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Odour discrimination in the olfactory bulb of goldfish: contrasting interactions between mitral cells and ruffed cells

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Anatomical differences characterizing mitral cells and ruffed cells have been published by T. Kosaka and K. Hama in three teleost species. Physiological responses from both types of relay neurons were recorded extracellularly and simultaneously in the plexiform layer, using a single tungsten microelectrode. During interstimulus intervals mitral cells responded with higher, frequently burst-like impulse rates triggered by the activity of epithelial receptor neurons. Mitral cell activity could be totally suppressed by local anaesthesia of the olfactory epithelium. Ruffed cell impulse rates were low, and each action potential triggered a long-lasting (3–5 ms), continuously varying, summed granule cell potential. During olfactory stimulation with non-familiar stimuli and important biological stimuli such as amino acids, preovulatory and ovulatory pheromones, and a probable alarm pheromone, contrasting interactions between mitral cells and ruffed cells were recorded frequently, which resulted in a drastic intensification of centrally transmitted information. An excitation of mitral cells' activity via granule cells laterally inhibited the ruffed cells' activity, and an inhibition of mitral cells' activity simultaneously 'released' an excitation of ruffed cells.

Keywords: goldfish; olfactory bulb; mitral cells; ruffed cells; electrophysiology

1. INTRODUCTION

Goldfish as experimental animals offer a number of physiological advantages: in higher vertebrates mitral cells and tufted cells are located in different layers; in goldfish the two classes of bulbar relay neurons lie close to each other in the plexiform layer. Simultaneous extracellular recordings from both types of relay neurons can be made with a single tungsten electrode. Whether a direct lateral inhibition via granule cells present in goldfish also exists between different relay neurons in mammals (Nickell & Shipley 1992) is unknown.

In contrast to mitral cells (Kosaka & Hama 1982), ruffed cells' glomerular dendrites have no input from epithelial sensory neurons (Kosaka & Hama 1981, 1982–1983), while sensors from the olfactory epithelium innervate both mitral and tufted cells. Anaesthesia of the olfactory epithelium in goldfish therefore results in a blockade of the driving input from spontaneously active receptor neurons, and the activity of mitral cells is reversibly blocked. Consequently, a partial or total blockade of lateral inhibition from granule cells activated by mitral cells results in a high and frequently rhythmic activity of ruffed cells (Zippel *et al.* 1999).

Each ruffed cell forms many initial pedunculate protrusions (IP 'ruff') from a long (70–250 m) non-myelinated portion of the axon (Kosaka & Hama 1979; Kosaka 1980). 'The number of synapses on the IP is roughly estimated to be 1.000 to 2.000 . . . asymmetrical' (excitatory for granule cells)' synapses from the IP, 63%; symmetrical' (inhibitory) 'synapses onto the IP, 12%, and reciprocal synapses, 25%' (Kosaka 1980, p. 119). Therefore, from mitral cells, single action potentials (1–1.5 ms) or bursts were recorded, whilst a 1.0–1.5 ms ruffed cell action potential always triggered a summed granule cell potential of 3 ms duration. Steps in the summed granule cell potential were never recorded, i.e. the ruffed cells' action potential always triggered via the IP a smaller or greater pool of granule cells. Triggering of the granule cell potentials by the ruffed cells' action potential resulted in a constant 3 ms interval from peak to peak. The significantly different morphological features, excellently investigated by T. Kosaka and K. Hama, and their physiological consequences (Zippel *et al.* 1999), make it easy to discriminate mitral cell and ruffed cell potentials during extracellular (not, however, during intracellular) recordings.

2. MATERIAL AND METHODS

Potentials from the two different types of relay neurons could be recorded extracellularly and simultaneously in the plexiform layer, using a single tungsten microelectrode (AM systems 5770; S. W. Everett, WA, USA; 5–7 MΩ). In contrast to higher vertebrates, in goldfish a great number of non-familiar and important natural stimuli, including pheromones, are structurally known (Zippel *et al.* 1993) and can be applied in defined molarities: (non-familiar stimuli: amyl acetate, β-ionone, 2-phenylethanol 10^{-6}; relevant natural stimuli: four amino acids (L-Ala, L-Glu, L-Arg, L-Lsy) 10^{-6} to 10^{-10} M; pre-ovulatory pheromones: in

