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Taste Responses to Binary Mixtures of Amino Acids in the sea catfish, *Arius felis*

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Abstract

In vivo electrophysiological recordings in the sea catfish, *Arius felis*, showed that the magnitude of the integrated facial taste responses to binary mixtures of amino acids was predictable with knowledge obtained from previous cross-adaptation studies of the relative independence of the respective binding sites of the component stimuli. Each component from which equal aliquots were drawn to form the mixtures was adjusted in concentration to provide for approximately equal reponse magnitudes. The magnitude of the taste responses to binary mixtures whose component amino acids showed minimal cross-adaptation was significantly greater than that to binary mixtures whose components exhibited considerable cross-reactivity. There was no evidence for mixture suppression. The relative magnitude of the taste responses in the sea catfish to stimulus mixtures is similar to that previously reported for olfactory receptor responses in the freshwater channel catfish and chorda tympani taste responses in the hamster. **Chem. Senses 21: 45–53, 1996.**

Introduction

In nature, chemical mixtures rather than sequentially spaced single chemicals are the primary stimuli that excite olfactory and taste receptors. Previous studies of aquatic organisms have reported that stimulus mixtures rather than individual components account for a significant percentage of the responsiveness to a natural extract or synthetic complex mixture (Hashimoto *et al.*, 1968; Carr, 1976; Carr and Chaney, 1976; Carr *et al.*, 1977, 1984; Adron and Mackie, 1978; Harada and Ikeda, 1984; Harada and Matsuda, 1984; Zimmer-Faust *et al.*, 1984; Elliott, 1986). That stimulus mixtures are processed differently by olfactory receptor neurons than their individually presented component stimuli is often indicated and purported to be due to the frequent occurrence of mixture interactions, i.e. mixture suppression and enhancement (synergism). For these cases, responses to the stimulus mixtures were reported to be unpredictable based on the responses to the components of the mixture tested individually (Derby and Ache, 1984b; Zimmer-Faust *et al.*, 1984; Derby *et al.*, 1985, 1991a, b; Gleeson and Ache, 1985; Johnson *et al.*, 1985; Borroni *et al.*, 1986; Carr and Derby, 1986a, b; Atema *et al.*, 1989; Johnson *et al.*, 1989). It was recently reported, however, that responses of a population of olfactory receptor neurons to binary mixtures in the spiny lobster can be predicted with considerable success utilizing a non-competitive model that incorporates multiple transduction pathways (Daniel and Derby, 1995).

Two reports (Caprio *et al.*, 1989; Kang and Caprio, 1991) from this laboratory clearly showed that the magnitude

of the electro-olfactogram (EOG) and integrated olfactory receptor neural activity of the freshwater channel catfish, *Ictalurus punctatus*, to binary and to more complex mixtures of up to ten amino acid components were predictable with knowledge of the relative independence of the receptor site types obtained from electrophysiological cross-adaptation studies (Caprio and Byrd, 1984; Michel *et al.*, 1993). Mixtures whose components showed little cross-adaptation generated 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. These investigations revealed 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. These reports also indicated that there was no evidence for mixture suppression. The present investigation extends the findings of the two previous studies on olfactory receptor responses to stimulus mixtures to the gustatory system. This study tested whether the basic principles learned from studying olfactory receptor responses to amino acid stimulus mixtures are directly applicable to peripheral gustatory nerve responses to these compounds in the marine catfish *Arius felis*. The results clearly indicate this to be the case; i.e. the relative magnitude of the integrated taste responses to binary mixtures of amino acids was predictable based on knowledge of the relative independence of the receptor sites for the component stimuli. Thus, similar principles underlie the processing of both olfactory and gustatory information concerning amino acid stimulus mixtures.

Materials and methods

Experimental animals

Thirty-six sea catfish (*A. felis*), 48–170 g in body wt and 18–26 cm in total length, were tested in the present experiments. The fish were captured by barbless hook and line at the Louisiana Universities Marine Consortium (LUMCON) facility at Port Fourchon, LA. The fish transported to the animal care facility at Louisiana State University from Port Fourchon were maintained in a 250 l fiberglass aquarium with artificial seawater (ASW; Instant Ocean) adjusted to the salinity of ambient seawater (25–30‰ at 25°C). The fish were fed daily with fresh shrimp and tested within 3 weeks after capture.

