Electro-Olfactogram and Multiunit Olfactory Receptor Responses to Complex Mixtures of Amino Acids in the Channel Catfish, *Ictalurus punctatus*

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ABSTRACT   In vivo electrophysiological recordings from populations of olfactory receptor neurons in the channel catfish, *Ictalurus punctatus*, clearly showed that both electro-olfactogram and integrated neural responses of olfactory receptor cells to complex mixtures consisting of up to 10 different amino acids were predictable with knowledge of (*a*) the responses to the individual components in the mixture and (*b*) the relative independence of the respective receptor sites for the component stimuli. All amino acid stimuli used to form the various mixtures were initially adjusted in concentration to provide approximately equal response magnitudes. Olfactory receptor responses to both multimixtures and binary mixtures were recorded. Multimixtures were formed by mixing equal aliquots of 3–10 different amino acids. Binary mixtures were formed by mixing equal aliquots of two equally stimulatory solutions. Solution 1 contained either one to nine different neutral amino acids with long side-chains (LCNs) or one to five different neutral amino acids with short side-chains (SCNs). Solution 2, comprising the binary mixture, consisted of only a single stimulus, either a LCN, SCN, basic, or acidic amino acid. The increasing magnitude of the olfactory receptor responses to mixtures consisting of an increasing number of neutral amino acids indicated that multiple receptor site types with highly overlapping specificities exist to these compounds. For both binary mixtures and multimixtures composed of neutral and basic or neutral and acidic amino acids, the receptor responses were significantly enhanced compared with those mixtures consisting of an equal number of only neutral amino acids. These results demonstrate that receptor sites for the basic and acidic amino acids, respectively, are highly independent of those for the neutral amino acids, and suggest that a mechanism for synergism is the simultaneous activation of relatively independent receptor sites by the components in the mixture. In contrast, there was no evidence for the occurrence of mixture suppression.

INTRODUCTION

In nature, animals generally detect complex mixtures of odorants rather than the single substances or simple mixtures that are usually presented to organisms in laboratory studies of olfaction. Knowing whether stimulus mixtures are treated differently by olfactory receptors than individually presented stimuli is critical to a better understanding of the olfactory process. Two types of mixture interaction, mixture suppression and synergism, have been frequently cited as occurring in a number of studies in the chemical senses involving stimulus mixtures (Derby and Ache, 1984; Bartoshuk and Gent, 1985; Carr and Derby, 1986a, b; Johnson, Voigt, and Atema, 1989). Mixture suppression occurs if the measured responses are significantly less than the predicted response, often calculated by simply summing the responses to the individual components in the mixture. Synergism occurs if the measured responses are significantly greater than predicted. Possibly due to these mixture interactions, and in some cases to the experimental paradigm itself, numerous studies indicated the difficulty in predicting the response to stimulus mixtures, even when the responses to the individual components of the mixtures were known (Derby and Ache, 1984; Zimmer-Faust, Tyre, Michel, and Case, 1984; Derby, Ache, and Kennel, 1985; Gleeson and Ache, 1985; Johnson, Borroni, and Atema, 1985; Borroni, Handrich, and Atema, 1986; Carr and Derby, 1986a, b; Atema, Borroni, Johnson, Voigt, and Handrich, 1989; Johnson et al., 1989). A recent report by Caprio, Dudek, and Robinson (1989), however, clearly showed that olfactory receptor responses to binary and trinary mixtures of amino acids were predictable with knowledge of the relative independence of the receptor site types obtained from electrophysiological cross-adaptation studies (Caprio and Byrd, 1984). Binary mixtures whose components showed little cross-adaptation initiated enhanced responses compared with those whose components were indicated to interact either with a common receptor site or with receptor sites having highly overlapping specificities. This same report (Caprio et al., 1989) indicated that one mechanism for the response enhancement evoked by particular stimulus mixtures is simply the simultaneous activation of independent receptor site types by different components within the mixture. In addition, the report indicated that evidence for mixture suppression was lacking and suggested that some of the previous reports of this phenomenon could be explained by simple competitive binding among stimuli of different potencies that share the same olfactory receptor binding site type (Gleeson and Ache, 1985; Bell, Laing, and Panhuber, 1987). Thus, the olfactory receptor cells of the channel catfish interacted predictably with mixtures of amino acids, and the observed enhancement of the electro-olfactogram (EOG) and integrated neural responses was dependent on the relative independence of the respective receptor sites for the component stimuli.

Evidence from previous studies (Caprio and Byrd, 1984; Caprio, Dudek, and Robinson, 1987; Bruch and Rulli, 1988; Caprio et al., 1989) indicated that olfactory receptor sites for basic, acidic, and neutral amino acids were different from each other. However, it was unclear, for example, whether neutral amino acids with long side-chains (LCNs) containing three or more carbons bound to a single LCN receptor site type, or whether the LCN receptor was actually a number of different receptor

site types having overlapping sensitivities for the LCNs. This study was designed to answer this question for the neutral, acidic, and basic amino acids and to determine whether the principles learned from studying the olfactory response to simple binary and trinary mixtures of amino acids (Caprio et al., 1989) would successfully predict olfactory receptor responses to more complex mixtures.

This report clearly indicates that (*a*) acidic, basic, LCN, and SCN (neutral amino acids with short side-chains, two carbons or less) amino acids, respectively, bind to a group of highly cross-reactive receptor site types, having highly overlapping specificities; (*b*) the receptor site types for basic and acidic amino acids, respectively, are highly independent of those for neutral amino acids; and (*c*) olfactory receptor responses to complex mixtures of amino acids are experimentally predictable.

MATERIALS AND METHODS

Experimental Animals

29 channel catfish, *Ictalurus punctatus*, ranging in weight from 12 to 150 g were obtained from a local hatchery, held in floating cages in a nearby university pond, and fed with commercial catfish chow. Animals brought into the laboratory holding facility were held in aerated, charcoal-filtered water in a 250-liter fiberglass aquarium at ~25°C. The fish were maintained on a 12:12 light-dark regime and were used experimentally within 2 wk of laboratory holding time (Tucker, 1973).

Animal Immobilization and Anesthesia

The catfish tested were immobilized with an intramuscular injection of Flaxedil (gallamine triethiodide; 0.05 mg/100 g body weight), wrapped in wet tissue paper, and secured to a wax block held in a Plexiglass container. The gills were irrigated throughout the experiment with aerated, charcoal-filtered tap water (artesian water) containing 0.005% (initial concentration) MS-222 (ethyl-*m*-aminobenzoate methane sulfonic acid). Supplemental doses of Flaxedil were applied to the fish as required.

Stimulus Compounds and Delivery

Nine LCNs (methionine [Met], histidine [His], norvaline [nVal], valine [Val], ethionine [Eth], norleucine [nLeu], leucine [Leu], glutamic acid-gamma-methyl ester [GME], phenylalanine [Phe]), five SCNs (glycine [Gly] and the L-isomers of alanine [Ala], serine [Ser], glutamine [Gln], and threonine [Thr]), two basic L-amino acids (arginine [Arg] and lysine [Lys]), and two acidic L-amino acids (glutamic acid [Glu] and aspartic acid [Asp]) were chosen as components to form various stimulus mixtures (Caprio and Byrd, 1984; Bruch and Rulli, 1988). Stock solutions of individual amino acids (Sigma grade; Sigma Chemical Co., St. Louis, MO) were prepared weekly in charcoal-filtered tap water (pH ~8.5) and stored at 4°C. The concentration of the amino acids in their respective stock solutions was 0.01 M, with the exception of that for Asp, which was 0.001 M. Charcoal-filtered tap water was used to dilute stock solutions to the desired test concentrations. Concentrations of all amino acid solutions used to form mixtures ranged from 1 to 1,000 μM. The pH values of all individual amino acid solutions tested remained between 8.0 and 8.5.

