Amino Acid Responses of Olfactory Bulb Cells in the Channel Catfish.

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Amino acid responses of olfactory bulb cells in the channel catfish

Thompson, Hilary Winfield, Ph.D.
The Louisiana State University and Agricultural and Mechanical Col., 1988
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ABSTRACT.

Amino acid (AA) responses of olfactory bulb (OB) mitral cells (MC) in the catfish, Ictalurus punctatus, were studied electrophysiologically. A total of 63 cells were recorded in 28 fish in two separate experiments. For the first study, 27 cells in 22 fish were studied with olfactory tract (OT) intact during stimulation with $10^{-6}$ M to $10^{-2}$ M concentrations of 11 AA. MC responses were quantified by interspike interval (ISI) variables sensitive to inhibition (maximum ISI) and excitation (number of action potentials (AP), minimum ISI). APs were also counted in subdivisions to produce a measure sensitive to MC response temporal patterning. These measures were applied during the rise and fall of olfactory receptor input, indicated by the summed receptor output, the electro-olfactogram (EOG). ISI variables, stable during background activity and well water applications, changed significantly upon AA stimulation. Stimulus concentrations regressed against ISI variables showed increases and decreases during stimulation with increasing concentrations. Regressions fitted to these dose-response curves were nonlinear. Responses to some AA were more
reproducible than responses to others. This phenomenon may be part of stimulus intensity representation in catfish OB. In study two, MCs were recorded with OT intact and transected, to isolate the OB from the central nervous system. Bulb isolation was conducted on 6 fish and 36 cells (18 different cells each from intact and isolated bulbs). All cells were stimulated with 5 AA at concentrations from $10^{-5}$ M to $10^{-3}$ M. Response changes following OT transection were increased ISIs and decreased maximum ISI. Three response classes (cluster analysis) of ISI variables from the first 1.5 seconds of the response were: inhibition, excitation and inhibition and excitation in one response. Inhibitory responses were rare after tract transection. Correlations of action potential counts evoked by different AA showed that the bulb cell population discriminated stimuli, but individual cells could not. AA discrimination was reduced after OB isolation, because responses became more alike. These data suggest that stimulus quality and intensity representation in the catfish olfactory system involves a central comparison of MC responses.
Introduction

Sensory systems transform information from the physical world into neural events through a rule of correspondence between physical and neural events. Many previous studies on the electrical activity of olfactory bulb cells have aimed at revealing this rule of correspondence between the physical event of odorant binding to olfactory receptor cells and the activity of the mitral cells, which are the output neurons of the olfactory bulb.

Mitral cells generate trains of action potentials representing the olfactory information and transmit these to other parts of the forebrain (Shepherd, 1979). The relationship of the peripheral olfactory system with the mitral cells and the two types of electrical signals (the electro-olfactogram or EOG and the mitral cell action potentials) recorded in this study are shown in Figure 1. The olfactory receptors are shown with their cilia or microvilli (Erickson and Caprio, 1984) which extend into the mucus of the olfactory epithelium. The EOG or electro-olfactogram represents the summed generator potentials from the olfactory receptors. This signal, which can be ob-
Figure 1. Diagram showing the parts of the olfactory system dealt with in this study, and the electrical signals that can be recorded from each level of the nervous system. The centrifugal neurons of the olfactory tract arise from more central parts of the nervous system.
tained for longer experimental periods than action potentials from the olfactory receptors, provides an indication of the onset and duration of olfactory receptor activation by the amino acids used in this study. At slow recording speeds, the mitral cell action potentials of 1 to 2 milliseconds duration appear as thin upright lines. This is the origin of the term "spike" for an action potential, and the term "interspike intervals" (ISI) is used here to describe the waiting times between these electrical signals.

Another aspect of olfactory bulb function represented in Figure 1 is the action of centrifugal neurons which project outward to the olfactory bulb from more central parts of the nervous system. The action of these centrifugal neurons is thought to be the excitation of granule cells (Yamamoto, Yamamoto and Iwama, 1963). The action of granule cells is to inhibit mitral cell activity (Mori, Nowicky and Shepherd, 1981). By cutting the olfactory tract which carries the centrifugal neuron axons to the olfactory bulb the action of these centrifugal neurons on mitral cell activity was evaluated in the present study.

Studies of the action potential output from the mitral cells have been undertaken in fish (MacLeod,
1976; Meredith and Moulton, 1978; Zippel and Voigt, 1982) amphibians (Døving, 1964; Kauer, 1974) reptiles (Shibuya, Aihara and Tonosaki, 1977). and mammals (Chaput and Holley, 1980; Mair, 1982; Meredith, 1986; Reinken and Schmidt, 1986; Harrison and Scott, 1986; Døving, 1987). These reports have shown that olfactory information processing, as represented by the output of mitral cells, involves different patterns of action potential occurrence. Mitral cell output may show increases or decreases in action potential frequency or both increases and decreases during the course of a response to a single stimulus. Individual mitral cells typically respond with different patterns of action potentials to a variety of different chemical stimuli. The exact manner in which response patterns of action potentials in olfactory bulb mitral cells represent odorant information is still unknown.

The results of these previous studies on mitral cells suggest that the information carried by the mitral cell axons to the more central parts of the nervous system is represented by the activity of the population of mitral cells and not the activity of individual cells. This general conclusion about olfac-
tory information representation, however, must be amplified, since some interesting differences between vertebrate species have emerged. Studies in the salamander (Kauer, 1974), rat (Mair, 1982) and mouse (Reinken and Schmidt, 1986) revealed mitral cells which showed only inhibitory responses to olfactory stimuli. These cells showed progressively longer ISI with increasing odorant concentrations, however this response pattern has not been described for mitral cells of all species studied. It was suggested that these cells may carry less information on odorant quality than cells which produced excitatory responses (Kauer, 1974). Such species differences indicate there may be no single uniform rule for the correspondence of stimulus and mitral cell output across vertebrates. If so, different species must be examined to find common modes of olfactory information representation. Another important question which proceeds from comparisons of the mitral cell activity of various species is whether or not there are discrete response types in mitral cell activity and, if they exist, whether response types are species specific or common to all vertebrates.

In the study of olfactory bulb responses, the stimuli used should have behavioral effectiveness for
the species tested. This provision makes it more likely that the mechanisms of information representation under study are those that the animal has evolved in the context of its environment, and thus more likely to reveal basic principles of olfactory information representation than responses to stimuli never experienced by the animal. Biologically "meaningful" stimuli could provide an understanding of olfactory information representation in one species and make cross-specific comparisons more valuable.

Another problem in the study of mitral cell activity is the proper way to characterize mitral cell responses for analysis. Earlier studies of mitral cell activity used action potential counts during stimulation corrected by subtraction of background action potential counts taken over some fixed time period (Bodznick, 1978; MacLeod, 1976). This method of analysis focused on the predominance of either inhibition or excitation in mitral cell responses. The shortcoming of this analysis method was its inability to deal with responses which had similar counts of action potentials prior to and during stimulus presentation, but with a temporal pattern of action potential occurrence that changed upon stimulus presentation. Other
olfactory bulb studies attempted to use response variables which reflected more accurately the changes that occur during the time course of the response (Meredith and Moulton, 1978: Meredith, 1986). In these studies, action potentials were summed for short subdivisions of the response (called "bins") accumulated before and after stimulus application. The advantage of this method is that each of the ten second response periods yields the same number of bins in which to analyze for the occurrence of action potentials, whereas the number of ISI in each response usually differs. This is useful for statistical tests involving the calculation of correlation coefficients between responses, since each correlation is based on the same number of time bins in every response.

In the present study, I applied and compared several different types of statistical analysis to mitral cell responses of the channel catfish. One method, the multiway analyses of variance (MANOVA), was chosen for examining the interrelationship of the number of action potentials, the shortest and longest ISI between them, and amino acid stimuli. These variables were chosen to indicate the response magnitude (number of action potentials), the intensity of ex-
citation (shortest ISI) and the intensity of inhibition (longest ISI) with three numbers only, rather than a list of all ISIs.

A second form of analysis applied were counts of mitral cell action potentials in short subdivisions of the observed responses (bins). These subdivided (binned) counts were used for constructing correlations between responses to different amino acid stimuli. The response interval variables and the binned interval counts were used to examine the question of whether there are discrete or continuous graded response types in mitral cells of the catfish olfactory bulb. Such response classes were found to be present, but it remained to be determined whether the response classes were associated with responses to particular stimuli or were preferentially produced by particular cells. An answer to this question will lead to a better understanding of the processing of olfactory information in the channel catfish and may provide the necessary information that will relate the processing of olfactory information in all vertebrates.

The analytic methods applied here have the advantages of being sensitive to different aspects of
the response, its magnitude and degree of inhibition and excitation as well as the pattern of ISI occurrence over time. The analysis methods are based on programs in a standard and widely available computer statistical analysis language (the Statistical Analysis System, SAS). Similar methods could be applied by any investigator with access to mainframe or microprocessor versions of this language.

The multivariate statistical methods applied took into account repeated stimulus applications and thereby allowed for the investigation of another important issue in the processing of neural information, i.e. response variability. Studies on information processing in other sensory systems revealed that response variability is a basic property of central and peripheral neuron populations (Werner and Mountcastle, 1963; van der Molen, Nederstigt and Veenman, 1985). Psychophysical studies have indicated that olfactory response variability was greater than could be accounted for by the variability of stimulus delivery to the olfactory organ (Cain, 1977). Variability was hypothesized to play a role in the accurate following of repetitive stimuli by photoreceptors (Knight, 1972). Variability among the thresholds
of individual receptors ensures that they will fire in a distributed manner throughout the time course of a changing stimulus, rather than firing in synchrony when the stimulus intensity reaches a common threshold magnitude. A similar hypothesis was advanced to explain the role of random ISI fluctuations in pacemaker neurons (Perkel et al., 1964). More recently, neuronal modeling of sensory systems indicated that cells with random membrane potential fluctuations (noise) are necessary for effective information processing in model neural networks (Buhmann and Schulten, 1987).

