

Electro-Olfactogram and Multiunit Olfactory Receptor Responses to Binary and Ternary 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 responses to binary and ternary mixtures of amino acids were predictable with knowledge obtained from previous cross-adaptation studies of the relative independence of the respective binding sites of the component stimuli. All component stimuli, from which equal aliquots were drawn to form the mixtures, were adjusted in concentration to provide for approximately equal response magnitudes. The magnitude of the response to a mixture whose component amino acids showed significant cross-reactivity was equivalent to the response to any single component used to form that mixture. A mixture whose component amino acids showed minimal cross-adaptation produced a significantly larger relative response than a mixture whose components exhibited considerable cross-reactivity. This larger response approached the sum of the responses to the individual component amino acids tested at the resulting concentrations in the mixture, even though olfactory receptor dose-response functions for amino acids in this species are characterized by extreme sensory compression (i.e., successive concentration increments produce progressively smaller physiological responses). Thus, the present study indicates that the response to sensory stimulation of olfactory receptor sites is more enhanced by the activation of different receptor site types than by stimulus interaction at a single site type.

INTRODUCTION

In contrast to most experimental studies, olfactory and gustatory receptor cells rarely encounter sequentially spaced, single chemicals under natural circumstances. The normal chemical world is a complex mixture of odorants and tastants varying in concentration and potency. Although exceptions exist (Pawson, 1977; Mackie and Adron, 1978; Mackie et al., 1980), numerous reports concerning chemoreception in aquatic organisms indicate that mixtures of substances rather than individual com-

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ponents account for a significant percentage of the responsiveness to a natural extract or synthetic mixture (Bardach and Case, 1965; Hashimoto et al., 1968; McLeese, 1970; Mackie, 1973; Carr, 1976; Carr and Chaney, 1976; Carr et al., 1977; Adron and Mackie, 1978; Carr et al., 1984; Harada and Ikeda, 1984; Harada and Matsuda, 1984; Zimmer-Faust et al., 1984; Ellingsen and Døving, 1986; Elliott, 1986). The concept that chemical mixtures are treated differently from individually presented stimuli by chemosensory systems comes from recent studies which indicate that responses to mixtures cannot be predicted from knowledge of the responses to individual components (Derby and Ache, 1984; Zimmer-Faust et al., 1984; Derby et al., 1985; Gleeson and Ache, 1985; Johnson et al., 1985; Borroni et al., 1986; Carr and Derby, 1986 *a, b*). This is generally attributed to both mixture suppression and synergism occurring at peripheral and possibly central neural levels among different components in a mixture. Thus, the general message relayed by the previous studies is that mixture suppression and synergism are common features of chemosensory systems of aquatic organisms and that these processes are unpredictable and the mechanisms unknown.

To some extent the previous results concerning mixture interactions were possibly confounded by the experimental conditions of testing stimuli of varying concentrations and potencies without consideration for the number of possible site types involved in the detection of a particular stimulus mixture. Receptor sites for olfactory stimuli in both fish (Kalinowski et al., 1987; Novoselov et al., 1988) and terrestrial vertebrates (Chen et al., 1986; Fesenko et al., 1988) are hypothesized to be portions of specific glycoprotein molecules that are partially embedded in the apical portions of the olfactory receptor cell dendrite, including the olfactory cilia (Getchell et al., 1980; Rhein and Cagan, 1983). Estimates of possible site types, defined by their respective chemical specificities, are obtained from cross-adaptation (i.e., competition) studies (Caprio and Byrd, 1984). To circumvent problems associated with testing stimuli of heterogeneous potencies, the stimuli are tested at concentrations that provide for approximately equal response magnitudes. With this information and an appropriate receptor biosassay, a better understanding of mixture-receptor interactions and estimates of the extent of mixture suppression and synergism occurring at the periphery may be achieved.

Olfactory and gustatory systems of teleosts are generally highly sensitive to L-alpha-amino acids (see Caprio, 1984, 1988 for reviews). Recent electrophysiological cross-adaptation (Caprio and Byrd, 1984) and biochemical receptor binding (Bruch and Rulli, 1988) experiments indicate that olfactory receptors of the freshwater, channel catfish, *Ictalurus punctatus*, contain different receptor sites for the acidic, basic, and neutral amino acids, respectively. In the present electrophysiological experiments, responses of populations of vertebrate olfactory receptors to mixtures of amino acids that interact with the same or different site types were studied. "Within-group" mixtures refers to mixtures of amino acids whose components were identified by both the electrophysiological and receptor binding experiments to interact with the same receptive process and possibly the same receptor site. Thus, components of "within-group" binary mixtures were both acidic, both basic, or both neutral amino acids. Because of the relatively high degree of cross-reactivity between the two proposed "N" sites (one for short side-chain and the other for long side-chain, neutral amino acids) (Caprio and Byrd, 1984; Bruch and Rulli, 1988),

neutral L-amino acids were considered as a single category in the present experiments. The "within-group" trinary mixture consisted of only neutral amino acids, since there are only two commonly occurring acidic (L-aspartic and L-glutamic) amino acids, and of the three basic amino acids (L-arginine, L-lysine, and L-histidine) (Lehninger, 1981), histidine acted as a neutral amino acid in both the electrophysiological cross-adaptation and the binding experiments. "Across-group" mixtures refer to mixtures of amino acids whose components were indicated to interact with relatively independent receptor sites (Caprio and Byrd, 1984; Bruch and Rulli, 1988). Thus, components of "across-group" binary mixtures were composed of the various combinations (acidic and basic, acidic and neutral, and basic and neutral) of the three groups of amino acids taken two at a time; components of an "across-group" trinary mixture consisted of a single amino acid from each of the three groups (acidic, basic, and neutral). In addition, "binary triplets," mixtures of three amino acids (neutral, neutral, and acidic; neutral, neutral, and basic), which were indicated by cross-adaptation experiments to interact with only two receptor site types, were also tested.

