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Responses of Olfactory Forebrain Units to Amino Acids in the Channel Catfish

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Nikonov AA, Caprio J. Responses of olfactory forebrain units to amino acids in the channel catfish. *J Neurophysiol* 97: 2490–2498, 2007. First published January 24, 2007; doi:10.1152/jn.01198.2006. A paucity of information exists concerning the processing of odorant information by single neurons in any vertebrate above the level of the olfactory bulb (OB). In this report, odorant specificity to four types of L-α-amino acids (neutral with long side-chains, neutral with short side-chains, basic and acidic), known biologically relevant odorants for teleosts, was determined for 217 spontaneously active forebrain (FB) neurons in the channel catfish. Group I FB units were identified that were excited by only one of four types of amino acids; no Group I unit was encountered that was excited by an acidic amino acid. The Group I FB units exhibited similar preferences as described previously for OB neurons, suggesting that no major modifications of olfactory information for at least some of these units occurred between the OB and FB. Evidence, however, for the convergence of odor information between the OB and FB was suggested by Group II FB units that exhibited a broader excitatory molecular receptive range (EMRR) than those of previously recorded types of OB units or the Group I FB units. Group II FB units were excited by both neutral and basic amino acids and a few also by acidic amino acids, EMRRs not observed previously in OB units. Stimulus-induced inhibition, likely for contrast enhancement, was also often observed for the many of the FB units encountered. The observed EMRRs of the FB units presently identified and those of the OB units previously studied are consistent with the ability of catfish to behaviorally discriminate these compounds.

INTRODUCTION

Much has been learned about the initial steps in olfaction and the manner in which olfactory information is represented and processed in the vertebrate olfactory bulb, the first olfactory relay in the brain (Axel 2005; Buck 2005). Axons of ORNs expressing the same ORs converge onto specific target glomeruli in the OB (Mombaerts et al. 1996; Strotmann et al. 2000; Vassar et al. 1994) where they synapse onto apical dendrites of mitral/tufted relay neurons, forming a functional (i.e., chemotopic) map relating general chemical features of odorant structure to particular glomerular fields. The unique combination of glomeruli activated in response to an odor is thus thought to define (i.e., code for) the odor.

How odor information is processed at the next ascending level above that of the OB in the vertebrate olfactory system is currently a major topic of contemporary research. Recently in spite of the apparent extensive overlap of olfactory tract terminals (Bass 1981; Finger 1975), a chemotopic map was identified in the portion of the forebrain (FB) that receives input from the olfactory tract in the channel catfish (Nikonov et al. 2005), providing additional evidence for odor quality being encoded by spatial patterns (Axel 2005; Buck 2005). This FB map in the channel catfish is highly related to that found in the OB (Nikonov and Caprio 2001) but was shown to also have some distinctive features, likely due for some FB neurons to the convergence of separate streams of odor information (Nikonov et al. 2005). An understanding of the function(s) of each CNS nucleus in the processing odor information requires knowledge of the excitatory molecular receptive range (EMRR) (Mori and Yoshihara 1995) of single cells at each neuronal level.

The present report is an electrophysiological analysis of the EMRR of single FB neurons in channel catfish to amino acids, biologically relevant odorant stimuli that can be used as feeding cues by teleost and how the EMRR compares to that of single neurons within the OB of the same species (Nikonov and Caprio 2004). The present results indicate that unit types for particular amino acids that were previously observed in the OB also occur in the FB. Further, the FB Group I units whose individual EMRRs are narrowly tuned to type of amino acid mirror those of Group I OB units. In contrast, FB Group II units exhibited a broader EMRR than either Group I or Group II OB units and Group I FB units and provided evidence for the convergence within the FB of separate amino acid odor pathways that had been distinct at the OB level. These data confirm the recently reported finding of the integration of olfactory information related to food odors within the teleost FB (Nikonov et al. 2005).

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-l 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 >27°C during the spring and summer and <20°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

The preparation of the animals was the same as that described previously (Nikonov and Caprio 2005). Each catfish was initially immobilized with an intramuscular injection of the neuromuscular blocking agent, gallamine triethiodide (Flaxedil, 0.03 mg/100 g).

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During the experiments, additional injections were applied as needed via 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, charcoal-filtered city tap water that initially contained the anesthetic, 50 mg/l MS-222 (ethyl-*m*-aminobenzoate methane sulfonic acid). 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. The right FB was exposed by removing ~1 cm^2 of skin at the midline at the top of the skull immediately caudal to the position of the eyes. After the removal of the underlying bone and cartilage, the open space was filled with freshwater teleost Ringer solution.

Odorant stimuli and delivery

Amino acids, nucleotides, and bile salts were obtained commercially (Sigma Chemical). Purity of the amino acids was stated to be a minimum of 98% to >99% (TLC); purity for both the bile salts and nucleotides was ≥97%. The stimuli selected were to provide a basis for the direct comparison of the EMRR of FB units to that recently determined for OB units in the same species (Nikonov and Caprio 2004). Stock solutions of a quaternary mixture of amino acids (each at 10^{-3} M) and individual amino acids were prepared weekly in charcoal-filtered city tap water (CFTW); log step dilutions in CFTW were made daily. Interstimulus intervals were ≥2 min. Stock solutions (10^{-2} M) of a ternary mixture of nucleotides previously shown to be stimulatory to olfactory receptor neurons of channel catfish (Nikonov and Caprio 2001) [adensosine triphosphate (ATP), inosine triphosphate (ITP), and inosine monophosphate (IMP)] dissolved in CFTW were prepared individually; 1 ml of each stock solution was placed into cryovials and frozen at −20°C. Log step dilutions of nucleotides to 10^{-6} M in CFTW were made daily. Stock solutions (10^{-4} M) of a ternary mixture of bile salts [Na^+ salts of taurocholic (TCA), taurolithocholic (TLC), and lithocholic (LCA)] were prepared weekly. TLC and LCA were indicated to activate olfactory receptor neurons of catfish (Sorensen, unpublished); TCA was also included due to its known stimulatory action on olfactory receptor neurons in goldfish. TCA and TLC (both water soluble) were prepared weekly and log step dilutions to 10^{-6} M in CFTW were made daily; 10^{-3} M LCA was prepared in ethanol weekly, and log step dilutions in CFTW were made daily. The concentration of methanol to water was <1: 10,000, below the olfactory threshold for this compound (20). Control solutions included: CFTW obtained from the same water source as that used to prepare the test solutions and ethanol at the appropriate dilution for testing LCA. Interstimulus intervals were ≥2 min.

