Electrophysiological Evidence for a Chemotopy of Biologically Relevant Odors in the Olfactory Bulb of the Channel Catfish

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Nikonov, Alexander A. and John Caprio. Electrophysiological evidence for a chemotopy of biologically relevant odors in the olfactory bulb of the channel catfish. *J Neurophysiol* 86: 1869–1876, 2001. Extracellular electrophysiological recordings from single olfactory bulb (OB) neurons in the channel catfish, *Ictalurus punctatus*, indicated that the OB is divided into different functional zones, each processing a specific class of biologically relevant odor. Different OB regions responded preferentially at slightly above threshold to either a mixture of *1*) bile salts (10^{-7} to 10^{-5} M Na^+ salts of taurocholic, lithocholic, and taurolithocholic acids), *2*) nucleotides [10^{-6} to 10^{-4} M adenosine-5′-triphosphate (ATP), inosine-5′-monophosphate (IMP), and inosine-5′-triphosphate (ITP)], or *3*) amino acids (10^{-6} to 10^{-4}M L-alanine, L-methionine, L-arginine, and L-glutamate). Excitatory responses to bile salts were observed primarily in a thin, medial strip in both the dorsal (100–450 μm) and ventral (900–1,200 μm) OB. Excitatory responses to nucleotides were obtained primarily from dorsal, caudolateral OB, whereas excitatory responses to amino acids occurred more rostrally in the dorsolateral OB, but continued more medially in the ventral OB. The chemotopy within the channel catfish OB is more comparable to that previously described by optical imaging studies in zebrafish than by field potential studies in salmonids. The present results are consistent with recent studies, suggesting that the specific spatial organization of output neurons in the OB is necessary for the quality coding/decoding of olfactory information.

INTRODUCTION

Axons of olfactory receptor neurons (ORNs) of vertebrates comprise the first cranial nerve and project to the olfactory bulb (OB), the first processing center of odorant information within the CNS. ORN axons terminate in OB glomeruli where they synapse with apical dendritic projections of the mitral/tufted neurons, the output neurons of the OB, and with associated intrinsic bulbar neurons. The ORN projection in rodents is to discrete glomerular regions of the OB, based primarily on the specific molecular olfactory receptors expressed within each of the respective ORNs (Mombaerts et al. 1996; Ressler et al. 1994; Vassar et al. 1994). The projection map to the mammalian OB is a functional map relating general chemical features of the odorant structure to specific glomerular fields within the OB (Bozza and Kauer 1998; Cinelli et al. 1995; Guthrie and Gall 1995; Johnson and Leon 2000; Johnson et al. 1998; Mori and Yoshihara 1995; Rubin and Katz 1999; Stewart et al. 1979; Uchida et al. 2000; Xu et al. 2000). A similar organization of ORN projection to the insect analogue of the OB, the antennal lobe, has recently been described in the honeybee (Galizia et al. 1998, 1999; Joerges et al. 1997).

Since the organizational parameter for the ORN projection to the OB is functional rather than anatomical (i.e., a somatotopical map), questions arise as to the precise organization of the OB. For example, are there different portions of the OB that process specific classes of biologically relevant odorants? It is known that different ORNs that express different molecular receptors which detect similar types of odorants terminate in closer OB regions than those that express receptors to detect chemically different types of odors (Bozza and Kauer 1998; Buonviso and Chaput 1990; Friedrich and Korsching 1997, 1998; Imamura et al. 1992; Katoh et al. 1993; Mori et al. 1992). Are there sharp boundaries between these functional regions? Is the OB chemotopic map bilaterally and dorsoventrally symmetric? Although the answers may vary depending on the species selected and the specific odorants and their concentrations tested, most are probably based on common principles across animal phyla (Hildebrand and Shepherd 1997). A key, however, to understanding any sensory system is deciphering what the biologically relevant stimuli are. Unfortunately, the olfactory capabilities of most mammals appear to be so broad that effective odorants do not fall neatly into a few chemical classes. In contrast, the olfactory system in teleosts responds to fewer odorants, and their behavioral significance often is known. Three classes of biologically relevant odorants known for teleost fish are amino acids, nucleotides, and bile acids (Carr 1988; Michel et al. 1988; Sorensen and Caprio 1998). Fish use olfaction to behaviorally discriminate among odorants (Valentincic et al. 1994) and use amino acids and nucleotides as feeding cues. Bile acids, produced by the biliary system to function as digestive detergents (Haslewood 1967), are released into the water in both urine and feces (Polkinghorne 1997) where they serve as potent olfactory stimuli and play a role in identification of conspecifics, apparently functioning as nonsexual attractants (Li et al. 1995).

