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Un-filtered recordings from insect taste sensilla

Frederic Marion-Poll[1] & Jan van der Pers[2]
1INRA Station de Phytopharmacie, route de Saint Cyr, 78206 Versailles Cedex, France
2Syntech P.O. Box 1547, NL-1200 BM Hilversum, The Netherlands

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Introduction

Electrophysiological recording from insect taste receptors has become a classical technique since the work of Hodgson *et at.* (1955), It consists of recording the electrical activity of sensilla as soon as they contact an electrolyte. Although such recordings lack information about the unstimulated and poststimulated state of the taste receptors, no alternative technique has yet emerged Side-wall recordings (Morita & Yamashita, 1956) or tungsten recordings from the base of the hairs have been obtained but they necessitate both an adequate insect preparation and delicate manual skills; in addition, the preparations tend to deteriorate rapidly.

Amplifiers designed for Hodgson type recordings must cope with two technical constraints. First, action potentials recorded extra-cellularly from insect sensilla have a low amplitude level and must be amplified to allow good recordings and reliable analysis. Secondly, taste sensilla exhibit a large DC offset relative to the ground, which would saturate the amplifier at such amplification factors. This problem is usually approached by using a high-pass filter (de Kramer & van der Molen, 1980; Frazier & Hanson, 1986) or by manually compensating for the DC signal (G6dde & Krefting, 1989; Schnuch & Hansen, 1990, 1992). Both approaches have limitations. In the first case, strong filtering modifies the spike waveforms and smoothes out baseline variations. This makes spike separation more difficult. The second approach requires recording the DC offset before measuring the response.

We have designed a new amplifier, TastePROBE, that permits reliable DC recordings with the Hodgson technique. This new amplifier performs three functions: contact detection, DC compensation and amplification,

Principle of operation

Extra-cellularly recorded action potentials exhibit an amplitude of 150 µV to 2 mV. Insect sensilla present a large DC offset relative to ground, typically 50 to 200 mV. These voltages are converted into numbers (digitisation), by an analogue-to-digital conversion *(AID).* At the present time most converters have an input range of + 1 0 to -10 V with a precision of 12 bits. This means that the maximal precision achieved by such AID converters is 20 V $/2^{12}$ or 4.9 mV. To keep this 4.9 mV precision limitation (' graininess') at an acceptable level (e.g., one percent) so that it does not significantly affect subsequent spike analysis, an amplified spike should be approx. 490 mV. This high spike amplification (ca. 3000 for small spikes) would amplify the DC offset to 15-60 V. This is well beyond the limits of physiological amplifiers and input ranges of AID converters. Thus, given the amplitude of action potentials, 12 bits is not sufficient to encode both the DC offset and the small spikes. The DC offset can be suppressed by high-pass filtering (fc = 10-300 Hz) but this introduces an artefact at the beginning of the recording, overriding the first spikes (Figure 1).

To alleviate these problems, TastePROBE achieves automatic DC compensation by measuring the sensillum offset at the beginning of the recording and subtracting it from the incoming signal. Our amplifier has three functional blocks: a probe, a differential amplifier and a signal detection unit (Figure 2).

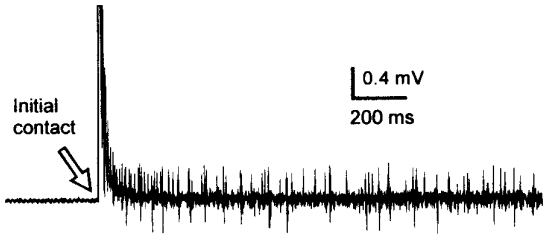

Figure 1. Sample response to KCl 10^{-3} M, using high-pass filter *(Ostrinia nubilalis,* prothoracic leg, CRb contact chemoreceptor).

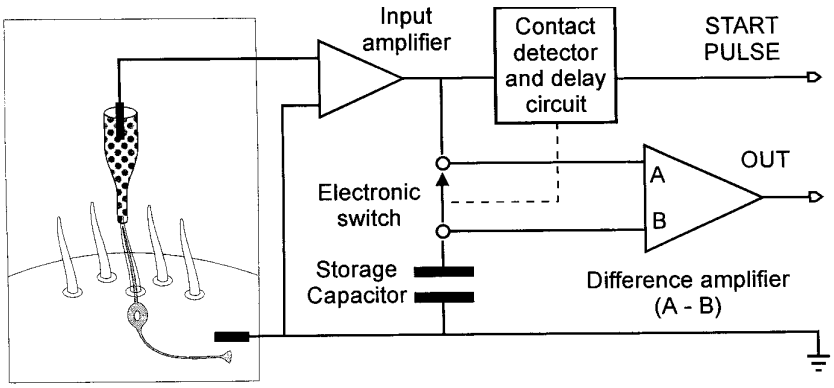

Figure 2. Recording set-up and TastePROBE diagram.

