

Pheromone Discrimination Ability of Olfactory Bulb Mitral and Ruffed Cells in the Goldfish (*Carassius auratus*)

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Abstract

Significant anatomical differences characterizing mitral cells and ruffed cells were published by Kosaka and Hama in three teleost species. Physiological responses from both types of relay neurons have now been 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. Ruffed cell impulse rates were low, and each action potential triggered a long-lasting, continuously variable, integrated granule cell potential. During olfactory stimulation with important biological stimuli such as preovulatory and ovulatory pheromones, a probable alarm pheromone and amino acids contrasting interactions between mitral cells and ruffed cells resulting in a drastic intensification of centrally transmitted information were frequently recorded. Individual neurons excellently discriminated stimuli. Irrespective of the physiological relevance of stimuli, however, similarities were recorded in the distribution of excitatory, inhibitory and indifferent responses.

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, mitral cells and ruffed cells, lie comparatively close to each other in the plexiform layer. Simultaneous extracellular recordings from both types of relay neurons therefore can be made with a single tungsten electrode.

In contrast to mitral cells (Kosaka and Hama, 1982) ruffed cells glomerular dendrites have no input from epithelial sensory neurons (Kosaka and Hama, 1982–1983), whilst mitral and tufted cells are both innervated by sensors from the olfactory epithelium. Anesthesia of the olfactory epithelium in goldfish results in a blockade of the driving input from spontaneous active receptor neurons, and the activity of mitral cells is reversibly blocked (Korff and Zippel, 1997; Zippel *et al.*, 1999). In consequence a partial or total blockade of lateral inhibition via granule cells activated by mitral cells results in a high and frequently rhythmic activity of ruffed cells (Zippel *et al.*, 1999). Whether a direct lateral inhibition via granule cells present in the goldfish also exists between different relay neurons in mammals (Nickell and Shipley, 1992) is unknown.

Three series of experiments using biologically important stimuli structurally known in goldfish were made:

1. A comparison of the effectiveness of preovulatory phero-

mones which result in significantly different amplitudes in the electro-olfactogram (EOG).

2. A comparison of effectiveness of structurally and chemically different naturally important stimuli, preovulatory and ovulatory pheromones, and amino acids.
3. Effects of stimuli which do not result in a recordable EOG in any concentration, a probable alarm pheromone [hypoxanthine-3(*N*)-oxide], hypoxanthine and hydrocortisone were compared with effects recorded during the application of a number of important (see 2) olfactory stimuli.

The present experiments should also present evidence for two major points: (i) does the sensory input of the olfactory system, in contrast to other sensory systems, really discern biologically highly relevant and irrelevant stimuli resulting in significantly different EOG amplitudes? and (ii) does no recordable EOG potential really mean that a stimulus applied to the olfactory epithelium is ineffective for the olfactory system?

Materials and methods

Animals and experimental procedure

In three series of experiments goldfish (1.5–2.0 years old, 10–12 cm in length, 30–45 g) reared in the laboratory were used. Fish were anesthetized with tricaine (3-amino benzoic

Table 1 Compounds tested

Abbreviation	Chemical name	Common name
4-Pregnen 21-carbon steroids (progesterone-like compounds)		
20 α P	4-pregnen-20 α -ol-3-one	4-pregnen-20 α -ol-3-one
20 β P	4-pregnen-20 β -ol-3-one	4-pregnen-20 β -ol-3-one
17,20 β P	4-pregnen-17 α ,20 β -diol-3-one	17 α ,20 β -dihydroxyprogesterone
17,20 α P	4-pregnen-17 α -diol-3-one	17 α ,20 α -dihydroxyprogesterone
17,20 β ,21P	4-pregnen 17 α ,20 β ,21-triol-3-one	17 α ,20 β ,21-trihydroxyprogesterone
19-carbon steroid (androgene)		
AD	4-androsten-3,17-dione	androstenedione

acid ethyl ester, A5040 Sigma, 300 mg/l tap water) and the cranium over the olfactory bulbs and tracts removed. To avoid reflex movements during the experiment fish were immobilized with Horvi curarine (tubocurarine chloride 0.5 mg/ml; 2 μ g/g) and the gills perfused with tap water. In each of the three series of experiments ~60 recordings from mitral cells and 45 recordings from ruffed cells were made. Simultaneous extracellular recordings were made with a single tungsten electrode (A-M systems, cat. no. 5770; 5–7 M Ω).

Stimuli were repetitively applied for 15 s to the olfactory epithelium. Stimulus applications were interrupted by 180 s tap water application of similar (1 ml/min) velocity during the interstimulus phases. These long interstimulus intervals are essential for functional recovery of epithelial receptor neurons (Zippel *et al.*, 1995a). Recordings were made on-line using 'Discovery' (Brainwave software), and evaluations with Discovery subprograms which enable discrimination of individual and simultaneously recorded cells. Stimulus effectiveness was tested visually and with the Cox-Stuart *U*-test. [For further details see (Schild, 1985; Schild and Zippel, 1986; Fischer and Zippel, 1989).]