Previous electrophysiological (Døving et al. 1980; Hara and Zhang 1996, 1998; Thommesen 1978) and optical imaging (Friedrich and Korsching 1997, 1998) investigations in salmonids and zebrafish, respectively, were consistent in indicating a coarse chemotopic organization in the OB for biologically relevant odorants. Electroencephalographic (EEG) recordings, which are the summed field potentials from an undefined volume of OB, were the sole source of the electrophysiological evidence for OB chemotopy in salmonids. It is, however,

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unknown as to what percentage of the underlying individual bulbar neurons reflected the identical odorant specificity of the gross EEG signal (i.e., within a particular bulbar region were there neurons with selectivities different from that observed in the integrated EEG signal?). Also, the calcium- and voltage-imaging studies in the zebrafish were able to clearly visualize only portions of the ventral OB with respect to identifying functional regions. The present electrophysiological evidence for chemotopy in the OB of the channel catfish is derived primarily from single-unit analysis of the responses of neurons located throughout the OB of the channel catfish.

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 above 27°C during the spring and summer and below 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 1993). 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 previously described (Kang and Caprio 1991). Each catfish was initially immobilized with an intramuscular injection of the neuromuscular blocking agent, gallamine triethiodide (Flaxedil; 0.03 mg/100 g). 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, CFTW 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 pedunculated OB was also exposed by removing an approximate 1-cm section of skin and subcutaneous fat at the midline of the fish caudal to the nasal capsule. Following the removal of the underlying bone and cartilage, suction was applied to remove adipose tissue from the cranial cavity, and the open space was filled with freshwater teleost Ringer solution.

Odorant stimuli and delivery

The chemical stimuli (amino acids, bile salts, and nucleotides) were obtained commercially (Sigma Chemical) and were the purest available. Stock solutions (10^{-3} M) of a quaternary mixture of representatives of four different classes of amino acids {L-Na^+glutamate (acidic), L-arginine (basic), L-methionine [neutral with a long side-chain (LCN)], and L-alanine [neutral with a short side-chain (SCN)]} that were previously shown (Kang and Caprio 1997) to be potent stimuli to ORNs of channel catfish were prepared weekly in CFTW; log step dilutions in CFTW to 10^{-6} M were made daily. Stock solutions (10^{-2} M) of a ternary mixture of nucleotides previously shown to be stimulatory to ORNs of channel catfish (Michel et al. 1988) [adenosine-5′-triphosphate (ATP), inosine-5′-triphosphate (ITP), and inosine-5′-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 ORNs of catfish (P. W. Sorensen, unpublished observations); TCA was also included due to its known stimulatory action on ORNs in goldfish. TCA and TLC (both water soluble) were prepared weekly, and log step dilutions to 10^{-7} 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 (Sorensen et al. 1990). Control solutions included: *1*) CFTW obtained from the same water source as that used to prepare the test solutions and *2*) ethanol at the appropriate dilution for testing LCA. Interstimulus intervals were at least 2.5 min.

Stimulus delivery was via a "gravity-feed" system employing 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 separate Teflon tubes (0.79 mm diam) at a rate of 6–8 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 cavity between stimuli (3- to 5-min interstimulus intervals). A foot switch connected to an electronic timer (model 645, GraLab Instruments Division, Dimco-Gray, Centerville, OH) triggered the valve to introduce the odorants for a 5-s stimulus duration. without a change in either pressure or temperature and without dilution (Sveinsson and Hara 1990).

Recording techniques

ELECTROOLFACTOGRAM (EOG). The underwater EOG is an odorant-induced, slow negative potential measured in the water immediately above the olfactory mucosa that is thought to reflect summated olfactory receptor generator potentials (Caprio 1995; Ottoson 1971). The EOG was recorded in vivo with calomel electrodes via Ringer-agar–filled capillary pipettes as reported previously (Silver et al. 1976). The EOG signal was amplified (Grass P-18 dc amplifier), printed on a chart recorder, 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.

