

Highly Specific Olfactory Receptor Neurons for Types of Amino Acids in the Channel Catfish

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Submitted 15 May 2007; accepted in final form 2 August 2007

Nikonov AA, Caprio J. Highly specific olfactory receptor neurons for types of amino acids in the channel catfish. *J Neurophysiol* 98: 1909–1918, 2007. First published August 8, 2007; doi:10.1152/jn.00548.2007. Odorant specificity to L- α -amino acids was determined electrophysiologically for 93 single catfish olfactory receptor neurons (ORNs) selected for their narrow excitatory molecular response range (EMRR) to only one type of amino acid (i.e., Group I units). These units were excited by either a basic amino acid, a neutral amino acid with a long side chain, or a neutral amino acid with a short side chain when tested at 10^{-7} to 10^{-5} M. Stimulus-induced inhibition, likely for contrast enhancement, was primarily observed in response to the types of amino acid stimuli different from that which activated a specific ORN. The high specificity of single Group I ORNs to type of amino acid was also previously observed for single Group I neurons in both the olfactory bulb and forebrain of the same species. These results indicate that for Group I neurons olfactory information concerning specific types of amino acids is processed from receptor neurons through mitral cells of the olfactory bulb to higher forebrain neurons without significant alteration in unit odorant specificity.

INTRODUCTION

Olfactory receptor neurons (ORNs) are the windows to the odorant world for all osmotic organisms. These ORNs express G-coupled-7-transmembrane molecular receptors at their apical ciliary or microvillous surface that evolved to detect specific natural odorants (Buck and Axel 1991). Often in experimental studies using common laboratory animals the behavioral significance of the selected test odorants is unknown. A major exception to this trend are studies of the response properties of ORNs in teleosts where different classes of biologically relevant odorants are well documented (Sorensen and Caprio 1998). Some fishes, such as catfish, are well-known experimental animals in the study of vertebrate olfaction (Sorensen and Caprio 1998). Results of the present investigation provide the opportunity to determine the logic of how specific biologically relevant odorants, such as amino acids, are processed from being initially detected at the level of single ORNs (present report), through the olfactory bulb (Nikonov and Caprio 2004) to higher forebrain neurons (Nikonov and Caprio 2007; Nikonov et al. 2005), which allows for their behavioral olfactory discrimination (Valenticic et al. 2000).

Numerous studies since the early 1970s documented that amino acids are highly potent, food-related, olfactory stimuli for fishes with electrophysiological thresholds for the more stimulatory compounds ranging from micromolar to nanomolar concentrations (Sorensen and Caprio 1998; Sutterlin and Sutterlin 1971; Suzuki and Tucker 1971). Electrophysiological

(Caprio and Byrd Jr 1984; Caprio et al. 1989; Kang and Caprio 1991; Ohno et al. 1984; Sveinsson and Hara 1990b) and biochemical (Bruch and Rulli 1988; Cagan and Zeiger 1978; Kalinoski et al. 1987; Rehnberg and Schreck 1986) studies indicated the existence of multiple olfactory receptor sites for amino acids; however, because the vast majority of the electrophysiological investigations of the amino acid specificities of ORNs in teleosts were performed in vivo by multiunit recordings, the distribution of these amino acid receptor sites across the individual ORNs was unknown. Some indication of the chemical specificities of individual ORNs in fish came not only from whole cell patch-clamp (Ivanova and Caprio 1993; Miyamoto et al. 1992; Nevitt and Moody 1992) but also from extracellular, electrophysiological recordings (Kang and Caprio 1995; Suzuki 1978, 1982) from single ORNs. Extracellular electrophysiological studies in the channel catfish indicated that many of the single ORNs encountered had ongoing spontaneous activity (~ 5 spikes/s) that could be elevated or inhibited in response to amino acids (Kang and Caprio 1995, 1997). Furthermore, response inhibition (25%) was the dominant response type (13% excited; 62% nonresponsive). In addition, a few single ORNs showed excitation to some amino acids and inhibition to others, which is similar to that reported recently for *Drosophila* ORNs (Hallem and Carlson 2006; Hallem et al. 2004). Calcium imaging experiments, however, did suggest that individual ORNs in fish respond to multiple amino acids (Restrepo and Boyle 1991; Restrepo et al. 1990), consistent with electrophysiological recordings in various species of tetrapods, suggesting that single vertebrate ORNs were not highly specific, but rather had a relatively broad response spectrum to olfactory stimuli (Blank and Mozell 1976; Gesteland et al. 1965; Holley 1991; Kauer 1987). In spite of using a variety of experimental techniques, none of these studies provided evidence for the existence of specific types of ORNs based on their odorant specificities to particular types of amino acids.

The present investigation probes the response specificities to amino acids of single ORNs in the channel catfish over a broad range of odorant concentrations, relevant to the aquatic natural world, and finds evidence for receptor units that are narrowly tuned to specific types (neutral with long side chains, neutral with short side chains, and basic) of L- α -amino acids. The present results parallel and were predicted by similar types of highly selective Group I units present in both the olfactory bulb (OB) and higher forebrain (FB) of this species.

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METHODS

Experimental animals

Channel catfish, *Ictalurus punctatus* (15–20 cm total length), obtained from a local hatchery, were maintained in floating cages held in ponds at the Louisiana State University Aquaculture Center facility. The fish were fed weekly with floating commercial fish chow. Each week catfish were transferred to an aerated, 250-liter polyethylene aquarium filled with charcoal-filtered city tap water (CFTW) at the Louisiana State University Animal Care Facility and maintained on a 12:12 light–dark regime. The temperature was held at $>27^{\circ}\text{C}$ during the spring and summer and at $<20^{\circ}\text{C}$ during the fall and winter to inhibit growth of the pathogenic bacterium, *Edwardsiella ictaluri*, which causes enteric septicemia and destroys chemosensory epithelia (Morrison and Plumb 1994). The fish were used experimentally within a 1-wk holding time and were not fed during this period.

Animal immobilization and anesthesia

Each catfish was initially immobilized with an intramuscular injection of the neuromuscular blocking agent Flaxedil (gallamine triethiodide, 0.03 mg/100 g). During the experiments, additional injections were applied as needed by a hypodermic needle embedded in the flank musculature. The immobilized fish was wrapped in a wet Kim-Wipe, placed into a Plexiglas container, and stabilized using a pair of orbital ridge clamps. The gills were irrigated using an orally inserted glass tube supplying a constant flow of aerated CFTW that initially contained the anesthetic ethyl-*m*-aminobenzoate methane sulfonic acid (50 mg/l MS-222). Surgical wounds were also bathed with 3% tetracaine. Once surgery was completed, the gill irrigation water was replaced with CFTW not containing MS-222.