Stimuli

In contrast to higher vertebrates, a great number of important natural stimuli, including pheromones, are structurally known in the goldfish (Stacey and Kyle, 1983; Stacey and Sorensen, 1986; Stacey *et al.*, 1986, 1987, 1989, 1994; Stacey, 1987; Sorensen *et al.*, 1988, 1990; Sorensen and Stacey, 1989; Sorensen, 1992; Zippel *et al.* 1993, 1995b; Sorensen and Scott, 1994), and therefore can be applied in defined molarities. Stimuli were purchased from Sigma (Munich, Germany), and the probable alarm pheromone from Menai Organics Limited. Stock solutions (1 ml) were made up at a concentration of 10^{-4} M and kept at -18°C . Because initial experiments have shown that goldfish probably release many hormones and hormonal metabolites (Van Der Kraak *et al.*, 1989) several precursors and metabolites of the preovulatory steroidal pheromones 17,20 β P (for definitions see Table 1) were investigated in a first series of experiments: 17,20 β P and a metabolite 17,20 β ,21P, both

of which are highly stimulatory steroids according to EOG recordings, three much less stimulatory (Sorensen *et al.*, 1990) 21-carbon steroids and one 19-carbon steroid (Table 1).

In a second series the effectiveness of two ovulatory pheromones (prostaglandin $F_{2\alpha}$ = PGF $_{2\alpha}$ and a metabolite 15-ketoprostaglandin $F_{2\alpha}$ = 15K-PGF $_{2\alpha}$, 10^{-7} , 10^{-9} , 10^{-11} M) were compared with the preovulatory pheromone 17,20 β P (10^{-9} , 10^{-11} , 10^{-13} M), and two amino acids, Arg and Pro (10^{-7} M). Amino acids represent food stimuli which are rapidly learned and long-term memorized (Zippel *et al.*, 1993; von Rekowski *et al.*, 1995). Amino acids were applied as control stimuli to investigate the stimulus specificity of mitral cells and ruffed cells. From EOG data, behavioral experiments and TEM anatomical findings following olfactory nerve axotomy there is evidence that microvillous olfactory receptor neurons mediate responses to pheromonal cues whilst ciliated receptor cells mediate responses during amino acid application (Zippel *et al.*, 1997a; Hansen *et al.*, 1999).

In a third series of experiments effects of stimuli which do not result in a recordable EOG (P.W. Sorensen, personal communication) were investigated in comparison with EOG-effective stimuli. Two purines, hypoxanthine and hypoxanthine-3(*N*)-oxide (10^{-7} , 10^{-9} , 10^{-11} , 10^{-13} M), a probable alarm pheromone [behavioral effects of the alarm pheromone 'Schreckstoff' were first published by von Frisch (von Frisch, 1938, 1941); thin-layer chromatography by Argentini (Argentini, 1976) showed identical values for the probable alarm pheromone hypoxanthine-3(*N*)-oxide and the natural product, and in comparison with several other compounds only hypoxanthine-3(*N*)-oxide was behaviorally active; see review by Pfeiffer (Pfeiffer, 1982)]. Hypoxanthine (10^{-7} , 10^{-9} M), which does not elicit a fright reaction, and hydrocortisone (10^{-9} M) do not result in a recordable EOG. 17,20 β (10^{-9} M) and PGF $_{2\alpha}$ (10^{-7} , 10^{-9} M) served as control stimuli.

Results

Response characteristics of mitral cells and ruffed cells

During extracellular recordings, potentials of mitral cells

and ruffed cells can easily be discriminated (Zippel *et al.*, 1999), and are in excellent agreement with detailed and careful anatomical investigations (Kosaka and Hama, 1979, 1982, 1982–1983; Kosaka, 1980). Each ruffed cell action potential triggers an integrated granule cell potential (Figure 1A–C). In cases where the recording electrode is located closer to the initial pedunculate protrusions, the ruffed cell's action potential is small and the amplitude of the summed up granule cell potential high (Figure 1A). In cases where the electrode is located close to the ruffed cell's cell body, the ruffed cells action potential is of greater amplitude (Figure 1B). In cases where the electrode is located extremely close to the ruffed cell's cell body itself, the action potential of the ruffed cell is greater than the summed up granule cell potential (Figure 1C). The delay between the peaks of the ruffed cell's action potential and the summed up granule cell potential is always 3 ms. In contrast, mitral cell potentials are single 1–1.5 ms action potentials (Figure 1D). If a small summed up granule cell potential is recorded it is always much smaller than the mitral cell action potential, and in a 4 ms peak-to-peak delay (Figure 1E).

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 (Zippel *et al.*, 1999). During application of an inhibitory olfactory stimulus (or anesthesia) (Zippel *et al.*, 1999), the driving activity of receptor neurons is blocked and the mitral cell inhibited. Consequently lateral inhibition of ruffed cells via granule cells activated by mitral cells is no longer present, and this results in a higher activity of ruffed cells. During epithelial application of an excitatory olfactory stimulus, increased activity of receptor cells results in the excitation of mitral cells which activate granule cells laterally, inhibiting ruffed cells (see Figure 4).

The interaction of mitral and ruffed cells can be seen clearly in Figure 2: Figure 2A shows the activity of a mitral cell and a ruffed cell during the interstimulus phase (0–180 s), and during the 15 s stimulus application. From the expanded views it is evident that, during interstimulus activity, ruffed cell potentials are recorded when pauses in the mitral cell activity are present (Figure 2B) and, during total inhibition of mitral cell activity with stimulus application, a simultaneous release of the ruffed cell's activity is recorded (Figure 2C).

