We are studying how odorant information is processed within the vertebrate central nervous system, the olfactory bulb (OB) and higher telencephalic nuclei. The experimental animal model tested in this study, the channel catfish, has an acute olfactory sense to known biologically relevant stimuli, such as amino acids, nucleotides and bile salts (Nikonov and Caprio, 2001). Extracelluar recordings from single neurons within the OB and within the cerebral lobes (CL), the next ascending olfactory nucleus were performed in vivo. We previously showed that olfactory receptor neural input to the OB is odotopically organized (Nikonov and Caprio, 2001), with the lateral OB processing food-related odors (amino acids and nucleotides) and the medial OB processing odors of more a social nature (bile salts). Neurons processing amino acid and bile salt odors are distributed in both dorsal and ventral OB regions, whereas neurons processing nucleotide information are localized to the dorso-caudal OB region. Our studies of odorant specificity focuses on excitatory responses of OB neurons located within the lateral amino acid zone of the catfish OB. Our previous behavioral studies clearly showed that although catfish both taste and smell amino acids, a functioning olfactory system is required for their discrimination (Valentinine et al., 1994). The present study is an attempt to determine the physiological basis for this discrimination, with emphasis on amino acids as odorants. Although it was previously documented (Kang and Caprio, 1995) that OB neurons in channel catfish respond to amino acids, the present report reinvestigated the amino acid selectivities of single OB units at lower stimulus concentrations than previously tested with a larger number of amino acid odorants. The majority of OB units recorded in the present investigation were likely mitral cells based on the distribution of recording depths in the OB being consistent with the locations of the majority of cell bodies of mitral cells identified previously in histological section and the large and relatively constant amplitudes of the action potentials suggestive of being elicted by the large mitral cells.

The specificity of 245 units located in the lateral, amino acid responsive portion of the OB was determined. Ninety-one OB units (Group I) were highly selective for a single type of amino acid (neutral, basic, acidic), compounds determined in previous electrophysiological cross-adaptation (Caprio and Byrd, 1984), amino acid mixture (Caprio et al., 1989; Kang and Caprio, 1991) and biochemical binding (Bruch and Rulli, 1988) studies to bind to relatively independent olfactory receptor sites in this species. None of the Group I units was excited by any of the other representative types of amino acids at odorant concentrations up to and including 10⁻⁹ M. Overall, 86% of the 245 OB units tested responded to ≥10⁻⁶ M amino acids. The majority of the Group I units were excited by either L-methionine (Met; n = 31; 34%) or L-arginine (Arg; n = 28; 31%); units excited by either L-alanine (Ala; n = 19; 21%) or monosodium glutamate (Glu; n = 13; 14%) were fewer. In contrast, the 154 OB units (Group II) were excited by a second type of amino acid, but only at a 10–100x higher odorant concentration. Since the selectivity of mitral cell responses to amino acids in zebrafish changed over 2.2 s of the response, which resulted in a declustering of the response types observed during the initial 500 ms of the response (Friedrich and Laurent, 2001), we addressed the question of whether our classification of response type based on an analysis of 3 s of response time would be significantly altered by analyzing different portions of the response to 3 s stimulus applications. Of the 78 Group I units analyzed (i.e. those that were originally determined to be selectively responsive to only Met, Ala, Arg or Glu over 3 s of response), 81 and 85% were similarly classified when analyzing the first and third seconds of the responses, respectively. The reason(s) for this discrepancy among odorant responses of bulbar neurons in the channel catfish and zebrafish is currently unknown.