Experimental procedures

The fish were immobilized with an i.m. injection of Flaxedil (gallamine triethiodide; ~0.3 mg/100 g body wt; Davis & Geck Dept, American Cyanamid, Pearl River, NY). After loss of body equilibrium and reduction in gill opercular movement, the fish was wrapped in wet tissue paper and secured to a wax plate held in a Plexiglas chamber. A gill irrigation flow of ~500 ml/min aerated ASW was provided by a recirculating system into which 0.005% ethyl-*m*-aminobenzoate methane sulfonic acid (MS-222) was added. Supplemental Flaxedil and MS-222 were provided as necessary to maintain animal immobility and a constant level of anesthesia respectively. A maxillary barbel was inserted into a tubular plastic sleeve and was continuously bathed with ASW without MS-222 at a flow rate of 12 ml/min. The local anesthetic tetracaine (3% w/v) was applied to the skin around the eye prior to deocculation. After deocculation, the ramus maxillaris branch of the facial–trigeminal nerve complex was teased into several nerve bundles, of which one that was highly responsive to amino acid stimulation was selected and placed over a platinum hook electrode. The nerve bundle on the electrode was placed under halocarbon oil to preserve it from drying during the experiment. Multi-unit neural activity was amplified, monitored aurally, displayed on a storage oscilloscope and recorded on analog tape. The peak integrated response (0.5 s time constant), recorded simultaneously on a chart recorder, served as the experimental response measure.

Stimuli

Amino acids (Sigma Chemical Co., St Louis, MO) were chosen as test stimuli based on previous taste recordings in this species (Michel and Caprio, 1991; Michel *et al.*, 1993). Six of the eight amino acid stimuli tested, L-alanine (Ala), D-alanine (DAla), glycine (Gly), L-arginine (Arg), L-histidine (His), L-proline (Pro) and L-glutamate (Glu), were indicated from electrophysiological cross-adaptation experiments to bind to at least some relatively independent binding sites (Michel *et al.*, 1993). Further, single taste fiber analysis indicated the existence of two major amino acid taste fiber types in the facial taste sytem of *A. felis* that were stimulated best by DAla and Ala/Gly respectively (Michel and Caprio, 1991). In addition, L-lysine (Lys), a basic amino acid, and L-aspartic acid (Asp), an acidic amino acid, completed the test stimuli. These Sigma grade amino acids were prepared at 10^{-2} M prior to each experiment. The pH range of the test stimuli and the ASW was 8.3–8.4. A 0.5 ml volume of

a stimulus or control ASW was applied to the maxillary barbel through a sample injection loop of a sample injector (Rheodyne model 50). The peak concentration reached was 75% of the concentration injected into the stimulus delivery system as determined by photodensitometry of dye studies. The stimulus concentrations indicated in the text were not corrected for dilution.

Experimental protocol

The following protocol is based on previous results indicating that the tested amino acids are characterized by having approximately parallel power functions [slope ~0.23; derived from log-transformed ordinate data from figure 3 in Michel *et al.* (1993)]. Two amino acids were chosen (a and b) and their concentrations were adjusted for each nerve bundle sampled to provide for approximately equal response magnitudes (Ra and Rb) [Table 1; the wide range of stimulus concentrations for some of the components of the tested binary mixtures in Table 1 were due to differences in the numbers and types of single taste fibers (Michel and Caprio, 1991) comprising each of the different nerve bundles sampled]. Next, the concentrations of the amino acids a and b were doubled (2a and 2b) and the respective responses (R'a and R'b) were recorded. The response (Rab) to the binary mixture consisting of equal aliquots of 2a and 2b was obtained. Two indices of response (Hyman and Frank, 1980; Caprio *et al.*, 1989), the mixture discrimination index (MDI; mean value of Rab/R'a and Rab/R'b) and the independent component index (ICI; Rab/(Ra + Rb) were calculated for each binary mixture tested. The MDI = 1 unless mixture suppression (MDI < 1) or mixture enhancement (MDI > 1) occurred. The ICI 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.