The present results clearly show that (a) both the underwater electro-olfactogram (EOG) and integrated neural responses from olfactory receptors of the channel catfish to binary and trinary mixtures of amino acids were predictable when the respective relative independence of the binding sites of the component stimuli obtained from cross-adaptation studies was known, (b) a mechanism for the response "enhancement" evoked by mixtures is the simultaneous activation of multiple types of receptor membrane binding sites by the different components of the mixture, and (c) "mixture suppression" was not evident, which suggests that some of the previous reports of this phenomenon may be explained by simple competitive binding among stimuli with differing potencies that share the same receptor membrane-binding site.

METHODS

Maintenance of Experimental Animals

Juvenile (100–200 g) channel catfish, *Ictalurus punctatus*, were obtained from a local catfish farm, held in floating cages in nearby ponds, and fed commercial catfish chow. Catfish from these cages were transferred regularly to the university animal holding facility, held in aerated, charcoal-filtered city water (i.e., artesian well water) in 75-liter aquaria, and maintained on a 12:12 light-dark regime. All fish were tested within 10 d after transfer to the animal facility (Tucker, 1973) and were not fed during this period. The catfish was immobilized with Flaxedil (gallamine triethiodide; 0.1 mg/100 g body weight; Davis and Geck Dept., American Cyanamid, Pearl River, NY) injected into the flank musculature, wrapped in damp tissue paper, and placed in a Plexiglass holding device. Before surgery, the anesthetic MS-222 (ethyl-*m*-aminobenzoate methane sulfonic acid; Sigma Chemical Co. St. Louis, MO; 0.01% initial concentration) was circulated in the gill irrigation fluid composed of aerated, charcoal-filtered water. Supplemental doses of Flaxedil and MS-222 were delivered to the fish as necessary throughout the experiment.

Chemical Stimuli

Stock solutions of Sigma grade (Sigma Chemical Co.) acidic, basic, and neutral amino acids were prepared weekly and stored at 4°C; dilutions (charcoal-filtered water) were made before

each experiment. The pH of all amino acid solutions at the concentrations tested ranged between 7.8 and 8.1. Stimulus samples (0.5 ml) at room temperature were hand injected uniformly from disposable Pasteur pipettes into the flow (14 ml/min) of charcoal-filtered water that continuously bathed the olfactory mucosa. The maximum concentration at the receptors was 42% of the concentration injected as determined by photodensitometry of dye solutions (values in the text are not corrected for dilution). The rise time of the dye was much faster than its decline and the pulse duration was ~5 s measured at 37% of the peak photocell response to the dye.

Electrophysiological Recordings

The olfactory lamellae were exposed by removing the skin, connective tissue, and cartilage dorsal to the nasal capsule. The underwater EOG was recorded in vivo from the water immediately above the midline raphe of the olfactory organ with calomel electrodes via Ringer-agar-filled capillary pipettes, amplified by a direct-coupled amplifier, and displayed on a pen recorder. The magnitude of the response was measured as the height of the phasic displacement from the baseline levels (Silver et al., 1976; Caprio, 1978). Absolute response values (in millivolts) were obtained by comparison with the deflection elicited by a known calibration voltage. Multiunit olfactory neural activity was recorded in vivo from the surface of the sensory region (Caprio and Raderman-Little, 1978) of an olfactory lamella with metal-filled glass capillary electrodes tip-plated with Pt-black (Gesteland et al., 1959; Caprio, 1978). The Pt-black electrode was resistance-capacitance coupled (220 pF capacitor, 20-M Ω resistor) to one input grid of a high impedance probe, and the other active input was grounded and connected to the reference electrode, a hypodermic needle embedded in the flank musculature. The multiunit neural activity was amplified (bandpass, 30–300 Hz), integrated (0.5-s rise time), and displayed on a pen recorder. The neural response magnitude was measured as the height in millimeters of integrated phasic displacement from the baseline level. The Pt-black electrode tips were estimated at having cross-sectional areas of ~300 μm^2 (ball diameters of ~20 μm). With the estimated olfactory receptor cell density of 31 neurons per 600 μm^2 of sensory area of a lamella in the channel catfish, the electrode could directly contact on the order of 15 olfactory receptor neurons (Erickson and Caprio, 1984). The degree to which receptor cells outside this immediate contact with the electrode tip contributed to the signal is unknown; however, it is likely that the neural activity was recorded from small, discrete epithelial regions, since neural signals recorded with this type of electrode abruptly cease when the electrode tip is moved from the sensory to the indifferent epithelium (Erickson and Caprio, 1984). In contrast, the recorded EOG activity was assumed to have originated from a much larger population of receptor neurons.