OLFACTORY BULB UNIT RECORDINGS. Unit/few unit activity (generally 350- to 1,000-μV peak-to-peak amplitude) was recorded extracellularly from the medial, middle, and lateral portions of the rostral, intermediate, and caudal portions of the dorsal and ventral OB (generally 3–3.5 mm in length and 1.8–2.0 mm in width at its mid-region). Each of these nine bulbar regions was approximately 600–700 μm in width and 800–1,000 μm in length, depending on the size of the fish. The electrode, a low-impedance (2–5 MΩ) platinum and gold-plated, metal-filled, glass micropipette (glass tip, 2 μm; ball diameter, 3–4 μm), was mounted on a hydraulic microdrive attached to a stereotaxic micromanipulator and advanced vertically downward from the dorsal surface of the OB. Stereotaxic methods were utilized in identifying the exact *x, y* positions of each recording position in the OB (Fig. 1). The *z*-position (depth) of the recording electrode was determined in micrometers directly from the scale on the hydraulic microdrive. The

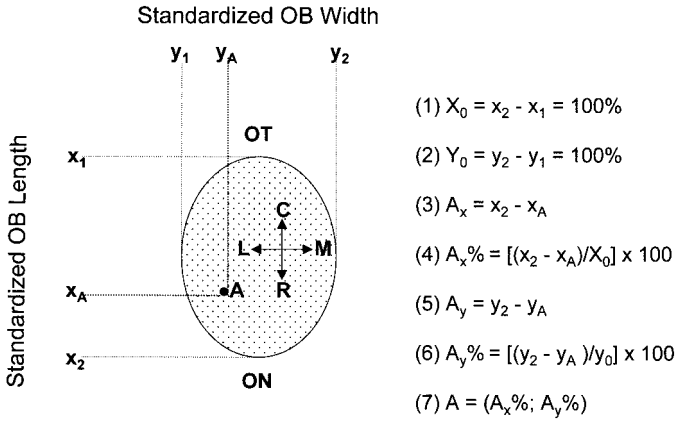

FIG. 1. Experimental protocol for the stereotaxic standardization of recording sites in the olfactory bulb of the channel catfish. The length (X_0; *Eq. 1*) and width (Y_0; *Eq. 2*) of the olfactory bulb of each fish are standardized to 100%. The position of recording position A along the *x*-axis (*Eq. 3*) and its standardized value (*Eq. 4*) are determined. The position of recording position A along the *y*-axis (*Eq. 5*) and its standardized value (*Eq. 6*) are determined. The standardized coordinates for each vertical electrode track where an olfactory bulb unit was recorded is defined in *Eq. 7*. ON, olfactory nerve; OT, olfactory tract; Std., Standardized; R, rostral; C, caudal; L, lateral; M, medial.

zero position for the *z*-axis was the point of contact of the electrode to the surface of the OB. Due to slight variations in the dimensions of the olfactory bulb across the different specimens tested, the position of each vertical electrode track was converted into relative units of % total length (*x*-axis) and % total width (*y*-axis). The *z*-axis total length was not determined due to the difficulty in obtaining an accurate measurement of the dorsoventral axis of the OB in vivo. Vertical (*z*-axis) electrode tracks were spaced 150 μm apart. During a single electrode penetration, the recording electrode often encountered regions of unit activity twice: once in the dorsal and once in the ventral OB. The majority of recordings of OB were obtained at primarily two ranges of depth, 150–400 μm and 700–1,000 μm where the cell bodies and dendrites of mitral cells are located in catfish (J. Kang and J. Caprio, unpublished observations). Units observed above 500-μm depth were defined to be within the dorsal bulb, while those below that depth were defined as within the ventral bulb. 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 each unit, each of the three odor mixtures (amino acids, nucleotides, bile salts) at each tested concentration was applied at least twice to the olfactory organ with at least a 2-min interstimulus interval. Initially, a moderate concentration of each of the three odor mixtures was tested (10^{-5} M amino acids, 10^{-6} M bile salts, 10^{-5} M nucleotides). For any odor mixture 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 odor mixtures, a log unit higher concentration of the respective odor mixture was tested. On the average, 3 to 4 bulb units were obtained from each of 37 fish tested; at the upper extreme, 8 units were recorded in each of 2 fish. The neural activity was amplified (Grass Instruments P511k; band-pass 30–10,000 Hz), observed with an oscilloscope, digitized, and stored on a video channel of a hi-fi VCR.

DATA ACQUISITION AND ANALYSIS. All recorded data from both the olfactory lamellae and OB 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 that were utilized 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 data files were displayed on a computer screen and viewed by Neuroexplorer (Nex Technologies, Lexington, MA) software.

Responses of single OB neurons to each of the three odor mixtures were classified as excitatory, suppressive, or null based on the interrupted time-series analysis (Crosbie 1993; Hudson 1977; Kang and Caprio 1995a–c, 1997). The interrupted time-series analysis was conducted on the number of action potentials occurring within successive 250-ms time bins for 5 s prior to and subsequent to the initial onset of the odor-induced EOG. Only those responses that were excitatory to at least one of the three stimulus solutions were used in constructing the chemotopic map of the OB.