'Within-group' binary mixtures are defined as mixtures of two amino acids whose components were indicated from electrophysiological cross-adaptation experiments to bind to the same or highly cross-reactive sites (Michel *et al.*, 1993). Within-group binary mixtures tested in the present study consisted of two acidic amino acids (A + A), two basic amino acids (B + B), two neutral amino acids (N + N) and a mixture of a neutral and an acidic amino acid (N + A) respectively. 'Across-group' mixtures are defined as mixtures of amino acids whose components were indicated to bind to at least some relatively independent receptor sites (Michel *et al.*, 1993). Across-group binary mixtures tested in the present study consisted of neutral + basic (N + B), acidic + basic (A + B), neutral + imino (N + I), acidic + imino (A + I) and basic + imino (B + I) amino acids respectively.

Table 1 Stimulus concentrations[a] that resulted in approximately equal potencies for the components of the tested binary mixtures

Mixture: components a + b	Type[b]	Range a (M)	Range b (M)
Within-group			
Asp + Glu	A + A	3×10^{-5} to 10^{-4}	10^{-5} to 10^{-4}
Glu + Ala	A + N	5×10^{-5} to 2×10^{-4}	2×10^{-8} to 10^{-7}
Glu + Dala	A + N	10^{-5} to 10^{-4}	10^{-7} to 10^{-6}
Glu + Gly	A + N	5×10^{-5} to 2×10^{-4}	2×10^{-8} to 2×10^{-7}
Arg + His	B + B	2×10^{-5} to 10^{-3}	2×10^{-6} to 5×10^{-4}
Arg + Lys	B + B	5×10^{-5} to 5×10^{-4}	10^{-4} to 10^{-3}
Ala + Dala	N + N	10^{-8} to 10^{-4}	2×10^{-7} to 10^{-4}
Ala + Gly	N + N	10^{-8} to 10^{-4}	5×10^{-9} to 5×10^{-4}
Dala + Gly	N + N	2×10^{-7} to 2×10^{-3}	5×10^{-9} to 5×10^{-4}
Across-group			
Asp + Arg	A + B	10^{-5} to 10^{-4}	5×10^{-5} to 5×10^{-4}
Asp + Lys	A + B	2×10^{-5} to 10^{-4}	2×10^{-4} to 5×10^{-4}
Glu + Arg	A + B	10^{-5} to 3×10^{-4}	10^{-5} to 5×10^{-4}
Glu + His	A + B	5×10^{-5} to 2×10^{-4}	10^{-5} to 10^{-4}
Glu + Lys	A + B	2×10^{-5} to 2×10^{-4}	5×10^{-5} to 10^{-3}
Glu + Pro	A + I	10^{-5} to 5×10^{-4}	2×10^{-5} to 10^{-3}
Arg + Pro	B + I	2×10^{-5} to 2×10^{-3}	5×10^{-5} to 10^{-3}
His + Pro	B + I	5×10^{-6} to 10^{-4}	5×10^{-5} to 10^{-3}
Arg + Ala	B + N	5×10^{-4} to 10^{-3}	3×10^{-8} to 10^{-5}
Arg + Dala	B + N	10^{-5} to 10^{-3}	2×10^{-7} to 3×10^{-3}
Arg + Gly	B + N	2×10^{-4} to 10^{-3}	2×10^{-8} to 10^{-4}
His + Ala	B + N	5×10^{-5} to 10^{-4}	2×10^{-8} to 10^{-7}
His + Dala	B + N	10^{-5} to 10^{-4}	10^{-7} to 2×10^{-6}
His + Gly	B + N	2×10^{-5} to 5×10^{-4}	2×10^{-8} to 10^{-6}
Pro + Ala	I + N	10^{-4} to 10^{-3}	3×10^{-8} to 2×10^{-7}
Pro + Dala	I + N	10^{-4} to 10^{-3}	2×10^{-7} to 6×10^{-6}
Pro + Gly	I + N	5×10^{-5} to 10^{-3}	10^{-8} to 3×10^{-7}

[a]Listed as undiluted values.
[b]A, acidic amino acid; B, basic amino acid; N, neutral amino acid; I, imino acid.

