Chem. Senses 26: 1023–1027, 2001

# Spiking Properties of Olfactory Receptor Cells in the Slice Preparation

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## Abstract

The whole-cell, patch-clamp method was applied to olfactory receptor cells in slice preparations made from bullfrog olfactory epithelium. Under voltage-clamp conditions, olfactory receptor cells showed a transient inward current followed by a steady outward current in response to depolarizing voltage steps, as has been shown in the isolated preparation. The input resistance was 5.4 ± 3.9 GΩ and capacitance 21.9 ± 9.7 pF. Under current-clamp conditions, depolarization of cells by current injection induced action potentials. In 13 out of 20, spike generation was repetitive with a maximum frequency of 24 Hz. The frequency of the repetitive discharges increased as the injected current was increased. The relationship between the size of the injected current and firing frequency could be well fitted by the Michaelis–Menten equation, indicating that the spike generation site lacks the non-linear boosting system. The slice preparation developed here would provide a powerful tool to study the spike encoding system of the olfactory receptor cells.

## Introduction

The study of electrical properties of vertebrate olfactory receptor cells has advanced significantly since the patch-clamp technique was applied to these small cells (Trotier, 1986; Anderson and Hamilton, 1987; Firestein and Werblin, 1987; Kurahashi, 1989; Schild and Restrepo, 1998). Olfactory signal transduction is locally carried out at the cilia where chemo-electrical transduction channels are localized (Kurahashi and Kaneko, 1991; Schild and Restrepo, 1998). The combination of two transduction channels—sequential openings of both cAMP-gated cationic and Ca-activated anionic channels (Kurahashi and Yau, 1993)—leads to establishing a strong non-linear amplification system (Lowe and Gold, 1993; Kurahashi and Yau, 1994; Frings *et al*., 2000). The dose–response relation could be fitted by the Hill equation with large Hill coefficients—4.4 (Firestein *et al*., 1993), 4.7 and 6.9 (Lowe and Gold, 1993) or 4.6 (Kurahashi and Menini, 1997).

Olfactory receptor cells also express diverse types of voltage-gated ion channels to determine their membrane activities (Firestein and Werblin, 1987; Lynch and Barry, 1989). One of the most important functions of the voltage-activated channels is the generation of action potentials that actually transmit the olfactory information to the brain (Getchell and Shepherd, 1978; Trotier and MacLeod, 1983; Masukawa *et al*., 1985a,b; Trotier, 1994; Kawai *et al*., 1996). Most of the studies investigating membrane channels were, however, performed on cells dissociated from the intact olfactory epithelium. In isolated preparations, most of the olfactory receptor cells lack abilities for generating repetitive action potentials, presumably due to the damage of the axon; repetitive firings were seen in only 10% of isolated cells (Anderson and Hamilton, 1987; Firestein and Werblin, 1987; Kurahashi and Shibuya, 1989; Kurahashi, 1989). To study the spike generation system of the olfactory receptor cell, in the present study we developed a method of making slice preparations containing cells that retain their functional axons. Sixty five per cent of cells discharged repetitive spikes when recorded under current-clamp conditions. The relation between the injected current and spike frequency was well fitted by the Michaelis–Menten equation. It seems likely that the spike generation site does not show any non-linear amplifications.

## Materials and methods

### Preparation of olfactory epithelium slices

Bullfrogs (*Rana catesbeiana*) were obtained from a commercial supplier and kept in a shallow tank with fresh tap water. The animals were anesthetized by cooling on ice and pithed, both rostrally and caudally. The olfactory epithelia were removed from the frogs and washed with normal saline solution (for compositions, see below). The epithelia were kept in cold normal saline solution until use.

A piece of the olfactory epithelium was placed on a circular filter paper (Nitrocellulose, pore size 3.0 μm, diameter 13 mm, Advantec Toyo) and the filter paper with the

epithelium was placed on a syringe filter holder (SX0001300, Millipore) with the surface side up. Suction was applied to the back of the filter paper so that the epithelium was firmly attached to the filter paper. The epithelium, together with the filter paper, was cut into 150 μm slices by a laboratory-made slicer equipped with a razor blade. Cut slices were transferred to the recording chamber. The strip of filter paper was fixed to the chamber with a small amount of silicone grease (Dow Corning, USA) at both ends of the strip in such a way that the cut surface was parallel to the bottom of the chamber. The chamber was mounted on the stage of a microscope (BX-FLA, Olympus) equipped with Nomarski optics and a ×40 water-immersion lens. Fluorescent images of Lucifer-yellow-filled cells were examined using an epi-fluorescent system and photographed with a cooled CCD camera (Nippon Roper). The images were recorded using a microcomputer (NEC Express 5800).