RESULTS

A total of 178 single OB neurons were excited by at least one of the three (amino acid, bile salt, nucleotide) solutions tested (Fig. 2). The vast majority [156 of 178 (88%)] of the neurons sampled were excited by only one of the three stimulus mixtures that were representative of the three different classes of odorants. Forty (93%) of 43 of the nucleotide-responsive OB neurons were excited solely by the nucleotide mixture and were located within a dorsal, caudolateral region of the OB; an additional three OB neurons in this region responded excitedly to all three odorant mixtures (Figs. 3*A* and 4). Fifty-five (90%) of 61 bile salt–responsive OB neurons were excited solely by the bile salt mixture and were located within a medial strip that extended the length of the OB both dorsally and ventrally; three additional neurons responded excitedly also to the amino acid mixure, and three other neurons responded to all three odorant mixtures (Figs. 3, *A–C*, and 4). Sixty-one (82%) of 74 amino acid–responsive OB neurons were excited solely by the amino acid mixture and were located lateral to the bile salt region in more rostral and intermediate OB regions both dorsally and ventrally; five additional neurons responded excitedly also to the bile salt mixture, and four other neurons responded excitedly also to the nucleotide mixture. Four additional neurons responded to all three odorant mixtures (Figs. 3, *A–C*, and 4).

DISCUSSION

The olfactory system of fishes responds to and distinguishes among a variety of biologically relevant stimuli, such as amino acids, nucleotides, and bile salts (Li and Sorensen 1997; Marui and Caprio 1992; Michel et al. 1988; Sola and Tosi 1993; Sorensen and Caprio 1998; Valentincic et al. 1994). Amino acids and nucleotides are feeding cues, whereas bile salts play a role in identification of conspecifics, apparently functioning as nonsexual attractants (Li et al. 1995). Each of these families of biologically relevant stimuli are detected via different molecular olfactory receptors (ORs) (Bruch and Rulli 1988; Caprio and Byrd 1984; Li and Sorensen 1997; Michel et al. 1988), which are broadly distributed in ORNs spread across sensory regions of the olfactory organ (Baier et al. 1994; Chang and Caprio 1996; Ngai et al. 1993; Vogt et al. 1995). ORNs expressing the same ORs are, however, excluded from close apposition (i.e., "near neighbor exclusion" of "like" ORNs) to each other (Baier et al. 1994).

In direct contrast to a random or at least a broad dispersal of ORNs expressing "like" ORs across the sensory epithelium in