Data analysis

All MDI and ICI values for the different binary mixtures (Figure 2) were combined by the specific category of mixture tested (Figure 3) and were analysed by one-way analysis of

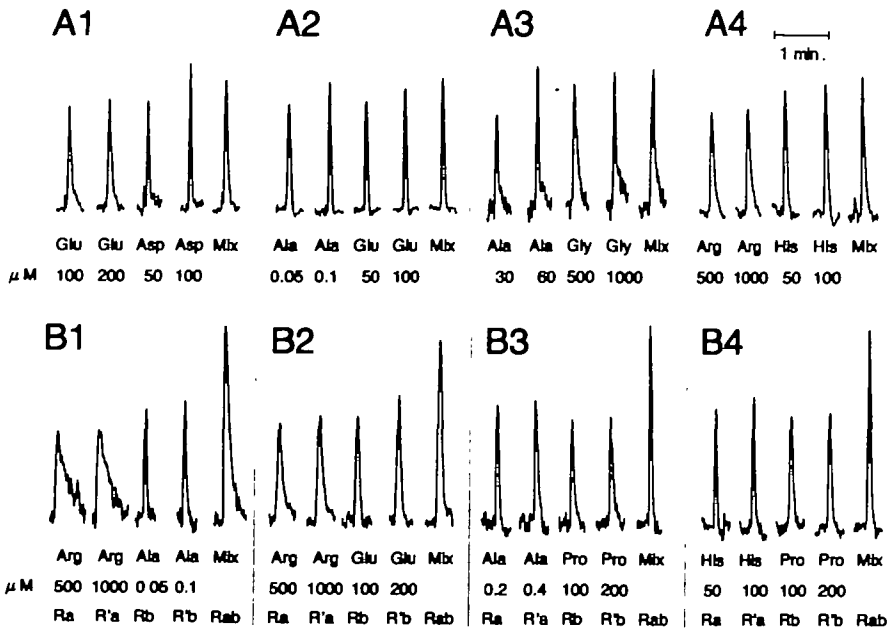

Figure 1 Representative integrated taste responses of *A. felis* to amino acids and their binary mixtures. **(A)** Responses to within-group mixtures and to their individual components. **(B)** Responses to across-group mixtures. Stimulus concentrations tested are listed below each amino acid component; the concentration for each component in the respective binary mixtures is identical to the lower of the two concentrations listed for each stimulus (e g. the concentrations of the components within the binary mixture in A1 are 100 μM LGlu and 50 μM LAsp)

variance (ANOVA). Subsequent comparisons of responses to the different binary mixture categories were performed using the Bonferroni test. Since MDI and ICI indices are ratio data, the respective values were also natural-log-transformed to calculate the proper variances required in the ANOVA. Since there were no significant differences between the analyses for the untransformed and the natural-log-transformed data for the tests indicated, all values reported in the text were derived from the untransformed data.

Results

A total of two hundred tests of binary mixtures comprising nine different within-group (A + A, A + N, B + B, N + N) and 17 different across-group (A + B, A + I, B + I, B + N, I + N) mixtures of amino acids were tested (Figures 1 and 2). Responses to the within-group and across-group mixtures, respectively, were not significantly different in magnitude from each other (Bonferroni; $P > 0.05$; Figures 1A1–4, 2 and 3). Responses to the across-group binary mixtures were significantly greater [1.41 ± 0.02 (SE); n = 107] in magnitude compared to the responses to the within-group mixtures (1.07 ± 0.01; n = 93) (ANOVA, $P <$ 0.0001; Figures 1, 2 and 3A). There was no overlap in the 95% confidence intervals for the MDI for the within-group (1.05–1.09) and the across-group (1.37–1.44) binary

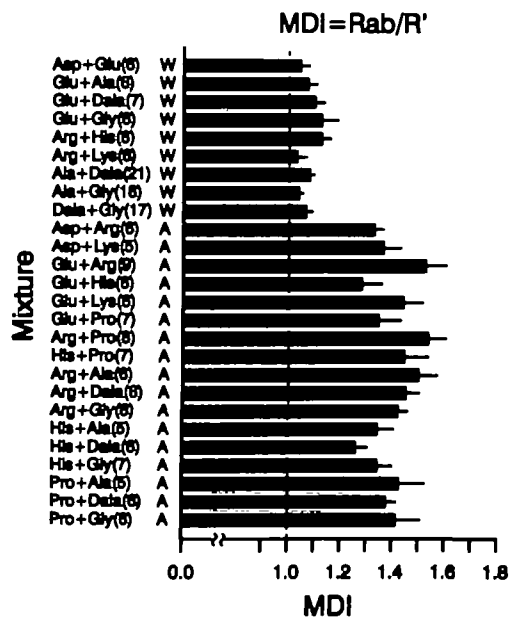

Figure 2 MDI of integrated multiunit taste responses to binary mixtures of amino acids. Rab is the response to the binary mixture; R' is the averaged response to the components at the isoresponse concentrations (twice their concentrations in the binary mixture). W, within-group mixture; A, across-group mixture; numbers in parentheses indicate the number of tests; bars indicate mean + SE.