30 animals were tested with mixtures of amino acids. For binary mixtures, EOGs and integrated neural activity were recorded in 21 and 6 animals, respectively; EOG responses to trinary mixtures of amino acids were observed in 3 animals.

Experimental Protocol

For testing each binary mixture and its components, two amino acids were chosen (a,b) and their concentrations were adjusted to provide for approximately equal response magnitude (R'a and R'b). The binary mixture (a + b) consisted of equal aliquots of a and of b, and the response (Rab) to the mixture was recorded. Analogously for trinary mixtures, R'a, R'b, R'c, and Rabc were recorded. Since the concentrations of a and of b in the binary mixture were halved, the solution 0.5a and 0.5b were also tested and their responses (Ra and Rb) were recorded. A trinary mixture consisted of equal aliquots of a,b, and c, and thus the solutions 0.33a, 0.33b, and 0.33c were tested and their responses (Ra, Rb, and Rc) were recorded. All stimulus concentrations tested in both binary and trinary mixtures ranged between 10^{-6} and

10^{-5} M, concentrations that are within the dynamic range of olfactory receptor dose-response (D/R) functions for amino acids (Caprio, 1978; Byrd and Caprio, 1982). Further, the slopes of these D/R functions for the EOG and for the neural responses, respectively, are approximately parallel (Caprio, 1978; Byrd and Caprio, 1982), a criterion necessary for using the following indices for characterizing the response to mixtures (Hyman and Frank, 1980).

Two indices of response (Hyman and Frank, 1980), the mixture discrimination index (MDI) and the independent component index (ICI) were calculated for each mixture tested. The MDI (R_{ab}/R'_a or R_{ab}/R'_b) equals 1 when the response to a binary mixture equals the response to either component at the concentration used to form the mixture (i.e., each component is tested individually at twice the concentration as its resulting concentration in the binary mixture). The MDI equals 1 unless mixture suppression ($MDI < 1$) or mixture enhancement ($MDI > 1$) occurred. The ICI ($R_{ab}/R_a + R_b$ for binary mixtures; $R_{abc}/R_a + R_b + R_c$ for ternary mixtures) measures the degree of independence of the components and equals 1 if the response to the mixture equals the sum of the responses to the components tested at their respective resulting concentrations in the mixture.

Data Analysis

The data were analyzed as a completely randomized design with a one-way treatment structure using SAS (1985; SAS Institute Inc., Cary, North Carolina). Treatment means were further analyzed using contrast statements and Tukey's Studentized Range test (honest significant difference procedure).

RESULTS

EOG: Binary Mixtures

Ten different "within-group" and twelve different "across-group" binary mixtures of amino acids were tested. EOG responses to all within-group mixtures were not significantly different ($P > 0.480$, $n = 228$) from those to either of the components at their concentrations that were used to form the respective mixtures (Figs. 1 A–3 A); thus, EOG responses to within-group mixtures did not vary significantly from an MDI of 1.0. MDI response indices of the across-group mixtures (Figs. 1 B, 2 A, 3 A; Table I) were significantly greater than those for the within-group mixtures (Figs. 1 A–3 A; Table I). Similarly, ICI values for across-group mixtures were significantly greater than those for within-group mixtures (Figs. 2 B, 3 B, Table I), but they were significantly less ($P < 0.05$, two-tailed t test) than an ICI of 1.0.

Neural: Binary Mixtures

To confirm that the EOG responses to mixtures of amino acids were of neural origin, tests of three different within-group and three different across-group binary mixtures were conducted while recording neural activity from olfactory receptor cells. As was found for the EOG data, integrated neural responses to within-group mixtures did not vary significantly ($P > 0.835$, $n = 30$) from an MDI of 1.0. MDI response indices derived from integrated, olfactory receptor neural recordings for across-group mixtures (Figs. 4 B, 5 A, Table I) were significantly greater than those obtained from within-group mixtures (Figs. 4 A and 5 A, Table I). Similarly, neural ICI values for across-group mixtures were significantly greater than those for within-group mixtures (Fig. 5 B; Table I), and they were not significantly different ($P > 0.05$, two-tailed t test) from an ICI of 1.0.

EOG: Trinary Mixtures

Eight different within-group and four different across-group trinary mixtures, and five different "binary triplet" mixtures of amino acids were tested. EOG responses to all within-group mixtures were not significantly different ($P > 0.094$, $n = 56$) from those to any of the three components at the concentrations used to form the respective mixtures (Fig. 6 A). Thus, as was found for both EOG and integrated