During repetitive stimulation, stimuli were applied three times for 15 s, with 180 s interstimulus phases during which water was applied. In the majority of relay neurons (75–80%) an immediate return to the interstimulus activity level after stimulus application was apparent. In 20–25% of mitral and ruffed cells, however, persisting or antagonistic 'off-effects' were recorded. Persisting inhibitions (Figure 3, M1) and excitations (Figure 3, M2), and antagonistic

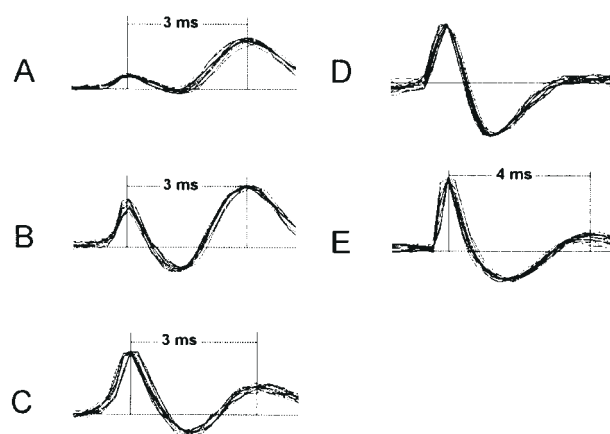


Figure 1 Superposed extracellular recordings from ruffed cells and mitral cells. (A–C) One-millisecond ruffed cell potentials permanently triggering an integrated granule cell potential of 3 ms duration (3 ms peak to peak). (D, E) One-millisecond mitral cell potentials. If a mitral cell triggers granule cell potentials, the integrated potential (E) has a small amplitude (4 ms peak to peak). For details see the text.

(excitation after inhibition during stimulus application—Figure 3, M3; inhibition after excitation during stimulus application—Figure 3, M4) effects were recorded up to 120–150 s during the subsequent interstimulus phases.

Probable alarm pheromone, control stimuli, preovulatory and ovulatory pheromones, and amino acid

From Figure 4 effects recorded from olfactory bulb relay neurons during epithelial application of stimuli which do not result in EOG responses are clearly visible. In Figure 4A application of all the stimuli results in a significant excitation of the mitral cell and a simultaneous total lateral inhibition of the ruffed cell. In Figure 4B,C inhibitions are recorded from the mitral cell and a release of activity is recorded from the ruffed cell; in Figure 4B application of hydrocortisone results in no modulation of activity. In Figure 4D slight inhibitions are present in the mitral cell during application of the probable alarm pheromone, hypoxanthine and Arg, excitations occur during application of PGF_{2α} and hydrocortisone, and no effect is evident during stimulation with the preovulatory pheromone. In the simultaneously recorded ruffed cell the respective contrasting responses are present.

Dose-response characteristics

During application of stimuli in different molarities, an unexpectedly large variety of response characteristics was recorded (Figure 5A): dose-dependent declining excitatory or inhibitory responses (Type I) were only apparent in 25–35% of recordings. In 10–20% of neurons similar excitations or inhibitions were recorded (Type II) independent from the stimulus concentration applied. In 20–30% of relay neurons indifferent responses (Type V) were found. In 20–30% of neurons unexpected effects, i.e. increasing

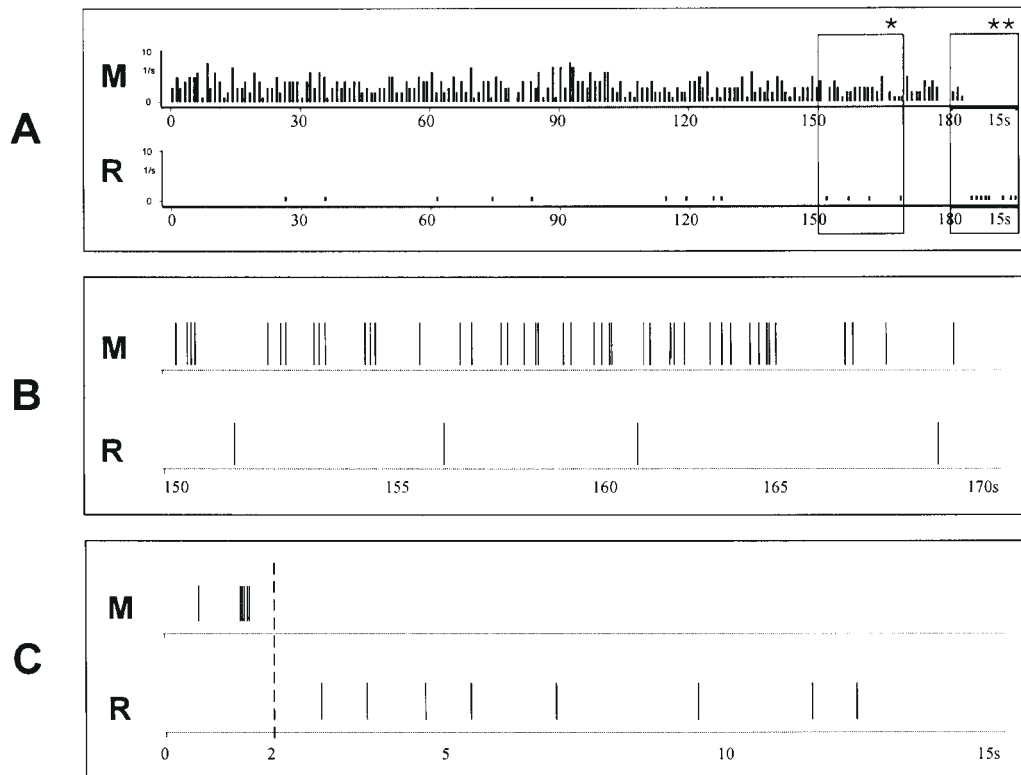


Figure 2 Simultaneously recorded potentials of a mitral cell and a ruffed cell. **(A)** The greater 180 s interstimulus activity of a mitral cell (M) and the low interstimulus activity of a ruffed cell (R). During the 15 s stimulus application, a total inhibition of M and an excitation of R is present. Vertical bars = number of potentials; bin width 1 s. **(B, C)** Expanded views of activity. B = Interstimulus activity of M and R (marked with one asterisk in A); C = activity during stimulus application (marked with two asterisks in A). Stimulus application to the olfactory epithelium starts with a 2 s delay after stimulus onset (dotted line). For details see the text, and in particular Materials and methods.