mixtures. The averaged ICI value for the across-group mixtures [0.86 ± 0.01 (SE); n = 107] was significantly greater than that (0.66 ± 0.01; n = 93) for the within group

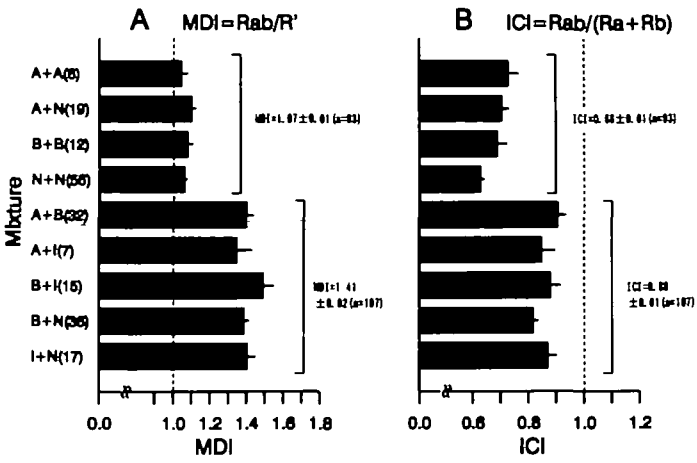

Figure 3 (A) MDI and **(B)** ICI summary indices for integrated taste responses to mixtures of binary amino acids listed in Figure 2, but grouped here by type of amino acid. A, B, I and N indicate acidic, basic, imino and neutral amino acids respectively Bars indicate means + SE. Numbers in parentheses indicate the number of tests

mixtures (ANOVA, $P < 0.0001$; Figure 3B). There was no overlap in the 95% confidence intervals for the ICI for the within-group (0.63–0.68) and across-group (0.84–0.89) binary mixtures.

Discussion

Mixture interactions

In any study on the effects of stimulus mixtures, the basis of whether mixture interaction occurred is whether the response to the stimulus mixture fell within the predicted value based on the responses to the components tested individually. If the response observed was significantly greater than that predicted, then mixture enhancement of chemosensory activity was said to occur. If the activity was significantly less than predicted, then mixture suppression was indicated. Obviously, how this prediction is made in any particular study necessarily affects the interpreted outcome.

Some studies predict responses to mixtures by calculations from different mathematical models (Daniel and Derby, 1987; Derby *et al.*, 1991a, b; Lynn *et al.*, 1994). Actual experimental results are then compared with the predicted values from the models to determine whether mixture suppression or enhancement occurred. In both the present report and in our previous studies (Caprio *et al.*, 1989; Kang and Caprio, 1991), responses to mixtures formed from mixing equal volumes of equally potent stimuli are compared directly with the responses to the individually tested components and decisions concerning whether a mixture interaction occurred and whether it was an enhancement or a suppression of activity is unambiguously observed. From previous findings for both taste and smell in animals of the genus *Ictalurus* (Caprio, 1978, 1980; Kanwal *et al.*, 1987) and *Arius* (Caprio, 1980; Michel *et al.*, 1993), dose–response relationships for different amino acids are approximately parallel. Thus, the relative potencies of the amino acids are preserved without radical shifts in relative effectiveness at different stimulus concentrations above threshold and below saturation. Therefore, equal mixtures of equipotent stimuli should, if no mixture interactions occur, result in a similar magnitude of response as the components (i.e. an MDI of 1.0). If the response to a stimulus mixture is significantly greater than the response to the components, then mixture enhancement clearly occurred (i.e. an MDI > 1.0). Thus, the operational definition of 'taste enhancement' in this report is a taste response to a stimulus mixture that is significantly greater (i.e. a greater peak of the integrated taste activity for multifiber preparations) than the response to the individual component stimuli. If the response to the mixture is significantly less than the response to the components, then mixture suppression clearly occurred.