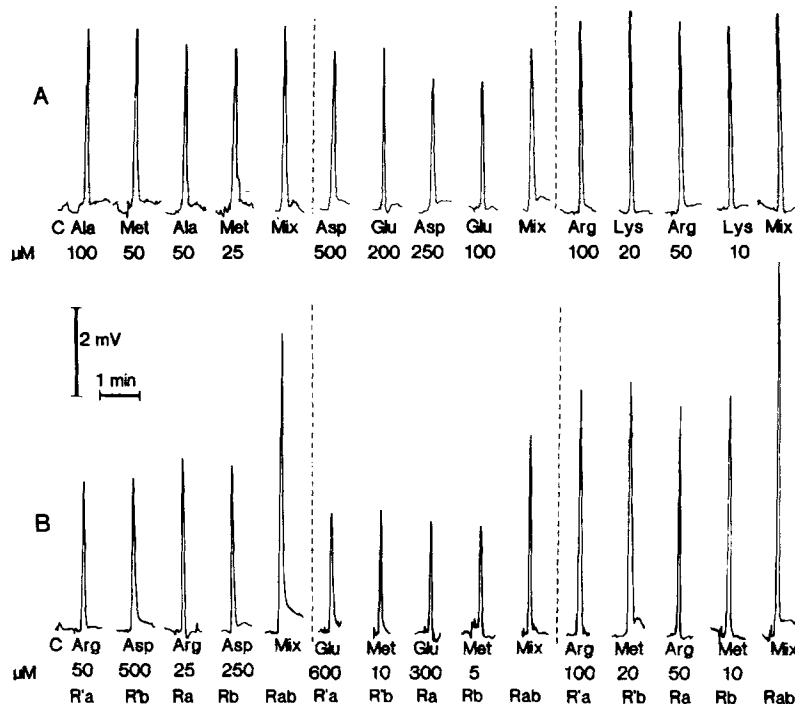


FIGURE 1. Representative EOG responses of the channel catfish to amino acids and their binary mixtures. (A) Responses to neutral (Ala, L-alanine; Met, L-methionine), acidic (Asp, L-sodium aspartate; Glu, L-sodium glutamate) and basic (Arg, L-arginine hydrochloride; Lys, L-lysine) amino acids, respectively, and to their binary within-group mixtures. (B) Responses to basic and acidic, acidic and neutral, and basic and neutral amino acids, respectively, and to their binary cross-group mixtures. R'a and R'b, responses to amino acids (*a* and *b*) that were adjusted in concentration to provide approximately equal EOG response magnitudes; Ra and Rb, responses to 0.5*a* and 0.5*b*, respectively, the resulting concentrations of each of the components in the binary mixture; Rab, response to the binary mixtures composed of equal aliquots of *a* and *b*.

neural responses to binary mixtures, EOG responses to trinary within-group mixtures did not vary significantly from an MDI of 1.0 (Table I). MDI values for across-group mixtures were significantly greater than those for within-group mixtures (Fig. 6 A, Table I). Similarly, ICI values for across-group mixtures were significantly greater than those for within-group mixtures (Fig. 6, A and B), but they were signif-

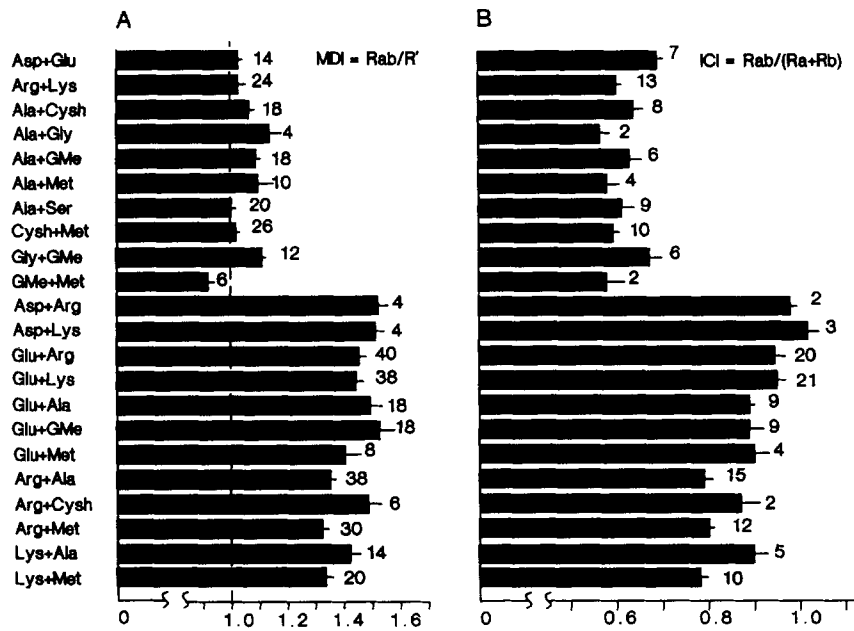


FIGURE 2. (A) MDI and (B) ICI of EOG responses to binary mixtures of amino acids. Rab is the response to the binary mixture; R' is the response to either stimulus at the isoresponse concentration (i.e., twice its concentration in the binary mixture). The broken line in A indicates the predicted MDI value of 1.0 (where Rab = R') for all within-group mixtures. Bars indicate mean + SE. Adjacent numbers indicate number of tests; Cysh, L-cysteine; Gly, glycine; GMe, L-glutamic acid gamma methyl ester; Ser, L-serine. Refer to Fig. 1 legend for additional stimulus abbreviations.