Charcoal-filtered tap water continuously bathed the olfactory mucosa at a flow rate of 12 ml/min for EOG recordings and 5 ml/min for integrated neural recordings. Stimulus solutions were introduced into a 0.5-ml Teflon loop of a manual sample injection valve (Omnifit USA Corp., Atlantic Beach, NY) and injected into the water bathing the olfactory organ. Photoden-

sitometry studies indicated that the maximum stimulus concentration delivered to the olfactory mucosa was 75% of the concentration injected (values in the text are the undiluted concentration). The water control was taken from the same charcoal-filtered tap water source as that used to prepare the stimulus solutions. Interstimulus intervals were 3 min. Due to the tendency of EOG responses to gradually increase over the course of the experiment, one amino acid (10 μM Met for the LCN tests and 10 μM Ala for the SCN tests) was chosen as the standard stimulus and was regularly applied to trace any changes in responsiveness in the electrophysiological preparation throughout the experiment.

Mixture Definitions and the Mixture Discrimination Index

The two types of mixtures in this report were multimixtures (M) and binary mixtures (B). Since competitive binding among the components of a mixture that have widely different binding affinities could possibly diminish the stimulatory potency of the more potent stimulus (i) (Gleeson and Ache, 1985), the components of both types of mixtures were adjusted in concentrations to provide approximately equal EOG and neural response magnitudes, respectively. In addition, within each mixture type there were "within-group" and "across-group" mixtures (Caprio and Byrd, 1984; Caprio et al., 1989). Further, dose–response power functions for EOG and integrated neural responses, respectively, to amino acids in the channel catfish (Caprio, 1978; Byrd and Caprio, 1982) were approximately parallel, a criterion necessary for using the mixture discrimination index (MDI) in the present experimental paradigm. Thus, equal dilutions of equipotent stimuli remained equally stimulatory within the range of concentrations (micromolar to millimolar) tested. The MDI, defined as the response to the mixture divided by the average of the responses to the approximately equipotent stimuli that were mixed in equal aliquots to form the corresponding mixture (Hyman and Frank, 1980; Caprio et al., 1989), was calculated for each mixture tested. Theoretically, the MDI equals 1 if the components of the mixture bind to the same olfactory receptor sites, and thus would not be distinguished by the system. An MDI significantly less than 1 indicates mixture suppression, whereas an MDI significantly greater than 1 indicates that the components bind to different receptor sites and thus may be distinguished by the system.

Multimixtures were formed by mixing equal aliquots of 3–10 equally stimulatory solutions of single amino acids. Thus, the concentration of each component within a multimixture composed of n amino acids was n^{-1} of its original concentration. Within-group mixtures of amino acids were mixtures whose components were indicated from previous electrophysiological cross-adaptation (Caprio and Byrd, 1984) and receptor binding (Bruch and Rulli, 1988) studies to compete for the same receptor site type or highly cross-reactive sites. All within-group multimixtures in these experiments were composed of three to nine LCNs and three to five SCNs, respectively. Across-group mixtures of amino acids were mixtures whose components were indicated to interact with relatively independent receptor site types (Caprio and Byrd, 1984; Bruch and Rulli, 1988). Across-group multimixtures consisted of a single basic (Arg or Lys), acidic (Glu or Asp), or SCN (Ala or Thr) amino acid with two to nine LCNs, or a single basic, acidic, or LCN (Met or His) amino acid with two to five SCNs.

Binary mixtures were tested to determine the effects on the MDI of multiple neutral amino acids substituting for a single neutral amino acid and were formed by mixing equal aliquots of two equally stimulatory solutions of amino acids. Solution 1 was composed of one to nine neutral amino acids, and solution 2 comprised a single across-group amino acid. Thus, the concentration of each neutral amino acid in a final binary mixture composed of n neutral amino acids was $(2n)^{-1}$ of its original concentration. The concentration of the single across-group amino acid in the final mixture was 0.5 of its original concentration. Tested in these experiments were within-group binary mixtures composed of two acidic, basic, LCN, and SCN amino acids, respectively. All across-group binary mixtures in this report consisted of (a) a

single basic (Arg or Lys), acidic (Glu or Asp), or SCN (Ala or Thr) solution combined with that of a single LCN or with an all LCN (n = 2–9) amino acid mixture, and (b) a single basic, acidic, or LCN (Met or His) solution combined with that of a single SCN or with an all SCN (n = 2–5) amino acid mixture.

Recording Techniques and the Response Measure

The olfactory lamellae were exposed by removing the skin, connective tissue, and cartilage dorsal to the nasal capsule. The underwater EOG, a slow negative potential change in response to chemical stimulation, was recorded in vivo in 24 catfish as described by Silver, Caprio, Blackwell, and Tucker (1976) with calomel electrodes via Ringer–agar-filled capillary pipettes in the water that continuously bathed the olfactory organ. The EOG response was amplified by a direct-coupled amplifier, displayed on an oscilloscope, and recorded on both video and chart recorders. In a subset of experiments, integrated neural activity was recorded in vivo in five catfish with metal-filled glass capillary electrodes as described previously (Erickson and Caprio, 1984; Caprio et al., 1989).

The magnitude of both the EOG and the integrated neural responses was measured as the peak height in millimeters of the phasic displacement from baseline, and all responses to amino acids were adjusted by subtraction of the mean control response. Prior experiments (Caprio, 1980; Evans and Hara, 1985) clearly indicated that differences in the waveform of the underwater EOG were due primarily to experimental variables affecting the duration of the stimulus flow over the receptors (i.e., both the EOG and the integrated neural activity show sustained tonic activity to the continuous presentation of a stimulus). Thus, the position of the fish in the recording setup along with the surgery to remove the tissue covering the olfactory organ could affect the clearance rate of the stimulus from the olfactory capsule and thereby change the duration of stimulus contact with the receptors. Although slight changes in the EOG and integrated neural recordings sometimes occurred during a recording session in the same fish, both the phasic EOG and the phasic integrated neural responses were the portions of the signal that were most reproducible.

Data Standardization

Over the course of the experiment (6 h, on the average), the EOG response to standard stimuli (either 10 μM Met for the LCN test or 10 μM Ala for the SCN test) gradually but steadily increased. The mechanism accounting for this phenomenon is unknown; however, it may have been due to the continuous rinsing away of ions from the mucous layer overlying the epithelium of the olfactory organ, thus increasing the resistance which resulted in a greater EOG magnitude. Also due to the slight movements of the electrode or the fish, the magnitude of the integrated neural responses to standard stimuli sometimes changed slightly over time. Thus, to minimize the possibility that any response enhancements observed were due to extraneous factors, two standardization procedures were developed to correct for these changes in responses over time. Both procedures used were based on the same assumption that the change in the response magnitude was parallel in all component amino acids tested at equipotency (i.e., amino acid stimuli have approximately parallel dose–response functions; Caprio, 1978; Silver, 1982). The first standardization procedure was used to adjust for slight changes (generally less than ±5% of the initial standard response) over a short period ($\leq$ 30 min) in the responses to stimuli which were bracketed by responses to two standard stimuli. For example, if there were N test stimuli bracketed by two standard stimuli and the response magnitude was X mm to the first standard stimulus and Y mm to the second, the response magnitude to the first test stimulus was standardized by subtracting $(Y - X)/N$ mm from the measured magnitude; the response magnitude to the second test stimulus was standardized by

subtracting $2[(Y - X)/N]$ mm, and so on up to the Nth test stimulus, in which $(Y - X)$ mm was subtracted from the measured response.

To calculate the MDI values for binary mixtures, the first procedure was sufficient since binary mixtures and their components were applied within two adjacent standard stimuli. However, multimixtures and their components were applied up to 6 h apart. During this time period, slight changes (generally less than ±10% of the initial standard response) in the amplitude of the responses to the standard stimuli occurred. Thus, the second standardization procedure was used to adjust the magnitudes among responses to the corresponding multimixtures and their components. The standardization procedure was the same as in the first procedure with the exception that the second procedure corrected for the changes in responses that were not bracketed by two adjacent standard responses. For example, if the response to the standard was X mm before testing the component amino acids, and the response to the standard was Y mm when applied close in time to the response to the multimixture, then the standardization procedure involved adding $(Y - X)$ mm to the average response magnitude for the component amino acids. This latter procedure ensured that the MDI value for the response to the multimixture was not artificially elevated by slow increases in response magnitudes that generally occurred in the preparation over time. All MDI values presented in this study were calculated from the corrected response magnitudes.

Data Analysis

The data were analyzed with a one-way ANOVA using SAS (1986, SAS Institute Inc., Cary, NC). Means were further analyzed using the Waller-Duncan K-ratio t test.