Surgical preparation

Access to the olfactory organ was achieved by removing skin and connective tissue between the incurrent and excurrent nares, superficial to the olfactory organ.

Odorant stimuli and delivery

The amino acids were obtained commercially (Sigma Chemical); purity was stated to be a minimum of 98% to $>99\%$ (by thin-layer chromatography) for the different tested amino acids. Stock solutions (10^{-3} M) were prepared weekly in CFTW; log-step dilutions in CFTW were made daily. Interstimulus intervals (ISIs) were ≥ 2 min. Stimulus delivery to the olfactory organ was by a “gravity-feed” system using a spring-loaded valve (Model 5301; Rheodyne, Cotati, CA) driven by a pneumatic actuator (Model 5300) at 40 psi. Stimulus solutions and the CFTW used to bathe the olfactory mucosa between stimuli were delivered through a Teflon tube (0.79-mm diameter) at a rate of 4–5 ml/min. The olfactory cavity was continuously perfused with CFTW to: 1) facilitate stimulus delivery, 2) protect the mucosa from desiccation, 3) avoid the introduction of mechanical artifacts associated with stimulus presentation, and 4) thoroughly rinse the olfactory organ between stimuli (3- to 5-min ISIs). A foot switch connected to an electronic timer (Model 645; GraLab Instruments Division, Dimco-Gray, Centerville, OH) triggered the valve to introduce the odorants generally for a stimulus duration of 0.8 s without a change in either pressure or temperature and without dilution (Sveinsson and Hara 1990a). The 0.8-s stimulus duration was chosen to correspond approximately with the time required for the stimulus to fill the total volume of the olfactory cavity ensuring stimulus contact with the entire complement of ORNs. For the experiments described in Table 2, stimulus duration was 1.5 s.

Recording techniques

THE ELECTROOLFACTOGRAM (EOG). The underwater EOG is an odorant-induced, slow negative potential measured in the water immediately above the olfactory mucosa, which is thought to reflect summated olfactory receptor generator potentials (Ottoson 1971). The EOG was recorded in vivo with sintered Ag–AgCl electrodes by Ringer-agar-filled capillary pipettes. The EOG signal was amplified (Grass P-18 DC amplifier), digitized, and stored on a video channel of a high-fidelity VCR recorder. The EOG signal served as an indicator of both the viability of the preparation and the response onset to the tested odorants.

ORN UNIT RECORDINGS. Unit/multiunit activity (75–150 μV peak-to-peak amplitude) was recorded in vivo from spontaneously active ORNs from the surface of the olfactory lamellae adjacent to the midline raphe of the olfactory organ of the channel catfish (Caprio and Raderman-Little 1978). The electrode, a low-impedance (50–150 k Ω) gold- and platinum-plated, metal-filled, glass micropipette (glass tip, 1.5–2.0 μm ; ball diameter, 10–15 μm), was mounted on a hydraulic microdrive attached to a stereotaxic micromanipulator for careful positioning onto the sensory surface of an olfactory lamella. Odor application began once a spontaneously active unit was encountered and was clearly isolated by fine-positioning the recording electrode by the remote fluid-filled microdrive. For any odorant that resulted in an apparent increase in activity, a log-unit lower concentration was also tested. If no apparent change in unit activity occurred to any of the moderate concentrations of the test odor, a log-unit higher concentration of the respective odor was tested. The neural activity was amplified (Grass Instruments P511k; band-pass 30–3,000 Hz), observed with an oscilloscope, and stored on an audio channel of a high-fidelity VCR.

Data acquisition and analysis

All recorded data were digitized at 32 kHz and analyzed off-line by Discovery software (Brainwave Systems Discovery package, version 5.0 with Autocut; DataWave Technologies, Longmont, CO) and printed. Some of the waveform parameters used by the software to identify and discriminate extracellularly recorded action potentials were peak amplitude, valley amplitude, spike height, spike width, spike time, and time between spikes. Spike events, EOG signals, and experimental parameters (i.e., beginning of a recording period, onset of stimulation, and end of the recording period) were time-stamped with a 32-bit 100- μs resolution value and saved in a data file. The Brainwave data files were displayed on a computer screen and viewed by Neuroexplorer (Winston-Salem, NC) software.

Responses of single ORNs were classified as excitatory, inhibitory, or null (not significantly different from prestimulus) based on the one-tailed interrupted time-series analysis (ITSA) (Crosbie 1993; Nikonov and Caprio 2004, 2007). The ITSA statistically compares the number of action potentials occurring within successive 250-ms time bins for 1 s before and subsequent to the initial onset of the odor-induced EOG. In a subset of experiments, the ITSA was used to analyze the number of action potentials occurring during 1 s before stimulation and the first and third 0.5 s of a 1.5-s response.

RESULTS

The present investigation profoundly expands our understanding of the specificity of amino acid-responsive ORNs in teleosts. For this report, excitatory responses were critically analyzed because it is the excitatory response that drives the response of postsynaptic neurons at the next ascending level in the olfactory bulb (OB). This is not to imply, however, that response inhibition (i.e., significant decline in the number of

action potentials from ongoing spontaneous activity) is not an important response type. The vast majority of inhibitory responses observed appeared to be for contrast enhancement among odor responses to specific classes of amino acids (Fig. 1; Table 1). Units excited by one type of L- α -amino acid were most commonly inhibited by other types of amino acids (i.e., those with very different side-chain structures).

The logic of the search paradigm used in this investigation was to identify and record from the more selective (i.e., Group I) ORNs to better understand the amino acid specificity of these receptor units that allowed for the catfish to behaviorally discriminate these odorants (Valenticic et al. 1994, 2000). All 93 Group I ORN units analyzed were selectively excited by specific types of amino acids within the stimulus range of 10^{-7} to 10^{-5} M.