Comparison of olfactory and taste responses to stimulus mixtures

The present report on facial taste responses of the sea catfish *A. felis* to binary mixtures of amino acids is consistent with the findings of two previous studies on the effects of amino acid mixtures on olfactory receptors in the freshwater channel catfish (Caprio *et al.*, 1989; Kang and Caprio, 1991). These studies clearly indicate that the response magnitude to mixtures of amino acids is predictable, that mixture suppression is not evident in electrophysiological recordings from populations of olfactory receptors or taste fibers in catfish, and that response enhancement is a frequent and predictable phenomenon. For all three studies, knowledge of the relative independence of the receptor sites for the component stimuli obtained from biochemical receptor binding (Cagan, 1986; Bruch and Rulli, 1988; Bryant *et al.*, 1989; Kalinoski *et al.*, 1989) and/or electrophysiological cross-adaptation (Caprio and Byrd, 1984; Wegert and Caprio, 1991; Michel *et al.*, 1993) studies was essential in order to be able to successfully predict the magnitude of both the electrophysiological olfactory and gustatory responses.

Analogous to the findings for olfaction (Caprio *et al.*, 1989; Kang and Caprio, 1991), binary taste mixtures whose components showed little cross-adaptation (i.e. the across-group mixtures) evoked enhanced taste activity in comparison with binary mixtures whose components cross-adapted

Table 2 Comparison of binary mixture parameters across different species and chemoreceptive systems

	Arius taste (VII)	Hamster taste (VII)[a]	*Ictalurus* olfaction (integrated)[b]	*Ictalurus* olfaction (EOG)[b]
MDI ± SE				
Within	1.07 ± 0.01	0.99 ± 0.04	1.09 ± 0.05	1.05 ± 0.01
	(93)[c][9][d]	(e)[4]	(20)[3]	(152)[10]
Across	1.41 ± 0.02	1.30 ± 0.04	1.58 ± 0.06	1.43 ± 0.01
	(107)[17]	[6]	(28)[3]	(238)[12]
ICI ± SE				
Within	0.66 ± 0.01	0.62 ± 0.03	0.62 ± 0.04	0.62 ± 0.01
	(93)[9]	[4]	(10)[3]	(67)[10]
Across	0.86 ± 0.01	0.83 ± 0.03	0.94 ± 0.04	0.88 ± 0.01
	(107)[17]	[6]	(14)[3]	(112)[12]

[a]Hyman and Frank (1980)
[b]Caprio *et al.* (1989).
[c]Number of tests.
[d]Number of different binary mixtures tested.
[e]Number not reported

(i.e. the within-group mixtures). However, the main difference between the previous olfactory reports and the present taste responses to binary mixtures of amino acids for these two catfish species was the response to the mixture of acidic and neutral amino acids. For olfactory receptors of the channel catfish, acidic and neutral amino acids resulted in little competitive binding (Bruch and Rulli, 1988) and minimal electrophysiological cross-adaptation (Caprio and Byrd, 1984). When applied to the olfactory organ, binary mixtures of acidic and neutral amino acids evoked enhanced responses (Caprio *et al.*, 1989) and were thus classified as across-group mixtures; however, for the facial taste system of the channel catfish (Wegert and Caprio, 1991) and the sea catfish (present report), acidic and neutral amino acid binary mixtures showed considerable cross-adaptation (Michel *et al.*, 1993) and did not evoke enhanced taste activity (present report). For these reasons, acidic and neutral amino acids were classified as within-group mixtures for the facial taste system. Significant interactions between neutral amino acids and Glu have also been reported for an amino acid transport system in yeast, where Glu transport was competitively inhibited by neutral amino acids (Rezková *et al.*, 1992). To indicate the differences between the within-group and across-group mixtures with respect to the results of reciprocal electrophysiological cross-adaptation tests between the components of each binary mixture (Michel *et al.*, 1993), the average percentage unadapted response [determined from figure 4 in Michel *et al.* (1993)] was 55.3 ± 3.1% (SE), $n = 26$, for the 13 across-group binary mixtures, which was significantly greater (i.e. significantly less reciprocal cross-adaptation between the components; $P < 0.05$, t-test) than the 22.4 ± 3.3%, $n = 14$, for the seven within-group mixtures. Thus, the generalized reduction in the responsiveness of multiunit taste activity between across-group components observed and discussed in previous studies of cross-adaptation (Wegert and Caprio, 1991; Michel *et al.*, 1993) also occurred here.