RESULTS

EOG: Within-Group Multimixtures

One to nine equipotent LCNs (Fig. 1 A) and one to five equipotent SCNs, respectively, were used to form mixtures with sequentially increasing numbers of components, in order to determine the relationship of EOG magnitude to the number of within-group components in the respective mixtures. Of three different orders (i.e., different arrangements) of LCNs and SCNs tested to form the series of within-group LCN (Table I) and SCN (Table II) mixtures, respectively, mean MDI values for the resulting mixtures consisting of an equal number of components were not significantly different. This occurred despite wide differences in the total molarity of the resulting amino acid mixtures. Thus, for the following determinations, all MDI values derived from the within-group LCN and SCN mixtures, respectively, across the three orders were pooled.

Mean MDI values of within-group mixtures comprising different numbers of LCNs and SCNs, respectively, were significantly different (Figs. 1 B and 2; Tables III and IV) (ANOVA, $P < 0.0001$). Further analysis (Waller-Duncan t test) indicated significant increases in the mean MDI values between the single LCN and the five-component LCN multimixtures. No further significant increase in the mean MDI values occurred for five- to eight-component LCN multimixtures. The MDI value for an LCN multimixture consisting of nine components ($M_{(9L)}$), the most complex within-group multimixture tested (Figs. 1 B and 2 A), however, was significantly larger than those for the binary LCNs and the three-, four-, and five-component LCN multimixtures. The regression equation derived from 92 tests (N) that describes the general increase

in MDI magnitude for the two- to five-component (n) within-group LCN multimixtures (including the binary LCN mixture) is:

$$MDI = 0.06n + 1.03 \qquad (n = 2\text{--}5; N = 92, P < 0.0001) \qquad (1)$$

Significant increases in the mean MDI values occurred between the single SCN and

A

B

c Met His nVal Val Eth nLeu Leu GME Phe Glu   Met   M(4L)   M(9L)

μM 10 200 5 400 20 5 100 20 800 500   10

C

D

M(3L) Glu B(3L),(1A)   M(8L) Glu B(8L),(1A)   Met   M(3L,1A)   M(8L,1A)

2 mV

1 min

μM   550   700   10
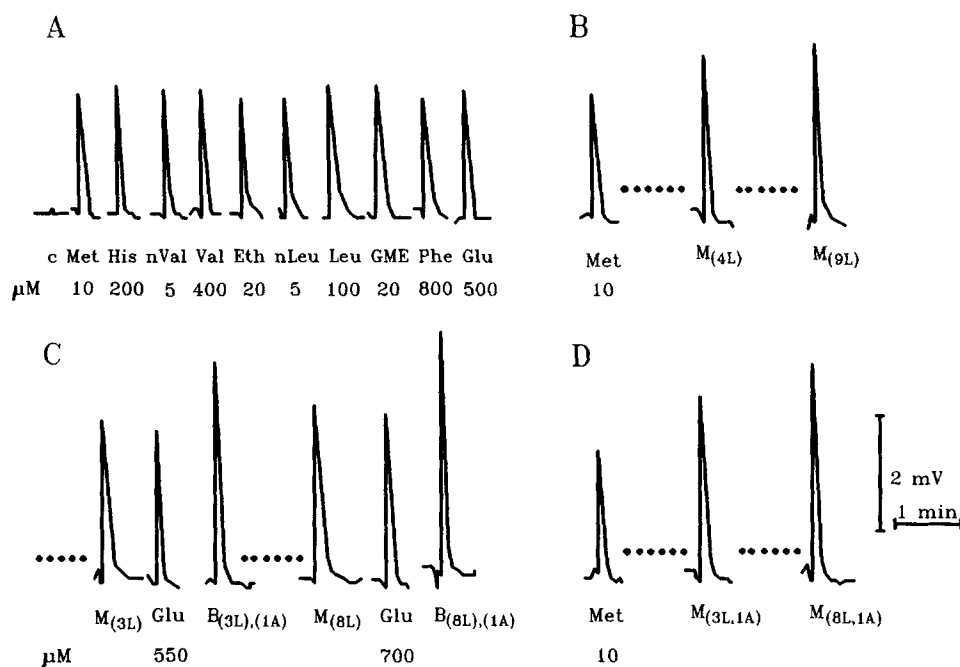
FIGURE 1.   Representative EOG responses to single amino acids and mixtures of amino acids. (*A*) EOG responses to control (*c*), L-isomers of nine single LCNs, and an acidic amino acid (Glu) that were adjusted in concentration (listed below each stimulus) to provide for approximately equal response magnitudes. (*B*) EOG responses to Met and two within-group multimixtures consisting of Met and three additional LCNs from *A* (i.e., $M_{(4L)}$), and Met and eight additional LCNs from *A* (i.e., $M_{(9L)}$), respectively. *C* illustrates how four-component ($B_{(3L),(1A)}$) and nine-component ($B_{(8L),(1A)}$) across-group binary mixtures were formed. For the four-component mixture, the acidic amino acid (i.e., Glu) was adjusted in concentration to approximately match the response of a three-component, within-group multimixture ($M_{(3L)}$); equal aliquots of the preceding two solutions were then mixed together and the EOG response to the resulting mixture was recorded. The nine-component, across-group binary mixture was similarly formed. (*D*) EOG responses to Met and two across-group multimixtures. A comparison of *B* and *D* illustrates the responses between within- (*B*) and across-group (*D*) four- and nine-component multimixtures composed primarily of LCN amino acids.

the four-component SCN multimixtures (Fig. 2 *B;* Table IV). The mean MDI for five-component SCN multimixtures was not significantly different from that for the four-component SCN mixture. The regression equation derived from 63 tests (*N*) that describes the general increase in MDI magnitude for the two- to four-component

TABLE I

One-Way ANOVA for the EOG-derived MDI Values Obtained from Three Orders of Within-Group LCN Mixtures

| Stimuli* | No. of LCNs | Orders | | | P |
		I	II	III	
Met	1	Met	Met	Met	—
$B_{(1L),(1L)}$	2	His	Phe	nVal	0.50
$M_{(3L)}$	3	nVal	GME	Eth	0.06
$M_{(4L)}$	4	Val	Leu	Leu	0.40
$M_{(5L)}$	5	Eth	nLeu	Phe	0.08
$M_{(6L)}$	6	nLeu	Eth	His	0.68
$M_{(7L)}$	7	Leu	Val	Val	0.82
$M_{(8L)}$	8	GME	nVal	nLeu	0.75
$M_{(9L)}$	9	Phe	His	GME	0.77
No. of tests		14	5	3	
No. of fish		11	5	3	

*$B_{(1L),(1L)}$, a binary mixture formed by mixing equal aliquots of two equipotent LCNs. $M_{(nL)}$, multimixtures formed by mixing equal aliquots of three to nine equipotent LCNs. Compounds used to form a stimulus mixture included the amino acids listed in each order column from the first LCN (i.e., Met) up to the LCN located in the same row as that mixture (e.g., $M_{(4L)}$ in order II was a mixture formed by mixing equal aliquots of equipotent solutions of Met, Phe, GME, and Leu).

²ANOVA shows that within each group of n LCNs, the mean MDI values for any of the three orders are not significantly different from those for mixtures consisting of an equal number of components.

(n) within-group SCN multimixtures (including the binary SCN mixture) is:

$$MDI = 0.06n + 0.97 \qquad (n = 2\text{--}4; N = 63, P < 0.001) \qquad (2)$$

The probability values for the regression equations (Eqs. 1 and 2) indicate that the MDI values reported here are due to the experimental manipulations, and that the

TABLE II

One-Way ANOVA for the EOG-derived MDI Values Obtained from Three Orders of Within-Group SCN Mixtures

| Stimuli* | No. of SCNs | Orders | | | P |
		I	II	III	
Ala	1	Ala	Ala	Ala	—
$B_{(1S),(1S)}$	2	Gly	Thr	Ser	0.43
$M_{(3S)}$	3	Ser	Gln	Thr	0.13
$M_{(4S)}$	4	Gln	Ser	Gly	0.21
$M_{(5S)}$	5	Thr	Gly	Gln	0.78
No. of tests		12	5	4	
No. of fish		9	4	4	

*$B_{(1S),(1S)}$, a binary mixture formed by mixing equal aliquots of two equipotent SCNs. $M_{(nS)}$, multimixtures formed by mixing equal aliquots of three to five equipotent SCNs. Compounds used to form a stimulus mixture included the amino acids listed in each order column from the first SCN (i.e., Ala) up to the SCN located in the same row as that mixture (e.g., $M_{(4S)}$ in order II was a mixture formed by mixing equal aliquots of equipotent solutions of Ala, Thr, Gln, and Ser).