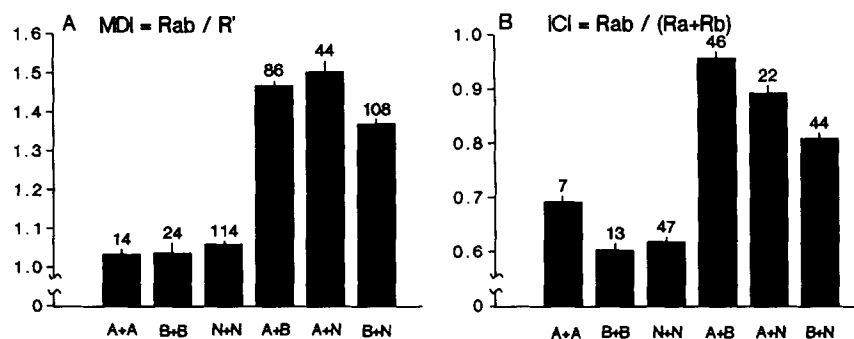


FIGURE 3. (A) MDI and (B) ICI summary indices for EOG responses to mixtures of binary amino acids listed in Fig. 2, but grouped here by type of amino acid. A, B, and N indicate acidic, basic, and neutral amino acids, respectively. Bars indicate means + SE. Adjacent numbers indicate number of tests. Refer to Fig. 1 legend for abbreviations.

TABLE I
MDI and ICI for Binary and Trinary Mixtures

| | Within-group mixture* | | | Across-group mixture* | | |
|----------------|-----------------------|---------------|---------------|-----------------------|---------------|---------------|
| | Binary | | Trinary | Binary | | Trinary |
| | EOG | Neural | EOG | EOG | Neural | EOG |
| MDI ± SE | 1.051 ± 0.008 | 1.092 ± 0.053 | 1.064 ± 0.013 | 1.426 ± 0.010 | 1.576 ± 0.059 | 1.363 ± 0.016 |
| n [†] | 152 | 20 | 42 | 238 | 28 | 42 |
| ICI ± SE | 0.622 ± 0.008 | 0.621 ± 0.038 | 0.487 ± 0.008 | 0.884 ± 0.009 | 0.941 ± 0.042 | 0.689 ± 0.010 |
| n | 67 | 10 | 12 | 112 | 14 | 9 |

* $P < 0.0001$, one-way anova for all comparisons of MDI and ICI values between within-group and across-group mixtures of the same category (i.e., binary EOG, binary neural, and trinary EOG). For example, the 1.051 ± 0.008 MDI value for EOG recordings of binary within-group mixtures is significantly less ($P < 0.0001$) than the 1.426 ± 0.010 MDI value for EOG recordings of binary across-group mixtures.

†Larger n for MDI than ICI due primarily to two R' values for a binary mixture and three R' values for binary triplets and trinary mixture.

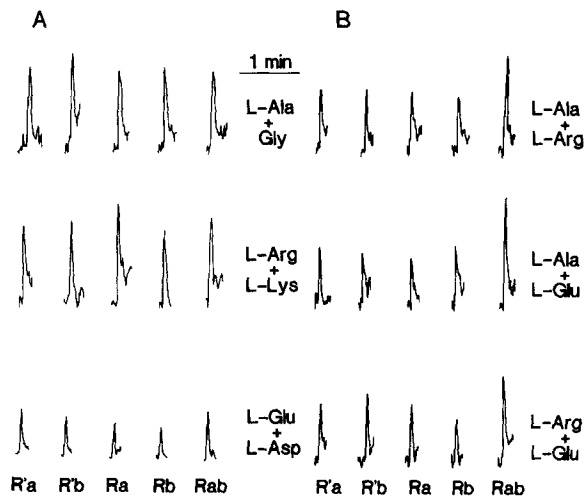


FIGURE 4. Representative integrated neural responses of the channel catfish to amino acids and their binary mixtures. For each stimulus pair listed, the top component is stimulus a and the lower component is stimulus b. (A) Responses to neutral (top row), basic (middle row), and acidic (bottom row) amino acids, and to their within-group binary mixtures. (B) Responses to neutral and basic (top row), neutral and acidic (middle row), and basic and acidic (bottom row) amino acids and their respective

across-group binary mixtures. Since the log-log dose-response functions for integrated neural responses of the channel catfish to amino acids are characterized by a slope of ~ 0.1 (Caprio, 1978), halving or doubling stimulus concentrations results in only minimal change in the magnitude of the integrated neural response. Thus, Ra and Rb may in some cases be larger than R'a or R'b due to the inherent variability of the electrophysiological preparation; however, Rab for across-group mixtures was clearly larger in magnitude than Ra, Rb, R'a, or R'b. Refer to Fig. 1 legend for abbreviations.

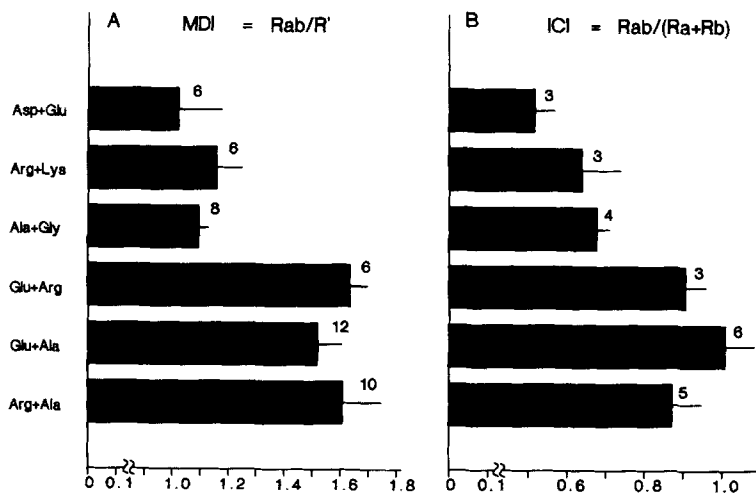


FIGURE 5. (A) MDI and (B) ICI indices for integrated olfactory receptor responses to mixtures of binary amino acids listed in Fig. 4, A and B. Bars indicate mean + SE. Adjacent numbers indicate number of tests.