The normal saline solution contained (in mM) 110 NaCl, 3.7 KCl, 3 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 15 glucose and 1 Na-pyruvate. The normal pipette solution contained (in mM) 119 KCl, 1 $CaCl_2$, 5 EGTA, 10 HEPES and 0.2 % Lucifer yellow. All solutions were adjusted to pH 7.2 with NaOH.

### Whole-cell recordings

Electrophysiological recordings were made from cells in fresh slices without staining. Patch pipettes were made of borosilicate tubing with filament (outer diameter, 1.2 mm; inner diameter, 0.68 mm; World Precision Instruments) by using a two-stage vertical patch electrode puller (PP-830, Narishige). The volume of the recording chamber was ~1 ml. Pipette resistance was 10–15 M. Experiments were performed at room temperature (23–25°C).

The debris on the surface of the slices was removed with a jet of normal saline solution ejected from a glass capillary placed near the slice. After cleaning, the whole-cell recording configuration was established. The recording pipette was connected to a patch-clamp amplifier (Axopatch 200B, Axon Instruments). The signal was low-pass filtered at 1 kHz, digitized by an A/D converter (sampling frequency, 10 kHz) connected to a computer. Simultaneously, signals were monitored on an oscilloscope. Generation of the command voltages and data acquisition were controlled by pClamp software (version 7, Axon Instruments). The results were analysed by an offline computer and plotted using Microcal Origin 6.0 software (Microcal Software).

## Results

Whole-cell, patch clamp was made on olfactory receptor cells in the slice preparation (Figure 1A). Under voltage-clamp conditions, depolarizing voltage steps to more positive than –40 mV induced a transient inward current (reaching a peak in ~15 ms), followed by an outward current (Figure 1B). The current–voltage (I–V) relation (Figure 1C) showed remarkable voltage- and time-dependence. The size of the inward current was –408.9 ± 172 pA (mean ± SD, $n$ = 20) at –30 or –40 mV. The outward current increased monotonically up to +50 mV (702.1 ± 331.5 pA, $n$ = 20). The resting potential was –53.2 ± 12.1 mV ($n$ = 18, two cells showing spontaneous firings were omitted from the analysis—see below), input resistance was 5.4 ± 3.9 GΩ ($n$ = 20) and the membrane capacitance was 21.9 ± 9.7 pF ($n$ = 20).

Under current-clamp conditions, olfactory receptor cells showed action potentials in response to the injection of depolarizing current. Thirteen out of 20 cells generated more than two action potentials, leading to the generation of repetitive discharges. Among these, two cells generated rhythmical spontaneous firings (Figure 2A). Two out of 20 cells showed only one action potential. The remaining five cells did not show any action potentials, even with very strong stimuli exceeding 10 pA. There were indications that spike-generation ability was dependent on the resting membrane potential under recording. In fact, cells showing no action potential showed positive resting membrane potentials (–42.0 ± 10.5 mV, $n$ = 5). This matter further discussed below. We attempted to find a correlation between the ability for the repetitive firing and the presence of the axon. However, because of the very fine structure of the axon (diameter, <0.1 μm), the spatial resolution of our system was insufficient to confirm the detailed axonal structure.

Amplitude of the action potential gradually decreased while the depolarizing current was injected ($n$ = 11) (Figure 2B). In two cases, however, the amplitude was constant during the current step (Figure 2C). The inter-spike intervals gradually increased during the current step. This may indicate that the spike generation machinery has an ability to be desensitized. In the present study, however, no further analysis was made of this matter.

The firing frequency of the repetitive discharges increased as the size of injected current was increased (Figure 3A). The relationship between the injected current and the frequency of action potentials was well fitted by the Michaelis–Menten equation (Hill coefficient 1, when fitted by the Hill equation; Figure 3B). The Hill coefficient obtained from 12 samples was 1.01 ± 0.19. This finding indicates that signal processing at the spike-generating site is different from the transduction process in terms of signal amplification; the spiking site shows, rather, a linear process, while the ciliary part expresses strong non-linear amplification.