Response variability is, however, a problem for the experimenter, since neural activity described as a response must be verified as being different from the preceding background activity. Yet, it is desirable that the applied response criteria do not reject responses that could be detected by the nervous system. This is the reason that the overall multivariate ANOVAS were performed. If the population of responses can be verified as being distinct from background activity on the basis of several variables, then individual responses can be examined for their correspondence to stimulus parameters with less chance of rejecting a possible mode of response.
The olfactory bulb offers the possibility of understanding olfactory information contained in ISIs originating from the peripheral olfactory system and how this neural information is modified through interaction with higher order neurons within the central nervous system. Interactions of the olfactory bulb and more central parts of the olfactory system were studied previously (Kerr and Hagbarth, 1955; Mancia, Green and von Baumgarten, 1962; Carreras, Mancia and Mancia, 1967). These studies measured electroencephalograms (EEG) in mammalian preparations evoked by electrical stimuli and EEG changes upon transection of the olfactory tracts that transmit higher order neuronal activity back to the olfactory bulb from more central parts of the olfactory system. More recent studies on the mammalian olfactory bulb have attempted to discern the effects of centrifugal neuron activity on the ISIs of the mitral cells (Potter and Chorover, 1976; Chaput, 1983; Cattarelli, 1982). In fish, the influence of the more central parts of the olfactory system on the activity of bulb cells was studied using electrical and chemical stimulation, with cooling of the olfactory tract to suppress its influence on the bulb (Døving and Hyvarinen, 1969; Døving and
Gemmne, 1966). In the present study, the activity of mitral cells in the olfactory bulb of the channel catfish was observed with the olfactory tract both intact and transected in order to determine the influence of other parts of the central nervous system on the activity of the mitral cells.

The channel catfish (Ictalurus punctatus) provides an excellent model to study the physiology of olfactory bulb neurons. The major advantages are the peripheral location of the olfactory bulb and demonstrated high sensitivity of the olfactory system to amino acids (Caprio, 1978; Byrd and Caprio, 1982). The olfactory bulbs are displaced from the telencephalon proper, to which they communicate through elongated olfactory tracts and are positioned immediately ventrolateral to the olfactory mucosa (i.e. a "pedunculate" condition). This anatomical organization permits access to the olfactory bulbs by minor surgery without disruption of other central nervous system structures. Amino acids as olfactory stimuli are available in pure form and can be delivered to the fish nose in aqueous solution. In comparison, many of the volatile organic odorants used in the study of olfaction in airbreathers (Døving, 1987; Mair, 1982;
Kauer, 1974; Meredith, 1986) generally have no clear relevance to the biology of the organism and require a more sophisticated experimental arrangement to deliver known concentrations of odorants in the vapor phase. The correlation of unit activity from the mitral cells with respiratory activity, which adds complexity to the analysis of recorded neural activity in mammals (Macrides and Chorover, 1972; Chaput and Holley, 1980) is not a problem in teleosts, where the olfactory chamber is not connected to the respiratory system. Finally the ease of obtaining well-resolved, single unit activity from neurons within the olfactory bulb of the channel catfish with the olfactory tract intact or transected makes this a favorable preparation in which to address the problems of response variability, appropriate response parameters, and the interactions of the olfactory bulb with other forebrain centers. These are problems relevant to the study of the processing of olfactory information in both aquatic and terrestrial vertebrates.
MATERIALS AND METHODS.

Channel catfish, *Ictalurus punctatus*, of post fingerling size (100-200 g, 15-25 cm in length) were transported to L.S.U. from local fish farms in oxygenated well water or natural pond water. They were maintained in floating cages in university ponds and fed commercial catfish chow. Fish used in these experiments were moved to laboratory holding facilities and held without feeding in 75 liter aquaria on a 12:12 light dark cycle for no longer than two weeks before use or they were released (Tucker, 1973).

Charcoal-filtered Baton Rouge city tap water was used in the aquaria as well as for preparing stimulus solutions. Baton Rouge city water, obtained from deep artesian wells, has a basic pH (7.8-8.2) due to a naturally occurring sodium bicarbonate (3 mM/L) buffer system. Because of this buffering an adjustment of the pH of the amino acid solutions used in the present experiments was not required. Baton Rouge artesian well water is hereafter referred to as "well water".

Olfactory Bulb Preparation and Stimulation Techniques.

Fish were immobilized by intramuscular injection of Flaxedil (gallamine triethiodide, 0.1 mg/100 g body weight; Flaxedil is a trademark of the Davis and Geck
department of American Cyanimid, Pearl River, NY) wrapped in moist tissue paper and clamped into a metal and plexiglass support. Centrally acting anesthetic agents could not be used because of their suppression of the olfactory bulb activity being studied (Stewart and Scott, 1976; Chaput and Holley, 1979). Gill irrigation with aerated well water maintained respiration over the three to nine hour recording periods. The olfactory capsule was opened by the removal of a flap of skin overlying the olfactory epithelium, and a flow of well water (flow rate 8 ml/minute) carried 0.5 ml aliquots of amino acid stimuli from a stimulus injection port to the olfactory mucosa. Stock solutions were prepared weekly at $10^{-2}$ M in well water and stored at 4 degrees centigrade. Stimulus solutions were diluted from these stock solutions on the days of the experiments in log steps from $10^{-5}$ M to $10^{-3}$ M. In some experiments, a more extensive concentration series of $10^{-6}$ to $10^{-2}$ M was tested. Dilution in the delivery water reduced the actual concentration of amino acid at the olfactory mucosa to approximately 40% of the applied concentration. Stimulus concentrations are listed as applied concentrations throughout this report.
The olfactory bulb was exposed by the removal of the soft tissue and bone overlying it. The surgical field of the olfactory bulb was immediately caudal and medial to the opened olfactory capsule. A thin strip of skin and underlying tissue was left intact between the bulb and mucosa to prevent the stimulus flow over the mucosa from contacting the olfactory bulb. When the olfactory tract was cut as an experimental treatment, the circulation of blood to the olfactory bulb and mucosa remained intact. Any disruption of this circulation led to rapid paling of the bulb and mucosa, rapid loss of bulbar and mucosal electrical activity and termination of the experiment.

**Histological Techniques.**

In order to confirm the types of olfactory bulb cells present at the depths recorded from in this study, selected olfactory bulbs were removed and placed in Bouin's solution. Specimens were dehydrated in an alcohol series after fixation, taken through a xylene-paraffin series, embedded in paraffin, and cut into 10 micrometer sections. Sections were stained with hemotoxylin and eosin and photographed with 35 mm photomicroscopy on a Zeiss Research microscope. All histological techniques were performed as described by
Humason (1979).

**Electrophysiological Recording Techniques.**

The underwater electro-olfactogram (EOG, Silver et al., 1976), a negative slow potential believed to be the summed generator potentials of the olfactory receptor cells (Ottoson, 1956), was recorded on analog tape (tape recorder channel 1) simultaneously with action potentials from neurons within the olfactory bulb (tape recorder channel 2). Action potentials were recorded extracellularly with an AC amplifier (bandpass 0.1 to 10 kilohertz). A voice commentary of the experimental procedure was also recorded (tape recorder channel 3). Unit activity from the olfactory bulb was recorded extracellularly with low impedance (5 to 8 megohm), 3 M NaCl-filled micropipettes, which were advanced into the olfactory bulb with a hydraulic microdrive. Only spontaneously active units were studied, since in pilot experiments silent areas rarely showed evoked activity upon amino acid presentation to the olfactory organ. To identify amino acid sensitive bulb cells, spontaneously active neurons were tested with single applications of $10^{-5}$ M solutions of the L-isomers of alanine, arginine, cysteine, glutamic acid and methionine.
To ensure that the action potentials from the olfactory bulb originated from a single mitral cell, waveforms of action potentials were superimposed in 50 or more successive sweeps of the oscilloscope screen in order to check for similarity of the waveforms (Figure 2). A minimum interspike interval of at least one millisecond was also a criterion for single unit activity, since, even with rapid firing, action potentials never followed each other within this time due to the duration of the absolute refractory period of the neurons (Mair, 1982). Responses from single units identified by these criteria were converted to standardized computer compatible pulses by a window discriminator (Bak Dis-1). Timings between these pulses represented ISI and lists of such timings were accumulated for each response to stimulation, as well as for the time that proceeded stimulus application. A microprocessor was used to write ISI to computer disk files. The audio commentary on tape recorder channel three indicated the point of stimulus injection, which preceded the EOG by 3 seconds. The point of separation of ISI before stimulation from those after the onset of stimulation was a marker placed in the interval file. The point of stimulus arrival at the receptors
Figure 2. Superimposed extracellularly recorded waveforms from a mitral cell of the channel catfish illustrating the method used to verify that extracellular recordings originated from a single neuron. A. Superimposed waveforms from 50 action potentials selected by amplitude and waveform discrimination. This degree of waveform similarity suggests the waveforms originated from one neuron. B. Overlay of the same action potentials at slower speed to illustrate that a minimum interspike interval was maintained between adjacent action potentials. This minimum interspike interval would not be maintained if the action potentials arose from two different neurons.
was estimated by the observation of recorded responses and by observing the time after the audio markers of stimulus injection. Lists of the waiting times between action potentials were from 10 seconds of ISI before stimulation and 10 seconds after the stimulus was applied. From these ISI lists other parameters were derived by programming statements, such as total number of action potentials before and after stimulation, and minimum and maximum ISI. ISI and the parameters derived from them were analyzed by Statistical Analysis System (SAS) language programs (SAS Institute, 1985).

Experimental Design.