Given that during this study we searched specifically for ORNs characterized by having a narrow excitatory molecular

TABLE 1. Distribution of response types of Group I ORNs

Concentration/Stimulus	Amino Acid Unit Type								
	Met			Ala			Arg		
	+	-	n	+	-	n	+	-	n
10^{-7} M									
Met	15	0	0	0	9	0	0	16	0
Ala	0	11	4	9	0	0	0	12	4
Arg	0	14	1	0	6	3	16	0	0
10^{-6} M									
Met	22	0	0	0	11	1	0	19	0
Ala	0	17	5	12	0	0	0	15	4
Arg	0	21	1	0	8	4	19	0	0
10^{-5} M									
Met	32	0	0	0	27	1	0	32	1
Ala	0	27	5	28	0	0	0	28	5
Arg	0	31	1	0	26	2	33	0	0

+, excitatory; -, inhibitory; n, no significant change.

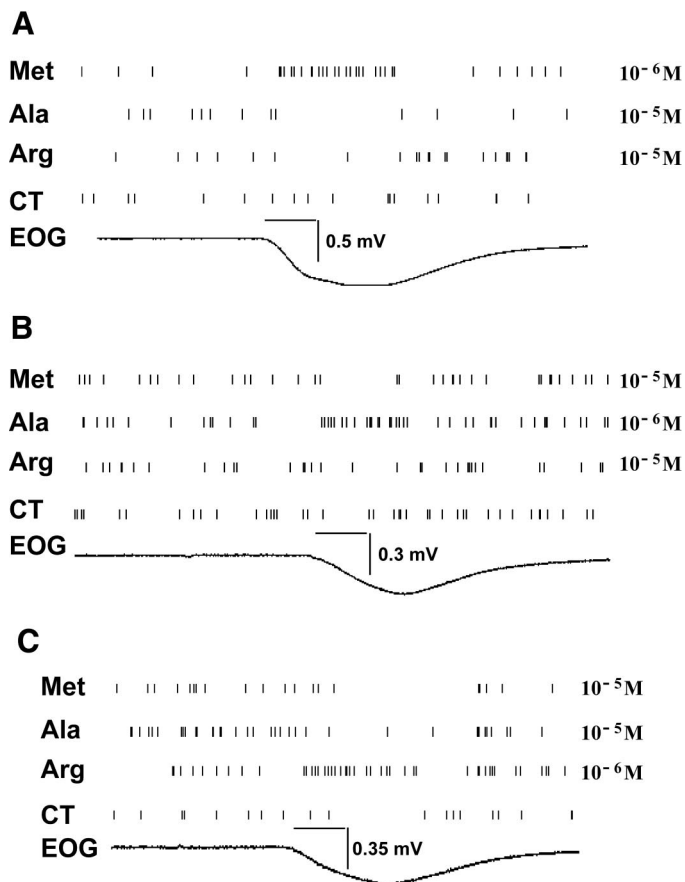


FIG. 1. Extracellular unit activity of responses of representative Group I olfactory receptor neurons (ORNs) to odorants representing 3 major types [long-chain neutral (LCN): L-methionine (L-Met); short-chain neutral (SCN): L-alanine (L-Ala); and basic (B): L-arginine (L-Arg)] of amino acids and to control water (CT). A representative electroolfactogram (EOG) trace is shown below the unit traces in A–C that was recorded simultaneously with the unit responses. Unit A is excited by Met and inhibited by Ala and Arg; unit B is excited by Ala, inhibited by Met and nonresponsive to Arg; unit C is excited by Arg and inhibited by Met and Ala. Stimulus concentrations listed adjacent to each record of digitized action potentials indicate that the stimuli that resulted in unit inhibition were tested at a 10-fold higher concentration than that of the stimulus that yielded excitation to illustrate the selectivity of the excitatory response. Odorant onset and duration (0.8 s) are indicated by the horizontal line below each series of responses; vertical lines indicate the calibration signals for the respective EOG traces.

response range (EMRR) to amino acids (i.e., Group I units), we initially failed to document the occurrence of units that exhibited a broader response specificity. However, to obtain an estimate of the percentage of Group I units, we performed a subset of experiments consisting of 164 different electrode positions within the olfactory mucosa. From these sites, 22 single ORN units were observed that were excited by the test amino acids. Of these, only 5 were Group I neurons, indicating that Group I ORNs are $\leq 25\%$ of the spontaneously active ORNs that are excited by amino acids.

Group I ORN unit selectivity to amino acid type

Ninety-three ORNs were recorded in 43 catfish that were excited by only one of the four types of amino acids tested at $\leq 10^{-5}$ M odorant concentrations (Fig. 1; Table 2). Approximately one third each of these neurons was selectively excited by either L-methionine (Met), a representative neutral amino acid with a long side chain (LCN); L-alanine (Ala), a representative neutral amino acid with a short side chain (SCN); or L-arginine (Arg), a basic (B) amino acid (Group I units). None was excited specifically by the acidic amino acid L-glutamic acid (Glu). From 20 to 50% of the ORNs in each category were selectively activated at 10^{-7} M amino acid, but the majority of the ORNs were activated by 10^{-6} M amino acid. All 93 of these receptor units were excited by 10^{-5} M of their respective stimulatory amino acid.

ORN unit selectivity to an expanded search strategy

We explored further the amino acid specificities of 37 of the Group I units to additional related amino acids.

SELECTIVITY OF GROUP I MET UNITS. Although there was some overlap in excitatory responses of single ORN units to the tested neutral amino acids, the 15 Group I units were separated into three general categories: 1) seven units whose excitatory thresholds were lowest to long, linear side chains (ILCNs) consisting of three to four methylene groups (i.e., nVal, nLeu, and/or Met) (Figs. 2 and 3A); 2) six units whose excitatory thresholds were lowest to branched side chains (bCNs) (Val and/or Leu) (Fig. 3B); and 3) two units whose highest sensitivity to amino acids included both ILCN and bCN groups (Fig.

TABLE 2. Responses of Group I ORNs

Response Type	Stimulus Concentration	Stimulus and Number (%) of Units Excited		
		Met	Ala	Arg
Excitatory	10^{-7} M	Met 15 (47%)	Ala 9 (32%)	Arg 16 (49%)
	10^{-6} M	Met 22 (69%)	Ala 12 (43%)	Arg 19 (58%)
	10^{-5} M	Met 32 (100%)	Ala 28 (100%)	Arg 33 (100%)
No excitatory response	10^{-6} to 10^{-5} M	Ala	Met	Met
		Arg	Arg	Ala
		Glu	Glu	Glu

3C). However, with increasing amino acid concentration, the majority of these units broadened such that they responded to both ILCNs and bCNs, but not to amino acids with acidic (Glu), basic (Arg), or SCN (Ala) side chains. Receptor units were also evident that were most sensitive to different amino acids within a category. For example, of the bCNs, unit 13 had the lowest threshold to Val; of the ILCNs, unit 3 had the lowest threshold to nVal (Fig. 3).