Stimulus delivery simultaneously to both olfactory organs was via a "gravity-feed" system employing a spring-loaded valve (model No. 5301, Rheodyn, Cotati, CA) driven by a pneumatic actuator (model No. 5300) at 40 psi. Stimulus solutions and the CFTW used to bathe the olfactory mucosae between stimuli were delivered through separate Teflon tubes (0.79 mm diam) at a rate of 4–5 ml/min. The olfactory cavities were continuously perfused with CFTW to facilitate stimulus delivery, protect the mucosa from desiccation, avoid the introduction of mechanical artifacts associated with stimulus presentation, and thoroughly rinse the olfactory organ between stimuli (3- to 5-min interstimulus intervals). A foot switch connected to an electronic timer (model No. 645, GraLab Instruments Division, Dimco-Gray, Centerville, OH) triggered the valve to introduce the odorants generally for 0.8-s stimulus duration without a change in either pressure or temperature and without dilution (Sveinsson and Hara 1990). The 0.8-s stimulus duration was chosen to correspond approximately with the time required for the phasic portion of the stimulus-driven EOG response to peak. For the experiments described in Table 2, stimulus duration was 1.5 s.

Recording techniques

ELECTROOLFACTOGRAM. The underwater electroolfactogram (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 (Caprio 1995; Ottoson 1971). The EOG was recorded in vivo with sintered Ag/AgCl electrodes via Ringer-agar-filled capillary pipettes. The EOG signal was amplified (Grass P-18 DC amplifier), digitized and stored on a video channel of a hi-fi VCR recorder. The EOG signal served as an indicator of both the viability of the preparation and the response onset to the tested odorants.

FB UNIT RECORDINGS. Action potentials (generally 75–300 μV peak-to-peak amplitude; Fig. 1) were recorded extracellularly from the lateral portions of the FB. The electrode, a low-impedance (2–5 MΩ) platinum and gold-plated, metal-filled, glass micropipette (glass tip, 1.5–2.0 μm; ball diameter, 2–3 μm), was mounted on a hydraulic microdrive attached to a stereotaxic micromanipulator and advanced vertically downward from the dorsal surface of the FB. Odor application began once a spontaneously active unit was encountered and was clearly isolated by fine-positioning the recording electrode via 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 30–10,000 Hz), observed with an oscilloscope, and stored on an audio channel of a hi-fi 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 (Fig. 1). The majority of the recordings involved software isolation of a single unit from few unit recordings based on software analysis of waveforms. Some of the waveform parameters that were utilized to identify and discriminate extracellularly recorded action potentials were peak amplitude, valley amplitude, spike height, spike width, spike time, and time between spikes. The units categorized in this report were, however, selected from those parameters that exhibited clearly different action potentials that were extracted (cluster cut) from mostly two unit recordings that often included a large and a small unit (Fig. 1*B*). Waveforms of low-amplitude action potentials were not selected for analysis due to possible errors in unit discrimination (i.e., interpreting somewhat similar waveforms emanating from different cells as the same unit). 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 FB neurons were classified as excitatory, suppressive or null (not significantly different from prestimulus) based on the one-tailed interrupted time-series analysis (ITSA) (Crosbie 1993; Nikonov and Caprio 2004). The ITSA compares statistically the number of action potentials occurring within successive 250-ms time bins for 1s prior to and subsequent to the initial onset of the odor-induced EOG. In a subset of experiments (Table 2), the ITSA was used to analyze the number of action potentials occurring during 1 s prior to stimulation and the first and third 0.5 s of a 1.5-s response.