icantly less than an ICI of 1.0 ($P < 0.05$, two-tailed t test). Further, the ICI responses to the "binary triplets" were not significantly different from each other, but were significantly less than the across-group and significantly greater than the within-group trinary mixtures (Tukey's Studentized Range test, $P < 0.05$).

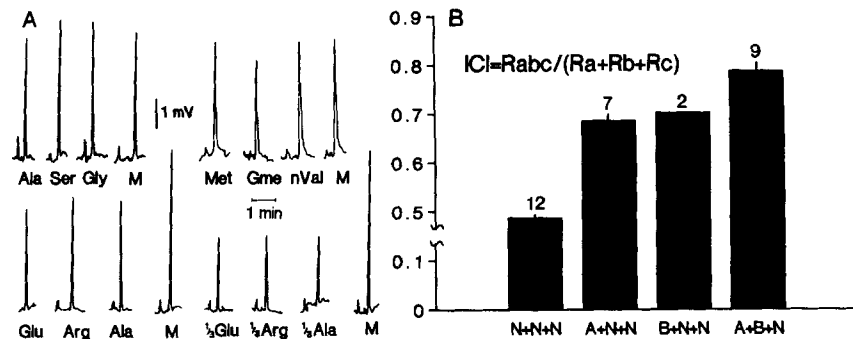


FIGURE 6. EOG responses to amino acids and their trinary mixtures. (A) Responses to representative neutral amino acids with short and long side chains, and to their corresponding within group trinary mixtures (M) (top row). Responses to a representative acidic, basic, and neutral amino acid, to their across-group trinary mixture (M), and to the component amino acids at their resulting concentrations in the mixture (bottom row). (B) ICI index summary of EOG responses to trinary mixtures of amino acids grouped by types of component amino acids. Responses to within-group trinary mixtures ($N + N + N$), binary triplet mixtures ($A + N + N$; $B + N + N$) and across-group trinary mixtures ($A + B + N$) are indicated. Bars indicate means + SE.

DISCUSSION

General Considerations

The data reported here show conclusively that knowledge of the specificity of the respective binding sites for the components of the mixture is essential to begin to understand the effects of mixtures on chemosensory systems and to successfully predict the response magnitude observed experimentally. Although the majority of the data presented in this report is obtained from EOG recordings, neural responses from olfactory receptors to selected within-group and across-group binary mixtures (Fig. 4) confirmed the EOG results and ensured that the larger amplitude EOG responses observed in response to across-group mixtures were of neural origin. These results provide evidence that the EOG, a negative slow potential caused by summed current flow through the extracellular resistance of the olfactory epithelium (Ottoson, 1956, 1971; Getchell, 1974; Getchell and Getchell, 1977), is a reliable indicator of olfactory receptor cell neural activity to amino acids in fish (Caprio, 1978; Silver, 1982). Further, evidence also exists to show that the electrical activity indicated by the EOG in fish is transmitted to the olfactory bulb (Byrd and Caprio, 1982; Kobayashi and Goh, 1985).

Predicting Responses to Mixtures

Behavioral (Carr and Derby, 1986 *a, b*), psychophysical (Bartoshuk and Gent, 1985) and physiological (Derby and Ache, 1984) studies have documented the occurrence of mixture interactions termed "mixture suppression" and "synergism" that result in opposite effects. The basis for classification of a response to a chemical mixture has generally been whether the response to the mixture was significantly less than (i.e., mixture suppression) or significantly more than (i.e., synergism) that expected based on simple additivity of the responses to the individual components in the mixture (Cain, 1975; Bartoshuk and Gent, 1985). Using the criterion of "additivity" to identify mixture interactions without consideration of the D/R functions of the components of the mixture will often lead to erroneous conclusions. Unless the slopes of the D/R functions of the stimuli are unity in either linear or log-log coordinates, the responses to the mixture will not sum by simple additivity (Cain, 1975, Rifkin and Bartoshuk, 1980; Carr and Derby, 1986 *a, b*). Electrophysiological (Caprio, 1978; 1982; Derby and Ache, 1984; Johnson et al., 1985) and behavioral (Borroni et al., 1986; Carr and Derby, 1986 *a, b*) D/R functions for amino acids in fish and decapod crustaceans, D/R functions for olfactory (EOG) responses in amphibians (Drake et al., 1969), and peripheral taste responses in mammals (Hyman and Frank, 1980), as well as magnitude estimation functions in human psychophysical studies (Cain, 1975; Bartoshuk and Cleveland, 1977), are considerably less than unity and are therefore characterized as exhibiting sensory compression. This results when successive increments of stimulus concentration produce progressively smaller increments in response magnitude. By the "additivity" criterion, recent reports in aquatic organisms may have overestimated the relative occurrence of mixture suppression, since a substance does not even add to itself by a simple addition when the slope of D/R function is less than unity (Rifkin and Bartoshuk, 1980).