Two types of experiments were conducted which gave rise to two sets of results. One set of results (data A) was made up of data from 27 cells recorded from 22 fish. All of these cells were obtained from fish with the olfactory tract intact. The stimuli applied in the first experiment were 11 amino acids: the L-isomers of alanine, arginine, cysteine, glutamic acid, methionine, asparagine, glutamic acid gamma methyl ester, lysine, norleucine, norvaline and threonine. Each stimulus was applied for two to nine repeated applications over the concentration range
from $10^{-6}$ M to $10^{-2}$ M.

For these experiments, ISI differences before and after stimulation were examined in a multiway analysis of variance (MANOVA) which considered the variability due to differences between fish, cells within fish, and the reproducibility of responses from each cell. These sources of variability were examined before testing the ISI differences related to the application of different amino acids and concentrations. MANOVAS were also applied to assess the constancy of background ISIs and the responses to well water controls. As is typical in physiological experiments, these data were not balanced because equal numbers of responses could not be obtained to each stimulus from each cell and unequal numbers of cells were recorded from in each fish. The proper model for the MANOVA was to analyze fish, cells within fish (a nested effect) and repeated stimulus applications as blocking factors and amino acids and concentrations as the main effects of interest (Millikin and Johnson, 1984).

ISIs were different in duration during different times in the response of the peripheral olfactory organ (EOG). Therefore, ISIs were subdivided into periods which corresponded to the rise to peak (1.5 S)
and fall to baseline (3.5 s) of the average EOG. These periods of the response are called EOG segments. Figure 3 shows these subdivisions of the ISIs relative to the EOG.

The second experiment conducted on the catfish olfactory bulb preparation took into account the problems which arose during the analysis of the unbalanced data set produced in the first experiment. The second experiment (data B) was conducted so that the same number of responses were obtained from each fish and cell. Six cells were recorded in each of six fish. Three of the cells within each fish were recorded in an intact bulb preparation and three different cells per fish were recorded after olfactory bulb isolation (olfactory tract transected). Each stimulus (L isomers of alanine, arginine, cysteine, glutamic acid and methionine) was applied twice to each cell at each stimulus concentration (10⁻⁵ M, 10⁻⁴ M and 10⁻³ M).

The question this experiment was designed to answer was whether olfactory bulb isolation through transection of the olfactory tract affected the representation of olfactory information as reflected by a change in the mitral cell action potential activity. The analysis of data B was accomplished with a split
Figure 3. The response of an olfactory bulb cell from data set A marked to indicate the data subdivisions used in this study. The period after stimulus administration was divided into three segments correlated with the magnitude of the electro-olfactogram.
stimulus onset

[AP 10 seconds pre stimulus]

[EOG]

1.5 second 3.5 second 5.0 second

EOG segment I EOG segment II EOG segment III
plot design MANOVA (Cochran and Cox, 1957). In the MANOVA, the multiple dependent variables were three interval parameters: number of intervals, maximum ISI and minimum ISI. The main experimental effect tested was the transection of the olfactory tract. The MANOVA was also used to assess the constancy of ISIs during background activity (Werner and Mountcastle, 1963) and after well water controls. The MANOVA significance test applied was Wilk's Lambda (Tabachnick and Fidell, 1983).

After the MANOVAS were used to demonstrate overall significant differences between response related ISIs and background ISIs, types or classes of responses in the mitral cell action potential activity were tested for. Response type refers to groups of responses which had a common ISI feature such as "inhibitory" in which the response was dominated by long interspike intervals, or "excitatory" with very short ISIs such as appear when action potentials occur at a high rate and consequently close together in time. Inhibitory and excitatory ISIs also occurred in the same responses, making another response type.

Response types were sought by the application of cluster analyses to the interval parameters associated
with the rising and falling phases of the EOG for each amino acid and the well water controls. A separate cluster analysis was conducted at each concentration of amino acid, this was done to observe how response types differed between concentrations. Number of intervals, minimum and maximum ISIs drawn from the rising and falling phases of the EOG were the six variables used to characterize each response in one set of cluster analysis. For comparison of the effectiveness of EOG segment 1 variables in classifying responses, the number of action potentials, minimum and maximum ISI from segment 1 only were also used as variables in another cluster analysis.

Principle component scores were clustered rather than the raw variables to remove correlations among the ISI parameters, since correlation inflates the value of the cubic clustering criterion which is used to evaluate the goodness of cluster analysis solutions (Aldenderfer and Blashfield, 1984). Cluster analyses were first performed using a hierarchical, agglomerative cluster technique, and the cubic clustering criteria were plotted vs the number of clusters in order to choose a reasonable range for the number of clusters (Sarle, 1983). The cubic clustering criterion
is a statistical test of the goodness of a cluster solution fit to a given body of data. In a good cluster solution, one where the cluster analysis presents information about a body of data that "makes sense" or is rationalizable to the investigator, the cubic clustering criterion will be positive and greater than one with larger values representing better cluster solutions (Aldenderfer and Blashfield, 1984). The hierarchical cluster analysis was performed first to reduce computing time which would have been prohibitively long for the calculation of solutions for all possible numbers of clusters using a non-hierarchical cluster method. Once the hierarchical cluster method indicated a reasonable number of clusters, a nonhierarchical cluster method was performed in order to fit responses into clusters that are not nested within each other as is assumed in hierarchical cluster techniques. This last step was necessary, because it was not logical to assume that response types are necessarily subsets of one another.

The responses of the mitral cells to increasing stimulus concentrations for each amino acid stimulus were investigated by regression analysis (Neter and Wasserman, 1974). In the first experimental data set
(data A), where the olfactory bulb's connection to the rest of the brain remained intact, four cells from four different fish were used in a study of the regression of stimulus concentration and the ISI. These four cells were tested with two different amino acid stimuli applied over a range of concentrations from $10^{-6}$ M to $10^{-2}$ M, for as many as nine replications. Since the ISI tended to show decreases as well as increases during the application of increasing concentrations, the dose-response curves were rarely linear. Because of the non-linear form of the dose-response relationships, polynomial regression equations with linear, quadratic and cubic terms were tested on these non-monotonic dose-response relationships.

The importance of temporal patterns in the bulb cell responses, as indicated by groups of ISIs of particular lengths appearing together during a response, was not tested by any of the statistical tests described above. This was because none of the tests considered the order of ISI occurrence, but only the number of action potentials and the maximum and minimum ISI. For the purpose of studying temporal patterns, the data from experiment two were used, because an
equal number of responses to each stimulus was obtained from each cell examined and an equal number of cells was recorded from each fish studied.

In the method applied to test for temporal patterns, ISIs were divided into time subdivisions of 500 milliseconds or "bins", (Glaser and Ruchkin, 1976), and the number of action potentials which occurred in each bin was counted. This procedure provided an equal number of bins in each response so that correlations based on an equal number of values were obtained to compare responses with unequal numbers of action potentials. Correlations were calculated between responses to different amino acid stimuli. Where the correlations were high, the stimuli were considered to evoke similar temporal response patterns, low correlations indicated different temporal patterns of response for the two stimuli.

For each of the three stimulus concentrations (10^{-5} M, 10^{-4} M and 10^{-3} M), correlations were obtained between the pattern of responses to each pair of the amino acid stimuli and between each amino acid and well water. These correlations were used as input to factor analysis (Harman, 1967), which reduced the pairwise stimulus correlations to a series of factors
which were interpreted as describing the relatedness of responses to the stimulus pairs. This scheme indicated similarities in the way the olfactory system responded to the two amino acids of each pair. Since receptor site types for amino acids in the channel catfish have been proposed (Caprio and Byrd, 1984), it is reasonable to ask whether such associations hold in the output of the olfactory bulb and how they change when the bulb is isolated by cutting the olfactory tract.
Results.

The responses of 63 cells in 28 fish were recorded in the two experiments of this study. In the first experiment (data set A) 1408 twenty second samples were collected from 27 olfactory bulb cells in 22 fish. In the second experiment (data set B) the activity of cells in the intact olfactory bulb was compared to activity after the olfactory bulb was isolated by olfactory tract transection. This second data set, unlike the first, was collected in a balanced manner. This meant that each fish contributed measurements from an equal number of different cells before and after the olfactory bulb was isolated, and each cell was stimulated an equal number of times with the same amino acids and concentrations. This second data set consisted of 1080 twenty second samples from 6 fish and 36 total cells (6 different cells in each fish).

Well resolved, single unit responses were recorded from depths of 150 to 550 micrometers and 750 to 950 micrometers in the olfactory bulb. Regions of the bulb between these depth ranges, and areas above and below these depths, lacked electrical activity
that could be resolved by the electrodes used in this study. The amplitudes of extracellularly recorded action potentials ranged from 500 to 800 microvolts.

Histological Observations.

A transverse section through the olfactory bulb of the channel catfish (Figure 4) showed that mitral cells were the cell type present in the bulb regions that corresponded to both of the recording depth ranges of this study. The incoming bundles of olfactory neurons form a distinct layer in the olfactory bulb. Beneath this olfactory neuron layer is the glomerular layer. The glomeruli are comprised of the olfactory receptors ending on the spherical tangles of mitral cell dendrites and are outlined by encircling capillaries. Beneath the glomeruli are the 60 to 150 micrometer long mitral cell bodies (Figure 4). Mitral cell bodies predominate at the recorded depths in this study and likely accounted for the large amplitude action potentials that were recorded. Because of the concentric layers of the bulb, a region of mitral cell bodies is found both at the superficial depths recorded (150 to 550 micrometers) and on the other side (ventral) of the central granule cell region of the bulb (750 to 950 micrometers). The granule cells are
Figure 4. A mitral cell body from the catfish olfactory bulb. The dorsal surface of the olfactory bulb is towards the top of the page. Solid arrow: dendrites arising from the cell body that proceed dorsally to glomeruli. Outline arrow: axon going ventral to the olfactory tract. The letter c is next to a capillary. The size bar represents 150 micrometers.
the small central cells of the olfactory bulb, named for the granular appearance of their nuclei (Shepherd, 1979).