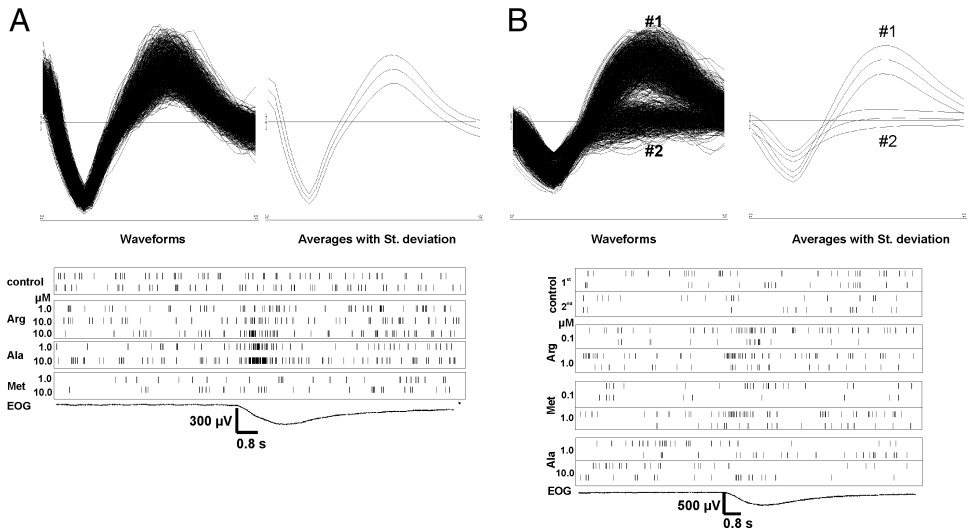

FIG. 1. Selection of extracellularly recorded forebrain (FB) units for analysis. *A*: single-unit preparation showing overlapping waveforms (n = 1,428) including the average waveform and its SDs from the mean (*top*). Unit is a Group II L-alanine (Ala) unit because it was excited at lowest concentration (1.0 μM) by Ala but also was excited by 10 μM Ala and 10 μM L-arginine (Arg) (applied twice). This unit was not excited by either of the 2 applications of the water control or 1 or 10 μM L-methionine (Met). A single electroolfactographic (EOG) response to 10 μM Ala is shown below the unit responses to indicate response onset. *B*: 2-unit preparation where the response specificity of *unit 1* is shown below. Right: means and SD of the waveforms (n = 1,756) for each of the units. *Unit 1* is a Group II Arg unit because it was excited at lowest concentration by Arg (0.1 μM) but was also excited by 1.0 μM Arg and 1.0 μM Met. This unit was not excited by either of the two applications of control water or 1 or 10 μM Ala. A single EOG response to 1 μM Met is shown below the unit responses. Stimulus application for units in *A* and *B* was for 0.8 s. Vertical calibration bars are for the respective EOG traces only.

RESULTS

All FB recordings were obtained from the ventrolateral telencephalon [areas Dpc and extending into the ventral part of DC-3 of Bass (1981) and the lateral terminal fields (both rostral and caudal parts) of Finger (1975)]. Only excitatory responses were analyzed because it is the excitatory response that drives the response of postsynaptic neurons at the next ascending level of the olfactory system.

All Group I FB units exhibited a narrow EMRR to type of amino acid, i.e., excited by a neutral amino acid with a long side-chain, a neutral amino acid with a short side-chain, or a basic amino acid. Group II units were excited by more than a single type of amino acid. The present investigation expands greatly our understanding of the EMRR of specific FB units in the channel catfish beyond that recently reported for the existence of the FB odotopic map (Nikonov et al. 2005). Due to the initial search paradigm in this investigation, all functionally isolated FB neurons were initially tested with a quaternary mixture of L-α-amino acids, a ternary mixture of nucleotides, and a ternary mixture of bile salts, whereby all components were at 10^{-6} M each in the respective mixtures. All units reported in this manuscript were excited by the mixture of amino acids but were either nonresponsive or suppressed by each of the other two stimulus mixtures, confirming their selectivity for amino acids. The number of Group I (n = 101) and Group II (n = 58) FB neurons reported herein, however, are not reflective of their relative distribution in the FB because a major emphasis of this report was to record from FB units showing a narrow EMRR to better understand the neural specificity of single FB units that allowed for the previously determined behavioral discrimination by catfish of these odorants (Valentincic et al.1994, 2000). We estimate for a random search of units located within the amino acid zone within the FB of the channel catfish that a ratio of ~6:1 Group II:Group I units would be observed (i.e., 3 Group I and 17 Group II FB units were recorded from an analysis of 10 electrode tracts in 5 fish). Further, all three Group I units were recorded within the same electrode tract where Group II units were also obtained; however, the Group II units were located closer together within the tract than the single Group I unit encountered.

EMRR of single FB Units to amino acids

GROUP I UNITS. *Results of primary search strategy.* One hundred and one FB units were identified in 43 catfish that were excited by the amino acid mixture but not by the mixtures of either bile salts or nucleotides; further, each of these units was excited by only one of the four amino acid components possessing different types of side chains representing the four different types of amino acids present within the mixture. Approximately a third each of these neurons was selectively excited by either Met, a representative neutral amino acid with a LCN, Ala, a representative neutral amino acid with a SCN, or Arg, a basic (B) amino acid (Group I Units; Fig. 2; Table 1). There was no evidence within the FB of a clustering of any of these types of amino-acid responsive units. No Group I Glu units (i.e., those solely excited by L-glutamic acid) were observed. The majority of the Met and Arg units, but only a third of the Ala units were activated by 10^{-8} M amino acid. The remaining units were excited by higher stimulus concentrations ($\leq 10^{-5}$ M). None of these highly selective units were activated by other representative types of amino acids at $\leq 10^{-5}$ M odorant concentrations.

Results of expanded search strategy. We explored further the EMRR of Group I units to amino acids and derivatives. Fifty-eight additional spontaneously active Group I units obtained from 23 catfish were located that were selectively excited by either 10^{-5} M Met, Arg, or Ala/Ser. No FB unit selectivity excited by glutamate was identified. The units were tested with 10^{-8} M to 10^{-5} M concentrations of the specific