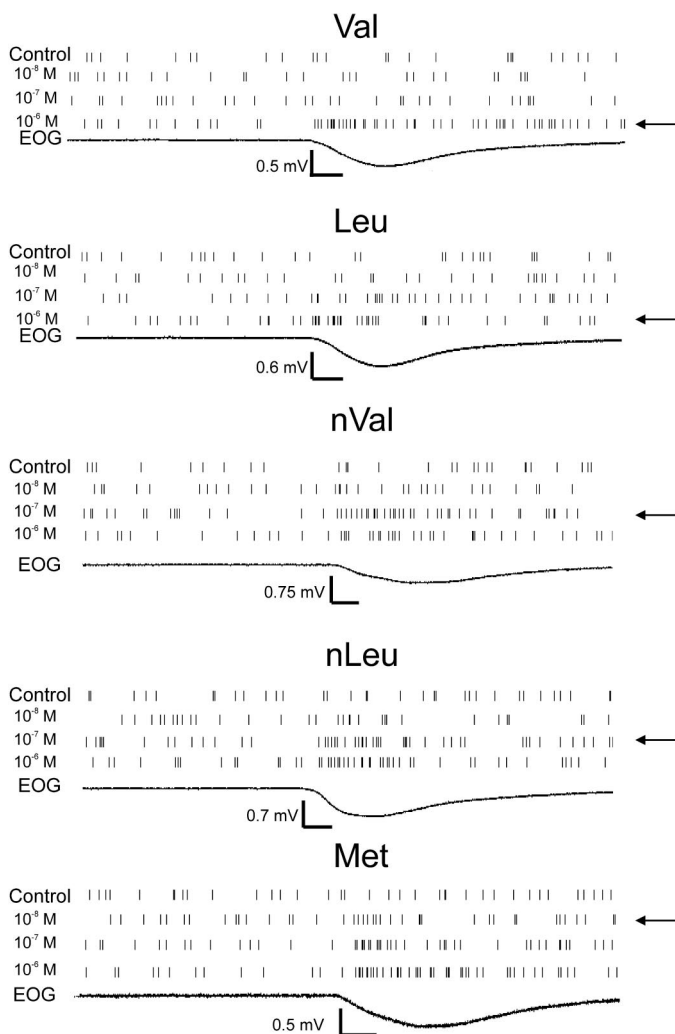


FIG. 2. Extracellular unit activity of responses of a representative Group I ORN unit (cell 1 in Fig. 3) to neutral amino acids with branched (bCNs: Val, Leu) and linear (ILCNs: nVal, nLeu, Met) side chains. Odorant concentrations are listed adjacent to each record; arrow indicates the lowest concentration that resulted in an excitatory response for that unit. Odorant onset and duration (0.8 s) are indicated by the line below each series of responses; vertical lines indicate calibration signals for the respective EOG traces.

SELECTIVITY OF SCN UNITS. Fourteen units were identified that were excited by the L-isomers of Ala and/or Ser (Figs. 4 and 5). Units were identified that were excited by only L-Ala (Fig. 4A; Fig. 5, units 1, 4, and 5), only L-Ser (Fig. 4B; Fig. 5, units 8, 11, and 12), or both (Fig. 4C, Fig. 5, units 2, 3, 6, 7, 9, 10, 13, and 14). Five cells were excited at a lower concentration by Ala (Fig. 5A), whereas seven cells were excited by a lower concentration by Ser (Fig. 5B); two other cells (Fig. 5C) showed thresholds similar to those of both compounds.

SELECTIVITY OF GROUP I B UNITS. Three additional basic amino acid odorants related to Arg [i.e., lysine (Lys), homoarginine (HArg), and ornithine (Orn)] were tested on 13 of the Group I ORN units that were selectively excited by 10^{-5} M Arg, but not by Met, Ala, or Glu (Figs. 6 and 7). Six (46%) were highly sensitive and were excited by 10^{-8} M basic amino acid (Fig. 6; Fig. 7, units 1–6). Of the 11 units that were excited by either of the two common basic amino acids, three units had lower electrophysiological thresholds to Lys (Fig. 7, units 1–3), five (Fig. 7, units 4–8) had lower thresholds to Arg, two (Fig. 7, units 10 and 12) had similar thresholds, and two (Fig. 7, units 11 and 13) were not excited by either of these two basic amino acids at stimulus concentrations $\leq 10^{-6}$ M, but were excited at lower stimulus concentration by Orn than by HArg. By increasing the stimulus concentration to 10^{-6} M, 9 of the 13 units were excited by both Lys and Arg, and two units (Fig. 7, units 11 and 12) were more responsive to Orn than to the other basic amino acids.

Analysis of response time with respect to unit classification

We addressed the question of whether a declustering of response types occurred from that determined early (first 0.5 s) compared with that during a later time period (1.0–1.5 s) of the response as reported for mitral cells in zebrafish (Friedrich 2006; Friedrich and Laurent 2001). Analyzed were the responses of 46 ORNs that were classified as having excitatory responses to either 10^{-6} M L-Met, L-Ala, or L-Arg during the 1.5-s stimulus application (Table 3). Eighty-three percent of the Met units, 55% of the Ala units, and 71% of the Arg units studied were classified similarly during the initial 0.5 s of the response (*time 0* was EOG onset) as they were during the entire time (1.5 s) of stimulus application. In an analysis of the response during the last 0.5 s of the 1.5-s stimulus application time, 72% of the Met units, 73% of the Ala units, and 82% of the Arg units were classified similarly as during the entire application time. To further justify that a significant change in response specificity over stimulus presentation time did not occur, none of the three types of units (Met, Ala, Arg) was excited by either of the other two amino acids during any portion of the 1.5-s stimulus application time.