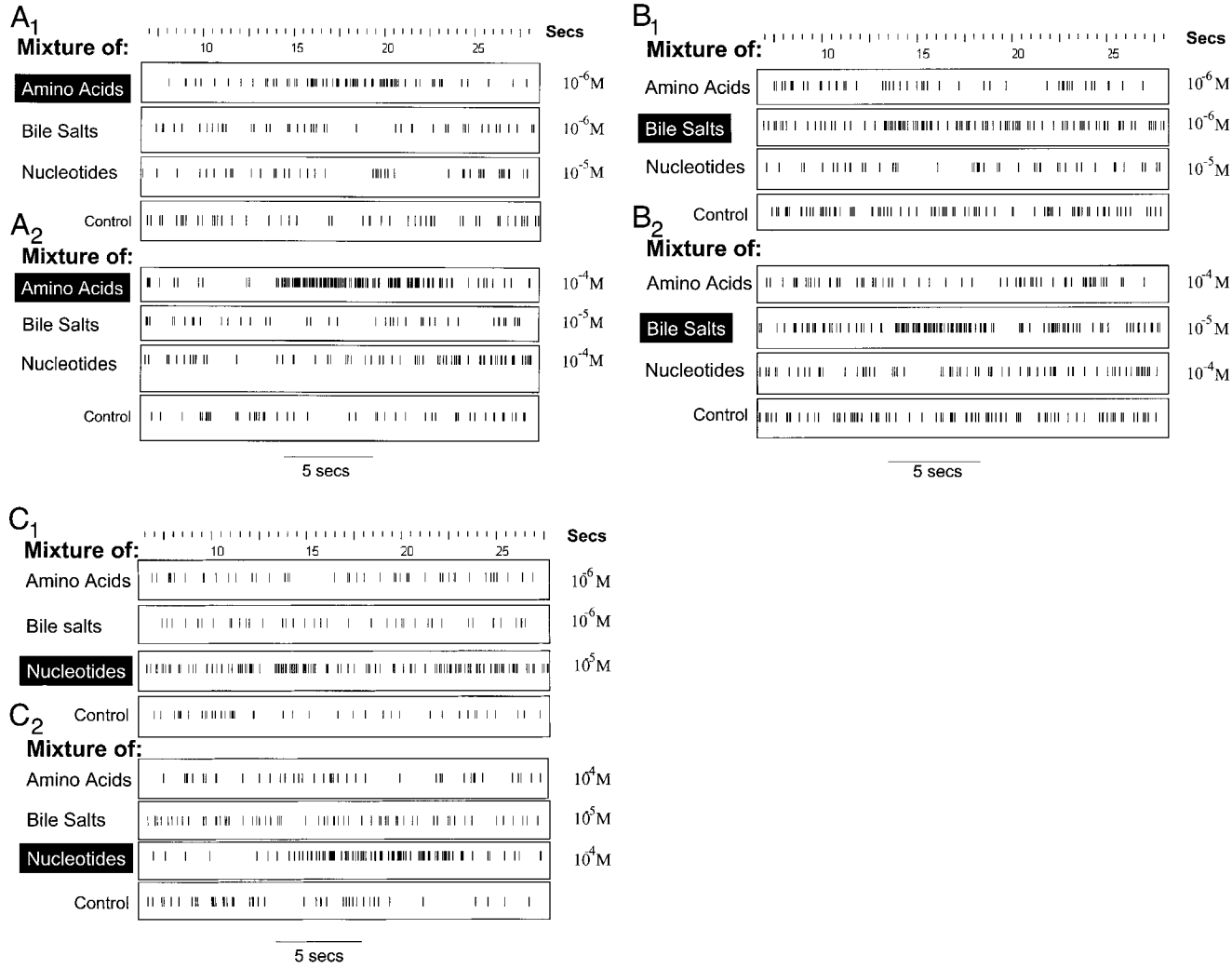

FIG. 2. Typical selectivity of single units recorded from olfactory bulb zones primarily responsive to amino acids (*A*), bile salts (*B*), and nucleotides (*C*). Two different test concentrations (*A1* and *A2; B1* and *B2; C1* and *C2*) per stimulus mixture are presented to show that selectivity is maintained at both low and high odor concentrations. Amino acid mixture: L-isomers of alanine, methionine, arginine, and glutamate; bile salt mixture: Na^+ salts of taurocholic, lithocholic, and taurolithocholic acid; nucleotide mixture: adenosine-5′-triphosphate (ATP), inosine-5′-triphosphate (ITP), and inosine-5′-monophosphate (IMP). Control: charcoal-filtered tap water. Five-second time bar starts at the initiation of the electroolfactogram (EOG; not shown) for each trace.

fish, odor representation within the OB, which contains neural circuitry fundamentally similar to that of mammals (Kosaka and Hama 1982), is chemotopically organized. On their course to the OB, axons of ORNs remain parallel to the long axis of the nerve until being redistributed by extensive sorting as they enter the OB (Riddle and Oakley 1992). This axon sorting is consistent with anatomical findings in rodents showing that ORNs expressing similar ORs project to the same glomeruli within the OB (Ressler et al. 1994; Vassar et al. 1994) and support anatomical (Guthrie and Gall 1995; Guthrie et al. 1993; Jourdan et al. 1980; Onoda 1992; Sharp et al. 1975; Stewart et al. 1979) and physiological (Buonviso and Chaput 1990; Mori and Yoshihara 1995; Uchida et al. 2000) studies indicating that glomeruli, which receive input mostly from ORNs expressing a common OR (e.g., Vassar et al. 1994), are the primary coding units for odorant discrimination.

The present report, which is the first study using single-unit electrophysiology to define OB chemotopy in a teleost, indicates that the OB in the channel catfish is divided into different functional zones, each processing a specific class of biologically relevant odor. Different OB regions responded excitedly and preferentially at slightly above threshold to either a mixture of bile salts, nucleotides, or amino acids. Excitatory responses to bile salts were observed primarily in a thin, medial strip in both the dorsal (100–450 μm) and ventral (900–1,200 μm) OB. Excitatory responses to nucleotides were obtained primarily from dorsal, caudolateral OB, whereas excitatory responses to amino acids occurred more rostrally in the dorsolateral OB, but continued more medially in the ventral OB.