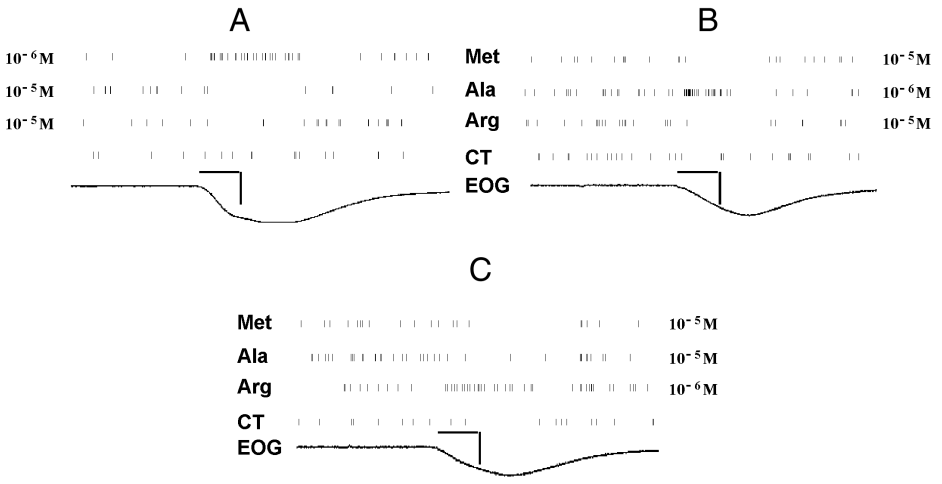

FIG. 2. Extracellular unit activity of responses of representative Group I FB units to odorants representing 3 major types [long-chain neutral (LCN): L-Met; short-chain neutral (SCN): L-Ala; and basic (*B*): L-Arg] of amino acids and to control water (CT). A representative EOG trace is shown below the unit traces in *A–C* that was recorded simultaneously with the excitatory response. *Unit A* is excited by Met and inhibited by Ala and Arg; *unit B* is excited by Ala but inhibited by Met and 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 was 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 CT trace; vertical line indicates a calibration signal for the EOG traces (750 μV in *A,* 500 μV in *B,* and 300 μV in *C*).

amino acid that activated the unit. FB units of each type were then tested with related compounds (Figs. 3–7). The classification of the type of amino acid unit identified was based on the lowest effective odorant concentration and numbers of such cases.

EMRR of Group I LCN units. Although there was some overlap in excitatory responses of single FB units to the tested neutral amino acids, two general groups emerged comprising units most responsive to amino acids with either long, linear side chains (lLCN) or with branched side-chains (bCN; Figs. 3 and 4). These 31units were separated into three general categories: 10 units the excitatory thresholds of which were lowest to lLCNs possessing side-chains consisting of three to four methylene groups (i.e., nVal, nLeu and/or Met; Fig. 4*A*), 17 units the excitatory thresholds of which were lowest to bCNs (Val and /or Leu; Fig. 4*B*), and 4 units the EMRRs of which included both lLCN and bCN groups (Fig. 4*C*). Interestingly, nVal, the lLCN possessing the shortest linear side-chain consisting of three methylene groups, activated the majority of OB (Nikonov and Caprio 2004) and FB units as both an lLCN and a bCN. However, with increasing amino acid concentration, the EMRR broadened such that these single FB neurons responded generally to both lLCNS and bCNs, but not to amino acids with acidic (Glu) or basic (Arg) side chains nor to SCNs (e.g., Ala). FB units were encountered that were most excited by different amino acids within each category. For example, of the bLCNs, *unit 15* had the lowest threshold to Val, whereas of the lCNs, *unit 5* had the lowest threshold to Met. (Fig. 4).

EMRR of SCN units. Fifteen additional units were identified that were excited by the L-isomers of Ala and/or Ser (Figs. 5 and 6). Four cells were excited by 10^{-8} M or 10^{-7} M Ala and not by Ser $\leq 10^{-5}$ M (Figs. 5*A* and 6*A,* 1–4). Eight cells were excited by 10^{-8} or 10^{-7} M Ser, but not by Ala (Figs. 5*B* and 6*B,* 9–16). Three cells were excited by Ala and Ser (Figs. 5*C* and 6*A,* 5–7).

TABLE 1. *Responses of Group I FB units*

	Stimulus and No. of Units Excited		
Response Type	Met	Ala	Arg
Excitatory			
10^{-8}M	Met 29 (78)	Ala 11 (34)	Arg 26 (81)
10^{-7}M	Met 33 (89)	Ala 29 (91)	Arg 28 (88)
10^{-6}M	Met 37 (100)	Ala 32 (100)	Arg 32 (100)
No Excitatory	Ala	Met	Met
Response	Arg	Arg	Ala
10^{-6}M - 10^{-5}M	Glu	Glu	Glu

Parentheses enclose percentages.

EMRR 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 11 additional Group I FB units that were selectively excited by 10^{-5} M Arg, but not by Met, Ala, or Glu (Fig. 7). Seven (64%) were excited by 10^{-8} M basic amino acid (Fig. 7, 1–7). Of the 11 units that were excited by either of the two common basic amino acids, 3 units had lower electrophysiological thresholds to Lys (Fig. 7*A*) and 6 to Arg (Fig. 7*B*). With increasing the stimulus concentration to 10^{-6} M, 7 of the 11 units were excited by both Lys and Arg. Three units were more responsive to Orn than to either Lys or Arg (Fig. 7, 9–11).

Analysis of response time with respect to unit classification. We addressed the question of whether a declustering of response types occurred during the response of FB units in catfish as reported for mitral cells in zebrafish (Friedrich and Laurent 2001; Friedrich 2006). Of 101 total Group I FB units that were classified originally as Met, Ala, or Arg units based on the number of action potentials elicited during the entire 1.5-s response period, 54% of the Met units, 47% of the Ala units, and 50% of the Arg units were so classified based solely on the initial 0.5 s of the response time (*time 0* was EOG onset; Table 2). However, during the last 0.5 s of the 1.5-s response time, 92% of the Met units, 84% of the Ala units, and 81% of the Arg units were so classified. The improvement in unit classification with additional time post the initial 0.5 s is likely due to the necessary time for a FB unit to evoke a sufficient number of action potentials to allow for a comparison of responses across different stimuli. A portion of the initial 0.5-s post EOG onset, where unit classification was the poorest, is involved with activating appropriate receptor and bulbar neurons and initiating responses in specific FB neurons.