**Background Activity and Control Responses.**

The mean background rate of action potentials recorded from bulb cells in data set A was \( 8.7 \pm 1.9 \) Hertz (+ the standard error of the mean). This mean was calculated over 704 ten second background periods measured in the 27 cells of data set A. Action potential counts for the ten second background periods ranged from 9 spikes to 283 spikes.

To determine if the background activity of olfactory bulb cells in data set A remained constant before stimulus application, a MANOVA was conducted on the ten second background time periods of data set A. The significance of differences in the background activity that preceded responses to stimulation was tested in this MANOVA by the effect labelled "periods of background activity" in Table 1. The differences between "periods of background activity" showed that background action potential counts preceding the response periods were not significantly different. This result is important because it would be difficult to apply an adequate criterion of response if the background rate
Table 1. Multiple ANOVA on the Background activity From Data Set A.

Number of observations = 630.

<table>
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<tbody>
<tr>
<td>Fish</td>
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<td>2529</td>
<td>8051202</td>
<td>10.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Periods of Background Activity</td>
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<td>400</td>
<td>252466</td>
<td>0.8</td>
<td>0.4630</td>
</tr>
<tr>
<td>Cells Within Fish</td>
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<td>34865</td>
<td>1636</td>
<td>2217423</td>
<td>12.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>1507</td>
<td>154</td>
<td>259070</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
of action potential occurrence or the minimum and maximum ISI were changing while the activity of a neuron is under observation.

For the MANOVA results of Table 1 and the MANOVA tables that follow, fish and cells within fish (a nested effect) are significant. This demonstrates a level of variability among individual fish as experimental animals and a heterogeneity of neurons recorded from within each fish. These sources of variation would be a problem in the detection of significant differences in ISI variables due to stimulation if animals and cells within animals were not taken into account.

The typical control effect was a slight rearrangement of background activity (Figure 5). The impression that ISIs during controls did not change from background activity was tested in a MANOVA on the controls applied in data set A. Table 2 shows that repeated control applications and ISI variables compared before and after control well waters are not significantly different. There are significant differences due to fish and cells within fish as expected, but EOG segments (subdivisions of the ISI within one 10 second time period) were also sig-
Figure 5. Responses to control well water applications from two different cells from data set A. AP, action potentials from the mitral cell (500 microvolt calibration bar). EOG, the electro-olfactogram (2 mV calibration bar). The EOG magnitude was small as was typical for well water control applications.
Table 2. MANOVA on the well water controls from data set a.

Number of observations=130.

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<td></td>
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<td>654</td>
<td>99021</td>
<td>558346</td>
<td>5.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Repeated control applications</td>
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<td>33</td>
<td>88361</td>
<td>96073</td>
<td>0.4</td>
<td>0.6931</td>
</tr>
<tr>
<td>Cells within fish</td>
<td>1</td>
<td>111</td>
<td>191902</td>
<td>188554</td>
<td>3.8</td>
<td>0.0121</td>
</tr>
<tr>
<td>Background vs. control application</td>
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<td>0.7</td>
<td>59090</td>
<td>10411</td>
<td>0.7</td>
<td>0.5524</td>
</tr>
<tr>
<td>EOG segments</td>
<td>1</td>
<td>2826</td>
<td>221020</td>
<td>3152487</td>
<td>78.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>EOG segment by cell interaction</td>
<td>1</td>
<td>374</td>
<td>302492</td>
<td>986511</td>
<td>3.9</td>
<td>0.0105</td>
</tr>
</tbody>
</table>
nificantly different. The interaction between the EOG segments and the individual cells was significant as well. This interaction between cells and EOG segments was due to the heterogeneous cell backgrounds demonstrated by the significant differences between cells in Table 1. The analyses in the first two MANOVA tables have shown that the background activity of particular mitral cells was stable, that the mitral cells differed from each other in background ISI, and that no change was evoked in the background activity by the application of control well waters.

Evaluation of Factors Affecting Responses.

After examining background activity and control responses for data set A, amino acid responses were analyzed in a similar way. In order to apply a MANOVA to data set A, balance in the data set was obtained by dropping all but 2 repeated stimulus applications and considering only two cells from each fish. In addition, only 5 amino acid stimuli (alanine, arginine, cysteine, methionine and glutamic acid) and three concentrations \(10^{-5}, 10^{-4} \text{ and } 10^{-3} \text{ M}\) were considered. These selection procedures were necessary to obtain a balanced data subset so that variability in the mitral cells could be analysed from the standpoint of the
population, rather than by conducting tests on individual cells. Four cells from data set A, where different amino acids were applied over a wide range of concentrations for three or more replications, were analyzed in the regressions of dose-response relationships described below.

Sources of error examined in the background and control MANOVAS, such as fish to fish differences and cells within fish, ISI changes before and after stimulation and EOG segments, were all tested as in the MANOVAS on background and control activity. Added to the analysis were the effects of different amino acids, the effects of different amino acid concentrations, and the interactions of amino acids, concentrations and EOG segments with mitral cells. The interaction terms are included in the analysis to determine whether or not cells were homogeneous in their amino acid responses. If significant interactions of cells and amino acids indicate cells responses to amino acids are not similar, then it is probable that an important aspect of information representation is based upon comparison of different cell's responses at a higher level in the nervous system.

In Table 3, the MANOVA reveals that the differences due to fish and cells within fish are sig-
Table 3. MANOVA on the amino acid responses from data set a.

Number of observations=1528.

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<td>6365</td>
<td>790864</td>
<td>4550852</td>
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</tr>
<tr>
<td>Repeated stimulus applications</td>
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<td>2683</td>
<td>158901</td>
<td>523207</td>
<td>8.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cells within fish</td>
<td>1</td>
<td>2347</td>
<td>672262</td>
<td>4614924</td>
<td>19.9</td>
<td>0.0001</td>
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<tr>
<td>Amino acids</td>
<td>4</td>
<td>83</td>
<td>198910</td>
<td>281513</td>
<td>1.2</td>
<td>0.2538</td>
</tr>
<tr>
<td>Concentrations</td>
<td>2</td>
<td>382</td>
<td>254203</td>
<td>276045</td>
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<td>0.0139</td>
</tr>
<tr>
<td>Before &amp; after stimulation</td>
<td>1</td>
<td>262</td>
<td>1177881</td>
<td>1095652</td>
<td>4.9</td>
<td>0.0021</td>
</tr>
<tr>
<td>EOG segments</td>
<td>1</td>
<td>53591</td>
<td>3238341</td>
<td>11025483</td>
<td>468.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>Amino acid by cell interaction</td>
<td>4</td>
<td>260</td>
<td>631505</td>
<td>762595</td>
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<tr>
<td>Concentration by cell interaction</td>
<td>2</td>
<td>750</td>
<td>164541</td>
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<td>0.0032</td>
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<tr>
<td>EOG segment by cell interaction</td>
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<td>1470</td>
<td>1404915</td>
<td>159059</td>
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<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>1434</td>
<td>122</td>
<td>89315</td>
<td>135870</td>
<td></td>
<td></td>
</tr>
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</table>
significant. Similar patterns of response were frequently evoked by the repeated application of an amino acid stimulus (Figure 6). In the population of responses in data set A, however, repeated applications of an amino acid were significantly different. This suggests that in spite of high reproducibility for some cells, the population of mitral cells examined in data set A were not reproducible in ISI variables from one application of a stimulus to another. This may have been due to the wide variety of stimuli and concentrations applied, to some cells failing to give reproducible responses at all, or to all cells giving poor responses to some amino acids and reproducible responses to others. This latter possibility is demonstrated to be the case in the analysis of data set B, where cells were selectively sensitive to amino acids.

Amino acids are not significantly different in the MANOVA tests of Table 3. The implication of this lack of significance is that the ISI, without specifying which cell has given rise to them cannot represent stimulus quality. This point is also considered in the cluster and factor analysis described below.

In Table 3, amino acid concentrations are significantly different, as are the ISI prior to and dur-
Figure 6. Two responses from the same olfactory bulb cell in data set A. The stimulus in both pairs of traces was $10^{-5}$ M glutamic acid. Inter-stimulus interval was one minute as described in materials and methods. AP, action potentials from mitral cell (500 microvolt calibration bar). EOG, the electro-olfactogram (2 mV calibration bar).
ing stimulation. Thus stimulus intensity might be represented by the ISI without any further information, such as the identity of the cell giving rise to the response (Kauer, 1974). The coding of intensity is considered further in the analyses of dose-response relationships in data set B. The significant differences in ISI before and after stimulation is a result of fundamental importance to the study of the neural representation of olfactory information. If there had been no significant difference in the ISI prior to and during stimulation, then it would not be clear how the cells receiving mitral cell output could detect stimulus onset. The significant statistical difference between ISI before and after stimulation suggests that if a coding scheme used information from the mitral cell population, as this analysis has, it would be possible to distinguish background from stimulated ISIs.

The EOG segments are significant in the MANOVA (Table 3), confirming the suggestion of Figures 5 and 6, that different ISI patterns occur during the rising and falling phases of the EOG. This point is explored in the cluster analyses, where the response classes
suggested by variables from the initial EOG segment are compared with the response types suggested by ISI from the rising and falling phases of the EOG.

All the significant interactions of individual cells and amino acids, amino acid concentrations and segments of the EOG are indicative of the heterogeneity of the mitral cell responses. This large degree of difference between individual mitral cells suggests that these differences may play a role in the coding of olfactory information.

Bulb Activity and Olfactory Tract Transection.

There is the possibility that interactions between the olfactory bulb and other parts of the telencephalon might play a role in olfactory information processing. Therefore, responses were recorded in the olfactory bulbs of fish with the olfactory tract intact and transected. Due to the amount of variability among fish and the significant differences between cells within fish seen in data set A, this second experiment was designed to account for these sources of variation and allow testing of ISI differences due to the isolation of the olfactory bulb from telencephalic inputs. The differences due to cells within fish were important, since the effect of tract transection had
to be recognized over and above the cell to cell differences.