## Discussion

In the present study, we employed a slice preparation made from the olfactory epithelium of the bullfrog in order to examine the action-potential-generation property. The fraction of cells showing repetitive discharges in the slice preparation was 65%, which is higher than that observed in

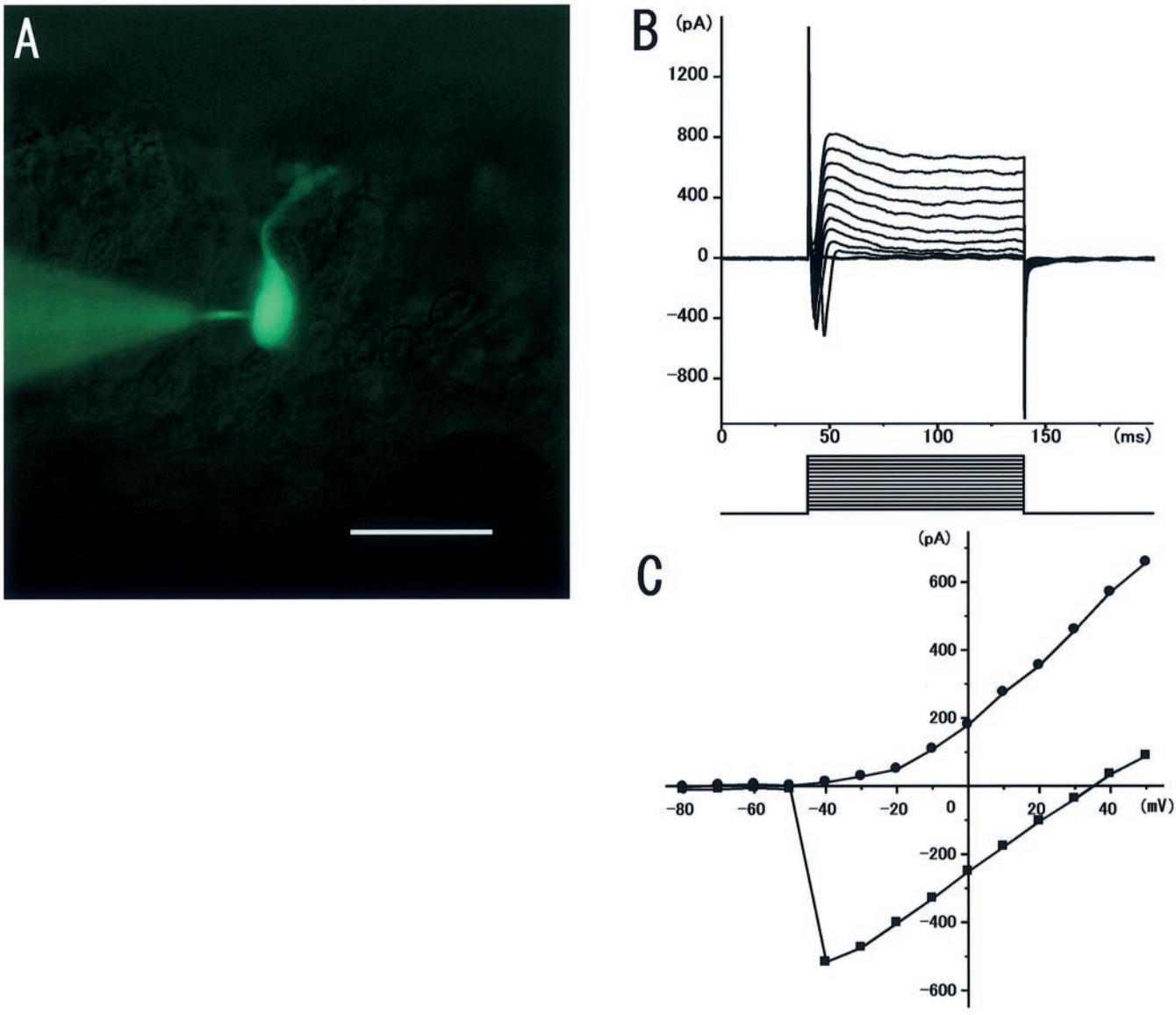


**Figure 1** Whole-cell recording from the olfactory receptor cell in the slice preparation. **(A)** Olfactory receptor cell under recording. The cell was visualized using Lucifer-yellow staining loaded from the whole-cell, patch-clamp pipette. Spherical cell body and one dendrite extending toward surface display a typical morphological feature of the olfactory receptor cell. The axon was not recognized in this preparation. Scale bar = 25 μm. **(B)** Current responses of the olfactory receptor cell in the slice preparation; different cell from (A). Recording mode was set to the voltage-clamp. Holding potential was –90 mV. Depolarizing voltage steps were from –80 to +50 mV and duration was 100 ms. The timing of stimulation is indicated by the upward deflection of the lower traces. **(C)** Current–voltage (I–V) relationship. The same cell as in (B); ●, outward current measured at 100 ms after the step initiation; ■, peak of inward current.

the solitary preparation (Anderson and Hamilton, 1987; Firestein and Werblin, 1987; Kurahashi, 1989).