The present results confirm suggestions based on psychophysical (Cain, 1975; Bartoshuk and Cleveland, 1977) and behavioral (Carr and Derby, 1986 *a, b*) experiments concerning which of the two specific models are appropriate predictors of responses to stimulus mixtures and defines the respective criteria for both of their applications in a predictive model. Responses to within-group mixtures (characterized by a MDI of 1) are consistent with predictions based on the "stimulus-addition" model (Cameron, 1947; Bartoshuk and Cleveland, 1977), which is also referred to as the "input-summation" (Cain, 1975) or "stimulus-summation" (Carr and Derby, 1986 *a, b*) model. In this model, the predicted response to a mixture, whose components are suggested from cross-adaptation studies to bind to the same receptor site, is based upon the D/R functions of the component stimuli. Thus, stimuli are expressed as "equivalent" concentrations of one of the components (i.e., the reference compound) of the mixture. These "equivalent" concentrations are summed and the response is predicted by the D/R function at the higher resulting concentration of the reference compound (Cameron, 1947). The present results confirmed the psychophysical finding that the response to a mixture of substances, whose individual D/R functions show sensory compression, result in an intensity less than the sum of the responses to the individual components (Bartoshuk, 1975, 1977; Bartoshuk and Cleveland, 1977). However, the hypothesis that sensory compression pre-

dicts mixture suppression (Bartoshuk, 1975; Bartoshuk and Cleveland, 1977) is justified only if the "additive" criterion for mixture suppression is observed. Since no within-group or across-group binary mixture tested in this study resulted in MDI values significantly <1.0 , no mixture suppression was actually observed. Thus, it is probably that some of the previous examples of "mixture suppression" observed in other studies might be explained simply by the rigid application of the "additivity" criterion to the experimental data and/or by the experimental procedure itself, which resulted in competitive binding among stimuli having significantly different relative potencies (Gleeson and Ache, 1985; Bell et al., 1987). Thus, the stimulatory effects of an effective stimulus could be diminished (i.e., diluted) in a mixture by competitive binding with a weak agonist, as indicated for taurine and glycine for the antennular chemoreceptors in *Panulirus argus* (Gleeson and Ache, 1985).

The "response-summation" model (Carr and Derby, 1986 *a, b*), also referred to as the "sum-of-perceived-intensity" (Bartoshuk, 1977) or "output-summation" (Cain, 1975) model, better predicted the responses to across-group mixtures (characterized by an ICI approaching one) than did the "stimulus-addition" model. In the "response-summation" model, the response to a stimulus mixture is predicted to equal the sum of the responses to the unmixed components of the mixture. The significant increase in the neural activity of olfactory receptor cells observed for all across-group binary mixtures is direct evidence that sensory stimulation of different types of receptor sites, each varying in chemical specificity, is transduced into a greater magnitude of neural activity than by stimulus interaction at a single-site type. Although in only two cases (Asp and Lys, EOG; Ala and Glu, neural) did averaged ICI values for particular binary mixtures actually attain unity, 42.9% of all neural responses to across-group mixtures were characterized as having an $ICI \geq 0.90$, whereas 50% had an $ICI \geq 0.85$. ICI percentages for EOG binary mixtures were, however, somewhat lower (i.e., 13.2% of the ICI values for the EOG responses were ≥ 0.90 , whereas 42.9% were ≥ 0.85). A possible reason for the majority of responses to across-group mixtures not attaining an ICI value of 1 is that different receptor site types present on the same receptor cell may not be as independent as different receptor site types on different cells. This may also be an explanation for why the ICI values for across-group trinary mixtures were less than those for the across group binary mixtures. With increasing numbers of stimuli in a mixture that bind with relatively independent receptor site types, the chances become greater that the respective site types for these stimuli may be located on the same receptor cell. Thus, the "sum" of generator potentials and spike activity that results from the binding of two or more amino acids on two or more different site types on the same cell may be less than that attained from independent cells. Nevertheless, these results are remarkable in that the responses to across-group binary mixtures, whose component D/R curves are characterized by power function exponents of ~ 0.10 (neural; Caprio, 1978) and 0.22 (EOG; Byrd and Caprio, 1982), which are identical to those for components of the within-group mixtures, may approach and sometimes attain additivity. Thus, for across-group mixtures, sensory compression does not predict mixture suppression (Bartoshuk, 1975), but predicts quite the opposite effect, i.e., response "enhancement." This is strong evidence that the "psychophysical model" (Bartoshuk, 1975; Bartoshuk and Cleveland, 1977)

should be applied only to mixtures of stimuli that interact at a single site. In summary, both the "stimulus-addition" and the "response-summation" models are more than just of historical value (Frijters, 1987), as they are essential for the successful prediction of neurophysiological responses to stimulus mixtures.