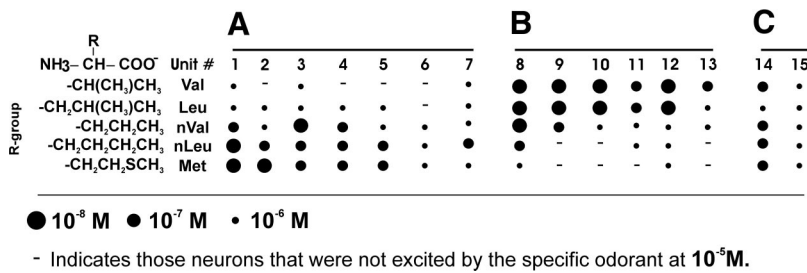


FIG. 3. Electrophysiologically derived excitatory thresholds of 15 Group I ORN neurons obtained from 7 fish to bCNS and ILCNs. Dots indicate the threshold concentration for each stimulus that resulted in an excitatory response for each unit analyzed. A: 7 ORNs (1–7) with lowest thresholds to ILCNs. B: 6 ORNs (8–13) with lowest thresholds to bCNS. C: 2 units (14, 15) that failed to show such a distinction. —, not excited by the specific odorant at 10⁻⁵ M. None of these units was excited by 10⁻⁸ to 10⁻⁵ M Ala [short side chain neutral (SCN)], Arg (Basic), or Glu (Acidic).

DISCUSSION

Olfactory unit selectivity over three levels of neural organization

It is exceptionally rare in the chemical senses to possess information concerning the specificity of odorant-responsive neurons across multiple levels of neural organization. Only two previous studies in vertebrates compared quantitatively the odorant specificity of single ORN and OB neurons to the same stimuli. For both tortoise (Mathews 1972) and frog (Duchamp 1982) there was little evidence for particular unit types based on response specificity of either ORNs or OB neurons. Recently, information concerning the odorant specificities of ORNs and antennal lobe (AL) neurons in *Drosophila* were obtained (Couto 2005; Ng et al. 2002; Wang et al. 2003). The results for *Drosophila* were similar to those reported for channel catfish (present report; Nikonov and Caprio 2004) where odorant specificity for single ORNs and OB/AL neurons was highly correlated with little transformation of information occurring between input and output.

The present report on the amino acid specificity of single ORN units along with previously published studies of amino acid specificities of OB (Nikonov and Caprio 2004) and FB (Nikonov and Caprio 2007) neurons in the channel catfish provide the first quantitative information on how odors are processed across three levels of neural organization in a vertebrate. The odorant selectivity of individual molecular olfactory receptors expressed in the ciliary/microvillous (Hansen et al. 2003) membranes of ORNs to type of amino acid is faithfully transmitted from Group I ORNs (present report) through Group I mitral cells (Nikonov and Caprio 2004) to Group I FB units (Nikonov and Caprio 2007). These results are

essentially similar to those indicated for the processing of odor activity through three levels of the olfactory system of *Drosophila* (Couto 2005; Keller and Vosshall 2003; Ng et al. 2002; Wang et al. 2003), but dissimilar to those reported for both the rabbit (Yokoi et al. 1995) and honeybee (Sachse and Galizia 2003). For the latter two species, odorant selectivity of the OB/AL output neurons was reported to be sharpened by neural circuitry within the OB/AL. Note, however, that the results for *Drosophila* are contradicted in other studies in the same species where projection AL neurons were reported to have a broader response profile than that of ORNs that innervate the same glomerulus (Shang et al. 2007; Wilson et al. 2004). A most recent report in *Drosophila* indicated that ORN responses are preserved in AL projection neurons and that there is no mechanism for broadening the tuning of these neurons (Root et al. 2007). Despite the confusion with *Drosophila*, the present results are clear in that the EMRR of ORN units in the present report were mirrored by both OB (Nikonov and Caprio 2004) and FB (Nikonov and Caprio 2007) Group I units in the same species. That the specificities of Group I ORNs in catfish are maintained at higher (OB and FB) levels within the CNS suggests that odorant information concerning the identity of specific types of amino acids is critically important to the life history (e.g., nutrient acquisition) of these fish. If the EMRRs of ORNs in mammals are also mirrored by neurons within the FB, it may help explain how bulbectomized rats are able to perform olfactory discriminations due to only ORN input innervation of the olfactory cortex (Slotnick et al. 2004). The present results also show that narrowly tuned pathways from the receptor through to the forebrain are not characteristic of only pheromonal channels, but are also used by neural channels related to feeding.

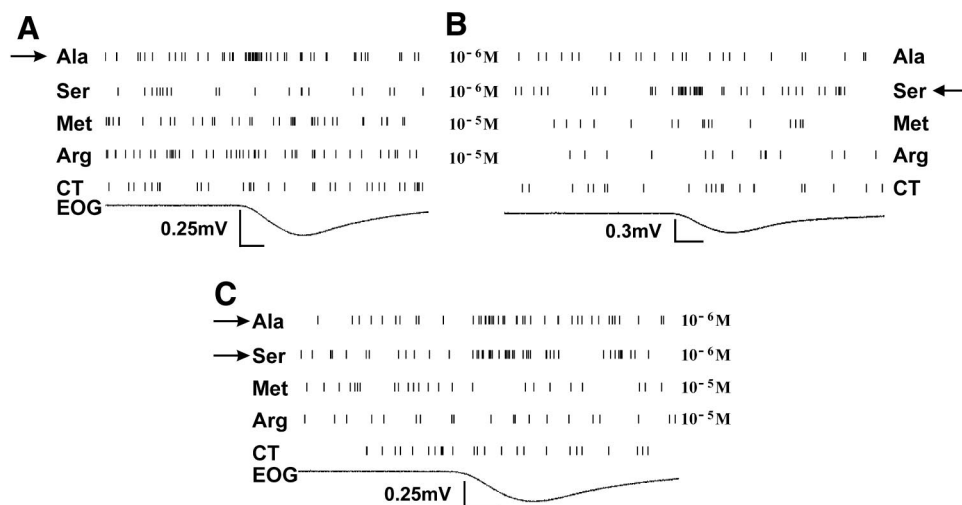


FIG. 4. Extracellular unit activity of responses of representative Group I ORNs to SCNs. A: unit excited by L-Ala. B: units excited by L-Ser. C: unit excited by both L-Ala and L-Ser. CT, control response. Odorant onset and duration (0.8 s) are indicated by the line below each series of responses; vertical lines indicate calibration signals for the respective EOG traces.