The experiment was conducted by recording from an equal number of different olfactory bulb cells in each fish before and after the olfactory tract was sectioned. Since the experiment contained the same amount of information from each cell in each fish, all statistical comparisons were made at the same confidence level and all values, such as correlation coefficients, were based on equal-sized groups. All measurements derived from this balanced data set were more reliable when used in multivariate analyses than values obtained from an unbalanced data set (Tabachnick and Fidell, 1983).

Six cells were recorded in each of six fish, three cells prior to olfactory bulb isolation and three different cells after transection of the olfactory tract resulting in the isolation of the olfactory bulb. The experiment was analyzed as a split plot design (Cochran and Cox, 1957) MANOVA. ISIs from EOG segments were not analyzed in this design to reduce the complexity of the MANOVA and computation time. The ISI variables used as multiple dependent variables were drawn from the 10 seconds of stimulus time. These
deletions were considered conservative, since they reduced the overall degrees of freedom and enlarged the error term.

Table 4 shows the MANOVA on background activity in data set B. The only significant factor is cells within bulb condition. As seen in all the analyses cells within fish are significantly different from each other. Bulb condition (tract intact or isolated by tract cutting) was not significant. Some cells, however, did show an increased background rate after tract transection while others showed reduced rates. Neither type of change was common enough to carry the significance for the population, or the changes canceled out. Just as cells did not respond in a homogeneous manner to amino acid stimulation in data set A, neither was the background activity of all cells in data set B affected to the same degree by tract transection.

Analysis of the well water control applications on data set B (Table 5) shows that fish and cells within condition of the olfactory bulb are significantly different as is consistent with all other analyses presented here. The MANOVA test for tract condition is significant with the well water controls.
Table 4. Multivariate ANOVA on the Background activity From Data Set B.

Number of observations in data set = 1080.

<table>
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<tr>
<th>Source</th>
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<td>Fish</td>
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<td>216300</td>
<td>457879</td>
<td>66398865</td>
<td>4.6</td>
<td>0.0001</td>
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<tr>
<td>Periods of Background Activity</td>
<td>1</td>
<td>7443</td>
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<td>882176</td>
<td>0.1</td>
<td>0.9039</td>
</tr>
<tr>
<td>Bulb condition</td>
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<td>3997</td>
<td>102480</td>
<td>281021</td>
<td>0.9</td>
<td>0.4430</td>
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<tr>
<td>Cells within bulb condition</td>
<td>4</td>
<td>57030</td>
<td>164159</td>
<td>31722713</td>
<td>1.8</td>
<td>0.0523</td>
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<tr>
<td>Error</td>
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<td>482</td>
<td>45931</td>
<td>923821</td>
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Table 5. Multivariate ANOVA on the effects of olfactory bulb isolation on well water controls, split plot arrangement of treatments.

Number of observations in data set = 130.

<table>
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<td>18573</td>
<td>70375</td>
<td>8555214</td>
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<tr>
<td>Repeated control applications</td>
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<td>156</td>
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<td>4557547</td>
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<td>Before and after stimulation</td>
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<td>37063</td>
<td>1063895</td>
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<td>0.5364</td>
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<tr>
<td>Bulb condition</td>
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<td>9.9</td>
<td>5973</td>
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<tr>
<td>Cells within bulb condition</td>
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<td>0.0203</td>
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<tr>
<td>Error</td>
<td>41</td>
<td>95</td>
<td>12682</td>
<td>426145</td>
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</tr>
</tbody>
</table>
Since there was no significant difference between control applications and background activity in Table 5, the significant difference due to tract transection may be the result of background changes. It is, however, inconsistent that the analysis of the data B background activity (Table 4) showed no significant change with olfactory tract cutting. This inconsistency may be due to the unequal number of well water controls analyzed from each cell, and therefore a sampling problem. The analysis of the amino acid responses of data set B, due to its improved sampling, resolves the question of the significance of olfactory bulb isolation on mitral cell responses.

Table 6 summarizes the results from the MANOVA tests on the amino acid responses from data B. Fish and cells within a condition of the tract (intact with the bulb connected to the CNS or transected with the bulb isolated) are both significant. Amino acids are significantly different and amino acid concentrations are not. The conflict between these results and those of the analysis on the amino acid responses from data set A, where amino acid concentrations were significantly different but amino acids were not, is due to differences in experimental design between the two
Table 6. Multivariate ANOVA on the effects of olfactory bulb isolation on amino acid responses, split plot arrangement of treatments.
Number of observations in data set = 1080.

<table>
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<tr>
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<td>Periods of Background Activity</td>
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<td>2037104</td>
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<td>Cells within bulb condition</td>
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data sets. In data set A, more different fish were tested, but fewer cells in each fish were recorded so that the differences between mitral cells were not distinguishable from fish to fish differences. It is the cell to cell differences that make amino acids distinguishable. In data B more cells were recorded in each fish and that allowed amino acids to be distinguished. In addition, fewer concentrations were applied which reduced the effect of stimulus intensity. The exact nature of the differences between responses to various amino acids and the effect of tract transection on these differences is further examined and clarified in the multivariate cluster analysis on response groupings described below. An important aspect of these results is the significance of the isolation of the olfactory bulb by transection of the olfactory tract. Overall, the number of action potentials increased, and the maximum ISI decreased as a result of tract transection. These conclusions are verified by inspection of the plots of ISI variables presented with the cluster analysis (Figure 8).

In two cases, electrophysiological recordings strongly indicated that the same cell was recorded from both before and after bulb isolation. Evidence
that the cells were maintained through olfactory tract transection was that the waveforms of the recorded action potentials were identical before and after transection of the olfactory tract and the records were obtained in regions of the bulb devoid of other neural activity. Table 7 gives the results of the MANOVA on these two cells. Repeated stimulus applications did not differ significantly (these data had one post tract transection replicate missing on one of the cells). Tract cutting was highly significant in its effect on these two cells. The cells within tract condition were significantly different due to the consistently observed cell to cell differences in all tests conducted here. Both prior to and during stimulus application, ISI values were significantly different, but concentrations were not. There was a significant difference between the ISI in response to different amino acid stimuli depending on whether the tract was cut or not, shown by the significant bulb condition by amino acid interaction. This suggests that the representation of information on amino acid stimuli by the olfactory bulb is different when the olfactory tract is cut from that when the olfactory tract is intact.
Table 7. Multivariate ANOVA on two mitral cells held before and after bulb isolation.
Number of observations in data set = 180.

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<th>Pr&gt;F</th>
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</thead>
<tbody>
<tr>
<td>Repeated stimulus applications</td>
<td>1</td>
<td>2173</td>
<td>216</td>
<td>690992</td>
<td>2.3</td>
<td>0.0816</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1841</td>
<td>1780</td>
<td>330957</td>
<td>8.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>Before and after stimulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulb condition</td>
<td>1</td>
<td>21110</td>
<td>57</td>
<td>14443863</td>
<td>31.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cells within bulb condition</td>
<td>1</td>
<td>47254</td>
<td>112</td>
<td>10868361</td>
<td>44.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>Amino acids</td>
<td>4</td>
<td>6485</td>
<td>482</td>
<td>883994</td>
<td>5.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>Concentrations</td>
<td>2</td>
<td>172</td>
<td>53</td>
<td>16094</td>
<td>0.3</td>
<td>0.9207</td>
</tr>
<tr>
<td>Bulb condition by amino acid interaction</td>
<td>4</td>
<td>1854</td>
<td>113</td>
<td>349687</td>
<td>2.4</td>
<td>0.0046</td>
</tr>
<tr>
<td>Error</td>
<td>165</td>
<td>407</td>
<td>106</td>
<td>187744</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
For one of these two cells held before and after tract transection, two applications of each stimulus before and after tract cutting were recorded. The significant difference in responses after tract transection for this cell is due to an increase of the maximum interspike interval. Thus, for this cell, the effect of olfactory tract cutting was opposite that seen for the population of bulb cells. This is consistent with the heterogeneity of the population of bulb mitral cell responses seen in all the analyses presented here.

**Dose-Response Functions.**

In order to better understand how information on stimulus concentration is represented in the olfactory bulb, regression analyses were applied to the responses of cells to increasing concentrations of amino acids. The results of the MANOVA on data A suggested that the ISIs might represent concentrations, thus the regression of the ISI variables on amino acid concentrations was examined. Some of the possibilities considered for the neural representation of information on stimulus concentration were that (1) all mitral cells were equally good at representing this information through some or all of the ISIs, (2) that
only a few cells were capable of representing information on concentration, (3) that many different cells could each code for the concentration of one or a few stimuli. The results of this study support the latter possibility, that particular cells "specialize" in accurate representation of information about the concentration of one or a few stimuli. This was shown by the results of the regression analyses in which a cell had significant regressions to some, but not all of the amino acid concentration series applied to it. The cell to cell differences in amino acid responses over different concentrations also explained the inconsistent pattern of significance for amino acids and concentrations in the MANOVAS on the amino acid responses of data sets A and B.

In data set A, regression analysis was applied to the responses of 4 cells. These cells were held for recording periods of 3 to 8 hours each. Stimulation was conducted with different amino acids for as many as 9 repeated applications over a range of concentrations from $10^{-6}$ M to $10^{-2}$ M. The ISI variables number of action potentials, maximum ISI or minimum ISI often did not increase in a simple linear fashion. With increasing stimulus concentration, the maximum ISI, for
example, might first increase then decrease as excitation and shorter minimum ISI became more evident at higher concentrations. Complex changes in all three ISI variables were seen. Simple linear increases over a wide range of concentrations were the exception rather than the rule. In order to fit such non-linear changes in the ISI variables, polynomial regression equations with linear, quadratic and cubic terms were used to study dose response-relationships.

