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Adaptation-induced Changes in Sensitivity in Frog Olfactory Receptor Cells

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Abstract

The suction pipette technique was used to study simultaneously the odour-induced action potential and receptor current responses in frog olfactory receptor cells, which were exposed to the odour cineole for 1 s by rapidly exchanging the solution bathing their cilia. The frequency of action potential firing increased as the odour concentration was raised and saturated within a 15-fold elevation above the odour threshold, while the number of spikes fired initially grew at low-to-intermediate concentrations but then declined at higher concentrations. The receptor current response rose steadily and showed no clear sign of saturation over the 300-fold range of cineole concentration employed. The effect of adaptation on the sensitivity of olfactory receptor cells was investigated by first exposing the cell for 4 s to an adapting pre-pulse and then stimulating with a 1 s test pulse. As the pre-pulse concentration was increased, adaptation led to a progressive shift of the dose–response relationships towards higher test pulse concentrations. This resulted in a steep decline in the sensitivity of the receptor current response, combined with an even more dramatic fall in the sensitivity of the spiking responses, since the higher pre-pulse concentrations prevented the generation of action potentials at test pulse concentrations which still evoked a receptor current response.

Introduction

Exposure of amphibian olfactory receptor cells to odour generates an inward receptor current (Firestein and Werblin, 1989; Kurahashi, 1989). The ensuing depolarization of the cell (Trotier and MacLeod, 1983) leads to the generation of action potentials, an increase in stimulus generally causing an increase in action potential frequency (Mathews, 1972; Getchell and Shepherd, 1978a). For high odour concentrations a progressive decline in the amplitude of individual spikes has been observed (Shibuya and Shibuya, 1963; Trotier and MacLeod, 1983), but neither the precise dose-dependence of the action potential response nor the decline in spike amplitude has been investigated in detail.

The responses to steady stimuli and their effect on sensitivity has been widely investigated in many sensory systems, but have received little attention in olfaction. The reason for this comparative neglect may lie in the difficulty of obtaining recordings of sufficiently long duration from olfactory receptor cells with the recording techniques which have hitherto commonly been employed. In contrast, the suction pipette technique (Baylor *et al.*, 1979; Lowe and Gold, 1991) allows stable recording of the odour response for periods of up to 4 h, making it the ideal recording configuration to investigate background-induced changes in sensitivity (Reisert and Matthews, 1999).

Materials and methods

The techniques for tissue preparations, electrophysiological recording and solution changes are described briefly below; a more detailed description can be found elsewhere (Reisert and Matthews, 1999).

Frogs (*Rana temporaria*) were killed by rostral and caudal pithing. The basal olfactory epithelia were placed in a Ringer-filled Petri dish and cut lightly until the tissue began to dissociate. Cell were transferred to the recording chamber, which was mounted on a inverted microscope with phase contrast optics.

Ringer solution contained 111 mM NaCl, 2.5 mM KCl, 1.6 mM $MgCl_2$, 1 mM $CaCl_2$, 0.01 mM EDTA, 3 mM HEPES, 10 mM glucose, pH adjusted to 7.7 with NaOH. Rapid solution changes for odour stimulation were carried out by stepping the interface between parallel streams of solution across the tip of the suction pipette (Hodgkin *et al.*, 1985). Four streams of solution emerged from grooves cut into the back of the recording chamber, which allowed multiple rapid solution changes (~70 ms for 90% relaxation of the junction current between bath and pipette solutions).

The suction pipette technique was used to record odour-induced electrical responses (Baylor *et al.*, 1979; Lowe and Gold, 1991). The cell body of an isolated olfactory receptor cell was drawn into a suction pipette, leaving the cilia exposed to the superfusing solution. The suction pipette

current was recorded with a patch clamp amplifier (Warner PC-501A, Warner Instruments, Hamden, CT) and digitized over a wide bandwidth (filtered DC-500 Hz, sampled at 1000 Hz) by an IBM PC-compatible microcomputer equipped with an intelligent interface card (Cambridge Research Systems, Rochester, UK). The firing frequency was calculated for each spike as the reciprocal of the mean of the two time intervals between that spike, its predecessor and its successor.