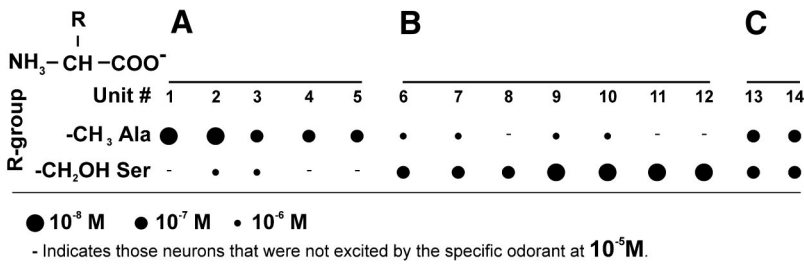


FIG. 5. Electrophysiologically derived excitatory thresholds of 14 Group I ORNs obtained from 8 fish to SCNs (Ala, Ser). Dots indicate the threshold concentration for each stimulus that resulted in an excitatory response for each ORN analyzed. A: 5 ORNs (1–5) with lowest thresholds to Ala. B: 7 ORNs (6–12) with lowest thresholds to Ser. C: 2 ORNs with similar thresholds to both amino acids. —, not excited by the specific odorant at 10⁻⁵M. None of these units were excited by 10⁻⁸ to 10⁻⁵ M Met (ILCN), Arg (Basic), or Glu (Acidic).

Logic for the selection of the tested amino acid odorants

The logic for the selection of the tested amino acid odorants was based on our previous understanding of the response specificity of both OB (Nikonov and Caprio 2004) and higher FB (Nikonov and Caprio 2007) neurons in the same species for the different classes of these biologically relevant (i.e., food-related) chemicals. This, in turn, evolved from numerous years of electrophysiological cross-adaptation (Caprio and Byrd Jr 1984; Michel and Derbidge 1997), biochemical binding (Brown and Hara 1981;

Cagan and Zeiger 1978; Lo et al. 1991; Rehnberg and Schreck 1986; Rhein and Cagan 1983), and calcium-imaging (Friedrich and Korsching 1997; Fuss and Korsching 2001) studies in teleosts that indicated the existence of relatively independent olfactory receptor sites for acidic, basic, and neutral amino acids.

Morphological types of ORNs responding to amino acids

Fish possess at least three morphological types of ORNs: ciliated, microvillous, and crypt neurons (Hansen et al. 2003).

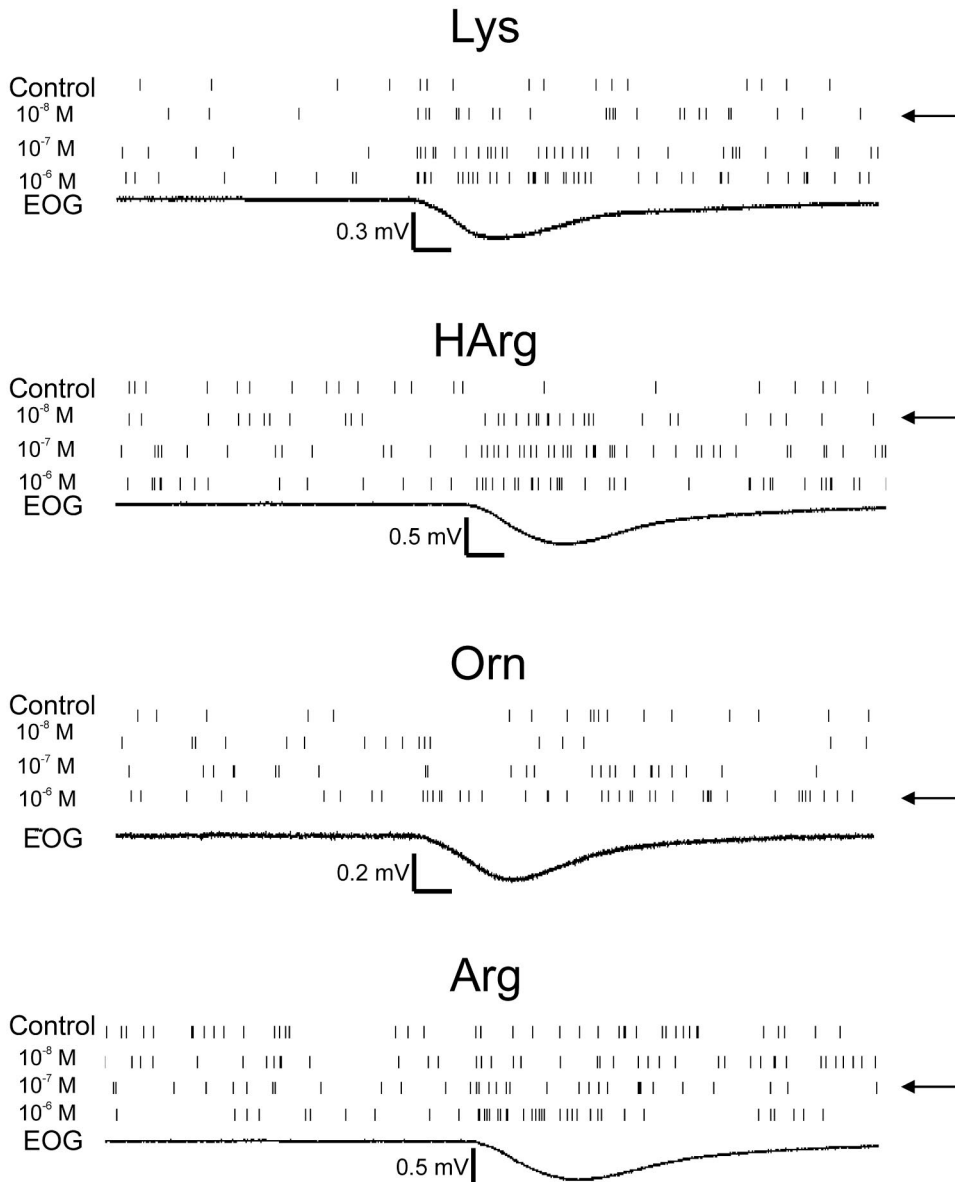


FIG. 6. Extracellular unit activity of responses of representative Group I ORN (cell #3 in Fig. 7) to basic amino acid odorants. Odorant concentrations are listed adjacent to each record; arrow indicates the lowest concentration that resulted in an excitatory response for that unit. Odorant onset and duration (0.8 s) are indicated by the line below each series of responses; vertical lines indicate calibration signals for the respective EOG traces.