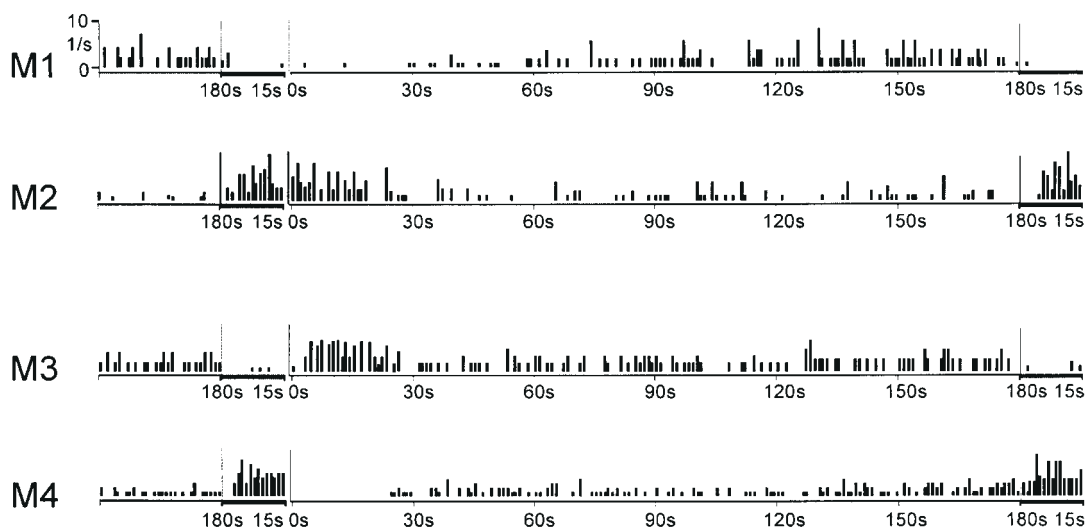


Figure 3 Persisting stimulus effects and antagonistic 'off'-effects. Mitral cell 1 (M1) is totally inhibited during stimulus application. During the following interstimulus phase inhibition persists for ~90 s. In mitral cell 2 (M2) an excitation during stimulus application persists for ~30 s during subsequent interstimulus phase. From mitral cell 3 (M3) a 30 s excitation is recorded after 15 s total inhibition during stimulus application during the subsequent interstimulus phase, and from mitral cell 4 (M4), a 25 s total inhibition is present during the subsequent interstimulus phase following a strong excitation during stimulus application. Time scale: interstimulus phases = 180 s, stimulus application = 15 s. Each recording starts with a 30 s (150–180 s) final cutting of a 180 s interstimulus phase, followed by the 15 s stimulus application, a second 180 s total interstimulus phase and a second repetitive 15 s application of the same stimulus; vertical bars = number of potentials per second.