GROUP II UNITS. Fifty-eight FB units obtained from 14 fish were excited by only one of the four types of amino acids tested at the lowest stimulus concentration (10^{-7} M) tested but importantly were excited by more amino acids than Group I units at stimulus concentrations $>10^{-7}$ M (Table 3). Approx-

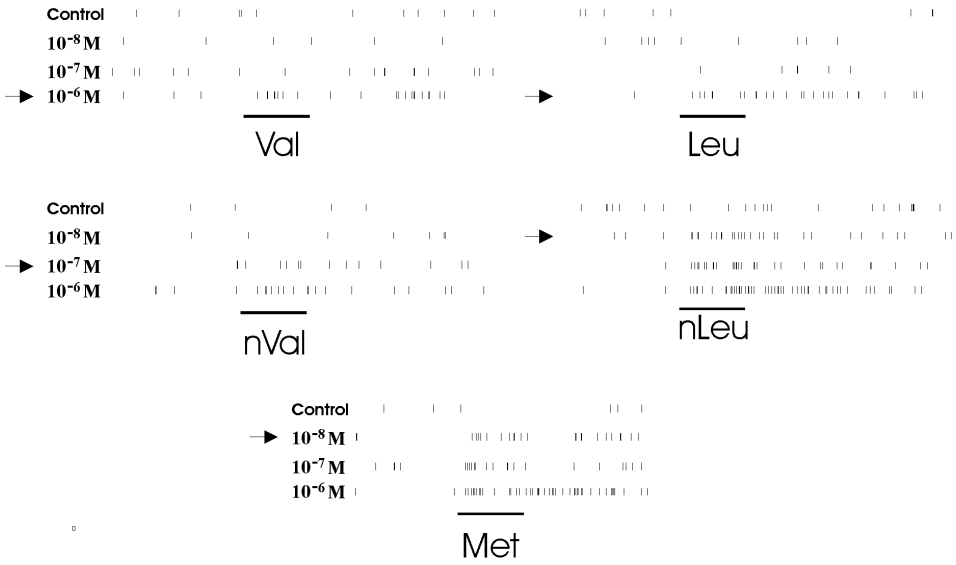

FIG. 3. Extracellular unit activity of responses of a representative Group I FB unit (*cell 3*) to neutral amino acids with branched (bCNs:Val, Leu) and linear (lLCNs: nVal, nLeu, Met) side chains. Odorant concentrations are listed adjacent to each record; →, lowest concentration that resulted in an excitatory response for that unit. Odorant onset and duration (0.8 s) are indicated by the line below each series of responses.

imately a third each of the Group II units was activated by 10^{-7} M Met, Ala, and Arg, respectively, and none was excited by either 10^{-7} M or 10^{-6} M Glu. The EMRR of the units broadened with increased stimulus concentration, such that the majority of the Group II Met units were also excited by Ala and Arg (Fig. 8), but not by Glu. The majority of the Group II Ala units were also excited by Arg (Fig. 1*A*) at a 10-fold higher concentration, and a few ($n = 7$) of these units were also excited by Glu; none of the Group II Ala units were excited by Met. The majority (13 of 15) of the Group II Arg units were activated by 10^{-6} M Met (Fig. 1*B*), and 6 units were excited by Ala; only at 10^{-5} M were three of the Group II Arg units also activated by Glu.

DISCUSSION

Amino acid-responsive olfactory areas of the teleost FB: homology with mammals

In contrast to all other vertebrates, the forebrain of ray-finned fishes develops as paired eversions that expand laterally and ventrally without an underlying ventricle (Liem et al. 2001). Recent molecular evidence, however, indicates that the location of the lateral pallium, which receives the densest olfactory input from the OB, is not everted, but is located laterally, as it is in the majority of vertebrates (Wullimann and Mueller 2004; Wullimann and Rink 2002). The amino acid-responsive region of the pallium in catfish is located in the ventrolateral telencephalon and in areas Dpc and extending into the ventral part of DC-3 of Bass (1981) and the rostral and caudal portions of the lateral terminal fields of Finger (1975) and Nikonov and Caprio (2005). These regions receive input from the OB via the lateral olfactory tract and are likely homologous to the olfactory cortex and possibly olfactory tubercle of mammals.

Excitation and inhibition of FB olfactory units

The present investigation centered solely on the excitatory responses of single catfish FB units; however, this is not to imply that inhibition (i.e., significant decline in the number of action potentials from ongoing spontaneous activity) is not important within the FB. Although olfactory cortical units in frog (Duchamp-Viret et al. 1996), rat (Giachetti and Macleod 1975), cat (Nemitz and Goldberg 1983), and monkey (Tanabe et al. 1975) were mostly excitatory, inhibitory odorant responses of units within the catfish FB were common. The majority of inhibitory responses observed in Group I neurons appeared to be for contrast enhancement among odor responses