First, the input probe amplifies and buffers the electrical voltage present at the recording electrode, It is built with an operational amplifier (Analog Devices AD515; gain: 10; input impedance: 10^{12} Ohms). Secondly, the instrumentation amplifier has two inputs (A and B), one of which (B) is continuously subtracted from (A) to produce the output. Input A receives the signal from the probe. Input B is connected to a capacitor (0.1 nF). Before recording, A and B are bridged by an electronic switch (normally closed) resulting in an output voltage (A-B) of zero volts. When this electronic switch is opened, the capacitor maintains the DC voltage that was present just before the change. Therefore the output signal will be A-B (B = constant), thus compensating for the initial offset.

The third functional block operates outside the main signal path and performs two functions: contact detection and control of the electronic switch. Contact detection is realized by filtering the electrophysiological signal with a powerful high-pass filter followed by an adjustable level discriminator. The output of this discriminator triggers a circuit that opens the switch after an adjustable delay of 5 to 20 ms. The switch returns to a closed state after an adjustable time.

Sample recording

Recordings were performed from CRb taste hairs from the legs of female *Ostrinia nubilalis* (MarionPoll *et al.*, 1992) and from blowfly labellum. The TastePROBE preamplifier was connected to a software adjustable amplifier with filters (CyberAmp 320, Axon Instruments, USA). Data were digitised on a compatible PC at 10 kHz using a 12 bits resolution AID card (DT2821, Data Translation, USA) driven by a custom DOS program (Marion-Poll & Tobin, 1992). The contact detection pulse delivered by the TastePROBE triggered the acquisition.

A typical DC recording is shown in Figure 3a. It demonstrates the initial compensation time, the DC signal and spikes superimposed on it. Two classes of spikes could be discriminated from this recording as shown in Figure 3b (using AWAVE: Marion-Poll, this volume).

TastePROBE has been routinely used in our laboratory for over one year. It was tested or contact-chemoreceptor sensilla of various preparations, Lepidoptera (adults and larvae; four species), Hymenoptera

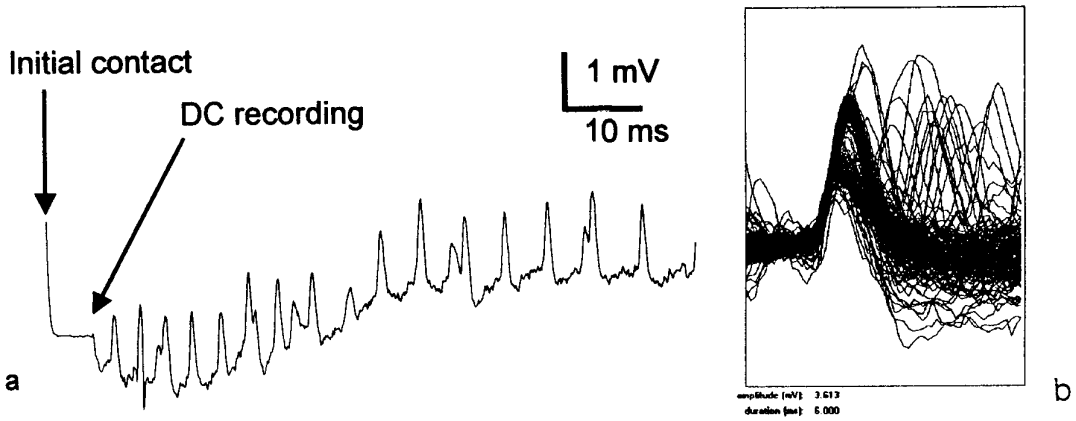

Figure 3. Response sample from a blowfly taste hair stimulated with sucrose 10^{-2} M diluted in KCl 10^{-3} M. (a) initial contact with delay for compensating DC offset; (b) superimposed spikes detected from the record (187 spikes).

(worker honeybees; legs and antennae) and Arachnida *(Tegenaria atrica).*

Conclusion

TastePROBE is a convenient and flexible electronic circuit designed to record action potentials from taste sensilla of insects. It facilitates the recording of slow potentials arising in taste sensilla, improves the signal to noise ratio, and preserves spike shapes. This new amplifier design combines excellent signal to noise ratio with complete compatibility as regards existing electrophysiological equipment.

DC recordings have higher information content than filtered recordings. With DC recordings, spike shapes are not modified and thus better sorting is possible. Moreover, slow variations in the transepithelial potential (i.e. less than 10 Hz) are preserved. Both aspects are of considerable importance when studying the physiology of taste receptors.

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