²ANOVA shows that within each group of n SCNs, the mean MDI values for any of the three orders are not significantly different from those for mixtures consisting of an equal number of components.
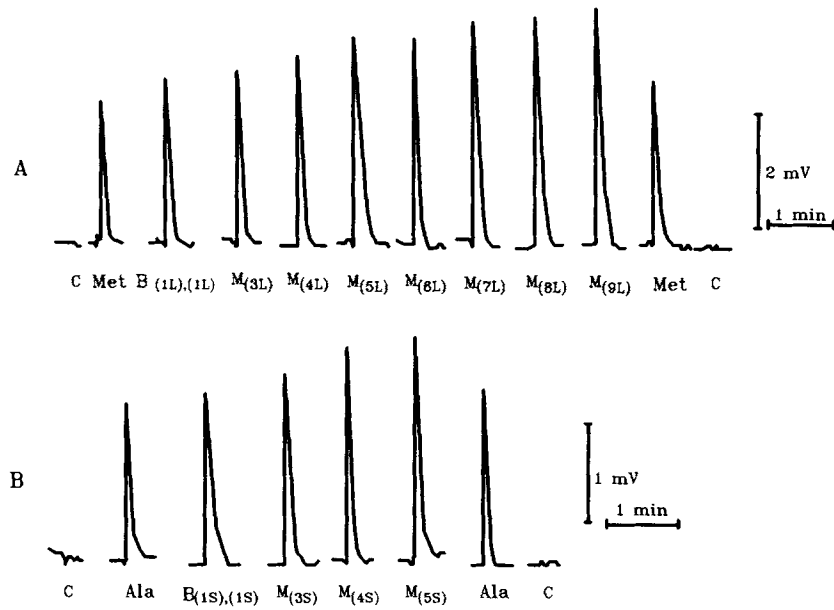
FIGURE 2.   Representative EOG responses to within-group binary mixtures and multimixtures of neutral amino acids. (*A*) EOG responses to Met, a binary LCN mixture, and sequentially increasing within-group LCN multimixtures. (*B*) EOG responses to Ala, a binary SCN mixture, and sequentially increasing within-group SCN multimixtures. C indicates the control response. All LCNs and SCNs used to form the respective mixtures were initially adjusted in concentration to result in approximately equal EOG response magnitudes.

TABLE III

Mean EOG-derived MDI Values for Within-Group LCN Mixtures

Stimuli*	No. of LCNs	MDI (mean ± SE)	t test[‡]	
Met	1	1.00 ± 0.00	A	
$B_{(1L),(1L)}$	2	1.14 ± 0.01	B	
$M_{(3L)}$	3	1.22 ± 0.02	C	
$M_{(4L)}$	4	1.27 ± 0.02	D	
$M_{(5L)}$	5	1.32 ± 0.02		E
$M_{(6L)}$	6	1.34 ± 0.02	F	E
$M_{(7L)}$	7	1.36 ± 0.02	F	E
$M_{(8L)}$	8	1.36 ± 0.02	F	E
$M_{(9L)}$	9	1.40 ± 0.02	F	

*Stimuli consisted of a single LCN (i.e., Met), a binary mixture, ($B_{(1L),(1L)}$), and multimixtures consisting of three to nine equipotent LCNs ($M_{(nL)}$). The MDI value for Met and each of the stimulus mixtures was based on 23 tests from a total of 13 fish.
[‡]One-way ANOVA indicates that there are significant differences ($P < 0.0001$) among MDI values for different stimuli. Further t test analysis shows that the mean MDI values with the same letter are not significantly different from each other, but are significantly different ($P < 0.05$) from the mean MDI values with different letters.

TABLE IV

Mean EOG-derived MDI Values for Within-Group SCN Mixtures

Stimuli*	No. of SCNs	MDI (mean ± SE)	t test[t]
Ala	1	1.00 ± 0.00	A
$B_{(1S),(1S)}$	2	1.09 ± 0.01	B
$M_{(3S)}$	3	1.15 ± 0.01	C
$M_{(4S)}$	4	1.21 ± 0.02	D
$M_{(5S)}$	5	1.22 ± 0.02	D

*Stimuli consisted of a single SCN (i.e., Ala), a binary mixture, $(B_{(1S),(1S)})$, and multimixtures consisting of three to five equipotent SCNs $(M_{(nS)})$. The MDI value for Ala and each of the stimulus mixtures was based on 21 tests from a total of seven fish. [t]One-way ANOVA indicates that there are significant differences ($P < 0.0001$) among MDI values for different stimuli. Further t test analysis shows that the mean MDI values with the same letter are not significantly different from each other, but are significantly different ($P < 0.05$) from the mean MDI values with different letters.

respective equations can be used confidently to predict the MDI values for LCN and SCN multimixtures for EOG responses in channel catfish.

EOG: Across-Group Multimixtures

Three sequences of across-group mixtures, $M_{(nL,1B)}$, $M_{(nL,1A)}$, and $M_{(nL,1S)}$, formed by mixing a basic (Arg or Lys), acidic (Glu or Asp), or SCN (Ala or Thr) amino acid with
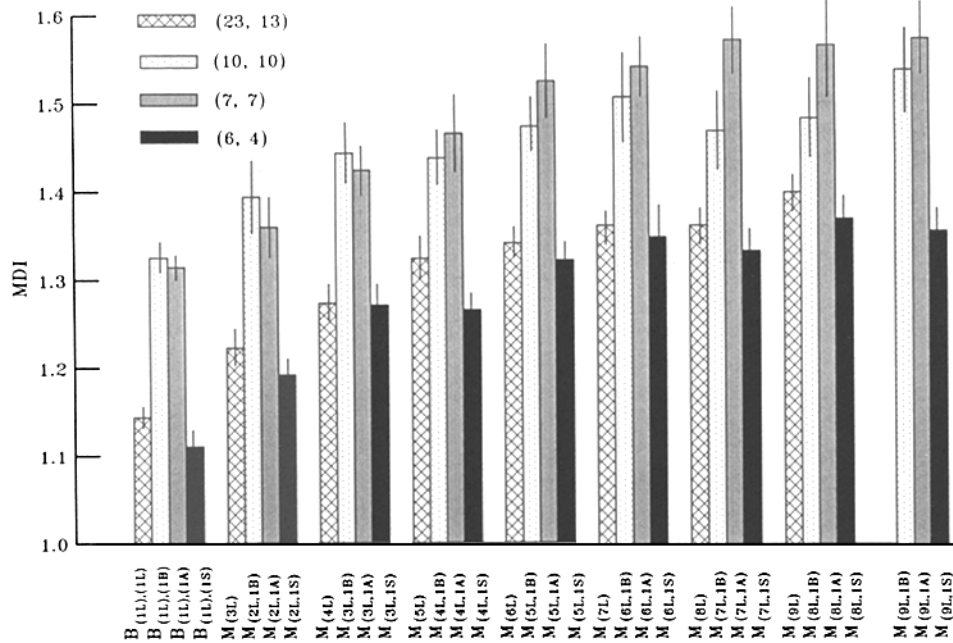

FIGURE 3. Comparison of mean (±SE) MDI values for two-component binary mixtures (B) and three- to ten-component multimixtures (M) composed primarily of LCN amino acids. Numbers in parentheses indicate number of tests and fish, respectively.