Results

Responses to brief odour stimuli

Figure 1 shows a dose–response family recorded from an olfactory receptor cell in response to a 1 s exposure to the odour cineole, recorded with the suction pipette technique. At 1 μM no response was elicited, while 2 μM generated a short train of action potentials after a considerable delay. The underlying receptor current became clearly visible at 5 μM and a longer spike train was fired at a higher frequency. Further increases in cineole concentration shortened the spike train to a high frequency burst early in the raising phase of the receptor current, which had now become quite prominent. At the highest stimulus concentrations the spike train shrank to 23 spikes fired at ~50 Hz at the onset of stimulation and the receptor current displayed two phases: an initial peak followed by a second plateau component, which could outlast the end of stimulation by several seconds.

The frequency of spike firing rose monotonically with increasing odour concentration; the dose–response relationship could be fitted with a Hill function, with Hill coefficients ranging from 1.2 to 2.8 (average 1.8, six cells), reflecting the relatively steep dependence of spike firing on the stimulus strength. Interestingly, the longest spike trains were fired during the steepest part of this dose–response relationship. The dynamic range of the receptor current response proved to be larger than the 300-fold increase of cineole concentration which was employed in this study since no clear sign of response saturation could be observed within this concentration range.

Adaptation of the odour response

Adaptation was investigated by exposing an olfactory receptor cell to a 4 s pre-pulse and subsequently stimulating with a 1 s test pulse. The receptor current response to a pre-pulse with the relatively low concentration of 5 μM (Figure 2B) was transient and terminated within the pre-pulse duration. The subsequent 20 μM test pulse evoked a short train of action potentials during the raising phase of the receptor current, but both spike frequency and peak receptor current were reduced in comparison with control conditions in the absence of the pre-pulse (Figure 2A). When the pre-pulse concentration was raised to 10 μM the receptor current did not recover before the onset of the test

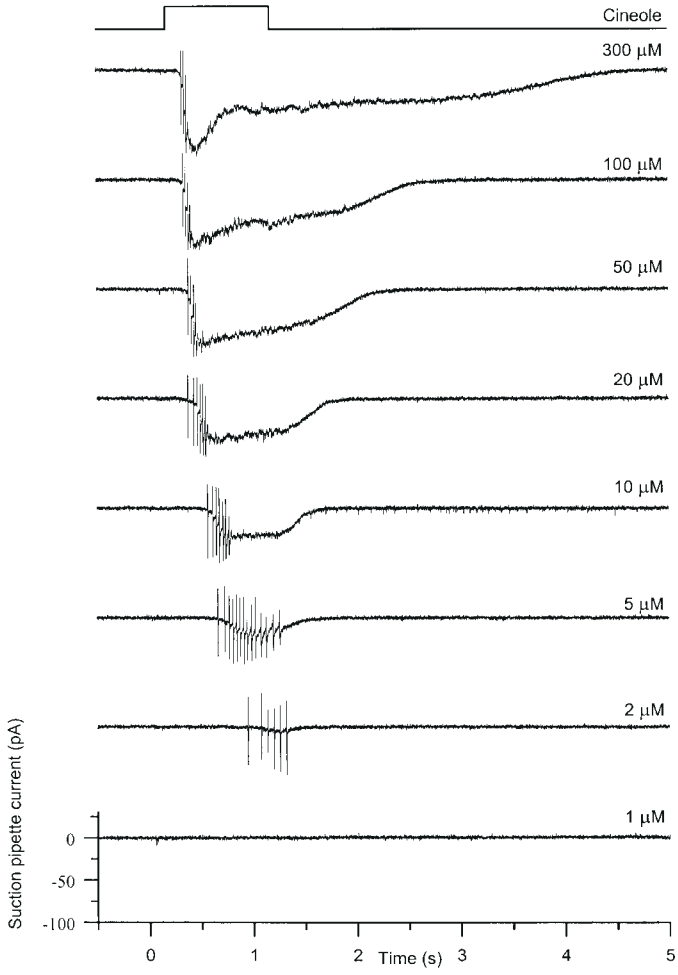

Figure 1 Dose–response family recorded from an olfactory receptor cell with the suction pipette technique. An isolated olfactory receptor cell was stimulated for 1 s as indicated by the solution monitor (upper trace) with the odour cineole at the concentration shown next to each trace. Reproduced from (Reisert and Matthews, 1999).