Although analyses of bulbar EEG responses from salmonid OBs and calcium- and voltage-sensitive dye imaging of the zebrafish OB were generally consistent with the present findings in channel catfish of a mediolateral distinction on OB responsiveness, some variations were evident. The bulbar chemotopy observed in the channel catfish (family siluriformes) is more similar to that previously described for zebrafish (family cypriniformes) (Friedrich and Korsching 1997, 1998) than indicated for salmonids (family salmoniformes) (Døving et al.

1980; Hara and Zhang 1996, 1998; Thommesen 1978). EEG recordings from the surface of the OB in salmonids, char (*Salvelinus alpinus*), trout (*Salmo trutta*), and grayling (*Thymallus thymallus*), indicated that bulbar neurons located primarily rostrolaterally responded with increased EEG activity to amino acids, and those mainly dorsomedial responded to bile acids (Døving et al. 1980). A more recent EEG study with six different species of salmonids [rainbow trout (*Oncorhynchyus mykiss*), Atlantic salmon (*Salmo salar*), Arctic char (*Salvelinus alpinus*), lake whitefish (*Coregonus clupeaformis*), brown trout (*Salmo trutta*), and lake char (*Salvelinus namaycush*)], where electrode bulbar positions included both surface and depth recordings, showed that responses to amino acids were most evident in the lateral-posterior OB, which is larger caudally and becomes smaller and more ventral rostrally. Responses to a bile acid (taurocholic acid), however, were centered in a narrow triangular surface area in the central region forming a thin sheet across the mid-OB over that of the amino acid–responsive region (Hara and Zhang 1998). This latter result concerning the more responsive bile acid region in salmonids is the most disparate on comparison with the bulbar chemotopic maps of zebrafish and rainbow trout. In both zebrafish and rainbow trout, the bile acid/salt region was localized to a