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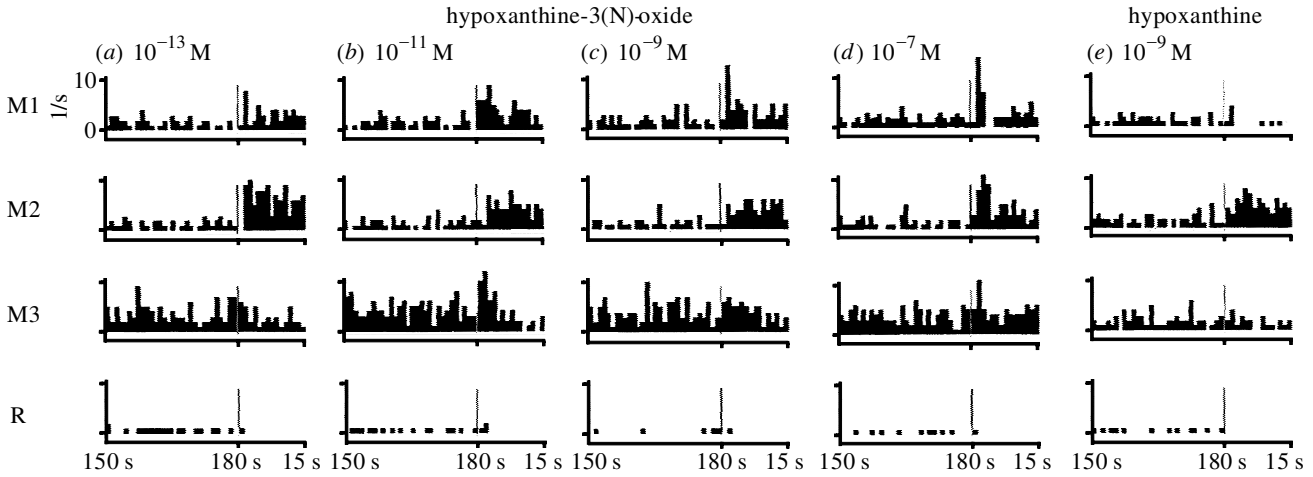

Figure 1. Simultaneous recordings from three mitral cells (M1–M3) and a ruffed cell (R) during application of hypoxanthine-3(N)-oxide, the probable alarm pheromone in four different molarities and hypoxanthine. (*a*) 10^{-13} M: tonic excitation of M1 and M2, R total inhibition. (*b*) 10^{-11}M: M1 phasic-tonic excitation, M2 strong excitation, M3 slight inhibition, R total inhibition; (*c*) 10^{-9}M: M1 phasic-tonic excitation, M2 tonic excitation, M3 indifferent, R total inhibition. (*d*) 10^{-7}M: all mitral cells (M1–M3) are excited phasic, M2 and M3 are phasic tonic, and R is totally inhibited. (*e*) hypoxanthine 10^{-9}M: inhibition of M1 and M3, tonic excitation of M2, R total inhibition. Recordings present the last 60 s of the 180 s interstimulus phase and the 15 s during stimulus application; vertical bars represent the number of potential recorded per second.

electro-olfactogram (EOG) (Sorensen *et al*. 1990) highly effective 17, 20β-dihydroxyprogesterone (10^{-9} to 10^{-11} M); 17, 20β-21-trihydroxyprogesterone (10^{-9} to 10^{-11} M), and in EOG (Sorensen *et al*. 1990) less effective 4-pregnen-20α-ol 3-one (10^{-7} to 10^{-9} M), 4-pregnen-20β-ol-3-one, dihydroxyprogesterol, and androstendione (10^{-7} to 10^{-9} M); two ovulatory pheromones: prostaglandin F_{2a} and 15 ketoprostaglandin F_{2a} (PGF_{2a}, 15K-PFG_{2a}; 10^{-7} to 10^{-11} M); hypoxanthine-3(N)-oxide (10^{-7} to 10^{-13} M), a probable alarm pheromone (Pfeiffer 1982), and hypoxanthine (10^{-7} to 10^{-9} M), which do not result in a recordable EOG during epithelial application (P. W. Sorensen, personal communication).