pulse response. Furthermore, although the test pulse still evoked a receptor current response, its amplitude was reduced still further and no action potentials were elicited. Even higher test pulse concentrations were also unable to evoke action potentials under these adapting conditions (not shown).

The progressive reduction of the receptor current response to the test pulse with increasing pre-pulse concentration translated into a progressive shift of the dose–response relationships derived from these responses to higher odour concentrations. A comparable shift in the dose–response relationship for spike frequency could only be observed at lower pre-pulse concentrations, since at higher pre-pulse concentrations the cell remained silent (see Figure 2C).

The dependence of receptor current sensitivity on the pre-pulse concentration is shown in Figure 3A. Sensitivity, the response magnitude per unit stimulus, was calculated

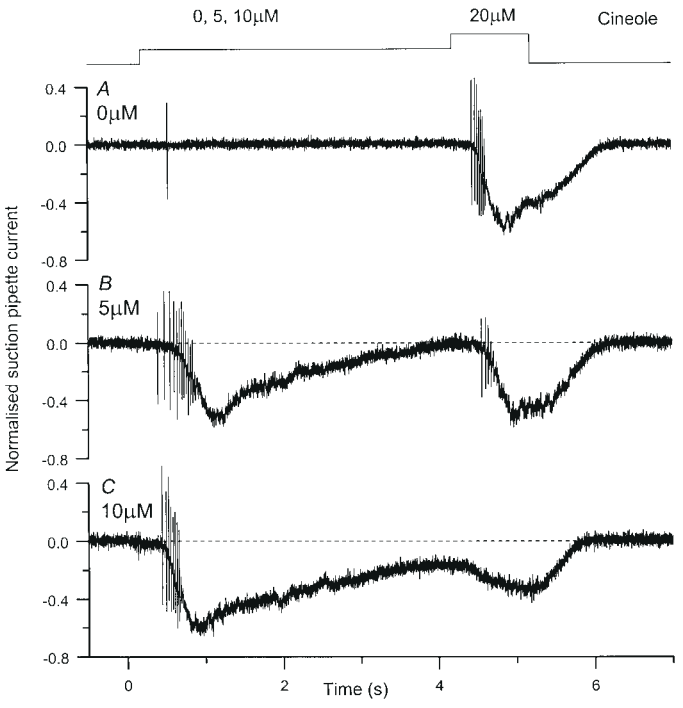

Figure 2 The effect of adaptation on the odour-induced response. Suction current response family to a 4 s pre-pulse of increasing concentration as indicated beside each trace, followed by a test pulse at a fixed concentration of 20 μM cineole. The upper trace is solution monitor. The suction current responses have been normalized to the response elicited by 300 μM cineole in the absence of the pre-pulse. Modified from (Reisert and Matthews, 1999).

from the corresponding dose–response relations as described in the legend of Figure 3 and in Reisert and Matthews (Reisert and Matthews, 1999). The fitted curve represents the modified Weber relation:

$$\frac{S}{S_0} = \frac{1}{1+\left(\frac{C}{C_{1/2}}\right)^n}$$

where C is the pre-pulse concentration, $C_{1/2}$ is the pre-pulse concentration which halves the sensitivity relative to its unadapted value, n represents the limiting exponent of the relation at high pre-pulse concentrations and S_0 is the sensitivity in the absence of the pre-pulse. The fitted curve, with a value for n of 2.4, reflects the steep reduction in sensitivity induced by only a small increase in background odour concentration. Figure 3B shows the corresponding decline in sensitivity estimated from the dose–response relations for spike firing frequency, which began to decline at even lower pre-pulse concentrations than the curve fitted to the receptor current sensitivity, reproduced from Figure 3A. At high pre-pulse concentrations the sensitivity of the spike firing mechanism actually fell to zero, representing the inability of olfactory receptor cells to generate spikes under these conditions.