Mitral cells responded to some stimuli in a highly variable manner that could not be fit with regression equations of any type. Under the same conditions highly reproducible responses to increasing concentrations of another amino acid could be obtained from the same cell. An example of this behavior is seen in Figure 7. Figure 7 A 1, A 2 and A 3 show the mean values and standard errors of the number of intervals, minimum and maximum ISIs from cell 1 in fish 12 of data set A to amino butyric acid. As the low values for the $R^2$ values on these plots suggest, no significant regression curves could be fitted to these responses. Cysteine on the same cell gave significant regressions to all of the ISI (Figure 7 B 1, B 2, B 3). This is shown with the higher and significant $R^2$ values and
Figure 7. Plots of the ISI variables for two stimuli applied to cell 1 of fish 12 data set A. R squared values, and where significant, regression equations and regression lines are shown on the graphs. The error bars represent standard errors. The extreme concentrations are single applications. A 1, number of intervals, A 2, minimum interval, A 3, maximum interval in response to amino butyric acid. B 1, B 2, B 3, the same ISI variables for responses of this cell to cysteine.
the smaller standard errors at concentrations where multiple applications were made.

In data set B, regression analysis confirmed the results of the regressions on data set A. The responses of a single neuron to different amino acids could not all be fit with significant regressions. Only three stimulus concentrations were applied in data set B, so there were not the required degrees of freedom to fit second and third order terms in regression models. Furthermore, the restricted ranges of concentrations used did not demonstrate the nonlinear curves that a wider range of concentrations might have indicated. Linear regression models were used in the analysis of data set B to determine whether cells responded in a predictable manner to the 3 concentrations of the 5 different amino acids applied to them. The ISI parameters used as dependent variables in these regressions were the number of action potentials, minimum and maximum ISI drawn from the two segments of the EOG. The ISI from 5 second time divisions of the 10 seconds of background ISI preceding stimulation were regressed against stimulus concentration as well to provide a check on the frequency of erroneous regressions due to nonspecific increases in background activity.
There were 540 regressions performed for each of the background and stimulus response segments. If these regressions were considered independent, 5% or 27 would be expected to be significant at random without any relationship of stimulus concentration and the ISI. A percentage of regressions close to the random expected percentage was seen in the first five seconds of background time both prior to and during bulb isolation (5% and 4.4% respectively, Table 8). Considering before and after bulb isolation, the frequency of significant regressions was greatest in the last 5 seconds of background activity and in EOG segments 1 and 2. The third EOG segment had a lower frequency, 5.9%, which approached that of the initial 5 seconds of background activity.

The regression frequencies indicated a decrease in the number of significant regressions after the olfactory tract was cut. Thus, olfactory bulb isolation disrupted the representation of information on stimulus quality. Another effect of olfactory bulb isolation was that the decrease in regression frequencies was due to the loss of regressions for glutamic acid and methionine. Both stimulus intensity representation and its specificity were altered upon isolation of the olfactory bulb.
Table 8.

Numbers of significant regression analyses for each amino acid in the background and EOG segments and for each condition of the olfactory bulb. Numbers are summed over all three interval measures.

A. Bulb Intact

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>background initial 5 seconds</th>
<th>background second 5 seconds</th>
<th>EOG seg. 1</th>
<th>EOG seg. 2</th>
<th>EOG seg. 3</th>
<th>amino acid sums</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>ARG</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>CYS</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>9</td>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>GLU</td>
<td>4</td>
<td>10</td>
<td>12</td>
<td>18</td>
<td>2</td>
<td>46</td>
</tr>
<tr>
<td>MET</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>27</td>
</tr>
</tbody>
</table>

Time segment sums

|              | 15 | 30 | 35 | 40 | 18 | 138 |

B. Bulb Isolated

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>background initial 5 seconds</th>
<th>background second 5 seconds</th>
<th>EOG seg. 1</th>
<th>EOG seg. 2</th>
<th>EOG seg. 3</th>
<th>amino acid sums</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>ARG</td>
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<td>4</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>CYS</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>GLU</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>MET</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>12</td>
</tr>
</tbody>
</table>

Time segment sums

|              | 12 | 16 | 26 | 8  | 10 | 72  |

Totals

|              | 27 | 46 | 61 | 48 | 28 |

% of 540: 5.0% 8.5% 11.3% 8.8% 5.2%
These data on the frequencies of significant regressions indicated that not all cells were equally effective at coding stimulus concentration for all the amino acids tested. Some cells appeared to specialize in the representation of concentration for 1 to 4 amino acids.

Classification of Responses.

The assignment of responses to response classes such as "excitatory", "inhibitory" or "complex" has been an important tool in attempts to understand olfactory information representation in the olfactory bulb. In the present, cluster analysis was utilized to attempt response classification on the basis of interspike interval variables drawn from the EOG segments. The application of cluster analysis is to some degree based on subjective decisions and its findings must be rationalized in physiological terms for meaningful application (Beiber and Smith, 1986). Hence to some degree the application of this multivariate classification technique suffers from the same limitations as the classification of responses performed by investigators without this analytical tool. The multivariate technique, however, has the advantage over subjective evaluation in that the cubic clustering criterion
(Sarle, 1983) provides an objective index for the comparison of different response groupings based on ISI from different parts of the response.

The cluster analyses applied here identified response groups in the mitral cell activity, which is in agreement with previous investigators (Meredith and Moulton, 1978; Kauer, 1974). The results also indicated that the initial portion of the EOG response, the first 1.5 seconds (EOG segment 1), provided the best classification in terms of cluster analysis and meaningful response classes. The analysis further indicated that response types were not associated with particular cells or stimuli.

The most suitable number of clusters was found to be three for these data by the initial hierarchical cluster solution. The first non-hierarchical cluster solution used six variables: the number of action potentials, minimum and maximum ISI drawn from the two EOG segments. The fits were poor as indicated by the cubic clustering criteria. The highest cubic clustering criterion found using six variables was 2.1 for the responses to $10^{-5}$ M amino acids before tract transection. Three of the six cubic clustering criteria (6 cluster solutions for the 3 concentrations
before and 3 after bulb isolation) were negative. The use of only the three ISI variables from EOG segment 1 greatly improved the cluster fits and made a more reasonable cluster solution with 4 fold higher cubic clustering criteria (8.2 for $10^{-3}$ M amino acids before tract transection).

The clusters found at each concentration before and after olfactory tract transection are shown in terms of the ISI variables in the three dimensional plots of Figure 8. Each point in these plots represents a single amino acid response of a mitral cell. The clusters, characterized by the ISI variables during EOG segment 1 (initial 1.5 seconds), are indicated by the three different symbols, excitation (pyramids), inhibition (balloons) and complex with both excitation and inhibition occurring (cubes).

The values of the ISI characterizing each cluster are seen by inspection of the plot axes. For instance, in the plot of the intact olfactory bulb at $10^{-5}$ M, pyramids representing the excitatory cluster occupy the part of the graph where the minimum ISI is never greater than 500 milliseconds and the maximum ISI is never more than 860 milliseconds. The greatest number of action potentials also characterize this excitatory
group, going as high as 39 action potentials. The balloons representing the inhibitory cluster occupy the sector of the graph where the minimum ISI is not less than 770 milliseconds, the maximum ISI is 940 milliseconds or greater and no more than five action potentials occurred. The cubes representing the complex cluster at $10^{-5}$ M before tract transection are found in the region with minimum ISI as short as 20 milliseconds, action potential counts as high as 11 and maximum ISI no larger than 1397 milliseconds. As shown in the plots of Figure 8 and as indicated by the high values of the cubic clustering criteria, these different response groups are quite distinct from each other in the response space.

Similar values are seen for the number of action potentials, maximum and minimum ISI in the pre tract transection clusters for the other stimulus concentrations (Figure 8 a,c,e). These three dimensional plots of the ISI parameters indicated that even though amino acid concentrations were significantly different in the MANOVAS, the ISI variables were not discriminating among the amino acids (Table 6). No stimulus appeared preferentially in any response cluster. This suggests, in conjunction with the MANOVA results, that the ISI
Figure 8. Plots of the ISIs for the response clusters based on the first EOG segment. Pyramids, excitatory clusters. Balloons, inhibitory clusters. Cubes, excitatory-inhibitory clusters. Note that the number of intervals scale is different for the post tract transection plots. The individual plots are: a, responses to $10^{-5}$ M amino acids with the olfactory bulb intact, b, responses to $10^{-5}$ M amino acids with the bulb isolated; c, responses to $10^{-4}$ M amino acids with the olfactory bulb intact, d, responses to $10^{-4}$ M amino acids with the olfactory bulb isolated; e, responses to $10^{-3}$ M amino acids with the olfactory bulb intact, f, responses to $10^{-3}$ M amino acids with the olfactory bulb isolated. The number of intervals is a count of action potentials, and the minimum and maximum interval are in milliseconds (mS).
CONCENTRATION $10^{-5}$ M.

**a** bulb intact

**b** bulb isolated

(number of intervals vs. maximum interval)

minimum interval

maximum interval
CONCENTRATION $10^{-4}$ M.

**c)** bulb intact

**d)** bulb isolated

number of intervals

maximum interval

minimum interval
CONCENTRATION $10^{-3}$ M.

**e**

- bulb intact
- number of intervals

**f**

- bulb isolated
- number of intervals

- maximum interval
- minimum interval
- mS
- 1500
- 1001
- 501
parameters, irrespective of cell, are not representing quality information directly.