a sequentially increasing number of LCN components, were tested (Fig. 3). For mixtures with an equal number of components, MDI values for $M_{(nL,1B)}$ and $M_{(nL,1A)}$ were significantly greater than those for $M_{(nL,1S)}$. In addition, MDI values for the former two sequences of mixtures were significantly greater than those for the within-group LCN mixtures, consisting of an equal total number of amino acids. The sole exception was that the MDI value for $M_{(8L,1B)}$ was not significantly different from those for $M_{(8L,1S)}$ and $M_{(9L)}$. However, the MDI values for the $M_{(nL,1S)}$ and within-group LCN mixtures consisting of an equal number of components were not significantly different from each other. Within each of the three sequences, MDI values generally increased with the increasing number of LCN components in the across-group mixtures (Figs. 1 *D* and 3). The regression equations derived from 50, 24, and 30 tests (N), which describe this increase in MDI magnitude for each of the three sequences of two- to six-component (n) across-group multimixtures, including the binary mixtures comprising one LCN and one across-group component, are, respectively:

$$\text{for } M_{(nL,1B)}, \text{ MDI} = 0.03n + 1.28 \qquad (n = 2\text{--}6; N = 50, P < 0.001) \tag{3}$$

$$\text{for } M_{(nL,1A)}, \text{ MDI} = 0.05n + 1.21 \qquad (n = 2\text{--}6; N = 24, P < 0.0001) \tag{4}$$

$$\text{for } M_{(nL,1S)}, \text{ MDI} = 0.05n + 1.03 \qquad (n = 2\text{--}6; N = 30, P < 0.0001) \tag{5}$$

Similarly, MDI values for the across-group mixtures, $M_{(nS,1B)}$ and $M_{(nS,1A)}$, were significantly greater than those for the across-group mixtures, $M_{(nS,1L)}$, and those for the within-group SCN mixtures consisting of an equal number of components (Fig. 4). However, MDI values for $M_{(nS,1L)}$ were not significantly different from those for the within-group SCN mixtures comprising an equal number of components. Within each of the three sequences, MDI values generally increased with an increasing number of SCNs in the across-group mixtures (Fig. 4). The regression equations derived from 40, 20, and 24 tests (N), which describe this increase in MDI magnitude for each of the three sequences of two- to five-component (n) across-group multimixtures, including the binary mixtures comprising one SCN and one across-group component, are, respectively:

$$\text{for } M_{(nS,1B)}, \text{ MDI} = 0.02n + 1.25 \qquad (n = 2\text{--}5; N = 40, P < 0.05) \tag{6}$$

$$\text{for } M_{(nS,1A)}, \text{ MDI} = 0.03n + 1.25 \qquad (n = 2\text{--}5; N = 20, P < 0.001) \tag{7}$$

$$\text{for } M_{(nS,1L)}, \text{ MDI} = 0.04n + 1.03 \qquad (n = 2\text{--}5; N = 24, P < 0.001) \tag{8}$$

The P values in Eqs. 3–8 indicate that the MDI values for across-group multimixtures are highly correlated to the number of amino acids forming the mixtures. Thus, these equations predict the MDI values for the across-group multimixtures composed primarily of LCN (Eqs. 3–5) and SCN (Eqs. 6–8) amino acids, respectively.

EOG: Within-Group Binary Mixtures

For acidic and basic amino acids only two-component within-group mixtures were tested. MDI means ($\pm$SE) for the EOG responses to within-group binary mixtures of acidic (L-glutamic and L-aspartic acids) and basic (L-arginine and L-lysine) amino acids, respectively, were 1.10 $\pm$ 0.02 ($n = 8$) and 1.16 $\pm$ 0.01 ($n = 8$), with both

values being significantly greater than 1. Similarly, MDI means ($\pm$SE) for the within-group binary mixtures composed of two LCN and two SCN amino acids, respectively, were 1.14 $\pm$ 0.01 (n = 23, Table III) and 1.09 $\pm$ 0.01 (n = 21, Table IV), which were also significantly greater than 1.

EOG: Across-Group Binary Mixtures

Of the three sequences of across-group binary mixtures, $B_{(nL),(1B)}$, $B_{(nL),(1A)}$, and $B_{(nL),(1S)}$, mean MDI values for the former two sequences of binary mixtures were significantly greater than those for the latter sequence, ranging between 1.19 and 1.32 (mean $\pm$ SE, 1.24 $\pm$ 0.01) for the $B_{(nL),(1B)}$ sequence, and between 1.26 and 1.31
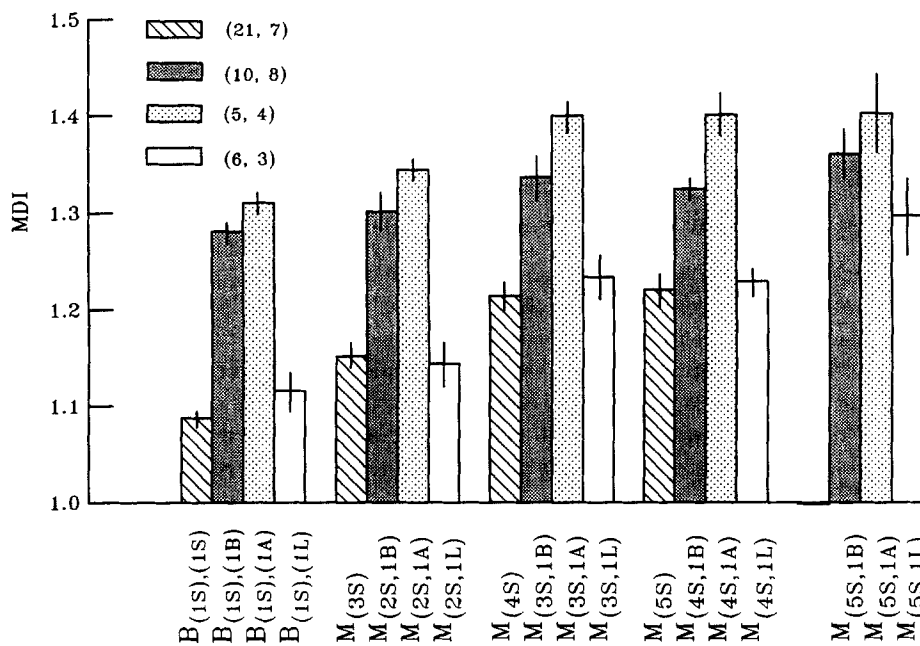

FIGURE 4. Comparison of mean ($\pm$SE) MDI values for two-component binary mixtures (B) and three- to six-component multimixtures (M) composed primarily of SCN amino acids. Numbers in parentheses indicate number of tests and fish, respectively.

(mean $\pm$ SE, 1.29 $\pm$ 0.01) for the $B_{(nL),(1A)}$ sequence (Fig. 5). Mean MDI values for the sequence of across-group binary mixtures consisting of a single SCN added to stimuli with an increasing number of LCN components (i.e., $B_{(nL),(1S)}$) ranged between 1.06 and 1.11 (mean $\pm$ SE, 1.09 $\pm$ 0.01). There were no significant changes (P > 0.05) among the respective MDI values for across-group mixtures formed by the binary mixing of sequentially increasing numbers of LCN components with a single acidic amino acid ($B_{(nL),(1A)}$) or SCN ($B_{(nL),(1S)}$); however, MDI values for the binary mixing of sequentially increasing numbers of LCN components with a basic amino acid ($B_{(nL),(1B)}$) decreased significantly (P < 0.001; Fig. 5). The MDI values for the mixtures

$B_{(2L),(1B)}$ and $B_{(3L),(1B)}$ were not significantly different from $B_{(1L),(1B)}$, but the MDI values for mixtures from $B_{(4L),(1B)}$ to $B_{(9L),(1B)}$ were all significantly smaller than $B_{(1L),(1B)}$ (Fig. 5).

