

Correlations between Olfactory Discrimination, Olfactory Receptor Neuron Responses and Chemotopy of Amino Acids in Fishes

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Olfactory discrimination of amino acids in catfish and zebrafish

Catfishes (genera *Ictalurus* and *Ameiurus*) discriminate nearly every conditioned amino acid stimulus from every other amino acid stimulus (Valentinčič and Caprio, 1994; Valentinčič *et al.*, 1994, 2000a). However, bullhead catfish were always unable to discriminate L-isoleucine (L-Ile) from L-valine (L-Val) and in some cases they were also unable to discriminate L-alanine (L-Ala) from L-serine (L-Ser) and glycine (Gly). We discovered that the olfactory discrimination capabilities of catfish and zebrafish (*Danio rerio*) are very similar. Knowledge of chemotopic projections of amino acid stimuli on the surface of the olfactory bulb studied with calcium labeling technique (Friedrich and Korsching, 1997) enabled us to predict which amino acids zebrafish can and cannot discriminate. Differential bulbar activity patterns should facilitate olfactory discrimination, whereas identical bulbar patterns should not enable olfactory discrimination. Largely different bulbar activity patterns for short-chain neutral and long-chain neutral, acidic and basic amino acids make their behavioral discrimination easy. Nearly identical bulbar activity patterns that in most zebrafish occur after stimulation with L-Val and L-Ile did not enable discrimination of these two amino acids irrespective of the discrimination training applied. The bulbar activity patterns that occur after stimulation with chemically similar amino acids, such as L-Ala and L-Ser and L-arginine (L-Arg) and L-lysine (L-Lys) are not identical, their fractional differences allowed zebrafish to discriminate these amino acid pairs. In conclusion, minor differences in bulbar activity patterns enable olfactory discrimination of amino acids; the more similar these patterns are, the less the chance of their olfactory discrimination. In individual zebrafish, the bulbar activity patterns after stimulation with the same amino acid are slightly different (Friedrich and Korsching, 1997), which makes discrimination capabilities of individual zebrafish different. In bullhead catfish, the individual differences in L-Ala/ L-Ser discrimination capabilities depend on phenotypic expression rather than genetic differences between individuals since the same catfish that did not discriminate L-Ala from L-Ser before olfactory organ extirpation started to discriminate these two amino acids subsequent to regeneration (Stenovec and Valentinčič, 2001).

Olfactory discrimination of amino acid mixtures in bullhead catfish

Bullhead catfish initially detect binary and ternary mixtures of amino acids as their more stimulatory components; five to 12 successive comparisons of a mixture and its more stimulatory component alone enable the catfish to discriminate the more stimulatory amino acid from the mixture (Valentinčič *et al.*, 2000b). The more stimulatory components of binary mixtures were prepared using amino acid concentrations that were 3–30-fold higher than their equal stimulatory effectiveness concentrations determined by equal amplitude of electroolfactogram (EOG). Multimixtures composed of seven and 12

amino acids were studied at component concentrations that resulted in equal EOG amplitudes. Catfish discriminated a conditioned seven amino acid mixture from their six-, five- and four-component counterparts. The conditioned mixture of 12 amino acids was discriminated from its nine- and 10-component counterparts; however, irrespective of the missing component, they were unable to discriminate the 12-component mixture from its 11-component counterparts.

Regenerated olfactory organs facilitate olfactory discrimination of amino acids

To study olfactory discrimination in catfish with regenerated olfactory organs, the fish were anesthetized with ethyl 3-aminobenzoate methanesulfonate salt (MS-222, dilution 1:8000) and olfactory organs were surgically excised (>95% of the olfactory organ was removed). The incompletely excised olfactory organs of bullhead catfish regenerated ~3 months after surgery. The olfactory discrimination capabilities of catfish were tested between 3 and 7 months post surgery. The regenerated olfactory roseta was always several times smaller than the intact olfactory roseta; in some cases, only few fingerlike lamellae had regrown. Olfactory discrimination capabilities of bullhead catfish with the intact and regenerated olfactory organs were identical and in some cases even better than the olfactory discrimination capabilities of the same catfish prior to extirpation. At the end of the 5–6 months period after surgery even a tiny single olfactory lamella enabled amino acid discrimination. We investigated the connectivity of the olfactory receptor neurons (ORNs) with different areas of the olfactory bulb (OB) in bullhead catfish with either intact or regenerated olfactory rosetae. In regener-

Ion concentrations in highly purified water

Ions	Sample 1 Highly purified water (HPW)	Sample 2 HPW delivered into nasal cavity	Sample 3 Drain from the nasal cavity	Artificial pond water
Na ⁺	7 ± 2 µg/l	8 ± 2 µg/l	30 ± 2 µg/l	39 mg/l
K ⁺	50 ± 5 µg/l	64 ± 5 µg/l	45 ± 5 µg/l	4 mg/l
Ca ²⁺	5 ± 2 µg/l	16 ± 2 µg/l	12 ± 2 µg/l	7 mg/l
Mg ²⁺	0,5 ± 0,1 µg/l	2 ± 0,1 µg/l	2 ± 0,1 µg/l	2mg/l

Figure 1 Normal physiological function of olfactory receptor neurons was maintained in highly purified water. Ion concentration in highly purified water is ~1000× smaller than ion concentration in artificial pond water.