3. RESULTS AND DISCUSSION

The interstimulus activity is characterized by the higher and frequently burst-like activity of mitral cells (mean value 2.7 s^{-1}) driven by the spontaneously active epithelial receptor neurons. During pauses in the mitral cell activity, ruffed cell potentials (mean value 0.75 s^{-1}) are present. During application of an inhibitory olfactory stimulus the activity of receptor neurons is blocked and the mitral cells are inhibited. Consequently, lateral inhibition of ruffed cells via granule cells activated by mitral cells is no longer present and results in a higher activity of ruffed cells. During epithelial application of an excitatory olfactory stimulus, increased activity of receptor cells results in excitation of mitral cells, which activate granule cells laterally inhibiting ruffed cells. Figure 1 shows responses of three mitral cells and one ruffed cell during application of hypoxanthine-3(N)-oxide and hypoxanthine in different molarities. These stimuli do not result in a recordable EOG during epithelial application (P. W. Sorensen, personal communication). Figure 1 presents a simultaneous recording from three mitral cells and from one ruffed cell. During application of the probable alarm pheromone in the highest concentration (10^{-7} M), an excitation is recorded from the three mitral cells and a total lateral inhibition from the ruffed cell. Concentrations of 10^{-7}, 10^{-9}, 10^{-11} and 10^{-13} M excite mitral cells 1 and 2, and again the ruffed cell is totally inhibited. Mitral cell 3 remains indifferent during application of the 10^{-9} and 10^{-11} molar concentrations and is slightly inhibited during application of the 10^{-13} molar concentration. The major interaction between mitral cell 2 and the ruffed cell can clearly be seen during application of hypoxanthine (10^{-9} M): only mitral cell 2 is excited and the ruffed cell is totally laterally inhibited.

Contrasting interactions between mitral cells and ruffed cells were present that even in the lowest concentration resulted in a drastic intensification of centrally transmitted information. An inhibition was recorded more frequently from mitral cells, and decreasing lateral inhibition via granule cells resulted in an activation of ruffed cells. Activation of ruffed cells resulted in an activation of pools of granule cells laterally inhibiting pools of mitral cells in their vicinity.

In contrast to EOG recording (Sorensen *et al*. 1990), application of highly effective and less effective preovulatory pheromone stimuli resulted in more similar responses recorded from olfactory bulb relay neurons. The EOG is a slow (DC) potential change recorded in teleosts from the water above the surface of the olfactory organ in response to chemical stimulation and is suggested to be the population average of receptor potentials responsible for the initiation of neural impulses. From the present recordings from olfactory bulb relay neurons, however, the EOG obviously is not a reliable indicator of olfactory organ sensitivity and specificity to odorants in fishes.

Olfactory bulb relay neurons frequently respond to a comparatively great number of olfactory stimuli: amino acids, preovulatory and ovulatory stimuli (figure 2), and contrasting interactions between mitral cells and ruffed cells were recorded frequently during stimulus application (figure 1). However, significant differences in the

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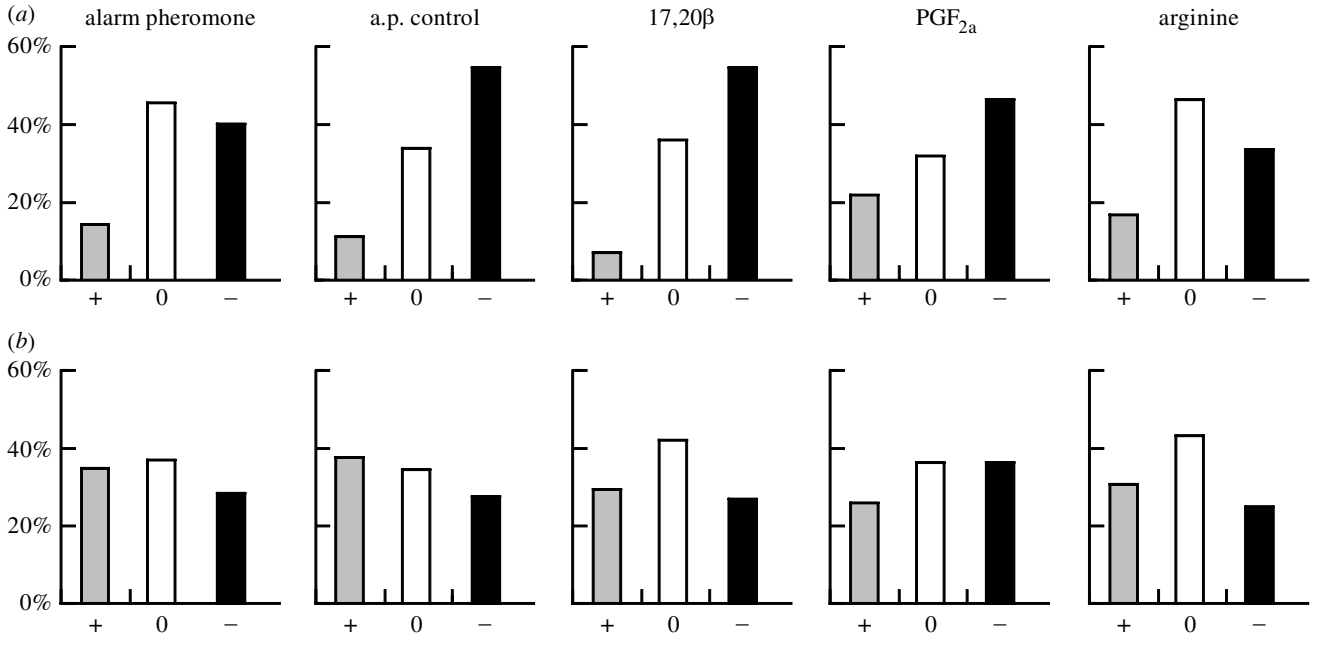