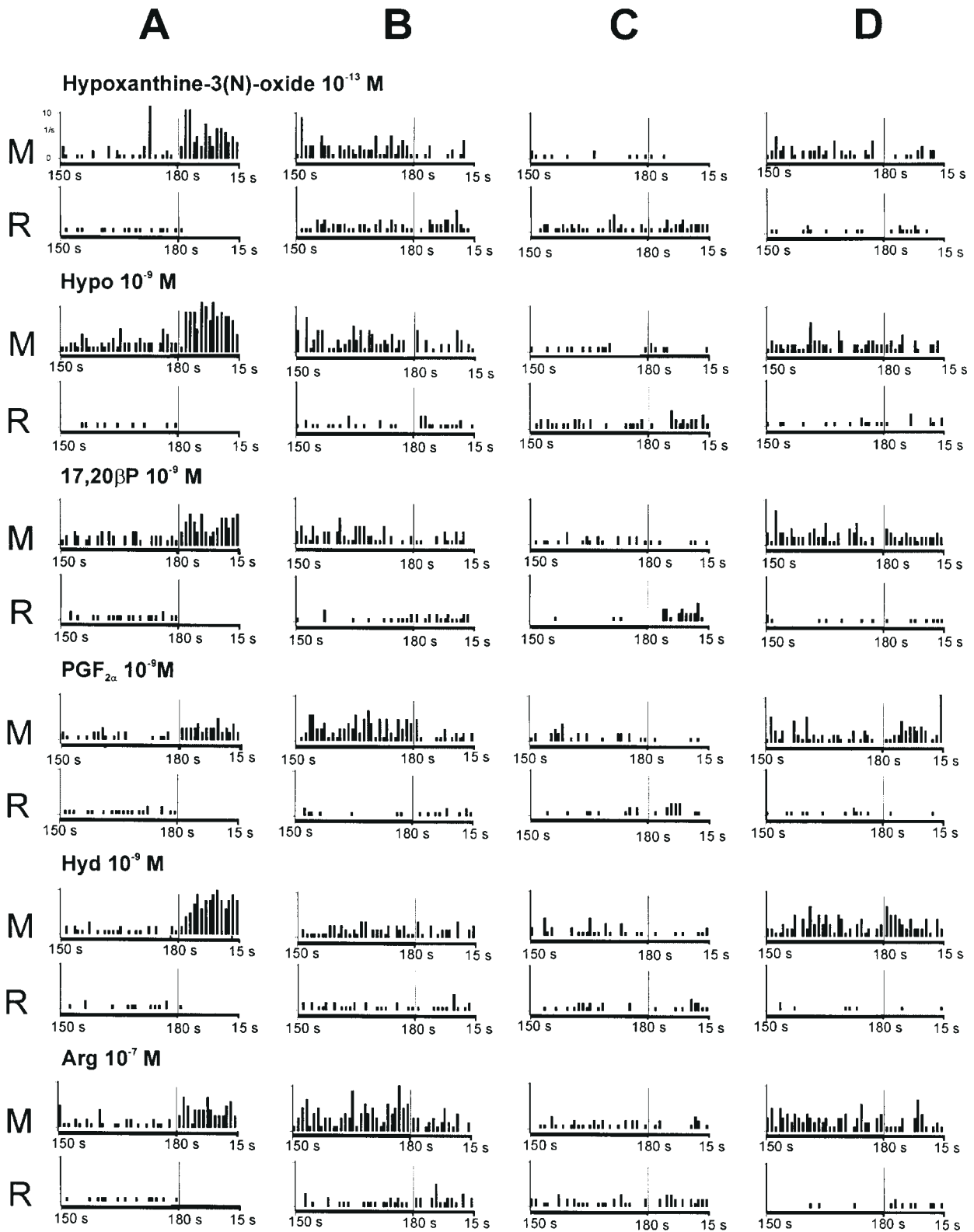


Figure 4 Activity patterns simultaneously recorded from four pairs of mitral cells and ruffed cells during stimulation with the probable alarm pheromone and several control stimuli. **(A)** Significant excitations of the mitral cell (slightly weaker PGF_{2 α}) and inhibitions of the ruffed cell; **(B)** slight inhibitions of the mitral cell and excitations of the ruffed cell (hydrocortisone indifferent); **(C)** inhibitions and slight inhibitions of the mitral cell, and excitations or slight excitations of the ruffed cell; **(D)** quantitatively different effects on mitral cells and ruffed cells during application of different stimuli. Pheromones and control stimuli 10^{-9} M. Recordings present the last 30 s of the 180 s interstimulus phase and the 15 s stimulus application; vertical bars represent the number of potentials per second; for stimulus abbreviations see Materials and methods.

excitation or inhibition during application of lower stimulus concentrations (Type III), were recorded. Finally, in 5% of neurons reversible response patterns (Type IV) were present.

From Figure 5B it is evident that the above distribution of percent values was recorded mainly from mitral cells. The selected examples in Figure 5B clearly demonstrate that in ruffed cells there is a greater variety in the percent values for the respective response characteristics.

Dose–response characteristics were investigated for all the stimuli applied during the different experiments. From the comparatively similar distribution of response types shown in Figure 5B, it is evident that there was no significant distribution of the response pattern for any of the stimuli for the probable alarm pheromone, the control stimulus, the ovulatory pheromone or the amino acid. In general there was no significant trend for the distribution of any response characteristic which might support evidence for the biological ‘importance’ of any of the applied stimuli.

Stimulus discrimination

During application of important natural stimuli three types of responses were recorded from single mitral cells and ruffed cells:

1. Significantly discriminative responses: in these recordings cells responded during the epithelial application of different stimuli with excitation or inhibition, or remained indifferent. A significant discriminative response was recorded from 30–40% of mitral cells in the different experiments.
2. Discriminative responses, i.e. similar (only excitations or only inhibitions) but quantitatively different responses, were recorded from 10–20% of mitral cells.
3. Non-discriminative effects, i.e. cells in which application of different stimuli resulted in quantitatively similar responses (excitations or inhibitions) or no response, were recorded from ~50% of neurons.

In ruffed cells the number of discriminative and significantly discriminative responses versus non-discriminative responses showed a greater variation than in mitral cells, which in part may be the result of smaller amounts of cells (Figure 6).

In Figure 6 the amount of discriminative and non-discriminative responses is shown in mitral cells during application of structurally similar stimuli, such as hypoxanthine-3(*N*)-oxide and hypoxanthine, and structurally significantly different stimuli, such as hypoxanthine-3(*N*)-oxide and the preovulatory pheromone, the ovulatory pheromone, a control stimulus and an amino acid. The number of discriminative and of non-discriminative responses is surprisingly similar (45–50 versus 50–55% respectively) in pairs of resembling and pairs of structurally very different stimuli. The just mentioned distribution was also apparent in series 1 (preovulatory pheromones) and