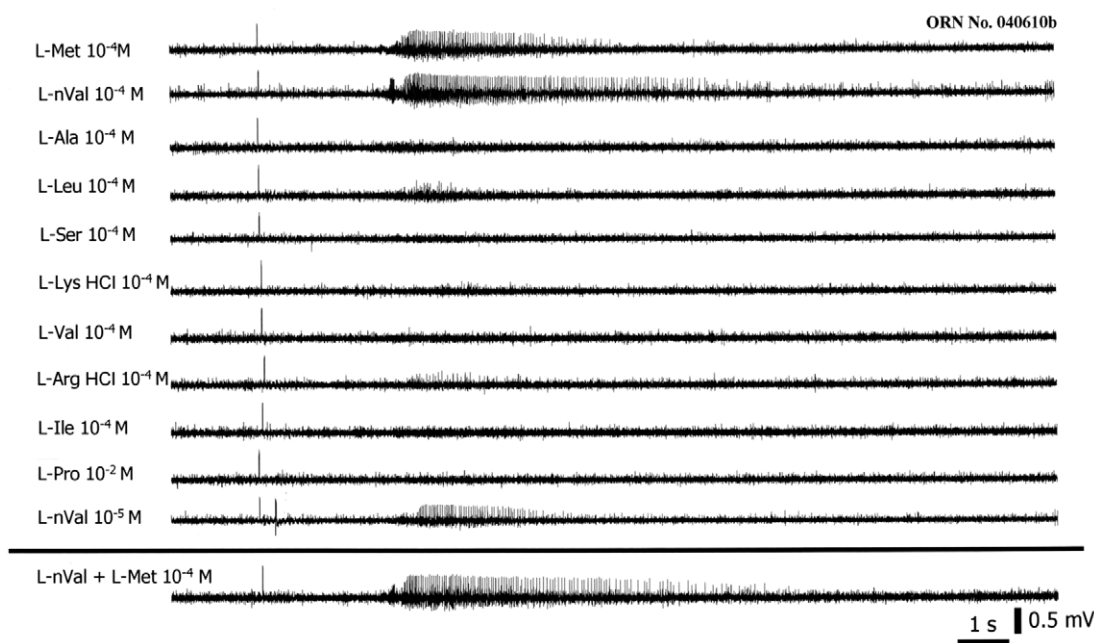


Figure 2 Greatest number of silent olfactory receptor neurons of bullhead catfish responded to two amino acids, L-methionine and L-norvaline.

ated olfactory rosetae, DiI crystals were introduced into either anterior ventral or lateral ventral sites of the bulb. In both, intact and regenerated olfactory rosetae, the insertion of the DiI into the anterior region of the ventral OB resulted in fluorescent labeling of tall (ciliated) olfactory receptor neurons (Morita and Finger, 1998; Hansen *et al.*, 2003) whereas lateral crystal insertion of the ventral OB resulted in labeling intermediate (microvillus) ORNs. Both, the fine structure of the olfactory organ and the olfactory discrimination abilities recovered during regeneration of the olfactory organs.

Electroolfactogram and single olfactory receptor neurons responses to amino acid stimuli

The cellular olfactory code is provided by a layer of ORNs. A subpopulation of ORNs is spontaneously active and a second much larger subpopulation of ORNs is not spontaneously active (silent ORNs) prior to stimulation. For technical reasons, only the physiological responses of the spontaneously active ORNs were reported previously (Kang and Caprio, 1995). The spontaneously active ORNs predominantly responded to amino acid stimuli with suppression. However neurons that code for amino acid odorants should respond to stimulation in a dose-dependent manner. For the electrophysiological experiments, we anesthetized the catfish with the anesthetic MS-222 and placed them into the recording chamber, perfused their gills with the anesthetic and immobilized them with an intramuscular injection of gallamine triethiodide (Flaxedil, 0.16 mg/100 g body wt). The concentration dependence of the suppressive response to amino acid stimuli was at best ordinal (Stevens, 1946, 1951; Velleman and Wilkinson, 1993).

We found no correlation between the ability of catfish to discriminate amino acids and the relative number of suppressive responses of ORNs. Among several hundreds of spontaneously active ORNs tested, few (<3%) cells responded to stimulation with dose-dependent excitation. Spontaneous activity is a likely property of young olfactory receptor neurons establishing synaptic connections with the olfactory bulb glomeruli.

Olfactory organs of fishes are fully functional in freshwater that contain very small ion concentrations. The physiological functions

of the entire olfactory organ and of individual ORNs were preserved for several hours even in highly purified water (HPW) that contained ~1000 times fewer ions than the artificial pond water (Figure 1). Due to the high resistance ($R > 18.2 \text{ M}\Omega\text{cm}$) there was little shunting of the electrophysiological signals in HPW. Responses to amino acid stimuli of numerous silent (non-spontaneously active) ORNs could be observed in these conditions. The number of lamellar locations where responses to amino acid stimuli were detected correlated highly with the amplitude of EOGs to the same stimuli. These results corroborate the assumption that the summed receptor potentials add up into the EOG amplitude. The silent ORNs responded to amino acid stimuli repeatedly and the duration of their responses was dose-dependent. Most ORNs (Figure 2) responded to one or two amino acid stimuli; the most numerous were the neurons responding to L-methionine (L-Met) and L-norvaline (L-nVal; 27% of all the tested cells). Responses to stimuli that elicit very small magnitude EOGs, such as L-proline (L-Pro), were also detected. At 23 recording locations, responses to four to eight amino acids were observed; some of these activities originated from single ORNs, whereas in other locations, single cell origin of action potentials could not be confirmed in our extracellular recordings.

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