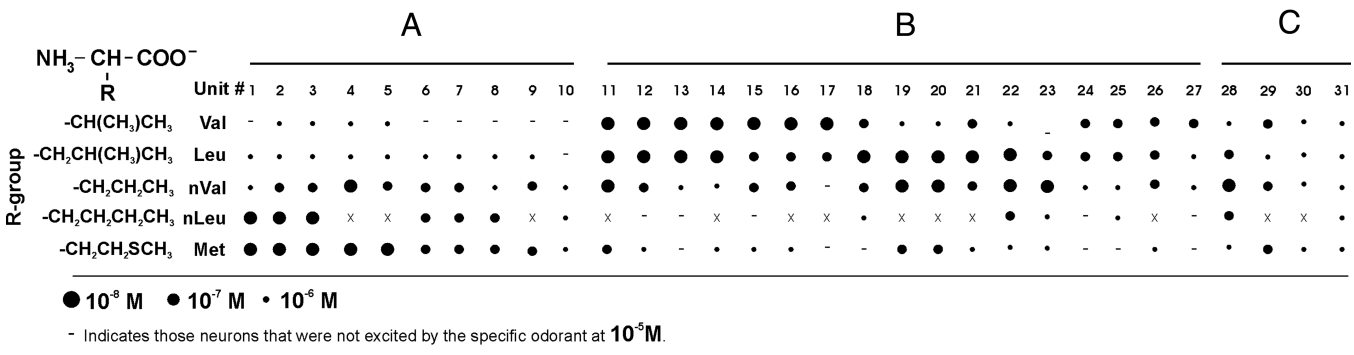

FIG. 4. Electrophysiologically derived excitatory thresholds of 31 Group I FB neurons obtained from 13 fish to bCNs and lLCNs. Dots within the shaded boxes indicate the threshold concentration for each stimulus that resulted in an excitatory response for each FB unit analyzed. *A*: 10 FB units (*1–10*) with lowest thresholds to lLCNs. *B*: 17 OB units (*11–27*) with lowest thresholds to bCNs. *C*: 4 units (*28–31*) that failed to show such a distinction. ×, not tested; minus, not excited by the specific odorant at 10^{-5} M. None of these units were excited by 10^{-8} M to 10^{-5} M Ala (SCN), Arg (basic), or Glu (acidic).

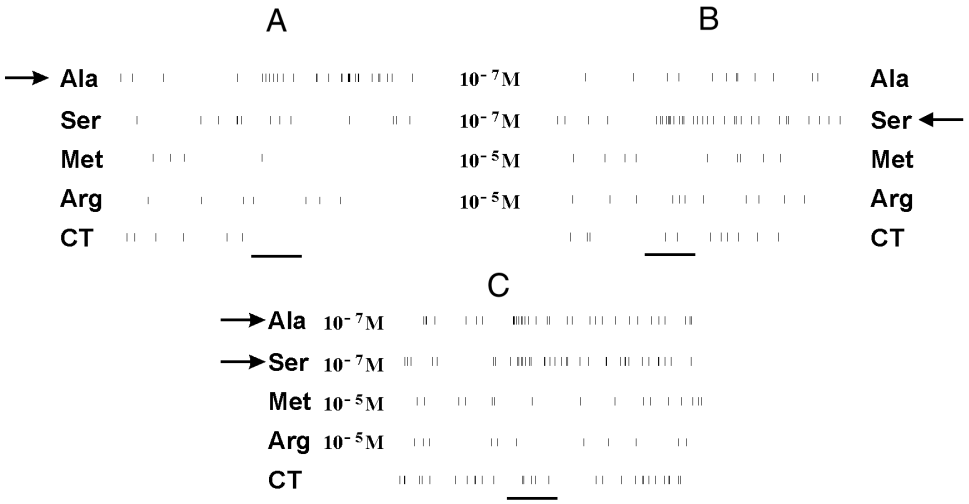

FIG. 5. Extracellular unit activity of responses of representative Group I FB units to SCNs. *A*: unit excited by L-Ala. *B*: units excited by L-Ser. *C*: unit excited by both L-Ala and L-Ser. Odorant onset and duration (0.8 s) are indicated by the line below each series of responses. CT, control response.

to specific classes of amino acids. For example, Fig. 2 shows the responses of FB 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 and Arg; similarly, the Arg unit is excited by Arg but is inhibited by Met and Ala. Suppression also occurred in responses to Group II neurons (not shown), but their functioning as contrast-enhancement was not as clear as that for Group I neurons.

EMRR of Group I FB units to amino acids

All 101 of these FB units were excited from threshold ($\sim 10^{-8}$ M to 10^{-7} M) to 10^{-5} M by only one of the four amino acid odorants representing four different categories of amino acid side-chains. The EMRR of catfish FB Group I units was indistinguishable from that previously determined for Group I OB units (Nikonov and Caprio 2004). These particular odorants were selected for the present FB and previous OB unit studies because electrophysiological cross-adaptation (Caprio and Byrd 1984), biochemical binding (Bruch and Rulli 1988), and mixture (Caprio et al. 1989; Kang and Caprio 1991) investigations in channel catfish and in other teleosts (Brown and Hara 1981; Friedrich and Korsching 1997; Fuss and Korsching 2001; Michel and Derbidge 1997; Rhein and Cagan 1983) indicated independent molecular olfactory receptors for these types of amino acids. One major difference between the results of specific responses for Group I units of the OB and FB is that FB units excited by an acidic amino acid (Glu) were not encountered; however, this may simply be related to the fewer number of units excited by Glu that exist in comparison to the units excited by other amino acids as determined from the previous analysis of OB units where only 14% (13 of 91) Group I OB units were specific to Glu (Nikonov and Caprio 2004). The present data clearly show that Group I FB units in catfish are not coincidence detectors as they do not require correlated input of mitral cells having differing specificities as proposed for a portion of the olfactory driven cortical neurons in mice (Zou and Buck 2006).

LCN UNITS. FB units excited by neutral amino acids with LCNs, analogous to Group I OB LCN units (Nikonov and Caprio 2004), were identified by being excited at lower stimulus concentrations by neutral amino acids with linear side chains (lLCNs) than those excited by neutral amino acids with branched side-chains (bCNs; Fig. 4, *A* vs. *B*). Prior studies also in the channel catfish provided evidence for the existence of olfactory receptor sites for LCNs being different from those for SCNs (Bruch and Rulli 1988; Caprio and Byrd 1984); however, electrophysiological evidence for independent olfactory receptor sites for bCNs was lacking. The broadening of the EMRR of both OB and FB units to neutral amino acids with increasing stimulus concentrations reflects the related finding of the recruitment of additional responsive OB glomeruli with increasing odor intensities (Friedrich and Korsching 1997; Joerges et al. 1997; Meister and Bonhoeffer 2001; Rubin and Katz 1999). The discrimination of bCNs and lLCNs were also indicated in a study of olfactory discrimination of amino acids in bullhead catfish (Valentincic et al. 2000), suggesting likely differences in the processing of odor information for these different types of neutral amino acids.