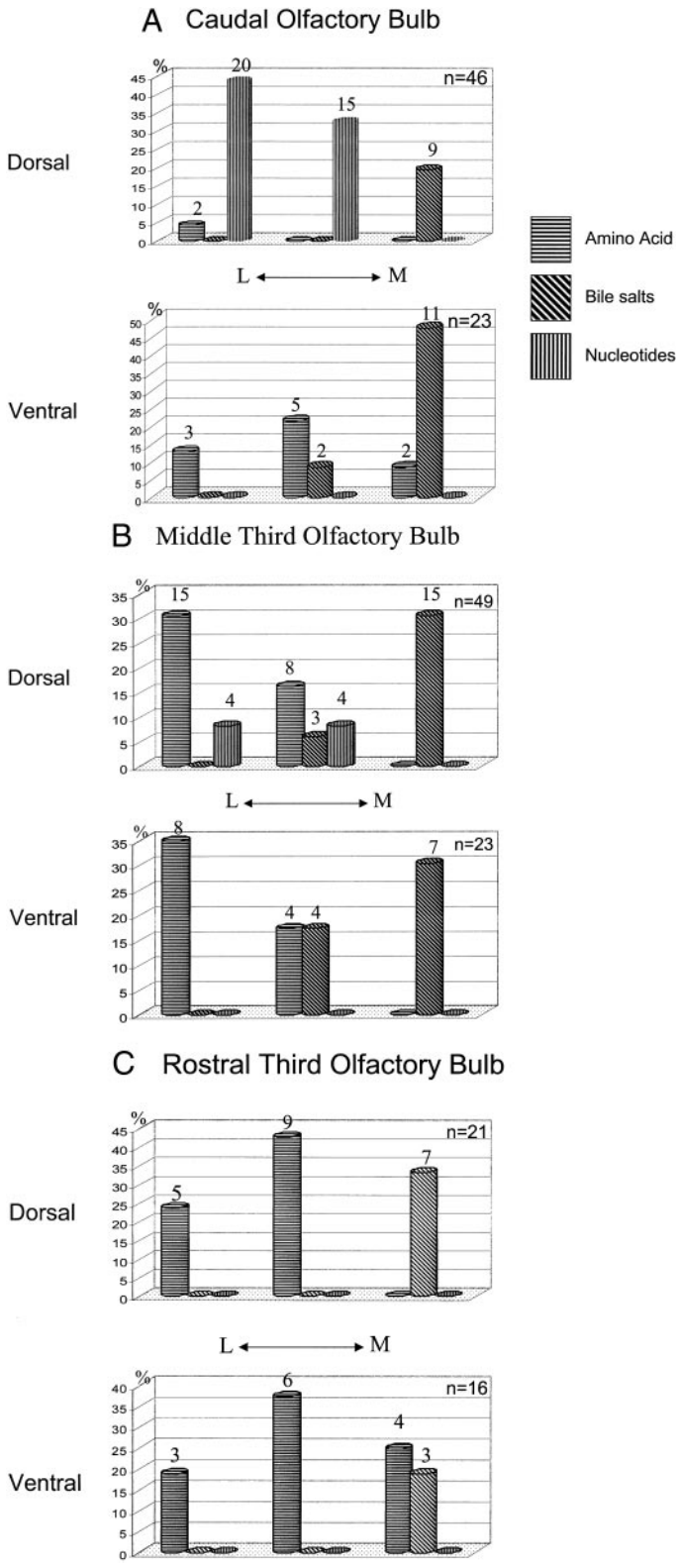

FIG. 3. Regional differences in responses of olfactory bulb neurons to amino acids, bile salts, and nucleotides. Indicated are dorsal and ventral regions of the caudal (*A*), intermediate (*B*), and rostral (*C*) thirds of the channel catfish olfactory bulb. Extracellular microelectrode recordings were performed in vivo from 178 olfactory bulb neurons from 37 channel catfish. The number of olfactory bulb neurons responding to stimulation of the olfactory organ by mixtures of amino acids, bile salts, and nucleotides, respectively, are indicated and listed above each bar. Ordinate: % of total number of neurons (100%) recorded in dorsal and ventral sections, respectively. Abscissa: relative position [from lateral (*L*) to medial (*M*)] of recorded olfactory bulb units.

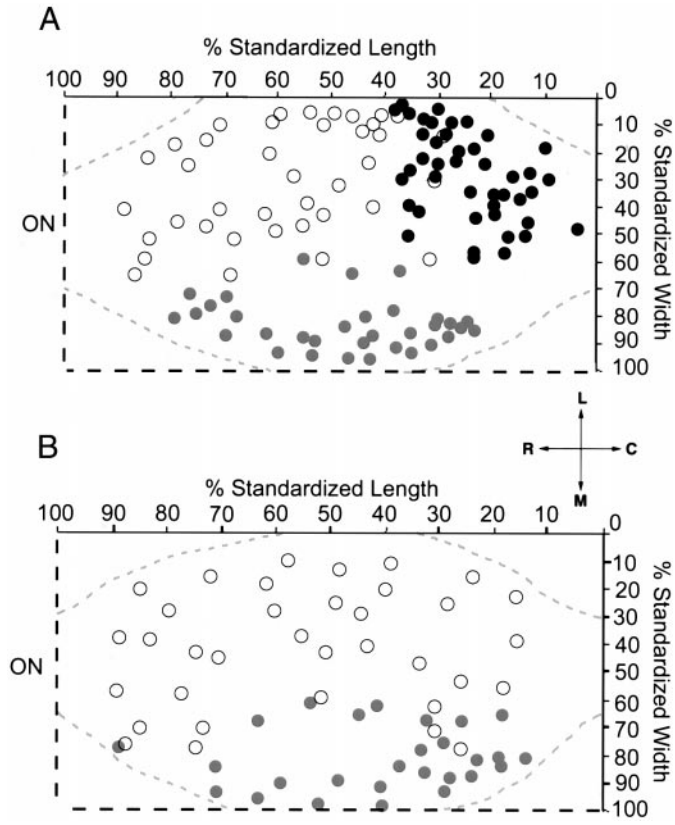

FIG. 4. Dorsal (*A*) and ventral (*B*) views of the channel catfish olfactory bulb indicating the standardized length (*x*) and width (*y*) positions (see Fig. 1) of the 178 olfactory bulb neurons recorded from 37 fish that process amino acid (○), bile salt (◎), and nucleotide (●) odor information. ON, olfactory nerve; OT, olfactory tract; R, rostral; C, caudal; L, lateral; M, medial.

medial OB region. Unfortunately, nucleotides were not tested in the salmonid studies.

For zebrafish, both calcium- (Friedrich and Korsching 1997) and voltage- (Friedrich and Korsching 1998) sensitive dye studies of responses of ORN terminals in the OB were consistent in identifying specific subregions of the OB that were preferentially activated by the different classes of biologically relevant odors. In results that were more consistent with the catfish than salmonid OB maps, both studies in zebrafish identified a rostrolateral OB region that was primarily responsive to amino acids; in addition, voltage-sensitive dyes indicated a primarily anterior-medial OB region responsive to bile acids and a caudolateral OB region responsive to nucleotides that overlapped with the posterior portion of the amino acid–responsive region. A shortcoming of visualizing activity of both dyes was that these studies were performed in an explant preparation of the olfactory organ and bulb and viewed ventrally; thus dorsal OB regions where neurons most activated by bile acids could not be resolved. The present electrophysiological study in the channel catfish, where unit responses of neurons in both the dorsal and ventral OB were accessible and were recorded were generally consistent with the optical studies in zebrafish and provided a clearer picture of the chemotopy of the dorsal OB.