Figure 2. Summary of responses recorded from (*a*) 81 mitral cells and (*b*) 68 ruffed cells. Alarm pheromone (a.p.) is hypoxanthine-3(N)-oxide; alarm pheromone control is hypoxanthine; 17, 20β is preovulatory pheromone 17, 20β-dihydroxyprogesterone; PGF_{2a} is ovulatory pheromone prostaglandine F_{2a}; stimulus concentration is 10^{-9} molar; +, excitation; 0, indifference; −, inhibition.

effectiveness, which are obvious in the EOG during application of different stimuli, were not present in recordings from olfactory bulb relay neurons.

The fish alarm pheromone system is characterized by distinctive epidermal club cells that contain the alarm pheromone, probably hypoxanthine-3(N)-oxide. In contrast to EOG recordings, application of the probable alarm pheromone resulted in a similar effectiveness to that of the preovulatory and the ovulatory pheromones, and the amino acid (figure 2). In summary, natural important stimuli are excellently discriminated by individual mitral and ruffed cells. During application of non-familiar stimuli, responses are slightly more similar, i.e. less discriminative. Contrasting interactions between mitral cells and ruffed cells were found frequently during simultaneous recordings. Details were recently published (Zippel *et al*. 1999, 2000). Dose-dependent declining excitatory or inhibitory responses were only apparent in 40–45% of recordings. In 25%, lowering the stimulus concentration resulted in increasing excitation or inhibition. In 30% of cells indifferent responses, and in 5% reversible response patterns, were recorded. Responses from mitral cells were more frequently inhibitions, and those from ruffed cells excitations. The dominance of mitral cell inhibition was high during non-familiar stimulus application, and less during application of biologically relevant stimuli. Details, however, of response characteristics warrant further investigation. Indifferent neurons are either specialized for application of different stimuli, or, like relay neurons in the rat (Ezeh *et al*. 1993), in which electrical stimulation of the olfactory nerve results in no effect during intracellular recording, are not responsible for olfactory stimuli. Information processing in the goldfish olfactory bulb offers some obvious similarities to the visual system: mitral and ruffed cells' interstimulus and stimulus interaction reminds one of 'on- and off-centre' ganglion cells in the retina, however, also like cortical complex neurons that heavily depend on the 'shape' of the stimulus applied in the retina. That is, an excitatory stimulus turns the mitral cell on and the ruffed cell off, and an inhibitory stimulus turns the mitral cell off and releases the activity of the respective ruffed cell. Therefore mitral cells (and ruffed cells) can become 'on- and off-centre' neurons as well, or remain indifferent depending on the stimulus applied.

In the three series of experiments in which recordings were made from a total of approximately 180 mitral cells and 90 ruffed cells we were not able to determine a 'specific' bulbar area (neither lateral or medial nor rostral or caudal) to which any of the non-familiar or pheromone stimuli was preferentially projected. Hypothetically, behavioural specificities recorded following dissection of different subtracts (see Zippel, this issue) result from stimulus-specific cells somewhere in the olfactory bulb projecting to the respective telencephalic nuclei via different subtracts (see Zippel *et al*. (2000) for details).