It may be argued that synergism in the present report should be limited to responses to within-group mixtures of amino acids that were characterized by MDIs significantly >1.0 and responses to across-group mixtures characterized by ICIs significantly >1.0 (see Carr and Derby, 1986*a, b*). However, neither occurred, which is consistent with the idea that synergism is an extremely rare event in the chemical senses (Rifkin and Bartoshuk, 1980; Engen, 1982; Gleeson and Ache, 1985). Another interpretation is that the criterion for synergism for the ICI to be >1.0 is invalid based on the compression functions of the component stimuli (Bartoshuk, 1975, 1977; Bartoshuk and Cleveland, 1977; Bartoshuk and Gent, 1985), and that synergism results when MDI values exceed 1.0, irrespective of the "type" of mixture. Thus, all across-group responses could be considered examples of synergism. Whether the activation of additional receptor site types, as observed in across-group mixtures resulting in response enhancement, is a mechanism of peripheral "synergism" (Zimmer-Faust et al., 1984) depends upon the working definition of the term. Therefore, caution is recommended in the application of terms, such as "excitants" and "suppressants" to stimuli and "synergism" and "mixture suppression" to the respective phenomena without any particular hypothesis to the causative mechanism(s).

Mixture Recognition at the Receptor Cell Level

A common physiological mechanism is indicated to be involved in the processing of chemical mixtures in vertebrates. For olfactory receptor responses in the channel catfish (present study) and chorda tympani recordings in the hamster (Hyman and Frank, 1980), ICI and MDI values for binary mixtures whose components were indicated to be the most independent (i.e., having relatively independent binding sites) of the chemicals tested were virtually identical between the two species. This occurred even though the power function exponent (0.33) characterizing the stimuli in the hamster study was larger than those for amino acid stimuli in the catfish. Mean ICI and MDI indices for across-group olfactory mixtures of amino acids in the catfish and for binary taste stimuli, such as D-phenylalanine mixed with either sodium chloride, calcium chloride, or ammonium chloride in the hamster, ranged from 0.88 to 0.94 (ICI) and from 1.33 to 1.58 (MDI) (Table I this report; Figs. 4 and 5 in Hyman and Frank, 1980). Analogously, ICI and MDI values for binary mixtures whose components were indicated to be interdependent (i.e., sharing the same binding site) were also virtually identical. ICI and MDI indices for within-group olfactory mixtures of amino acids in the catfish and for binary taste stimuli, such as mixtures of electrolytes in the hamster, averaged ~ 0.6 (ICI) and 1.0 (MDI). Thus, the fundamental basis for mixture discrimination at the receptor cell level may simply be the number of different receptor site types present to interact with the various components of the mixture. There was no discrepancy in the EOG or neural recordings to implicate nonspecific effects of the odorants (Price, 1987), nor

apparent problems with possible differential adsorption of the amino acid stimuli onto the sensory regions of the olfactory organ (Mozell and Hornung, 1985; Laing, 1987).

The greater response to across-group mixtures than to within-group mixtures observed in this study may be the electrophysiological correlate of the behavioral observations that mixtures are often more stimulatory than individual components (see introduction for references). The converse of this, where a single component or subset of components is more stimulatory than a complex mixture containing that (those) component(s) (Hamilton and Ache, 1983; Derby and Ache, 1984; Borroni et al., 1986) is, however, understandable in that certain components may reduce the overall effectiveness of the mixture by acting as either relatively poor agonists or as antagonists of the different receptor site types available. Further, the behavioral observation that the same chemical mixture may be a highly effective stimulus in one species and relatively poor in another (Carr and Derby, 1986b) can readily be explained by differences in specificity of the receptor site types in the two species.

Based on cross-adaptation evidence that indicated at least four different olfactory receptor site types for L-amino acids in the channel catfish (Caprio and Byrd, 1984), the present results suggest that a hypothetical mixture of the twenty common amino acids would be interpreted by the population of olfactory receptors as being composed of as few as four qualitatively different odorants. Amino acids that interact at the same site (i.e., within-group compounds) are interpreted as functionally identical odors, whereas those that bind to different, independent sites are sensed as different odor qualities. For this reason the term "binary triplets" was used in the present report to define a trinary mixture composed of two neutral and one acidic or one basic amino acid, since this mixture was sensed as only a binary mixture. Recent evidence to support this hypothesis was indicated for the coho salmon *Oncorhynchus kisutch*, where specific, individually presented amino acids, judged from competitive binding assays to compete for the same olfactory receptor binding site, were unable to be discriminated, but those that bound to relatively independent olfactory sites were discriminable in a whole animal behavioral bioassay (Rehnberg and Schreck, 1986). Whether whole animal behavior would mirror the predictions based on olfactory receptor considerations for mixtures of odorants is unknown. There is evidence, however, that mixture interactions are not solely limited to receptor cell processes, but extended to neural interactions within the central nervous system. Central nervous system components of mixture suppression have been suggested in psychophysical studies of both olfaction (Cain, 1975; Gillan, 1983; Derby et al., 1985) and taste (Lawless, 1979; Gillan, 1982; Kroeze, 1982), and indicated physiologically for olfaction in the spiny lobster *Panulirus argus* (Derby et al., 1985). However, the magnitude of the effect that the central nervous system may have in initiating mixture interactions could be quite variable for specific chemical mixtures and organisms. Thus, a basic understanding of stimulus-receptor interactions to rather simple mixtures will provide the foundation for assessing the degree of possible central neural effects and may allow for the successful prediction of responses of higher order neurons and even whole animal behavior to more complex stimuli.

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