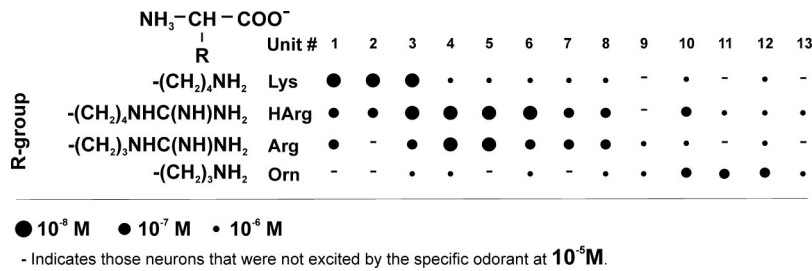


FIG. 7. Electrophysiologically derived excitatory thresholds of 13 Group I ORNs obtained from 4 fish to basic (B) amino acids. Dots indicate the threshold concentration for each stimulus that resulted in an excitatory response for each ORN analyzed. —, not excited by the specific odorant at 10^{-5} M. None of these units was excited by 10^{-8} to 10^{-5} M Met (LCN), Ala (SCN), or Glu (Acidic).

Identification of the morphological type of ORN that was excited by the amino acid odorants in the present in vivo recordings was not determined. In catfish and other fishes, both ciliated and microvillous ORNs were reported to respond to amino acids (Hansen et al. 2003; Lipschitz and Michel 2002; Sato and Suzuki 2001; Schmachtenberg and Bacigalupo 2004; Speca et al. 1999; Zippel et al. 1993). It is likely that some of both types of ORNs were recorded in the present study.

Excitatory and inhibitory ORN responses

The present report is consistent with previous findings that an individual ORN has two modes of response; i.e., a single ORN can respond to different odorants with either excitation or inhibition (Doolin and Ache 2005; Duchamp et al. 1974; Duchamp-Viret et al. 1999; Hallem and Carlson 2006; Hallem et al. 2004; Kang and Caprio 1995; Michel and Ache 1994; Michel et al. 1991; Morales et al. 1994; Sanhueza et al. 2000). Odorant-induced inhibition of neural activity observed at the level of the OB/AL within the CNS was suggested to be critical for increasing signal-to-noise activity as odorant information is passed to output neurons (Nikonov and Caprio 2004; Schild and Restrepo 1998); however, the present data clearly show that improvement in signal to noise is performed at the initial level of detection of the odorant by single ORNs. A single ORN that is excited by a specific type of amino acid was most often inhibited by other types of amino acids. For example, Fig. 1 shows the responses of ORN units described as Met, Ala, and Arg units. The Met unit is excited by Met (a neutral amino acid with a long side chain), but inhibited by Ala (a neutral amino acid with a short side chain) and Arg (an amino acid with a basic side chain). The Ala unit is excited by Ala, but is inhibited by Met; similarly, the Arg unit is excited by Arg, but is inhibited by Met and Ala. In only 42 (13%) of 330 tests with a type of amino acid that was different from that which excited the unit did an inhibitory response fail to occur; for these trials no significant change in the number of action potentials compared with prestimulus occurred. How both excitation and inhibition to odors is achieved at the level of a single ORN is unclear because it is assumed that only a single type of OR is expressed per individual ORN and there are no

inhibitory networks of neurons innervating ORNs. Recent studies in *Drosophila* (Hallem and Carlson 2006; Hallem et al. 2004), however, indicated that a single molecular OR can transduce both excitatory and inhibitory responses in a single ORN in which it is expressed.

For the present experiments, how an L- α -amino acid that binds to a particular molecular olfactory receptor could result in excitation or suppression of ORN activity remains an enigma; however, one speculative mechanism that could account for the present results is that the binding pocket for a specific type of amino acid requires a three-point binding. The positively charged α -amino group and the negatively charged α -carboxyl group of the amino acid bind within the receptor's proximal binding pocket, whereas the amino acid side chain binds to the receptor's distal binding pocket (Luu et al. 2004). This three-point attachment of the amino acid to its receptor results in an excitatory response by activation of a cation conductance and/or chloride conductance (Schild and Restrepo 1998). However, because all L- α -amino acids possess α -amino and α -carboxyl groups, a receptor site can likely interact with other L- α -amino acids, but only with two-point binding within the proximal binding pocket because structurally different side chains are precluded from binding to the distal site. Activation of only the proximal binding pocket is hypothesized to lead to inhibition by activation of a K^+ conductance (Lucero and Chen 1997; Michel et al. 1991; Morales et al. 1997) and/or the suppression of a steady-state Cl^- conductance (Doolin et al. 2001; Dubin and Dionne 1993; Dubin and Harris 1997) or to no activation, resulting in no significant change in spontaneous activity. Further, in this model, the selectivity of the distal binding pocket that determines the specificity of an L- α -amino acid must have a somewhat relaxed stringency compared with that of the proximal pocket. Thus different amino acids within a type (e.g., L-norvaline and L-norleucine) can activate the binding site for neutral amino acids possessing long, linear side chains with three-point binding as did L-Met (Fig. 3), although other L- α -amino acids with a different type of side chain are precluded from binding to this distal site.

The selectivity of Group I ORN units to amino acid members within a type

LCN (LONG SIDE CHAIN NEUTRAL). The majority of units investigated that were excited by neutral amino acids with long side chains could distinguish neutral amino acids with long, linear side chains (LCNs) from those with branched side chains (bCNs). These results are virtually identical to the specificities determined for LCNs of both Group I OB (Nikonov and Caprio 2004) and FB (Nikonov and Caprio 2007) neurons and are likely responsible for the ability of catfish to discriminate behaviorally between these two subtypes of neutral amino

TABLE 3. Classification of ORNs over response time (RT)

Unit Type	Number of Units Classified Based on		
	1.5-s RT at 10^{-5} M	First half second of response (% 1.5-s RT)	Last half second of response (% 1.5-s RT)
Met	18	15 (83)	13 (72)
Ala	11	6 (55)	8 (73)
Arg	17	12 (71)	14 (82)

acids (Valenticic et al. 2000). For Group I neurons at all three anatomical levels in the olfactory system of the channel catfish, bCNs and ILCNs were clearly discriminated at low concentrations, but with increasing stimulus strength, unit selectivity broadened to include representatives of both subtypes of LCN odorants. The broadening of the excitatory response spectrum with increasing odorant concentration is similar to that previously reported for olfactory neurons of other organisms (Friedrich and Korsching 1997; Joerges et al. 1997; Meister and Bonhoeffer 2001; Rubin and Katz 1999).