Similarly, for binary mixtures involving SCN stimuli, MDI values for $B_{(nS),(1B)}$ and $B_{(nS),(1A)}$ were significantly greater than those for $B_{(nS),(1L)}$, ranging between 1.27 and 1.29 (mean ± SE, 1.28 ± 0.00) for $B_{(nS),(1B)}$, and between 1.19 and 1.31 (mean ± SE, 1.25 ± 0.02) for $B_{(nS),(1A)}$ (Fig. 6). MDI values for the sequence of across-group mixtures consisting of a single LCN added to stimuli with increasing numbers of SCN components, $B_{(nS),(1L)}$, ranged between 1.08 and 1.12 (mean ± SE, 1.11 ± 0.01). There were no significant changes ($P > 0.05$) among the respective MDI values for across-group mixtures formed by the binary mixing of sequentially increasing
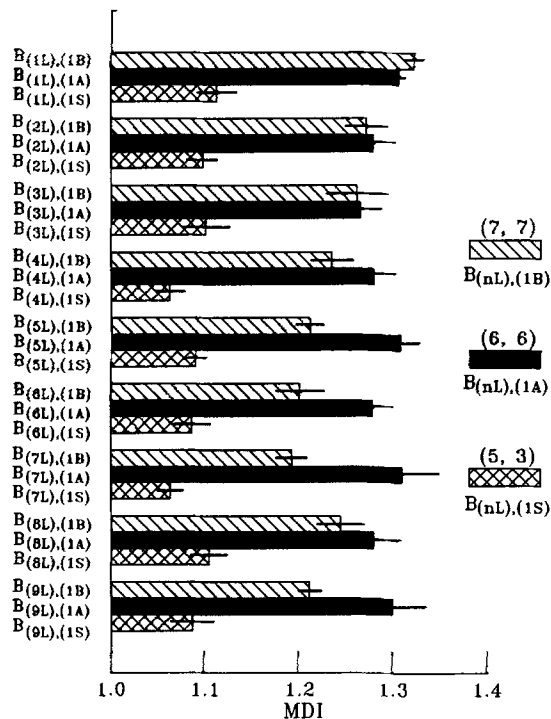

FIGURE 5. Comparison of mean (±SE) MDI values for two- to ten-component across-group binary mixtures: $B_{(nL),(1B)}$, $B_{(nL),(1A)}$, and $B_{(nL),(1S)}$. Numbers in parentheses indicate number of tests and fish, respectively.

numbers of SCN components with a single basic amino acid ($B_{(nS),(1B)}$) or LCN ($B_{(nS),(1L)}$); however, MDI values for the binary mixing of the SCN sequences with an acidic amino acid ($B_{(nS),(1A)}$) decreased significantly ($P < 0.01$). MDI values were not significantly different between $B_{(1S),(1A)}$ and $B_{(2S),(1A)}$, but the MDI values for $B_{(3S),(1A)}$, $B_{(4S),(1A)}$, and $B_{(5S),(1A)}$ were all significantly smaller than that for $B_{(1S),(1A)}$ (Fig. 6).

Neural Activity

11 different mixtures, two within-group and three across-group multimixtures, and one within-group and five across-group binary mixtures were selected to confirm that the enhanced EOG responses to mixtures were of neural origin and were mirrored in
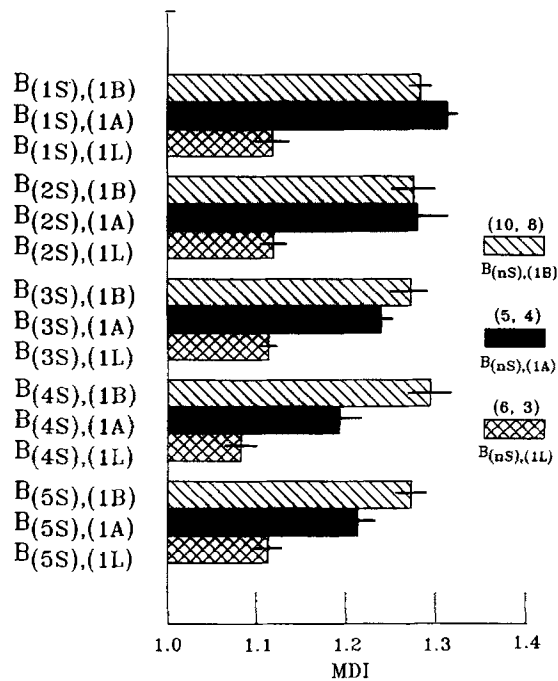
FIGURE 6. Comparison of mean ($\pm$SE) MDI values for two- to six-component across-group binary mixtures: $B_{(nS),(1B)}$, $B_{(nS),(1A)}$, and $B_{(nS),(1L)}$. Numbers in parentheses indicate number of tests and fish, respectively.

the action potential activities of olfactory receptor neurons. As previously indicated for the EOG recordings, across-group multimixtures and binary mixtures, composed of a basic or acidic amino acid and neutral amino acids, resulted in significantly larger MDIs than occurred for within-group multimixtures and binary mixtures,

TABLE V

Comparison of EOG- and Neurally Derived MDI Values

Stimuli	EOG			Neural		
	n_1*	n_2*	MDI (mean $\pm$ SE)	n_1*	n_2*	MDI (mean $\pm$ SE)
Multimixtures						
$M_{(4L)}$	23	13	1.27 $\pm$ 0.02	4	2	1.36 $\pm$ 0.03
$M_{(6L)}$	23	13	1.34 $\pm$ 0.02	13	3	1.36 $\pm$ 0.03
$M_{(3L,1B)}$	10	10	1.44 $\pm$ 0.04	4	2	1.57 $\pm$ 0.07
$M_{(5L,1B)}$	10	10	1.47 $\pm$ 0.03	4	2	1.63 $\pm$ 0.06
$M_{(5L,1A)}$	7	7	1.53 $\pm$ 0.11	5	3	1.65 $\pm$ 0.09
Binary mixtures						
$B_{(1L),(1L)}$	23	13	1.14 $\pm$ 0.01	5	3	1.20 $\pm$ 0.07
$B_{(1L),(1S)}$	6	4	1.11 $\pm$ 0.02	4	2	1.13 $\pm$ 0.07
$B_{(1L),(1A)}$	7	7	1.32 $\pm$ 0.01	3	2	1.61 $\pm$ 0.07
$B_{(4L),(1B)}$	7	7	1.23 $\pm$ 0.02	2	1	1.55 $\pm$ 0.05
$B_{(6L),(1B)}$	7	7	1.20 $\pm$ 0.03	6	3	1.56 $\pm$ 0.07
$B_{(6L),(1A)}$	6	6	1.28 $\pm$ 0.02	6	3	1.52 $\pm$ 0.06

*n_1, No. of tests; n_2 No. of fish.

respectively (Table V). The neurally derived MDI value for a within-group binary mixture (i.e., $B_{(1L),(1L)}$) was not significantly different than that for the across-group binary mixture, $B_{(1L),(1S)}$, as was also predicted by the EOG recordings. Further, the finding for the EOG-derived data that there was no significant change in the MDI values with increasing numbers of LCN compounds for the binary mixtures, $B_{(nL),(1A)}$, also occurred for the neurally derived MDIs (compare $B_{(1L),(1A)}$ with $B_{(6L),(1A)}$). For the binary mixtures involving a basic amino acid (i.e., $B_{(nL),(1B)}$) the neurally derived MDI values were also consistent with the EOG-derived data (compare $B_{(4L),(1B)}$ with $B_{(6L),(1B)}$). The neurally derived MDI values for three within-group mixtures (i.e., $B_{(1L),(1L)}$, $M_{(4L)}$, and $M_{(6L)}$) increased with increasing numbers of LCN compounds in the mixtures (Table V). However, significant changes in the neurally derived MDI values occurred only between those for $B_{(1L),(1L)}$ and $M_{(4L)}$, and $B_{(1L),(1L)}$ and $M_{(6L)}$, as in the EOG recordings, and not between those for $M_{(4L)}$ and $M_{(6L)}$ (Table V).

DISCUSSION

Receptor Site Types for Amino Acids

The question of how many different receptor site types for amino acid stimuli are present within the membranes of olfactory receptor neurons in fish is still unsolved and may possibly be species dependent; however, electrophysiological (Caprio and Byrd, 1984; Ohno, Yoshii, and Kurihara, 1984; Sveinsson and Hara, 1990a, b) and biochemical (Cagan and Zeiger, 1978; Cancalon, 1978; Novoselov, Krapivinskaya, and Fesenko, 1980; Brown and Hara, 1981; Rhein and Cagan, 1983; Rehnberg and Schrek, 1986; Kalinoski, Bruch, and Brand, 1987; Bruch and Rulli, 1988) studies have provided critical evidence indicating that multiple olfactory receptor sites for amino acids exist. Based on electrophysiological cross-adaptation experiments in the channel catfish, Caprio and Byrd (1984) suggested the relative independence of receptor sites for the acidic, basic, and neutral amino acids. The independence of the acidic and basic sites from the neutral sites was subsequently confirmed biochemically by receptor binding experiments (Bruch and Rulli, 1988). Bruch and Rulli (1988) also confirmed the existence of previously identified (Caprio and Byrd, 1984) receptor sites (SCN) that selectively recognize neutral amino acids with short side-chains. As a group, the LCN ligands (neutral amino acids with long side-chains) were significantly different from SCN ligands in their ability to compete with L-[^{3}H]alanine for specific binding sites in isolated cilia preparations from the olfactory epithelium of the channel catfish. However, the present findings of increasing MDI values for within-group LCN and SCN mixtures (Tables III and IV) suggest that there are multiple sites within both the LCN and SCN categories. Theoretically, if all the components of a mixture are equipotent and bind to the same site, the MDI should remain 1.0. This did not occur. The concept that both LCN and SCN amino acids bind to receptor sites with overlapping specificities is also consistent with previous cross-adaptation results, which showed that EOG responses to some amino acids thought to bind to the same receptor site type were differentially affected by the same adapting stimuli (Caprio and Byrd, 1984).