The reason why 25% of cells did not show repetitive discharges is still under speculation. It is possible that, even in the slice preparation, the axon was injured in those cells. Furthermore, low-seal recordings may affect the apparent resting membrane potential. Since the input resistance of the olfactory receptor cell is extremely high (>5 GΩ), even a giga-ohm seal (>1 GΩ) affects the resting membrane potential (Kawai *et al*., 1996). Such artificial depolarization would facilitate the inactivation of the voltage-gated Na and T-type Ca channels which are essential for the generation of action potentials in olfactory receptor cells (Kawai *et al*., 1996, 1997).

The relationship between the injected current and the frequency of action potentials could be fitted by the Michaelis–Menten equation (Ma *et al*., 1999). It has been shown that the olfactory transduction system shows a strong non-linear amplification (Hill coefficient of 4–7), most likely due to the sequential opening of cAMP-gated channels and Ca-activated Cl channels (Kurahashi and Yau, 1993, 1994; Lowe and Gold, 1993; Frings *et al*., 2000). Throughout the

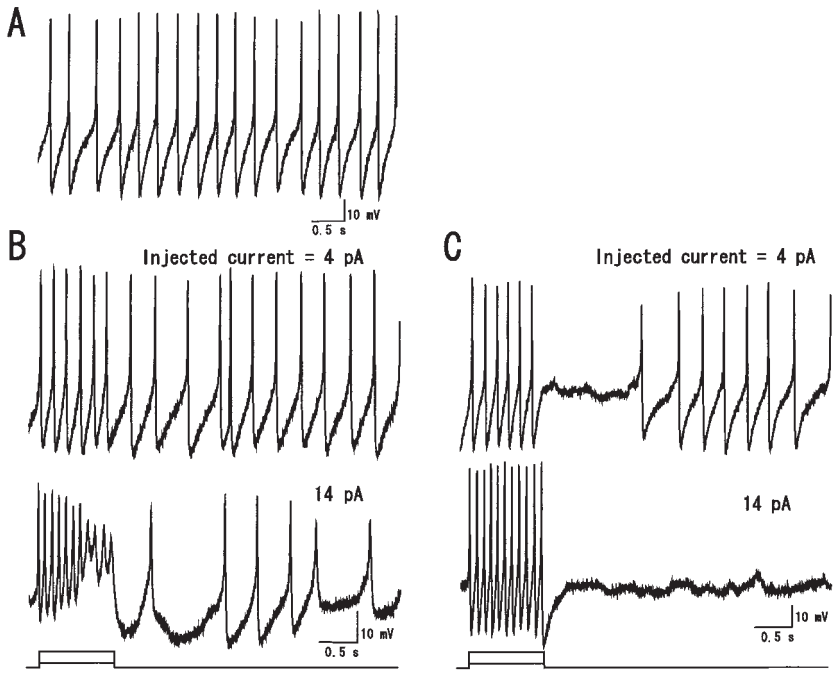


**Figure 2** Spike activities recorded under the current-clamp mode. **(A)** Repetitive firings spontaneously observed in a cell; Averaged firing frequency, 3.8 Hz. **(B)** Change in the firing frequency during the depolarizing current step (1 s duration); different cell from (A). Note that the spike amplitude decreased with increasing injected currents. **(C)** Example of cell showing spikes with constant amplitude; same cell as in (A).