The medial-lateral distinction in chemotopy (i.e., medial, bile salts; lateral, amino acids and nucleotides) in the OBs of channel catfish (present report), salmonids (Hara and Zhang 1998), and zebrafish (Friedrich and Korsching 1998) is consistent with mitral cell axons of the medial and lateral OB, respectively, projecting into the medial and lateral olfactory tracts (Dubois-Dauphin et al. 1980; Satou 1990; Sheldon 1912). The neuronal activities on one side of the fish OB are not influenced much by those in the opposite side and may be explained by limited dendritic fields of neurons in each part of the bulb (Satou 1990). In this respect, only the medial tract transmits pheromone information (Demski and Dulka 1984; Døving et al. 1980; Hamdani et al. 2000; Kyle et al. 1987; Sorensen et al. 1991; Stacey and Kyle 1983), whereas the lateral tract processes food-related odors (Døving et al. 1980; Stacey and Kyle 1983; Von Rekowski and Zippel 1993). These results of medial-lateral differences in bulbar unit specificities of teleosts to odorants are consistent with similar findings in mammals (Bozza and Kauer 1998; Imamura et al. 1992; Johnson and Leon 2000; Mori et al. 1992; Uchida et al. 2000).

The presumable function of the OB chemotopic map in the channel catfish is to enhance both the detection and discrimination of amino acids, bile salts, and nucleotides, respectively (Xu et al. 2000). In addition, it is likely that a finer map exists within each of the described OB functional zones for biologically relevant odorants for each of the three classes of stimuli; i.e., the response specificity of individual glomerular modules (Friedrich and Korsching 1998), and thus the individual neuronal elements within each respective zone can have different excitatory molecular receptive ranges (EMRR) (Mori and Yoshihara 1995). For example, with respect to the amino acid zone, single OB neurons residing within the amino acid zone can be excited by L-arginine (a basic amino acid) and either inhibited or nonresponsive to L-methionine (a neutral amino acid) and vice versa (unpublished observations). As proposed for mammals (Mori and Yoshihara 1995; Xu et al. 2000; Yokoi et al. 1995), the chemotopic organization of the OB minimizes the distance for lateral inhibitory bulbar circuitry, which is hypothesized to enhance contrasts in response specificity, thus sharpening the molecular receptive ranges of the olfactory inputs to the respective glomeruli. It is known for ictalurid catfish that the olfactory and not the taste system is required for the behavioral discrimination of amino acids (Valentincic et al. 1994, 2000a,b).

The map of chemotopy in the OB of the channel catfish (Fig. 4) is a schematic and not an absolute map. The designated portions of the OB that process amino acid, nucleotide, and bile salt odorants, respectively, represent the boundaries in which the OB units of different specificities were recorded. However, in addition to the three previous classes of odorant chemicals, fish are known to detect through olfaction pheromones, such as gonadal steroids and prostoglandins (Sorensen and Caprio 1998); these specific compounds, however, have yet to be identified for channel catfish. It is also probable that additional classes of chemicals may be found to be olfactory stimuli in fish. Thus, if additional classes of odorants are identified for the channel catfish, the bulbar chemotopic map described herein is likely to be modified. Further, although this report indicates that the spatial bulbar map is important for the quality coding of odorant information in the channel catfish, it does not address the role of precise timing of neural activity. It is reasonable to assume that temporal firing patterns of neurons within each of the three defined chemotopic regions might be important for a finer discrimination and identification of the specific members of each of the three major odorant classes tested here. The present results, however, do argue that the initial stage of odorant quality coding occurring within the OB is based on a spatial pattern of glomerular activation.

A key question is whether specific anatomical types of ORNs project to the presently defined specialized bulbar regions. Recent findings indicate that morphologically different types of ORNs (i.e., ciliated and microvillous) are intermingled in the olfactory epithelium of fish (Morita and Finger 1998) and other vertebrates (Miller et al. 1995; Moran et al. 1982; Morrison and Costanzo 1990, 1992; Rowley et al. 1989) and project to different regions of the OB. Future studies will explore whether the ciliated and microvillous ORNs, respectively, project primarily to any of the presently described chemotopic bulbar regions and thus respond preferentially to a specific class (or classes) of biologically relevant odor(s).

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