The remaining 154 (63%; Group II) of the 245 units tested had a broader specificity than those of the Group I units, but their sensitivities to the amino acid types were not randomly distributed (i.e. each unit type was not excited by other particular types of amino acids). Similar to the Group I units, the more numerous of the Group II units were those excited by ≥10⁻⁶ M Met (n = 83; 54%) or ≥10⁻⁵ M Arg (n = 46; 30%); units excited by ≥10⁻⁶ M Ala (n = 21; 14%) or Glu (n = 4; 2%) were fewer. The majority (70 of 83; 84%) of the Group II units were excited by ≥10⁻⁶ M amino acid; the remaining units responded to ≥10⁻⁵ M. Seventy-seven of 83 (93%) Group II units with lowest threshold to methionine, a neutral amino acid with a long side-chain, were also excited by Ala, a neutral amino acid with a short side-chain, but at a 10-fold higher stimulus concentration. The converse, however, did not occur, as Group II units with lowest thresholds to alanine were not excited by Met. This Met Group of OB neurons showed high specificity to neutral amino acids as none of the 83 Met units were excited by Arg or Glu. The Ala units were the least specifically tuned of the Group II OB neurons as 86% were also excited by ≥10⁻⁴ M Arg and 71% by ≥10⁻³ M Glu. Although only 7% (17 of 245) of the OB units analyzed (including Groups I and II) had the lowest thresholds to Glu, this amino acid at high stimulus concentrations stimulated the vast majority (60 of 67 units; 90%) of two of the three types of Group II units.

Because of their high selectivity, we explored further the response specificity of 69 additional Group I OB units to additional amino acids and derivatives at stimulus concentrations from 10⁻⁸ to 10⁻⁵ M. An additional 31 Group I units that were excited only by Met (not by Ala, Arg or Glu) were tested with an additional eight related odorants. Two Groups emerged, those most responsive (i.e. with lowest excitatory electrophysiological thresholds) to neutral amino acids with long, linear side-chains and those with branched side-chains. Of 12 additional Group I OB units that were initially excited only by Ala (not by Met, Arg or Glu), all were most sensitive to Ala, and 5 of the 12 were equally responsive to L-serine, another neutral amino acid with a short side-chain. An additional 26 Group I OB units that were initially excited only by Arg (not by Met, Ala or Glu) were tested with eight odorants related to Arg. These units were excited by amino acids that possessed in their side-chains at least three methylene groups and a terminal amide or guanidinium group. Group I OB units that were most selective to Glu were too few to study. Overall, these collective results are sufficient to account for many of the previous results of the behavioral discrimination of...
amino acids in this and related species of teleosts (see Valentincic in this issue).

The second portion of this ongoing study is to determine how odorant information arriving from the olfactory bulb via the medial and lateral olfactory tracts is represented in the cerebral lobes (CL) of the telencephalon in the channel catfish. Odor-responsive neurons in the CL were located in caudo-medial and lateral regions as predicted from previous anatomical studies in catfish (Finger, 1975; Bass, 1981). The lateral olfactory tract (LOT) projects to the ventrolateral wall of the telencephalon and extends dorsally and caudally in the CL, whereas the medial olfactory tract (MOT) projects medially, rostral to the anterior commissure; however, both lateral and medial termination zones receive input from the LOT and MOT. Although a segregation of function for olfactory pathways in the CNS has not been studied in most animals, functional differences are evident in fish between odorant information carried by MOT (food-related) and LOT (social stimuli). The medial–lateral distinction in odotopy of the OB in the channel catfish (Nikonov and Caprio, 2001) is consistent with this distinction. The initial question of the present investigation was to determine whether the chemotopic organization of the olfactory bulb to feeding (lateral OB) and social (medial OB) odors is maintained, altered or eliminated at the next ascending olfactory nucleus in the CL. A second question was whether the odorant specificities observed in the olfactory bulb to these three classes of odorant stimuli are maintained or altered at the next synaptic level.

Fifty-one CL neurons were excited and 22 were suppressed by odorants (amino acid or bile salt). Twenty-nine of the 35 units that were excited by amino acids were located more laterally in the CL and their odorant specificity was similar to Group I OB neurons. Sixteen units located more medially in the CL were excited by bile salts and were unresponsive to amino acids. Thus, the medial–lateral distinction between excitatory responses to bile salts and amino acids as observed in the OB is reflected in the CL; how nucleotide information is represented in the CL is currently being investigated. Further, evidence of a convergence of amino acid information that was previously separate in the OB was observed in six CL units that were excited by both neutral and basic amino acids. Ongoing studies are aimed at examining odorant specificities of CL neurons to a broader array of odorants.

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References