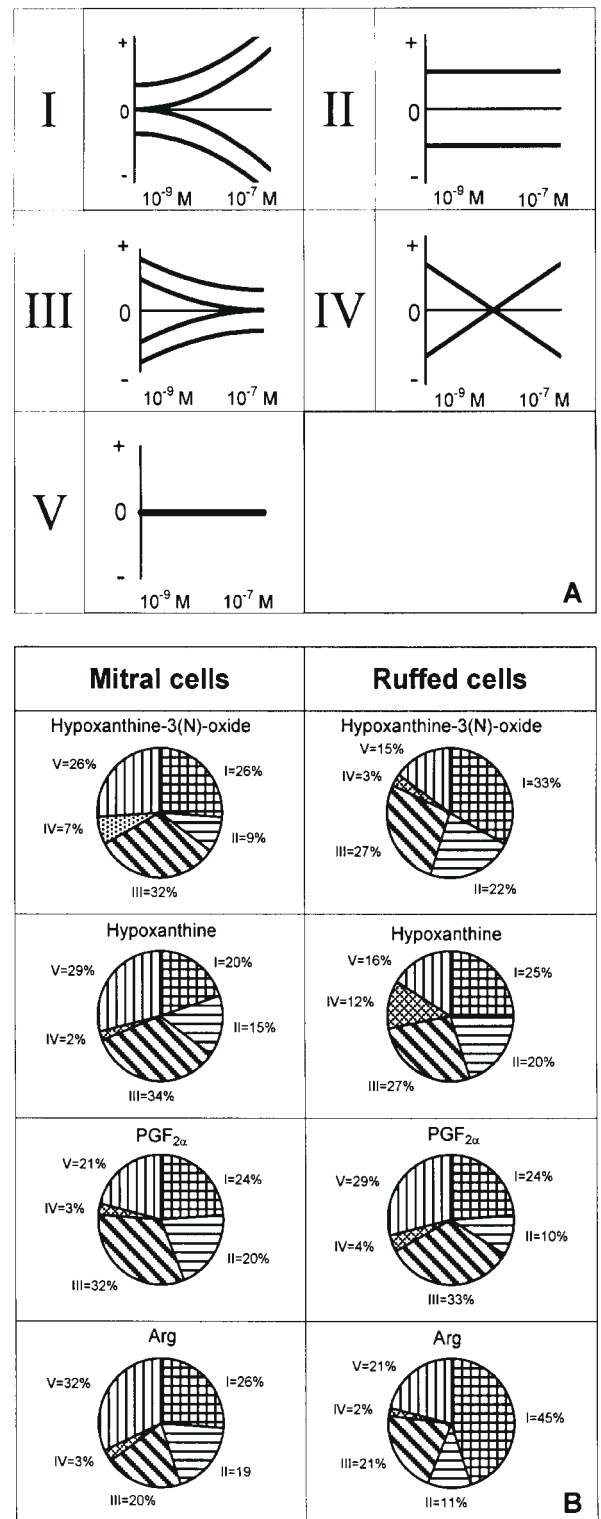


Figure 5 (A) Schematic representation of different dose–response patterns recorded from mitral cells and ruffed cells. I, decreasing excitations and inhibitions; II, excitations or inhibitions remain similar; III, increasing excitations or inhibitions; IV, excitation (or inhibition) during application of low, and reverse patterns recorded during application of high concentration; V, no response to any concentration = indifferent. (B) Some typical distributions of the different response patterns recorded from 65 mitral cells and 45 ruffed cells during application of different stimuli.

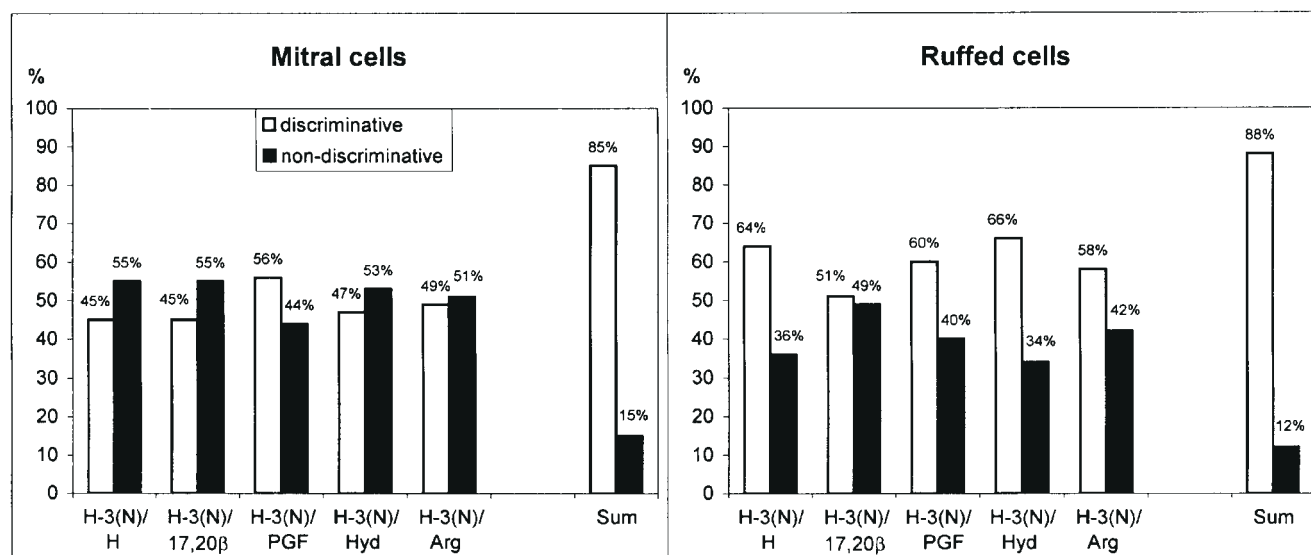


Figure 6 Stimulus discrimination by mitral cells and by ruffed cells. The figure presents some examples with respect to the probable alarm pheromone. Responses during application of structurally similar and dissimilar pairs of stimuli to 75 mitral cells and 50 ruffed cells are shown on the left hand side and effects recorded following stimulation with all the stimuli (Sum) are shown on the right. During application of pairs of stimuli 45–55% of mitral cells discriminate the respective stimuli, and in 45–55% of neurons non-discriminative responses were recorded. The discriminative activity of ruffed cells was more variable, and slightly greater (50–65%). The greater the number of stimuli the greater the number of discriminative responses: discriminative responses were recorded from 85% of mitral cells and 88% of ruffed cells. H-3(N) = hypoxanthine-3(N)-oxide; H = hypoxanthine; 17,20 β = preovulatory pheromone; PGF = PGF_{2 α} ; Hyd = hydrocortisone. For a definition of discrimination and further details see the text.

series 2 (ovulatory pheromones, preovulatory pheromone, and amino acids).