The effect of tract transection on the characteristics of the response groupings was to make the response classes less distinct. The change is reflected in the lower cubic clustering criteria for each of the post-tract transection plots. A greater similarity of responses after the olfactory bulb is isolated is shown in the cluster plots by the collection of more of the responses into the lower right hand section in the foreground of the plots. In addition to the responses becoming more alike, the inhibitory cluster at each concentration had fewer members (balloons). The number of action potentials are greater values for the post-tract transection clusters and responses had lower overall values for the maximum ISI. The loss of longer maximum ISI is shown by the movement of the responses out of the portion of the plots at the lower left hand corner, where the longest maximum interval values appear. These differences were noted the MANOVA on the effect of olfactory bulb isolation, where greater numbers of action potentials and lower values for the maximum ISI were indicated.

The implication of the cluster results for information representation in the olfactory system is
that the response types recognizable in the activity of either the intact or isolated olfactory bulb are not representing amino acids in a direct way (i.e. there is no association of response classes based on stimulus quality). The response classes also do not appear to be widely different at the three concentrations when the bulb is intact or isolated. This suggests that the response classes are not representing stimulus concentration either. These conclusions drawn from the cluster analyses support the conclusions from the MANOVAS on data A and B (Tables 3 and 6), that there was no consistent association between the ISI variables, number of action potentials, maximum or minimum ISI and either amino acids or the concentrations at which they were applied.

**Temporal Patterns in Bulb Cell Responses.**

The remaining possibility to be examined for information representation in the catfish olfactory bulb is that the order of ISI occurrence carries information, rather than only the number of action potentials, minimum or maximum ISI. This possibility was tested on data set B by counting the number of action potentials in 500 millisecond bins from the first 5 seconds of each response. The action potential counts
were averaged over the two repeated stimulus applications, since in data B these were not significantly different. The action potential counts were correlated with the lists of action potential counts from a cell's responses to other amino acids and well water controls. By this means, each cell's activity was expressed as a list of correlations that expressed how similarly that cell responded to each stimulus. Thus, if a particular cell always responded to both alanine and arginine with inhibition and then excitation, that cell's correlation between alanine and arginine would be high. However, if a cell's response to a compound was not recognizably different from background, then that response would be as correlated to the well water controls as to any stimulus.

There were 36 such lists of 15 correlations for the responses of each of the 36 cells of data set B (18 prior to tract transection and 18 post). This set of correlations also takes into consideration the particular response characteristics of each cell, since the individual cells are each characterized by one list of correlations. This body of correlations was used as the input to factor analysis (Harman, 1967), which was aimed at reducing the 15 correlations be-
tween pairs of stimuli to a set of "factors" which helped explain the large number of correlations by providing weights that associate the different correlations with the factors. As with an effective cluster solution, a good cluster set of factors make "sense" in terms of the information put into the correlations (Bieber and Smith, 1986). In the present case, the factors will make sense if they show associations or grouping of the stimuli. The meaning of such groupings would be that certain of the amino acids evoke similar temporal patterns of response in all the cells. Such similar response groups might be associated at a higher level of nervous system information processing.

The factor analysis results are summarized in the factor plots of Figure 9, where each individual symbol represents a correlation between the responses of all mitral cells to the indicated pair of amino acids or to the control and a single amino acid. The results of the factor analyses show amino acid pairs that have a common member tend to be "loaded" or weighted highly on one of the three factors. The fact that amino acid correlations have similar loadings on a factor causes them to appear close together or at the same level in
Figure 9. Plots of factor scores for correlations of action potential counts in 500 millisecond "bins". The symbols represent correlations between pairs of amino acids with the same or different side chain chemistry. Hearts: correlations of neutral and basic amino acids (alanine and arginine, cysteine and arginine, methionine and arginine). Crosses: correlations of well water controls with all other amino acid stimuli. The single spade: correlation of the basic amino acid arginine with the acidic amino acid glutamic acid. Clubs: correlations of two neutral amino acids (alanine and cysteine, alanine and methionine). Diamonds: correlations between neutral and acidic amino acids (alanine and glutamic acid, cysteine and glutamic acid, methionine and glutamic acid). The individual factor score plots are for the response correlations when: a, the bulb is intact and the stimulus concentration is $10^{-5}$ M; b, bulb isolated and concentration $10^{-5}$ M; c, bulb intact and concentration $10^{-4}$ M; d, bulb isolated and concentration $10^{-4}$ M; e, the bulb intact and concentration $10^{-3}$ M; f, bulb isolated and concentration $10^{-3}$ M.
a  bulb intact  CONCENTRATION $10^{-5}$ M.

b  bulb isolated
CONCENTRATION $10^{-4}$ M.

c  bulb intact

d  bulb isolated
the three dimensional plots of the factor scores. In Figure 9a, the diamonds represent correlations involving the acidic amino acid glutamic acid and the neutral amino acids alanine, cysteine or methionine, which have their highest weights on factor 1. The single spade, representing the correlation of arginine and glutamic acid also has its highest loading on factor 1. The common aspect of the correlations which have their highest loadings on factor 1 is that in each of the pairs, one of the members is glutamic acid, associated with either a neutral amino acid or arginine. Factor 2 has as its most highly weighted pairs alanine and cysteine, alanine and methionine, all neutral amino acids (represented as clubs). The pair arginine and methionine, a basic and a neutral amino acid also have their highest loading on factor 2, so that the common element of the correlations loaded on factor 2 is the neutral amino acid. Factor 3 in this plot is most highly weighted for well water correlations and one of the neutral amino acid pairs, cysteine and methionine. The factors are named in Figure 9a for these main loadings that make them distinct from the other factors. The factor solutions do not allow the complete distinction of glutamic acid,
neutral amino acids, arginine or well water, but they
do represent the only evidence of quality distinction
among the mitral cell responses drawn from any of the
analyses conducted in this study.

The factor weights change with concentration, but
in general, at each concentration, one of the factors
is most closely associated with correlations involving
glutamic acid, neutral amino acids or well waters.
Isolation of the olfactory bulb by olfactory tract
transection disrupts these organized loadings of the
three factors. In the case of each concentration, the
bulb isolated plot is characterized by a less
segregated appearance of the plot symbols, which
reflects the loadings of most amino acids on the first
factor, and only well water on the second or third
factor (Figure 9b, 9d, 9f).

This finding of more similar responses (as indi-
cated by all correlations loading on the same factor)
after the olfactory tract is cut, is comparable to the
cluster solutions, where responses became more similar
after tract cutting. The factor solutions provide fur-
ther information on olfactory information representa-
tion, because they indicate that the individual cells
make a unique contribution to information representa-
tion. The correlation patterns of each cell account for the weighting of the amino acid pairs onto the separate factors in accord with amino acid structure. The individual response of each mitral cell, shown consistently in each analysis conducted here as a significant cell to cell difference, appears most important to olfactory information representation in the channel catfish. This suggests that the coding of olfactory information by mitral cells involves the comparison of the unique patterns of responses of individual mitral cells across the population.
Discussion.

This study has shown that ISIs of neurons in the olfactory bulb of the channel catfish are constant in background activity with a higher rate than previously observed in other fish olfactory bulbs. High rates of background activity ensure that changes will be evident and departures from background more easily detected (Døving, 1987). Statistically recognizable variation from the background interspike intervals occurred when the olfactory mucosa was stimulated with amino acids. Responses characterized by number of action potentials, maximum and minimum ISI were not specific to amino acid stimuli or concentration. These results suggest that the coding of amino acid quality and quantity requires a mechanism for the comparison of the output of different mitral cells. In such a coding scheme each cell would be unique in its pattern of responses to the different stimuli applied. The responses of individual cells could also be compared to give concentration information on particular stimuli as well. Specialization in quantity coding for particular amino acids was shown by the differential accuracy of dose-response relationships among individ-
ual cells. The influence of more central parts of the nervous system is essential to the discriminatory capabilities of the catfish olfactory system, as demonstrated by the loss of response specificity when the olfactory tract was cut.

The histological observation that large mitral cells were predominant at the depths recorded in the olfactory bulb, as well as the fact that the electrodes used only registered neural activity at these specific depths, strongly indicate that the cells recorded from were mitral cells. Further, the large amplitude action potentials recorded (500 to 800 millivolts) are characteristic of the large cell bodies of the mitral cells, since large neurons produce large transmembrane currents. Further, cell types other than mitral cells of fish olfactory bulbs, were characterized by a higher degree of bursting activity (MacLeod, 1976) than observed for the mitral cells in the present study in channel catfish.

Background and Control Responses.

The 8.7 Hertz mean background rate of action potentials in the catfish olfactory bulb with the olfactory tract intact is high when compared to the 1 to 4.2 Hertz rates reported for olfactory bulb cells in
other teleosts (Salmo gairdneri 3.5 Hertz, MacLeod, 1976; Lota lota 4.2 Hertz, Døving, 1966; Oncorhynchus nerka 1.0 Hertz, Bodznick, 1978; Carassius auratus 11.7 Hertz, Meredith and Moulton, 1978). The rate in the catfish is more similar to the background rate of 14.6 Hertz in the rat (Døving, 1987). The high background rate of action potentials makes inhibitory responses more detectable, both by the experimenter and the central nervous system.

As the MANOVAS on background activity in the two data sets indicated (Tables 1 and 4), the background activity of individual mitral cells remained constant over the experimental periods. This is important for analysis of the data, since without constancy of the background action potential rate it is difficult to determine whether or not small fluctuations in inter-spike intervals represent low intensity responses or random changes. Different mitral cells had different background rates and this difference is an aspect of the cell to cell differences seen in every test conducted on the responses of catfish mitral cells.

**Control Effects.**

Well water controls were analyzed in separate MANOVAS for data sets A and B (Tables 2 and 5), and
were included in the clusters for response classification and in the factor analysis for temporal patterns of action potentials. The nonsignificant differences between background activity and well water control activity indicate that the population of mitral cells did not respond to the well water. The correlations between the interspike intervals during well water controls and the amino acids were generally low and tended to segregate together on the third factor in the factor analysis plots (Figure 9). This factor, which distinguished well water from other response correlations, did not do so absolutely. The correlations of well water and some of the amino acids separated from the other well water-amino acid correlations for some concentrations. The failure of the segregation of well water correlations from the stimulus correlations indicates that at these concentrations, amino acids evoked responses which were as correlated with well water as with any other stimulus and therefore were weak responses.