In contrast to EOG recordings, in which the importance of an olfactory stimulus at least partly results in significantly different amplitudes (Sorensen *et al*. 1990), a similar image cannot be recorded from olfactory bulb relay neurons. The present study does not contain original EOG data because a number of stimuli (the two purines and hydrocortisone) do not result in a recordable EOG in any concentration (P. W. Sorensen, personal communication), and the rest of the stimuli were applied in low concentrations, which do not result in a recordable EOG either. Significantly different EOG amplitudes recorded at high (10^{-3}, 10^{-4} M) concentrations of amino acids, and after axotomy, when the number of receptor neurons

is at a minimum (Zippel *et al*. 1997), are obviously artefacts, because lower (10^{-5}, 10^{-6} M) concentrations result in similar EOG amplitudes, and below 10^{-6} molar concentrations do not result in a recordable EOG (Zippel *et al*. 1997). The present recordings from olfactory bulb mitral and ruffed cells clearly indicate that many (and in the EOG highly effective and totally ineffective) stimuli in general elicit rather similar amounts of activation, depression or indifferent responses. In different relay neurons, however, responses to different stimuli can vary drastically, i.e. stimuli are excellently discriminated by individual relay neurons. The pheromonal or learned-by-experience importance of a stimulus probably is not detected by epithelial sensors but by central (telencephalic) nuclei, as in other sensory modalities.

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REFERENCES

Ezeh, P. I. & Wellis, D. P. 1993 Organisation of inhibition in the rat olfactory bulb external plexiform layer. *Neurophysiology* **70**, 263–274.

Kosaka, T. 1980 Ruffed cell: a new type of neuron with a distinctive initial unmyelinated portion of the axon in the olfactory bulb of the goldfish (*Carassius auratus*). II. Fine structure of the ruffed cell. *J. Comp. Neurol.* **193**, 119–145.

Kosaka, T. & Hama, K. 1979 Ruffed cell: a new type of neuron with a distinctive initial unmyelinated portion of the axon in the olfactory bulb of the goldfish (*Carassius auratus*). I. Golgi impregnation and serial thin sectioning studies. *J. Comp. Neurol.* **186**, 301–320.

Kosaka, T. & Hama, K. 1981 Ruffed cell: a new type of neuron with a distinctive initial unmyelinated portion of the axon in the olfactory bulb of the goldfish (Carrasius auratus). III. Three-dimensional structure of the ruffed cell dendrite. *J. Comp. Neurol.* **201**, 571–587.

Kosaka, T. & Hama, K. 1982 Structure of the mitral cell in the olfactory bulb of the goldfish (*Carassius auratus*). *J. Comp. Neurol.* **212**, 365–384.

Kosaka, T. & Hama, K. 1982–1983 Synaptic organization in the teleost olfactory bulb. *J. Physiol.* (Paris) **78**, 707–719.

Nickell, W. T. & Shipley, M. T. 1992 Neurophysiology of the olfactory bulb. In *Science of olfaction* (ed. M. J. Serby & K. L. Chobor), pp. 172–212. Heidelberg, Germany: Springer.

Pfeiffer, W. 1982 Chemical signals in communication. In *Chemoreception in fishes* (ed. T. J. Hara), pp. 307–326. New York: Elsevier.

Sorensen, P. W., Hara, T. J., Stacey, N. E. & Dulka, J. G. 1990 Extreme olfactory specificity of male goldfish to the preovulatory steroidal pheromone 17², 20β-dihydroxy-4-pregnen-3-one. *J. Comp. Physiol.* A **166**, 373–383.

Zippel, H. P., Voigt, R., Knaust, M. & Luan, Y. 1993 Spontaneous behavior training and discrimination training in goldfish using chemosensory stimuli. *J. Comp. Physiol.* A **172**, 81–90.

Zippel, H. P., Hansen, A. & Caprio, J. 1997 Renewing olfactory receptor neurons in goldfish do not require contact with the olfactory bulb to develop normal chemical responsiveness. *J. Comp. Physiol.* A **181**, 425–437.

Zippel, H. P., Reschke, C. H. & Korff, V. 1999 Simultaneous recordings from two physiologically different types of relay neurons, mitral cells and ruffed cells, in the olfactory bulb of goldfish. *Cell. Mol. Biol.* **45**, 327–337.

Zippel, H. P., Gloger, M., Lüthje, L., Nasser, S. & Wilcke, S. 2000 Pheromone discrimination ability of olfactory bulb mitral and ruffed cells in goldfish (*Carassius auratus*). *Chem. Senses* **25**, 339–349.

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