SCN (SHORT SIDE CHAIN NEUTRAL). Catfish ORNs that were excited exclusively by amino acids with short side chains (Ala, Ser) exhibited a selectivity similar to that of neurons identified within both the OB (Nikonov and Caprio 2004) and FB (Nikonov and Caprio 2007) in the same species. Further, the SCN class of catfish ORN showed a selectivity similar to that of those observed in the zebrafish OB (Fuss and Korsching 2001). Catfish ORNs having higher sensitivity to Ala than to Ser and vice versa were similar to catfish SCN FB neurons. Identified catfish OB SCN neurons that were more sensitive to Ser than to Ala were not observed, but likely do exist. The selectivity of eight ORNs identified in the present study that were excited by both Ala and Ser is similar to that obtained for OB neurons in both rainbow trout (MacLeod and Lowe 1976) and goldfish (Meredith 1981), where these two SCN amino acids shared a high degree of response similarity. The present results indicating that single ORNs are excited by both Ala and Ser suggest that these two amino acids may share some common molecular olfactory receptors as determined in previous biochemical binding (Brown and Hara 1981; Bruch and Rulli 1988; Cagan and Zeiger 1978; Rehnberg and Schreck 1986) and electrophysiological cross-adaptation (Caprio and Byrd Jr 1984) experiments. However, the current evidence that some ORNs were selective for only one of these two amino acids is similar to previous results obtained for particular FB units and also suggests the existence of independent receptors with high selectivity for specific SCNs.

BASIC. The ORN units showing high selectivity for Arg and other basic amino acids are similar to unit types found in both the OB (Nikonov and Caprio 2004) and FB (Nikonov and Caprio 2007) in the same species (i.e., channel catfish) and are consistent with the independence of olfactory receptor sites for basic from other types of amino acids in different species of fishes (Bruch and Rulli 1988; Cagan and Zeiger 1978; Caprio et al. 1989; Friedrich and Korsching 1997; Rehnberg and Schreck 1986). Direct evidence for the existence of a specific molecular olfactory receptor for Arg and other basic amino acids was previously described in goldfish (Specia et al. 1999). Additional evidence for the existence of independent receptor sites for basic from other types of amino acids is derived from behavioral studies with catfish (Valenticic et al. 2000).

Specialists versus generalists

Selection of the specific odorants to be tested is critical in determining whether a particular chemoreceptive neuron is classified as being “broadly” or “narrowly” tuned. Further, it may not be simply a matter of how many of the selected odorants activate a particular neuron that defines the odorant selectivity. Rather, it is likely that the selectivity of an odorant

across different types of compounds is more relevant to answering this question. For example, a neuron at either of the three levels of organization—receptor—bulb—forebrain—might be excited by multiple odorants as were those neurons in the channel catfish that were excited by a number of representative neutral amino acids. To designate such a neuron as “broadly tuned” would confuse the issue because these neurons were highly selective to amino acids that possessed long, hydrophobic side chains, but were not activated by all the rest of the amino acids—i.e., those possessing side chains that were acidic, basic, or even neutral with less of a hydrophobic basis (i.e., short side chains). One could make a similar argument for a neuron at any of these three levels that was selectively activated by basic amino acids, but not acidic or neutral amino acids. An additional consideration is whether the particular odorant is tested at a relatively high or low stimulus concentration because ORNs can respond to a number of odorants at high stimulus concentration, but become much more narrowly tuned at lower stimulus concentrations. The ORNs from channel catfish reported here are highly specific to type of biologically relevant odorants (i.e., amino acids) across multilog units of stimulus concentration. It is for these points that we classify these neurons as narrowly tuned.

Response time consideration

A basic question that is presently unknown is how much time is required for the olfactory systems of fishes to code for stimulus quality. Are fish as rapid as mammals and insects or do they require slowly evolving temporal codes to code for stimulus quality? Rats (Uchida and Mainen 2003) and mice (Abraham et al. 2004) required ≤ 200 ms to identify an odor. For the processing of odor information in the antennal lobe (AL) of locusts (Stopfer et al. 2003) 200–300 ms were required and in bees, 400 ms (Sachse and Galizia 2003, 2005). The onset of responses in Kenyon cells of the honeybee mushroom bodies occurred within < 200 ms of the initial responses of AL output neurons (Szyska et al. 2005).

In a recent study, responses of mitral cells in zebrafish to amino acids were reported to be nonstationary in that the response specificity of individual mitral cells changed over 2.2 s of the response, resulting in a declustering of the response types observed during the initial approximately 500 ms of the response (Friedrich and Laurent 2001; Laurent et al. 2001). The interpretation of these data is that responses of the population of mitral cells changed over time in a stimulus-specific manner, which provided a mechanism for the behavioral discrimination of the individual amino acids (Friedrich and Laurent 2001). This study, however, appeared to place fish, or at least zebrafish, in a category for the speed of olfactory coding much below that of insects and tetrapods. A reanalysis of zebrafish OB unit responses over the initial 400 ms of the response (Fig. 3 in Friedrich and Laurent 2004) was performed using the catfish scheme of categorizing the stimuli into their different types and not by the specific amino acid tested (Caprio, unpublished). The results suggested that 49 of the 58 units (84%) could be arranged into the same groups as reported for catfish; i.e., units that were preferentially excited by a neutral amino acid with a long side chain, a neutral with short side chain, an acidic, or a basic amino acid. In addition, a fifth group emerged that was detected in zebrafish, but likely missed

in channel catfish, i.e., units preferentially excited by aromatic amino acids. The responses of single bulbar neurons in both the zebrafish and catfish (Nikonov and Caprio 2004) and also catfish forebrain neurons (Nikonov and Caprio 2007) failed to show a significant declustering over time based on the EMRR of the recorded units for the coding of the specific types of amino acids.

GRANTS

This study was supported by National Science Foundation Grant IBN-0314970.

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