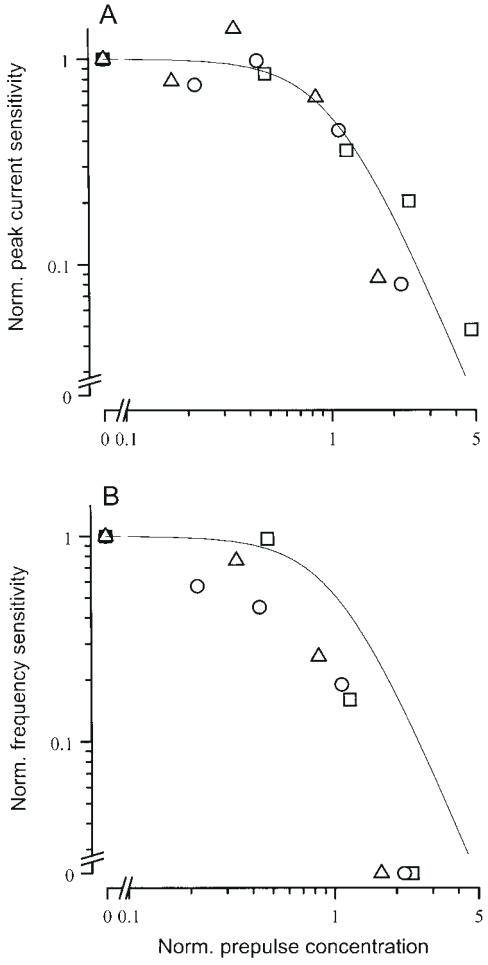

Figure 3 The decline of sensitivity with increasing pre-pulse concentration. Sensitivity **(A)** is of the receptor current response, **(B)** of the spiking response. For each cell sensitivity has been calculated from the dose–response relation by dividing a 20% response criterion by the increment above the pre-pulse concentration of the test pulse required to evoke it and subsequently normalized to the unadapted sensitivity. Peak current sensitivity data have been fitted for each cell by a modified Weber relation, and pre-pulse concentration has been normalized in both panels according to the pre-pulse concentration which reduced current sensitivity by a factor of two. The solid curve fitted to the data in (A) is a modified Weber relation with an exponent of 2.4 and normalized to unity and has been replotted in (B) to facilitate comparison. Reproduced from (Reisert and Matthews, 1999).

Conclusion

We used the suction pipette technique to record odour-induced responses from frog olfactory receptor cells. This recording technique enabled us not only to achieve stable recordings over extended periods, but also to record both the spiking and the receptor current responses simultaneously. The spiking responses which we have obtained are broadly similar to the excitatory responses which have previously been reported (O'Connell and Mozell, 1969; Getchell and Shepherd, 1978a,b; van Drongelen, 1978; Trotier, 1994). The steep dose-dependency of firing fre-

quency on stimulus concentration indicates the narrow dynamic range over which a given olfactory receptor cell is able to encode stimulus strength reliably. The receptor current did not show a clear sign of saturation with increasing stimulus concentration over the range of concentrations which we employed, which contrasts with the saturation observed when recording in the whole-cell voltage clamp configuration (Firestein *et al.*, 1993).

In many sensory systems [for reviews see (Shapley and Enroth-Cugell, 1984; Torre *et al.*, 1995)] adaptation leads to a progressive shift of the stimulus–response relationship to higher stimulus values with increasing levels of the adapting stimulus, and to an accompanying reduction in sensitivity. Odour-induced responses in olfactory receptor cells proved to be similarly affected by adaptation; however, sensitivity declined comparatively more dramatically with increasing steady stimulation within the time interval which we investigated than in many other systems. This is seen most strikingly in the complete absence of any spike output at high pre-pulse concentrations, thereby preventing the cells from encoding the odour signal into a spike output to be conveyed to the olfactory bulb.

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