Since the MDIs for within-group mixtures indicate multiple cross-reactive LCN and SCN receptor site types, the question still remains as to the total number of olfactory

receptor site types in the channel catfish for the neutral amino acids. Since the increase in the MDI values reached an approximate asymptote at approximately five LCN and four SCN components, at least four to five types of binding sites exist for these compounds. It is still possible that a receptor site type exists with the highest affinity for each of the neutral amino acids; however, the high degree of cross-reactivity for other neutral amino acids across these site types might have masked the appearance of additional types in both the biochemical and electrophysiological competition and the mixture experiments. The present study also indicated that MDI values for binary mixtures of acidic and basic amino acids, respectively, ranged between 1.1 and 1.2, suggesting more than one common receptor site for the two commonly occurring acidic and for the two commonly occurring basic amino acids.

Although in the present report MDI values for binary mixtures of equipotent within-group amino acids were somewhat similar to those in an earlier report (Caprio et al., 1989), the values in the present experiments were slightly larger and significantly greater than 1.0. This finding was surprising since in the earlier experiments with binary and trinary within-group mixtures MDI values were not significantly different from 1.0. This seeming conflict in the two studies probably arose due to the different within-group binary mixtures tested in the two experiments. For example, L-methionine and L-glutamic acid-gamma-methyl ester, LCN compounds that reciprocally cross-adapted each other (Caprio and Byrd, 1984) and in binary mixtures produced the smallest MDI value of all amino acids examined previously (Caprio et al., 1989), were not tested in the present experiments. Nevertheless, in both experiments EOG- and neurally derived MDI values for across-group binary mixtures were similar and significantly greater than those for the within-group binary mixtures.

Olfactory Receptor Responses to Complex Mixtures

The present results are in agreement with the earlier study of olfactory receptor responses to binary and trinary mixtures of amino acids (Caprio et al., 1989) in that evidence for mixture suppression was lacking. A number of contemporary reports of olfactory mixture suppression occurring in response to amino acid mixtures in decapod crustaceans (Derby and Ache, 1984; Johnson et al., 1985; Carr and Derby, 1986a, b) remain in sharp contrast to the present and past results for the olfactory receptors of the channel catfish. Either the olfactory mechanisms for olfactory mixture detection and receptor processing of amino acid information are different between these species (and possibly between aquatic invertebrates and vertebrates), or the differences in the experimental paradigms and/or theoretical basis for defining mixture interactions in the different laboratories involved are the basis for the profound differences reported. It is our present contention that a number of previous reports of mixture suppression in aquatic organisms were confounded by (a) the experimental conditions of testing stimuli of varying concentrations and potencies without knowledge of the number of relatively independent receptor sites involved, and (b) the criterion that less than an "expected" additivity of the responses to the individual components of the mixture signifies the occurrence of mixture suppression. As pointed out previously (Caprio et al., 1989), this criterion is valid only if the dose–response functions to the stimuli are linear. Since olfactory

dose–response functions for amino acids in both electrophysiological and behavioral assays in teleosts and decapod crustaceans are nonlinear, the additivity criterion is invalid and has possibly caused an overestimation of the occurrence of mixture suppression. As for the first possibility, competitive binding among stimuli having differing receptor affinities could probably have resulted in the mixture suppression reported (Gleeson and Ache, 1985; Bell et al., 1987). Competitive binding between a strong and a weak agonist for an olfactory receptor site, as reported for taurine and glycine in antennular chemoreceptors in *Panulirus argus* (Gleeson and Ache, 1985), would diminish the stimulatory effects of the stronger agonist and be one mechanism for mixture suppression. Since all components used to form mixtures in the experiments in the channel catfish were adjusted for equal potency, this mechanism for mixture suppression was experimentally eliminated.

The major finding by Caprio et al. (1989) of enhanced olfactory receptor activity to binary and trinary mixtures of amino acids whose components bound to relatively independent receptor sites (Caprio and Byrd, 1984) was confirmed here for more complex mixtures. An enhanced olfactory receptor response is defined in the present context as a response to a mixture that is larger than that produced by a within-group mixture consisting of the same total number of components. Whenever a basic or acidic amino acid was mixed either in a 50:50 proportion with an equipotent mixture of up to nine neutral amino acids (i.e., in "binary" mixtures) or in a declining proportion with increasing numbers of equipotent neutral amino acids (i.e., in multimixtures), the response to the resulting mixture was significantly larger than the magnitude of the component solutions used to form the mixture. As previously suggested (Caprio et al., 1989), this response enhancement that occurred by mixing stimuli that bind to relatively independent receptor sites may be one mechanism of synergism. Thus, much of the difficulty reported in earlier attempts to predict responses of stimulus mixtures from experimentally derived responses to the individual components may be attributed to insufficient information as to the relative independence of the respective receptor sites for the component stimuli.

Theoretically, the MDI of even a 10-component, binary across-group mixture, as formulated in the present experiments (i.e., the mixing of equivolumes of two equipotent solutions, one composed of a single basic or acidic amino acid and the second composed of two to nine neutral amino acids), should be similar (not significantly different) from that of a two-component, binary across-group mixture. Although this occurred for all binary across-group mixtures in which a solution of LCNs was mixed with an acidic amino acid, it did not occur for an LCN solution mixed with a basic amino acid. Conversely, MDIs were similar for binary across-group mixtures in which a solution of SCNs was mixed with a basic, but not with an acidic amino acid. In both of the exceptions, the MDI decreased with an increasing number of neutral amino acid components. Although the exact cause of the reduction in the MDI is presently unknown, the results of cross-adaptation experiments (Caprio and Byrd, 1984) and the results presented here provide for an interesting speculation. In the previous study, a principal components analysis related each test amino acid to all others on the basis of similarities of the EOG responses to the test stimuli across eight different amino acid adapting regimes. This type of analysis placed the SCNs close to the acidic amino acids, and the LCNs close to the basic amino acids. These results of

the cross-adaptation experiments indicated that although the SCN and acidic amino acids bound to relatively independent receptor sites, there were more similarities in the effects of the cross-adapting regimes on these test stimuli than were observed between SCN and basic amino acids; conversely, although the cross-adaptation results indicated that LCN and basic amino acids bound to relatively independent sites, there were more similarities in the effects of the cross-adapting regimes on these test stimuli than were observed between LCN and acidic amino acids. One possible explanation for these findings is that receptors for (a) SCN and acidic amino acids and (b) LCN and basic amino acids, respectively, have a greater probability of being located on the same receptor neurons than being segregated on different receptor neurons. It is also reasonable to assume that different receptor site types present on the same receptor cell when activated by a mixture cannot generate the same total amount of receptor potential as independent receptor sites located on different receptor cells. Thus, the reduction in the MDIs for the two types of across-group binary mixtures in the present results was possibly due to the limitation in output of the individual receptor cells containing relatively independent receptor sites for the respective stimuli.