As in a previous study of olfactory receptor responses to stimulus mixtures in the channel catfish (Kang and Caprio, 1991), the MDI for within-group binary mixtures in the present experiments was slightly greater than 1.0, suggesting some slight enhancement in taste activity to the mixture compared with the individual components. For the taste responses in the sea catfish, the lower limit of the 95% confidence interval for the within group MDI mixtures was 1.05. These combined results suggest that more than one common receptor site type exist for the components of the respective within-group mixtures (e.g. there are possibly different, but highly cross-reactive, amino acid receptor sites for different acidic, basic and neutral amino acids respectively). However, the slight enhancement in taste activity observed for the within-group binary mixtures is dwarfed by the substantial enhancement in taste activity recorded to across-group mixtures.

A common physiological mechanism is indicated to be involved in the processing of chemical mixtures by olfactory

and taste receptors in vertebrates. A 34% averaged enhancement (difference between the average within-group and across-group MDIs; Figure 3A and Table 2) in facial taste activity in the marine sea catfish *A. felis* was observed for binary across-group versus within-group mixtures. This enhancement is similar to the 38% enhancement observed for EOG responses for similar types of mixtures for olfactory receptors in the freshwater channel catfish (Table 2). The average enhancement (31%; mean MDI of binary mixtures of DPhe and sucrose, respectively, with the chloride salts of sodium, calcium and ammonium minus mean MDI of mixtures of sucrose and DPhe, sodium chloride and calcium chloride, sodium chloride and ammonium chloride, and calcium chloride and ammonium chloride) of taste activity in the hamster for binary mixtures of components that most likely bind to different receptor sites was also similar to both olfactory and gustatory results in catfish (Table 2). Thus, irrespective of the vertebrate class, fish or mammal, common biological principles underlie how olfactory and gustatory receptors respond to stimulus mixtures.

Degree of response enhancement to across-group mixtures

To better appreciate the magnitude of the response enhancement observed in the present study for the across-group binary mixtures, the response enhancement can be equated to the amplification factor necessary to obtain the same MDI value by increasing the concentration of only one of the equipotent components in a typical across-group binary mixture. Based on the definition of the MDI, the average MDI observed for across-group binary taste mixtures in *A. felis* was 1.41. An MDI of 1.41 indicates that the average response to the across-group binary mixtures in the present report was 41% greater than the response to the average equipotent component of an across-group mixture. In order to obtain a 41% response enhancement along the dose–response curve of the average component, the concentration of the component would have to be elevated by 446%, calculated from the dose–response function $R = k(10)^{\log C/\Gamma}$ [Γ = 4.35 (Caprio, 1978; J. Kohbara and J. Caprio, unpublished data)]. It is remarkable that this enhancement in gustatory neural activity in the present experiments resulted from stimulating taste receptors with an appropriate amino acid mixture and not necessarily by elevating stimulus concentration.

Dose–response functions for amino acids in the facial taste system of the sea catfish are characterized by power functions with an exponent averaging only 0.23 (i.e. 1/Γ). Although these dose–response functions are highly nonlinear, the enhancement observed for the across-group mixtures approached the sum of the responses to the individual components which is characterized by an averaged ICI (i.e. the response to the mixture divided by the sum of the responses to the components) for the across-group mixtures of 0.86. Within-group components were characterized by an ICI of only 0.66. These ICI values for the across-group and within-group binary mixtures in the sea catfish are amazingly similar to the ICI values calculated for the respective binary mixture types for both hamster taste responses (Hyman and Frank, 1980) and channel catfish olfaction (Caprio *et al.*, 1989), both for the EOG and integrated neural responses (Table 2). Arguments indicating that the term 'mixture enhancement' is appropriate for responses that are less than the arithmetic sum of the responses to the components of nonlinear systems characterized by power function exponents <1.0 have previously been presented (Caprio *et al.*, 1989; Kang and Caprio, 1991). The greater taste response to 'across-group' mixtures than to 'within-group' mixtures observed in *A. felis* may be the electrophysiological correlate of 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 these components (Derby and Ache, 1984a; Borroni *et al.*, 1986; Derby *et al.*, 1991b), is possible 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, behavioral observations that a particular chemical mixture may be a potent stimulus in one species and ineffective in another can be explained by differences in the specificities of the receptor sites across the different species.

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