Although differences were clearly evident in electrophysiological thresholds between lLCNs and bCNs in different groups of neurons in both the previous OB (Nikonov and Caprio 2004) and present FB studies, the majority of both OB and FB neurons were responsive to both types of amino acids,

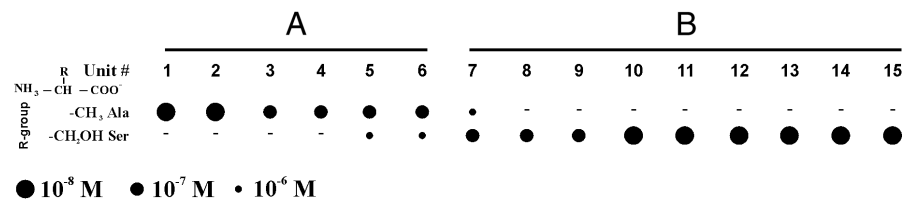

FIG. 6. Electrophysiologically derived excitatory thresholds of 15 Group I FB neurons obtained from 6 fish to SCNs (Ala, Ser). Dots within the shaded boxes indicate the threshold concentration for each stimulus that resulted in an excitatory response for each FB unit analyzed. *A*: 6 FB units (*1–6*) with lowest thresholds to Ala. *B*: 9 FB units (*7–15*) with lowest thresholds to Ser. minus, not excited by the specific odorant at 10^{-5} M. None of these units were excited by 10^{-8} M to 10^{-5} M Met (lLCN), Arg (basic), or Glu (acidic).

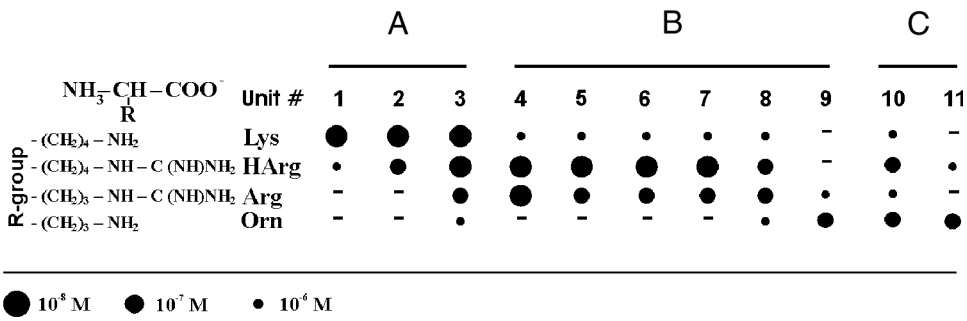

FIG. 7. Electrophysiologically derived excitatory thresholds of 11 Group I FB neurons obtained from 4 fish to basic (*B*) amino acids. Dots within the shaded boxes indicate the threshold concentration for each stimulus that resulted in an excitatory response for each FB unit analyzed. Of the 2 common basic amino acids (Arg, Lys), *A* indicates the 3 units (*1–3*) with lowest thresholds to Lys, whereas *B* indicates the 6 units (4–9) with lowest thresholds to Arg. *C*: 2 units (*10* and *11*) along with units *B5-7* that were most sensitive to other basic amino acids. minus, not excited by the specific odorant at 10^{-5} M. None of these units were excited by 10^{-8} M to 10^{-5} M Met (lLCN), Ala (SCN), or Glu (acidic).

albeit at different stimulus concentrations. This finding is similar to that observed for mitral cell responses in the rabbit to a homologous series of normal and iso-fatty acids (Imamura et al. 1992). In both catfish and rabbit, OB and FB neurons were activated by compounds comprising a 4–5 carbon skeleton that were either linear or branched. For both OB and FB neurons in catfish and OB units in rabbit that responded with excitation to branched amino acid and branched fatty acid molecules, respectively, these units were often also activated by the linear form of the molecule. Only 4 (13%) of the 31 Group I FB units recorded failed to show a clear distinction between their responses to lLCNs and bCNs. The EMRR of these units did not include representatives of the other classes of tested amino acids.

SCN UNITS. FB units were identified that were similar to units previously observed within the catfish OB in that they were excited by Ala, a SCN, and not by representatives of the other classes of tested amino acids. These results are similar to those obtained in the zebrafish using Ca^{2+}-imaging techniques in that some OB glomeruli were selectively activated by neutral amino acids with short side-chains (Fuss and Korsching 2001). An unexpected finding in the present report was the identification of FB units that were selectively excited by L-Ser. This olfactory unit type had not previously been identified in catfish at any neural level, although the majority of OB SCN units were excited by both Ala and Ser, and in five OB SCN units thresholds to both Ala and Ser were similar (in the 10^{-7} M to 10^{-6} M range).

B UNITS. FB units that were selectively activated by basic amino acid units were similar to a class of OB units previously identified (Nikonov and Caprio 2004). These electrophysiological results correlate well with results of behavioral studies in Arg- and Lys-conditioned catfish where neither SCN nor LCN amino acids were as effective in releasing swimming activity as were the basic amino acids (Valentincic et al. 1994, 2000).