An enhanced olfactory receptor response was also clearly evident in across-group multimixtures, where, with the increasing number of within-group components (neutral amino acids), the percent content of the basic or acidic amino acid (the single across-group component) declined. This diluting out of the across-group component would tend to compromise somewhat the response enhancement that results from activating a relatively independent amino acid site type, while the increase in the number of neutral amino acid components in multimixtures of increasing complexity would tend to increase the MDI (Tables III and IV). However, the MDI values for the across-group multimixtures were still significantly greater than those for the within-group multimixtures consisting of the equal total number of amino acids (Figs. 3 and 4). The sole exception, the nine-component across-group multimixture, $M_{(8L,1B)}$, which does not result in a significantly larger MDI than that for the within-group multimixture, $M_{(9L)}$, (Fig. 3), is currently unexplained, but may be due to the saturation of the multiple receptor sites.

With an increasing number of neutral amino acid components, the MDI values for the across-group binary mixtures remained approximately constant or decreased slightly (Figs. 5 and 6), while the MDI values for the across-group multimixtures increased (Figs. 3 and 4). However, due to differences in the construction of the respective mixtures, the actual EOG response magnitudes to across-group mixtures, consisting of neutral and basic or neutral and acidic amino acids, were greater to the binary mixtures than to the multimixtures. This, however, should be apparent when considering that in the 10-component across-group binary mixtures, the single basic or acidic amino acid was 50% of the solution, whereas it was only 10% of the solution in the multimixture.

Predicting Olfactory Receptor Responses to Complex Mixtures

Caprio et al. (1989) used two models, the "stimulus addition" (Cameron, 1947; Bartoshuk and Cleveland, 1977) model, also referred to as the "input summation" (Cain, 1975) or "stimulus summation" (Carr and Derby, 1986a, b) model, and the

"response summation" (Carr and Derby, 1986*b*) model, also referred to as the "sum of perceived intensity" (Bartoshuk, 1977) or "output summation" (Cain, 1975) model to predict olfactory receptor responses of channel catfish to stimulus mixtures. In the stimulus addition model, the components in a mixture, which bind to the same receptor site and are therefore indistinguishable by the system, are expressed as "equivalent" concentrations of one of the components, the reference compound. These values for each of the components are then summed and the mixture response is predicted by the dose–response function of the reference compound at the higher resulting concentration. For the response summation model, the components in a mixture activate independent receptor site types and initiate a mixture response that is the sum of the responses to each of the components applied individually. Based on the results of binary and trinary mixtures of amino acids (Caprio et al., 1989), the stimulus addition model appeared adequate to predict the responses to within-group mixtures, whereas the response summation model was a better choice for predicting across-group mixtures. However, since the present results indicate that a number of different and highly overlapping receptor site types, rather than a single receptor type, exist for neutral amino acids (indicated by the increasing MDI for within-group mixtures; Tables III and IV; Figs. 2–4), the stimulus addition model underestimates the responses to more complex mixtures of neutral amino acids. An accurate prediction of the response to a mixture consisting of equipotent, within-group neutral amino acids, however, can be formulated by using the experimentally determined regression equations (Eqs. 1 and 2). Also, Eqs. 5 and 8, which predict MDI values for across-group multimixtures composed of LCN and SCN amino acids, are similar to Eqs. 1 and 2, which is further evidence that the receptor sites for the LCN and SCN amino acids have highly overlapping specificities.

The results of the across-group mixtures in the present and previous reports (Caprio et al., 1989) indicate that although the responses to both the across-group binary mixtures and multimixtures consisting of neutral amino acids mixed with basic or acidic amino acids are significantly larger than those predicted by the stimulus addition model, the response summation model overestimates the mixture responses. A probable reason for the responses to these across-group mixtures not attaining the predicted values is that the receptors for the basic and acidic amino acids may not be as segregated on different receptor neurons as those for the neutral amino acid components of the mixture. For the multimixtures, a more accurate estimate for across-group multimixtures composed of neutral amino acids and a basic or acidic amino acid can be obtained using the experimentally determined regression equations (Eqs. 3, 4, 6, and 7) for the respective mixture types.

Implications for Natural Mixtures

With certainty, mixtures of amino acids found in nature would be more complex than those tested here. Rarely would a naturally occurring amino acid mixture be found in which the components would be in the appropriate concentrations that would be equipotent to the chemical receptors. Further, what might be equipotent concentrations to one chemosensory system, like olfaction, could be of quite different potency to another system, like taste, since the relative effectiveness for amino acids differs between the two chemosensory systems of teleosts (Caprio, 1977, 1978; Goh and

Tamura, 1980). However, what appears essential in this and previous work on amino acid mixtures in teleosts (Caprio et al., 1987, 1989), is that to be able to successfully predict the responses to any mixture, knowledge of the specificity of the respective binding sites for the components of the mixture is required. Among the components of a mixture, it is necessary to determine which amino acids bind to the same site, to highly overlapping sites, and to relatively independent sites in order to determine the appropriate amplification factor (i.e., the degree of enhancement if it occurs due to across-group components in the mixture). It also may be important to know how the independent sites are distributed, whether they are colocalized on receptor cells or are found on different cells. Possibly the amplification factor for two or more independent receptor sites located on the same olfactory receptor neuron would be less than if they were on different receptor cells. Currently, we lack this information. However, the present and previous (Caprio et al., 1989) reports provide the essential base of information for the further testing of mixtures on single olfactory neurons by patch clamp or other intracellular methods.

Although not yet found in teleosts, certain amino acids applied to antennular chemoreceptors in the southern spiny lobster, *Panulirus argus*, hyperpolarize the membrane and lead to a suppression of activity (Michel and Ache, 1990). Also, mechanisms of "synergism" other than the activation of relatively independent receptor site types may be found. Since under most conditions in nature chemoreceptors only detect stimulus mixtures, knowledge of the mechanisms of mixture detection are critical for beginning to understand the process of olfaction.

EOG and Neural Responses

Because of the relatively long-term stability of the preparation, the majority of the data presented were obtained from EOG recordings, although the neural spike activity data of relatively small populations of olfactory receptors to selected mixtures were also obtained. Even though EOG responses are composed of generator potentials from receptor neurons and nonneural events such as secretory potentials (Getchell, 1974), EOG responses to amino acids in teleosts have previously been shown to be reliable indicators of olfactory neural activity in response to both individual amino acids (Caprio, 1978; Silver, 1982) and to their binary mixtures (Caprio et al., 1989). As in the previous studies in fish, only the phasic displacement of the EOG from baseline was used as a reproducible and thus reliable measure of the magnitude of activation of the olfactory receptor neurons. The varying shape of the EOG and thus the area under the EOG waveform is a reflection of the tonic activity of the receptors, which is primarily only an indicator of the duration of stimulus activation of the receptors and the rate of stimulus clearance from the organ and can vary across preparations.

The results (Table V) indicated that although the MDI values derived from EOG and neural recordings were correlated, the response enhancement observed from the two different recording methods for many of the across-group mixtures was not linearly related. Since (a) the neurally derived MDI values are greater for across-group mixtures than the EOG-derived values, and (b) the slopes of the dose–response functions for amino acids based on neural recordings are significantly less than those for the EOG recordings (Caprio, 1978; Byrd and Caprio, 1982), the percent EOG

increase is a highly conservative indicator of the magnitude of the enhancement of action potential activity initiated by the olfactory receptor neurons. To better appreciate the difference in response enhancement attained in the EOG and integrated neural recordings, the response enhancement can be equated to the amplification factor necessary to obtain the same MDI value by increasing only the concentration of one of the equipotent components in an across-group mixture. For example, for the across-group binary mixture, $B_{(6L),(1B)}$, the EOG-derived MDI was ~ 1.2, whereas the MDI derived neurally was ~ 1.6 (Table V). Based on the MDI definition, an MDI of 1.2 indicates that the response to the mixture was 20% greater than the response to any of the equipotent components. Thus, to reach a 20% response enhancement level along the dose–response curve, the concentration of any of the components would have to be elevated by a factor of 2.3, calculated from the dose–response function $R = k(10)^{\log C/\text{gamma}}$ (EOG: gamma = 4.54; Byrd and Caprio, 1982). At the same time, this EOG increase is transduced into a neural MDI of 1.6, which indicates that the response to the mixture was 60% greater than the response to any of the equipotent components. To obtain a 60% increase in neural response, the stimulus concentration would have to be elevated at least 50 times (neural: gamma = 8.33, Caprio, 1978). It is remarkable that this enhancement in olfactory receptor neural activity in the present experiments resulted from stimulating the system with an appropriate amino acid mixture and not by elevating stimulus concentrations.

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