If, however, effects were compared during application of all the stimuli in the respective experimental series, the number of discriminative responses increased to values above 80% (e.g. Figure 6, Sum); i.e., 21-carbon steroids (progesterone-like compounds) applied during the first series (preovulatory pheromones), preovulatory, ovulatory pheromones and amino acid applied during the second series, and the probable alarm pheromone and diverse control stimuli (Figure 6) are excellently discriminated by olfactory bulb relay neurons.

Overall view on effects

Figure 7A,B summarizes the numbers of excitations, indifferent responses and inhibitions recorded from mitral cells and ruffed cells. The percent values are based on data from at least 60 mitral cells and 45 ruffed cells. During application of different naturally important stimuli the discriminative ability of both types of relay neurons is excellent (see above). The percent values for excitations, indifferent responses and inhibitions, however, were surprisingly similar for stimuli of significantly different amplitudes in the EOG, and also for those which were totally ineffective in the EOG. In comparison to the rest of the applied stimuli, suppressive responses were ~10% greater during application of the preovulatory pheromone 17,20 β P and the ovulatory pheromone 15K PGF_{2 α} in mitral cells, and the excitatory

responses were ~10% greater during application of AD (Figure 7A).

The probable alarm pheromone was most effective when applied at an extremely low molarity (10⁻¹³ M), resulting in 55% of suppressive responses. This value is similar during application of 10⁻⁹ M hypoxanthine (10⁻¹³ M not tested), and only slightly lower (49%) during application of the control stimulus hydrocortisone (Figure 7B). The alarm pheromone, hypoxanthine, and hydrocortisone do not result in a recordable EOG when applied to the olfactory epithelium in similar concentrations (P.W. Sorensen, personal communication).

Discussion

Contrasting interactions between mitral cells and ruffed cells were present during the epithelial application of pheromones, amino acids and control stimuli that even in the lowest concentration resulted in a drastic intensification of centrally transmitted information. More frequently an inhibition was recorded from mitral cells, and decreasing lateral inhibition via granule cells more frequently resulted in the activation of ruffed cells. The activation of ruffed cells resulted in the activation of pools of granule cells laterally, thereby inhibiting pools of mitral cells in their vicinity. Simultaneous and synchronous activation of great numbers of granule cells (Kosaka, 1980) possibly supports a long-lasting inhibition initially induced by application of an inhibitory olfactory stimulus.

