for later digitization and computer analysis.

Data Analysis

The analog data were digitized at 300 samples per
second using a Massey-Dickinson Analog to Digital Converter, which wrote on a computer tape. Previous digitization at 750 samples per second had shown that little neural activity was present above 150 cycles per second, so aliasing was not a problem with the sample rate used. The digitized records were 2,048 samples in length, corresponding to about 6.5 seconds of real time.

A time series analysis of the data was performed on a PDP-10 computer using a modified version of the BMDX92 (Dixon, 1969). The program produced a plot of the power spectrum (volts$^2$/cycle/second as a function of the frequency). The program was run with 8, 16, 32 and 64 frequency bands in order to judge the appearance of spurious peaks as the digital filter became narrower (Jenkins and Watts, 1969).

Limitations of computer time set an arbitrary limit of 4 spectra per condition or 12 per subject.
RESULTS

The shape of the spectra differed across animals and sites within the same animal, while within conditions at a site the spectra were relatively similar.

Large differences appeared for records which by visual analysis of the polygraph record seemed similar. This was particularly true for sites with prominent frequencies above 70 cycles per second, which was the damping frequency for motion of the pen.

In some graphs a major peak is present in the 56-60 cycle per second range. Earlier work had shown that line voltage will produce a peak in this range. Care, then, must be taken in attributing activity in this range to neural origin. In general, the experimenters dismissed as 60 cycle artifact a peak that appeared in all spectra with similar amplitude. Other control work indicated that there were no spurious peaks inherent in the recording system. Test inputs of sine waves from an audio oscillator produced a peak or peaks at the appropriate frequency, though with the record length employed the peak always appeared in two bands. Therefore, care must be taken in interpreting small peak shifts.

The data are presented graphically for each subject and consist of four spectra per condition, except for subject Y where two spectra per condition are shown for
multiple sites. Since quantitative comparisons are
difficult and often meaningless, the salient features
of the spectra will be described.

Subject C

The spectra for subject C show a peak between 0-
14 cps and another between 14- 28 cps. There are no
apparent differences between conditions (Fig. 2).

Subject Z

Air produced a peak at 9 cps, while camphor and
background spectra are similar and show less activity.
At higher frequencies, air and background have similar
spectra, with camphor showing a broad peak between 42-
60 cps. A 60 cycle artifact appears to be present.
Above 65 cps all spectra are similar (Fig. 3).

Subject B

The background spectra show a prominent peak
between 56-79 cps, while the pure air condition shows
a much smaller peak in the same range. The camphor
spectra have a peak between 56-70 cps suggesting a
peak shift in the EEG under camphor stimulation. In
all other frequency ranges the spectra are similar (Fig.
4).
Subject U

The spectra for subject U show a prominent 60 cycle artifact. The background spectra show no peaks. The air spectra show peaks between 0-4 cps and a broad peak between 14-28 cps. Above this frequency the spectra are similar (fig. 5).

Subject V

The air and background spectra have similar shapes, with the air spectra having a larger peak between 89-93 cps, while the camphor spectra have a less defined peak. There is evidence of a peak shift from 93 to 89 cps in the camphor EEG. In all other frequency ranges the spectra are similar (Fig. 6).

Subject Y

At sites 1, 2, and 3 the air spectra are characterized by a large low frequency peak. The background spectra are relatively flat. At site 4 the low frequency values are highly variable. A consistent difference does appear at 28 cps, where there is a large peak in the air spectra. The large 60 cycle peak in one of the background spectra is probably artifact (Fig. 7, 8, 9 and 10).
DISCUSSION

The spectra of the background activity revealed several interesting facts. There were only two cases in which the major frequency of the background activity was at or above 70 cps as would have been expected from the classical description of intrinsic or background activity (Adrian, 1942). It had been reported that the background activity was flat, with no prominent peaks (Hughes and Hendrix, 1967). In several cases in this experiment the background exhibited a major frequency component.

Stimulation with air often resulted in an increase in amplitude of a frequency already prominent in the background, rather than the appearance of a new peak, though this was the case for site 4 in subject X.

Addition of camphor to the airflow had varied effect. Subject B shows evidence of a downward shift in major frequency. Subject V shows a downward shift in frequency and a decrease in amplitude. Subject Z, however, shows the presence of a new peak of activity.

In subject C no effect is evident from the addition of camphor. Several explanations are possible. The stimuli may not have been reaching the mucosa, since this was a subject from which bursting could not be elicited. Secondly, the site may not have been receiving activity from camphor receptors. There is evidence for
a spatial distribution of odor responses in the bulb (Mozell and Pfaffmann, 1954). Thirdly, many sites may not participate in frequency coding.

In several subjects a large peak is present in the low frequency range. A record with substantial bursting would be expected to show such a peak due to the non-stationarity of the signal (Jenkins and Watts, 1969).

Hughes and Hendrix (1967) have suggested other reasons not to examine low frequency activity. They did not examine frequencies below 20 cps because of contamination of the record by d.c. shifting due to breathing and cardiac electrical activity. The spectra for subjects C, Y and U show that this may be a problem in ascribing low frequency peaks to local neural activity.

It is evident that changes in stimulation of the olfactory system can cause changes in the frequency characteristics of its response. In every case, except subject C, stimulation with air caused a change in the power spectra from the unstimulated or background condition. Camphor caused a downward shift in activity from the air stimulus condition, though not striking. The change in frequency could provide information to the central nervous system about odor quality.

Widespread application of this technique to the problem of olfactory coding will require several steps.
The study should be expanded to examine many odors at several sites per animal. Longer records should be employed to examine smaller frequency bands in order to detect more subtle changes. The inclusion of some chronically implanted, freely-moving rats might provide some information about the generality of the findings.

An elucidation of the mechanism for generation of the induced activity would be valuable. If the induced waves represent the summated cyclical mitral cell activation, then, the frequency coding theory will rest on firm ground. However, if the induced activity is a summation of action potentials and dendritic and somatic slow potentials from mitral, granule, tufted and periglomerular short-axonated cells, then the spectral analysis will be more difficult to interpret.
SUMMARY

Male rats (190-350 gm.) were anesthetized, tracheotomized and a tube placed rostrally in the trachea to provide nasal insufflation. The olfactory bulbs were exposed and the gross electrical activity (EEG) was recorded for three olfactory stimulus conditions, no nasal airflow, pure air and camphor. The activity was simultaneously displayed on a polygraph and stored on an FM tape recorder.

The analog data were digitized at 300 samples per second and written on a computer tape. A time series analysis of the data was performed to produce the power spectra.

The results indicated that the frequency characteristics of the EEG were dependent on the stimulus condition. The frequency coding theory of olfaction was supported.

Further systematic study of the theory is indicated.
Subject V

Upper trace: Olfactory bulb EEG with low frequencies filtered out. 20 microvolts per five divisions.

Bottom trace: Olfactory bulb EEG with high frequencies filtered out. 10 microvolts per five divisions.

An example of bursting with pure air as the stimulus. The time marker is one per second.
Figure 2
SUBJECT V

NATURAL LOG OF RELATIVE POWER IN VOLTS² PER CYCLE PER SECOND

- CAMPHOR
- AIR
- BACKGROUND

FREQUENCY IN CYCLES PER SECOND

Figure 6
Adrian, E.D., "Olfactory reactions in the brain of the hedgehog." J. Physiol., 100 (1942), 459-473.