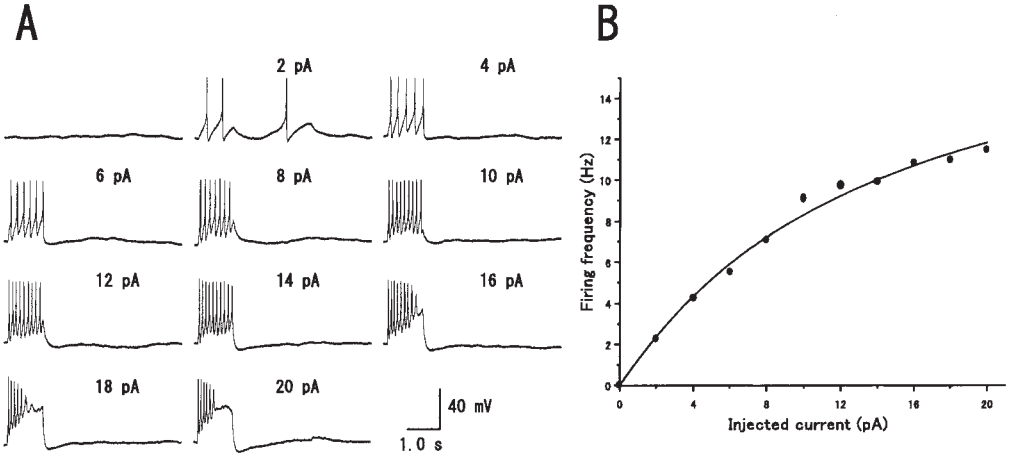


**Figure 3** Frequency–current relationship. **(A)** Spiking activities were recorded at different current steps (2–20 pA, 2 pA increments). **(B)** Relationship between the injected current and the mean firing frequency. The mean frequency was calculated from the inverse of the inter-spike intervals. For the recording at 2 pA, the frequency was calculated as an inverse of the inter-spike interval. For other recordings, voltage data were sampled for 300 ms and frequency value was calculated as an average from inter-spike intervals within a response. Solid line was drawn by the least-squares fitting using the Hill equation with a Hill coefficient of 1 (actually identical to the Michaelis–Menten equation). The Hill equation is $f = f_{max}\,[I^n/(I^n + K_{1/2}{}^n)\,]$, where $f$ is the firing frequency, $f_{max}$ is the maximal frequency, n is the Hill coefficient and $I$ is the injected current amplitude.

signal processing cascades in the olfactory receptor cell, transduction channels may be the most dominant part for non-linear signal amplification.

In the present study, cells were stimulated by current injection. In order to investigate signal processing in the olfactory receptor cell, however, it may be important to know how cells generate repetitive spikes when they are stimulated by native stimuli—odorants. There is a possibility that spiking properties could be changed by increase in membrane conductance resulting from the opening of transduction channels (Kurahashi, 1989; Lowe and Gold, 1993). This factor is not included in the present study. Experimental recording of spike activities under odorant stimulation may be technically difficult, especially under

whole-cell, patch clamp conditions. However, the experimental protocols developed here may be useful for such experiments.

The spiking rate was gradually decreased while cells were stimulated by prolonged current injection (Getchell and Shepherd, 1978; Lynch and Barry, 1989). It has been shown that the olfactory transduction system of the ciliary region expresses adaptation, which is regulated by Ca feedback (Kurahashi and Menini, 1997). Olfactory receptor cells may also express desensitization (or adaptation), even at the site of the spiking process. It would be worthwhile to further investigate the temporal pattern of spiking activities.

The slice preparation from the olfactory epithelium contained cells which were physiologically and morphologically reliable. Especially, olfactory receptor cells retained the ability to generate repetitive discharges. Since the slice preparation is also suitable for experiments using superfusion (compared with the *in vivo* preparation), it would be possible to examine the effect of endogenous factors which have been reported to affect olfactory signal encodings. Indeed, adrenaline (Getchell and Getchell, 1984; Kawai *et al*., 1999) and dopamine (Vargas and Lucero, 1999) have been shown to modulate olfactory cell activities.

In another set of experiments, we have obtained recordings from the supporting cell using the same preparation (Takeuchi *et al*., 2001). The experimental protocol established here would be a useful tool for the investigation of the olfactory system in greater detail.

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*Accepted June 28, 2001*