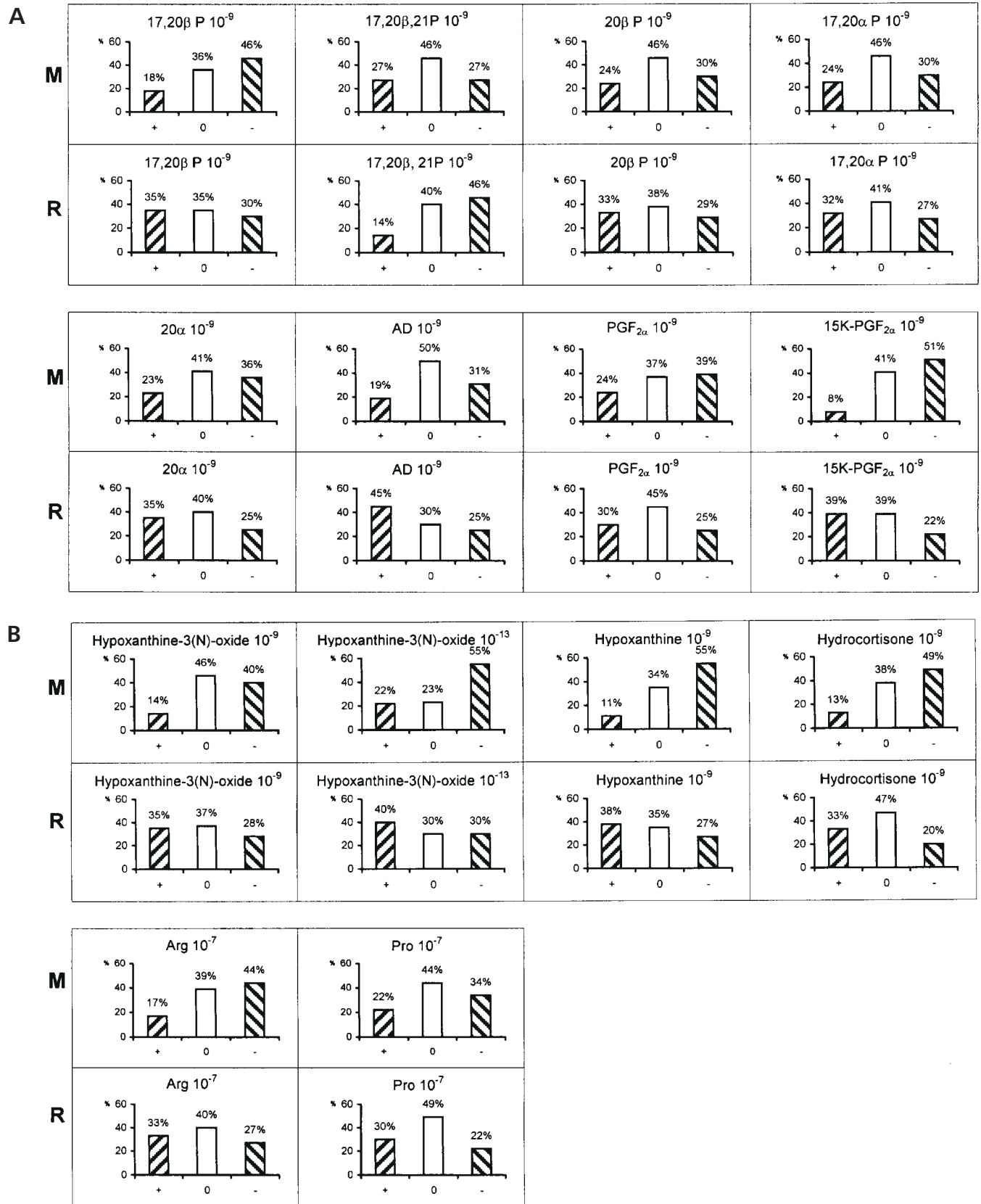


Figure 7 Summary of response patterns recorded in all the experiments from mitral cells and ruffed cells. Each response pattern is based on recordings from at least 60 mitral cells and 45 ruffed cells. **(A)** Preovulatory and ovulatory pheromones (10^{-9} M). **(B)** Probable alarm pheromone (10^{-9} , 10^{-13} M), control stimuli (10^{-9} M) and amino acids (10^{-7} M). For abbreviations see Table 1 and Materials and methods; for further details see text.

In contrast to EOG recordings (Sorensen *et al.* 1990), the 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 an excellent indicator of olfactory organ sensitivity and specificity to odorants in fishes.

Olfactory bulb relay neurons frequently respond to a comparatively large number of olfactory stimuli—amino acids, preovulatory and ovulatory stimuli—and contrasting interactions between mitral cells and ruffed cells were frequently recorded during stimulus application. Significant differences in the effectiveness, which are present during application of different stimuli in the EOG, were, however, not present in recordings from olfactory bulb relay neurons.

Sorensen *et al.* (Sorensen *et al.*, 1990) reported that low concentrations of preovulatory pheromones release endocrinological reactions; however, they do not result in a recordable EOG in the olfactory epithelium. Furthermore, a direct correlation between the amplitude of the EOG and the value of the physiological reaction has not yet been proved (Scott and Sorensen, 1994). Finally, there seems to be no significant correlation between the release of steroids by ovulatory female goldfish and the olfactory sensitivity of males (Sorensen and Scott, 1994).

The fish alarm pheromone system is characterized by distinctive epidermal club cells that contain the alarm pheromone, probably hypoxanthine-3(*N*)-oxide (Argentini, 1976; Pfeiffer, 1982). In contrast to EOG recordings, the present findings clearly show that application of the probable alarm pheromone resulted in a similar effectiveness as the preovulatory and ovulatory pheromones, amino acids, and the EOG-ineffective control stimuli (hypoxanthine, hydrocortisone).

Whether or not the above-mentioned probable alarm pheromone really is the only stimulus eliciting the fright reaction is unclear at present since a number of critical papers (Lebedeva *et al.*, 1975; Kasumyan and Lebedeva, 1979; Kasumyan and Pashchenko, 1982; Kasumyan and Ponomarev, 1987) have been published. The possibility that a combination of different chemical substances of different nature are necessary to elicit the full behavioral response, however, warrants further investigation.

In summary, natural important stimuli are excellently discriminated by mitral- and ruffed cells. During application of non-familiar stimuli, responses were slightly more uniform than for familiar stimuli, i.e. less discriminative (Zippel *et al.*, 1999). Contrasting interactions between mitral cells and ruffed cells, however, were frequently found during simultaneous recordings. The present findings clearly show

that olfactory bulb relay neurons excellently discriminate the naturally important stimuli, though they are not highly 'specialized' for a certain class of stimuli like lipid-pre-ovulatory pheromones, ovulatory pheromones or amino acids. The recently published findings that microvillous olfactory receptor cells mediate the response to pheromonal cues and that ciliated receptor cells are responsible for responding to food (amino acid) stimuli (Zippel *et al.*, 1997a; Hansen *et al.*, 1999) do not indicate that the axons of different types of receptor cells terminate in highly specific separate glomeruli at the bulbar surface in intact specimens. This question again warrants detailed investigations, e.g. recordings from single olfactory neurons in the epithelium before and after olfactory nerve axotomy. Dose-dependent declining excitatory or inhibitory responses were apparent in only 40–45% of recordings. In 25% lowering the stimulus concentration resulted in increasing excitation or inhibition. In 30% of cells indifferent responses were recorded, as were reversible response patterns in 5%.

In the three series of experiments in which recordings were made from a total of ~180 mitral cells and 135 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, behavioral specificities recorded following dissection of different subtracts (Døving and Selset, 1980; Stacey and Kyle, 1983; von Rekowski and Zippel, 1993; Zippel, 1993; Zippel *et al.*, 1993; Hamadami *et al.*, 2000) result from stimulus-specific cells somewhere in the olfactory bulb projecting to the respective telencephalic nuclei via different subtracts.

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 concentrations too low to produce a recordable EOG. Significantly different EOG amplitudes recorded during 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.*, 1997b, Hansen *et al.*, 1999) are obviously artefacts, because lower (10^{-5} , 10^{-6} M) concentrations result in similar EOG amplitudes, and below 10^{-6} M concentrations do not result in a recordable EOG (Zippel *et al.*, 1997b). 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 similar amounts of activation, depression or indifferent responses. In different relay neurons, however, responses to different stimuli can vary drastically, i.e. stimuli are discriminated excellently 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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