Olfactory Bulb Isolation.

The effect of cutting the olfactory tract and thereby isolating the olfactory bulb neurons from synaptic input from other parts of the central nervous
system was to reduce the maximum ISI and to increase the numbers of action potentials that occurred in a response. The significance of these changes is indicated by the olfactory bulb isolation factor in Table 6. The degree of changes in the ISI when the olfactory bulb is isolated is shown in the plots of the interspike interval variables of Figure 8. The upright axis representing number of interspike intervals has a different scale in the cluster plots for the isolated bulb to accommodate the greater numbers of action potentials (Figure 8 b, d, f). These changes in ISI when the bulb is isolated can be understood with respect to the neuronal circuitry of the olfactory bulb. The synaptic mechanisms which mediate the enhancement of inhibition in the olfactory bulb by centrifugal neurons from the forebrain have been examined by intracellular recording and electrical stimulation of the anterior olfactory nucleus from which some of the centrifugal fibers arise (Yamamoto, Yamamoto and Iwama, 1963; Mori and Takagi, 1981). These previous studies demonstrated that electrical stimulation of the anterior olfactory nucleus or the olfactory tract caused excitatory post-synaptic potentials in granule cells and inhibitory post-synaptic
potentials in mitral cells. Granule cells and mitral cells have reciprocal synapses. When a granule cell is excited its action is to inhibit the mitral cell it synapses upon. The mitral cell's synapse on the granule cell is excitatory (Price and Powell, 1970; Mori, Nowycky and Shepherd, 1981). Thus, the observed effects of olfactory tract transection can be rationalized in terms of the known synaptic physiology of the olfactory bulb through granule cell-mediated inhibition.

The increase in action potential frequency upon transection of the olfactory tract in the channel catfish is in agreement with earlier studies on centrifugal interactions between higher order neurons and the vertebrate olfactory bulb (Kerr and Hagbarth, 1955; von Baumgarten, Green and Mancia, 1962; Callens and Boisacq-Schepens, 1963). These studies used electrical stimulation and EEG recordings from the surface of the olfactory bulb rather than chemical stimuli and single neuron recording, but demonstrated increased response amplitude of background and stimulated activity after olfactory tract transection.

Both action potential rate increases and decreases in the responses of single mitral cells were
observed in the rabbit when central nuclei giving rise to the centrifugal fibers of the olfactory bulb were stimulated (Chaput, 1983). In fish, individual mitral cells sometimes gave inhibitory responses when the olfactory tract was intact and excitatory responses after the tract was cut (Døving and Hyvarinen, 1969). In mammals, tract sectioning uncoupled the synchronization of mitral cell activity with respiration and increased the number of effective stimuli (Chaput, 1983). In spite of individual cells which showed changes upon bulb isolation, neither of these studies demonstrated an overall statistically significant effect of olfactory tract transection on the population of bulb cell responses. In the present study there was a significant difference between individual mitral cells within the intact or isolated olfactory bulb and a significant effect of tract transection on amino acid responses (Table 6).

The variation in responses of cells upon olfactory tract transection indicates that the effect of olfactory bulb isolation was not a simple result displayed in the same manner on every cell. For example, one of the cells held before and after tract transection had longer maximum intervals in its responses, a
change opposite that shown by the population of bulb cells. Another consideration, is that the present experiments were performed on the acutely transected bulb. Since response patterns in the isolated bulb can resemble those in the intact bulb (Meredith, 1986), it is possible that the neuronal activity within the circuitry of the bulb reaches an equilibrium at some time after isolation in which the intrinsic inhibitory systems of the bulb replace the enhanced inhibition provided by the centrifugal fibers. The present study adds to the concepts for the action of the centrifugal fibers on the circuitry of the bulb by demonstrating the alterations of stimulus discrimination and response classes that occur upon olfactory bulb isolation. This is discussed in the next section.

Response Types and Olfactory Bulb Isolation.

Virtually every study on the activity of olfactory bulb neurons attempts to classify the responses obtained to odor stimulation, since it is evident that mitral cell responses fall into classes, and classification provides a powerful tool for understanding information representation in the olfactory system. One of the most complete and widely considered classification schemes for mitral cell responses is that of
Kauer (1974). In work on the salamander olfactory bulb, Kauer identified 5 response classes based on excitation, inhibition and the portion of the response that occurred during stimulation. The classes were: excitation, excitation then inhibition, a sequence of excitation, inhibition, excitation, inhibition, inhibition then excitation. These groups have been compared with similar classifications in the rat and mouse (Mair, 1982; Reinken and Schmidt, 1986), and the goldfish (Meredith and Moulton, 1978; Schild, 1987). Inhibitory responses were considered less specific to stimulus quality and were less common than excitatory response classes (Kauer, 1974). In addition to Kauer's scheme, more recent work classifies responses based on the change in spike activity during the initial period of stimulation (Meredith, 1986; Schild, 1987).

The classification of responses used in the present study is based on the success of clustering techniques at identifying possible groupings within the bulb responses. After such classifications have been constructed by statistical techniques, they are evaluated in biological terms. This avoids the subjective aspect of response classification, at least during the initial part of the classification process.
The remarkable aspect of the classification arrived at by this method was its excellent agreement with some aspects of previous classifications.

Like previous classification methods, the cluster analysis results indicated that the aspect of the response that established the best classification was the initial portion of the response. A clear separation of the responses into excitatory, inhibitory and inhibitory-excitatory was achieved by the cluster method. From inspection of the cluster plots of Figure 8, it is clear that some of the larger clusters, like the excitatory cluster represented by the pyramids in Figure 8a, or the complex response cluster identified by the cubes in Figure 8b, could be subdivided further. Yet it seems from the plots that all the responses are in a natural group (i.e. excitatory) that represents a continuum distinct from the inhibitory cluster (balloons, Figure 8a). The inclusion of ISI variables from the second segment of the EOG produced a less distinct response grouping.

As with the findings of other classification schemes for bulb responses, the cluster groupings were not associated with particular stimuli or cells (Meredith and Moulton, 1978; Kauer, 1974). The
response groups did, however, change in numbers of members upon olfactory bulb isolation. These changes were due to (1) a movement of responses from inhibitory to excitatory clusters after tract transection, and (2) an accumulation of greater numbers of responses into the excitatory-inhibitory clusters represented by the cubes (Figure 8). This shift occurred particularly among responses to $10^{-3}$ M and $10^{-4}$ M amino acids after tract transection (Figure 8 c vs 8 d and 8 e vs f). The lack of stimulus specificity for the response groups is not, however, changed by cutting of the olfactory tract.

Bulb isolation profoundly influenced the types of responses from the catfish olfactory bulb. When responses of the individual mitral cells were considered by factor analysis, the discriminatory capacity of the olfactory bulb was compromised as well. This was shown by the greater dispersion of the stimulus correlations on the factor space after the bulb was isolated (Figure 9 b, d, f).

When the population was analyzed, the amino acid responses of the mitral cells in the channel catfish were distinct from well water control responses and background activity. These responses upon stimulation
were not specific to particular stimuli or concentrations. The representation of olfactory information in this system must depend on a rule for the correspondence of olfactory receptor cell input and mitral cell output that compares the activity of the population of mitral cells and not on particular response classes or active cells. In the short term (up to 8 hours) of these experiments, the olfactory tract exerted an influence on mitral cell activity that heightened stimulus discriminability by the population.

This type of rule for the relationship of stimuli and responses has been pointed out as characteristic of sensory systems that do not use information about the exact location of stimulus application (Erickson, 1968). Such sensory systems are described as "non-topographic" in contrast to a sensory system, such as the sense of touch in the skin. In the skin, it is not only the stimulus quality that is of importance (light touch, tickling, injurious crush, burning), but its exact location on the skin as well. Non-topographic stimulus modalities are exemplified by color vision and olfaction. Both of these sensory mechanisms have sensitivity to each stimulus quality at each point in
the organ. There are three types of color sensitive cones at each point in the retina and many different "types" of olfactory receptor cells may exist at each point in the olfactory organ. This is not to say that there may not be some regionalization of receptors, but rather to point out that each receptor in non-topographic sensory modalities can respond in some degree to many different stimuli, such as the amino acids used in this study. It is in the comparison of the output from a number of receptor cells that the unique identification of the stimulus is suggested to occur. This type of code, called an "across-fiber pattern" was offered as possible coding scheme for peripheral taste fibers that were responsive to more than one taste quality (Erickson, 1968). More recently it has been proposed as a general mode of operation for many parts of the nervous system, including those not involved in sensory processing (Erickson, 1984). Such an interpretation fits the data observed for the mitral cell neurons of the channel catfish olfactory bulb.
REFERENCES


Døving, K.B. (1964) Studies of the relation between
the frog electro-olfactogram (EOG) and single unit ac-
tivity in the olfactory bulb. Acta Physiologica
Scandinavica 60:150-163.

Døving, K.B. (1966) Efferent influence upon the ac-
tivity of single neurons in the olfactory bulb of the

in the rat olfactory bulb to various parameters of
odor stimulation. Acta Physiologica Scandinavica
130:285-298.

Døving, K.B. and C. Gemne (1966) An elec-
trophysiological study of the efferent olfactory sys-
tem in the burbot. Journal of Neurophysiology 29:665-
674.

Døving, K.B. and J. Hyvarinan (1969) Afferent and
efferent influences on the activity pattern of single
olfactory neurons. Acta Physiologica Scandinavica
75:111-123.


MacLeod, N.K. (1976) Spontaneous activity of single neurons in the olfactory bulb of the rainbow trout (Salmo gairdneri) and its modulation by olfactory bulb stimulation with amino acids. Experimental Brain Research 25:267-278.


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