TABLE 2. *Classification of FB neurons over response time (RT)*

Group I Unit Type	No. Units Classified: Based on Entire 1.5-s RT	Based on Initial 0.5-s RT (% 1.5-s RT)	Based on Second 0.5-s RT (% 1.5-s RT)	Based on Final 0.5-s RT (% 1.5-s RT)
Met	37	20 (54)	30 (81)	34 (92)
Ala	32	15 (47)	27 (84)	27 (84)
Arg	32	16 (50)	26 (81)	26 (81)

EMRR of Group II FB units to amino acids

Although Group II FB units were selectively excited at 10^{-7} M by a single amino acid of the four types tested, their EMRRs were broader than those of Group I FB units, which is similar to previous findings of Group II OB units having a broader EMRR than Group I OB units in the same species (Nikonov and Caprio 2004). The EMRR of FB Group II units compared with Group II OB units is similar for the Ala units but is quite different for the Met and Arg units. Group II Met units within the OB were not excited by either 10^{-6} M to 10^{-4} M Arg or Glu, but the majority of the FB Met units were excited by 10^{-6} M and 10^{-5} M Arg. Further, Group II Arg units in the OB were not excited by either Met or Ala, but these stimuli did activate the majority of Group II FB Arg units. The EMRR of the FB Met and Arg Group II units is suggestive of a convergence of specific amino acid (i.e., food-related) odor pathways that were separate and distinct at the level of both the olfactory receptor (Bruch and Rulli 1988; Caprio and Byrd 1984) and bulbar neurons.

We previously reported that not only odorants of a similar class (i.e., amino acids) converge onto some single FB neurons in catfish, but even odorants of different chemical classes related to feeding (i.e., amino acids and nucleotides) converge in the FB onto "complex" neurons (Nikonov et al. 2005). This

TABLE 3. *Excitatory responses of Group II forebrain units to different amino acids*

Stimulus Concentration	Stimulus and No. of Units Excited: Met*,†	Ala*,‡	Arg*
n	18	21	19
10^{-7}M	Met 15 (83)	Ala 13 (62)	Arg 15 (79)
10^{-6}M	Met 18 (100)	Ala 21 (100)	Arg 19 (100)
	Arg 13 (72)	Arg 14 (67)	Met 13 (68)
	Ala 14 (78)		Ala 6 (32)
10^{-5}M	Arg 15 (83)	Arg 16 (76)	Met 14 (74)
	Ala 16 (89)	Glu 7 (33)	Ala 11 (58)
			Glu 3 (16)

Parentheses enclose percentages. *Unit type named after the amino acid that elicited an excitatory response at the lowest concentration. †Not excited by Glu. ‡Not excited by Met.

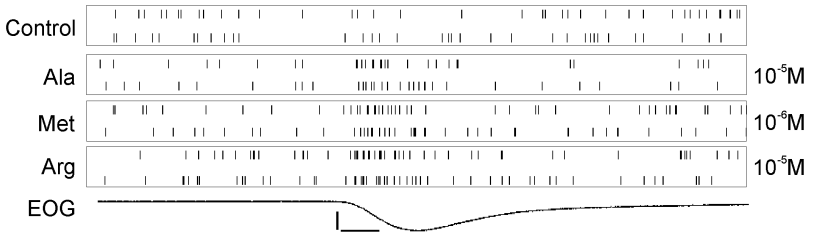

FIG. 8. Extracellular unit activity of excitatory responses of a representative Group II FB unit to a neutral amino acid with a short side-chain [L-alanine (Ala)], a neutral amino acid with a long side-chain [L-methionine (Met) and to a basic amino acid [L-arginine (Arg)]. Responses to two applications each are shown. Odorant onset and duration (0.8 s) are indicated by the line below the EOG trace; EOG calibration, 300 μV.

previous report also found no evidence for a convergence of odorants having distinctly different behavioral functions, i.e., there was no evidence for the convergence of the separate neural lines transmitting feeding (i.e., amino acid) and socially relevant (i.e., bile salt) odors.

Possible functional significance of Group I and Group II units

The EMRRs of Group I FB neurons are virtually identical to those of Group I OB units that relay amino acid odor information to the FB (Nikonov and Caprio 2004). The EMRRs of Group I OB and FB units are also similar to those of the amino acid-sensitive olfactory receptor neurons (ORNs) (unpublished data) and thus also to the ligand specificities of the molecular olfactory receptors that detect these compounds. The existence of Group I units at these three neural levels of organization (ORN, OB, and FB) within the olfactory system indicate an importance to the organism of reliably identifying and discriminating behaviorally among the different classes of amino acid odorants (Valentincic et al. 2000). It was previously demonstrated that olfaction and not taste is required for the learned discrimination of amino acids in catfish (Valentincic et al. 1994). The means to discriminate among the different classes of amino acids could provide the animal with the ability to develop a "chemical search image" of its prey (Atema et al. 1980) because aquatic organisms release amino acids in varying proportions and amounts into the surrounding water column (Johannes and Webb 1970; Olsen 1986). It is possible that different fishes and aquatic invertebrates that are potential food items release characteristically different proportions of amino acids that could be used by catfish to identify the type of prey organism. It is also feasible that the ability to discriminate amino acids is a basis for the recognition of conspecifics and possibly other teleost species due in part to the specific amino acid content exuded into the mucus that coats the external body of fish. For all the previous possibilities, the necessity to identify different classes of amino acids due to differences in side-chain structure requires the selective EMRRs exhibited by Group I FB units.

Group II FB units have a broader EMRR than either Group I OB and FB units and even Group II OB units (Nikonov and Caprio 2004; present report). Due to the convergence of previously separate streams of amino acid odor information in the FB, GP II FB units along with the more complex (even broader specificity extending to other types of biologically relevant compounds) FB units previously reported (Nikonov et al. 2005) do not have the role of identifying specific types of amino acids. These units, however, conceivably signal functional information which, depending on the environmental context, could be the presence of food or other organisms—but are not involved in the specific identification of the stimulus molecule other than it being an amino acid.

GRANTS

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