AN INTEGRATED MICROFLUIDIC DEVICE FOR HIGH-THROUGHPUT ELECTROPHYSIOLOGICAL ANALYSIS OF *C. ELEGANS*

Chunxiao Hu[1], Vincent O'Connor[2], Lindy Holden-Dye[2] and Hywel Morgan[1,2]
[1]*School of Electronics and Computer Science, University of Southampton, UK and*
[2]*Institute for Life Science, University of Southampton, UK*

ABSTRACT

The traditional method for recording electrophysiological signals from the *C. elegans* pharynx is called an electropharyngeogram (EPG). It provides an experimental platform for defining gene function, the impact of genetic mutations, and the effects of drugs on the nematode neural network. To date, the EPG has been a low-throughput assay. This paper describes a new microfluidic device that can sequentially trap worms and capture EPG activity combined with the capability to apply drug solutions. With the integration of micro-valves and embedded electrodes, we are developing a new tool for consistent, high-quality and high-throughput neurogenetic and neuropharmacological analysis of nematodes.

KEYWORDS: Microfluidic, *C. elegans*, Electropharyngeogram, High-throughput, Drug application

INTRODUCTION

The nematode *C. elegans* is a model organism widely used to study the development and function of the nervous system. It is the first multicellular organism to have its genome fully sequenced and neurons mapped.[1] The pharyngeal circuit of the worm is regulated by a microcircuit of 20 neurons and the activity of this network can be recorded by placing an extracellular recording electrode over the nose of the worm. This technique, called the electropharyngeogram, or EPG, is analogous to a human electrocardiogram (ECG) in that the shape of the waveform and the frequency of activity are dependent on a repertoire of ion channels and neurotransmitter receptors.

To date, the EPG has been a low-throughput assay, as each recording requires a stand-alone experiment in which a single glass microelectrode is used to make the recording. Microfluidics can provide a platform for processing hundreds of worms in high-throughput fashion and has been used for analyzing *C. elegans* for several years.[2] A microfluidic device for EPG measurement of the *C. elegans* has been recently demonstrated.[3] The device has eight parallel trapping channels and each channel has two side-arm channels on both sides for drug delivery. It collects individual electrophysiological recordings from up to eight worms using eternal recording electrodes. We also designed and fabricated a microfluidic chip to perform high-throughput EPG on whole worms. It is distinct from the earlier design [3] as it resolves discrete neural components of the EPG signal and thus has improved power for screening for neuroactive drugs and neural phenotypes. This chip is a combination of two PDMS layers (flow and control) with embedded platinum electrodes for electrical recording. The chip is capable of trapping worms, delivering drug and collecting whole worm EPG recordings. The integration of a fluid control layer and embedded electrodes simplifies the manipulation and increases the stability and resolution of the electrical recordings. This makes it possible to analyze a large number of worms sequentially in one chip. The chip has an unloading channel allowing the worm to be released unharmed for propagation, an important consideration for genetic screens, and additional the chip can be reused. With precise control of the micro-valves, we can sequentially trap the worm, deliver drugs, and obtain EPG recordings in an easy high-throughput fashion.

EXPERIMENTAL

In conventional EPG experiments, the worm's head is sucked into a glass capillary with a tip diameter of 12-15 μm. Two isolated electrical compartments are created by the seal between the capillary and the cuticle of the worm. With pharyngeal pumping, current transients are recorded by an amplifier.[4] A microfluidic device for EPG recoding must mimic the orifice of the glass capillary and provide a tight electrical seal around the worm's head. This was mimicked using a soft semi-circular PDMS channel as shown in figure 1. Several bypass channels were added into this layer to allow delivery of a worm and drugs. In order to maximise the orientation of the worm in the device, several posts were fabricated at the inlet port. This directs the worm into the trap with head to tail orientation in a ratio of 71%.[5] The control layer was coupled to pneumatic micro-valves, located underneath the flow layer to control the bypass channels. The control layer is made of softer PDMS to give a more elastic trapping channel, which provides a tight seal around the worm. Microfabricated platinum electrodes are embedded under the control channel on both side of the trap (Fig. 1A). For EPG recording, the worm flows from the inlet port and is pushed into the trap under a constant positive pressure (~ 0.3 mBar). Pharyngeal pumping of the worm is recorded as an EPG signal (Fig. 1B).

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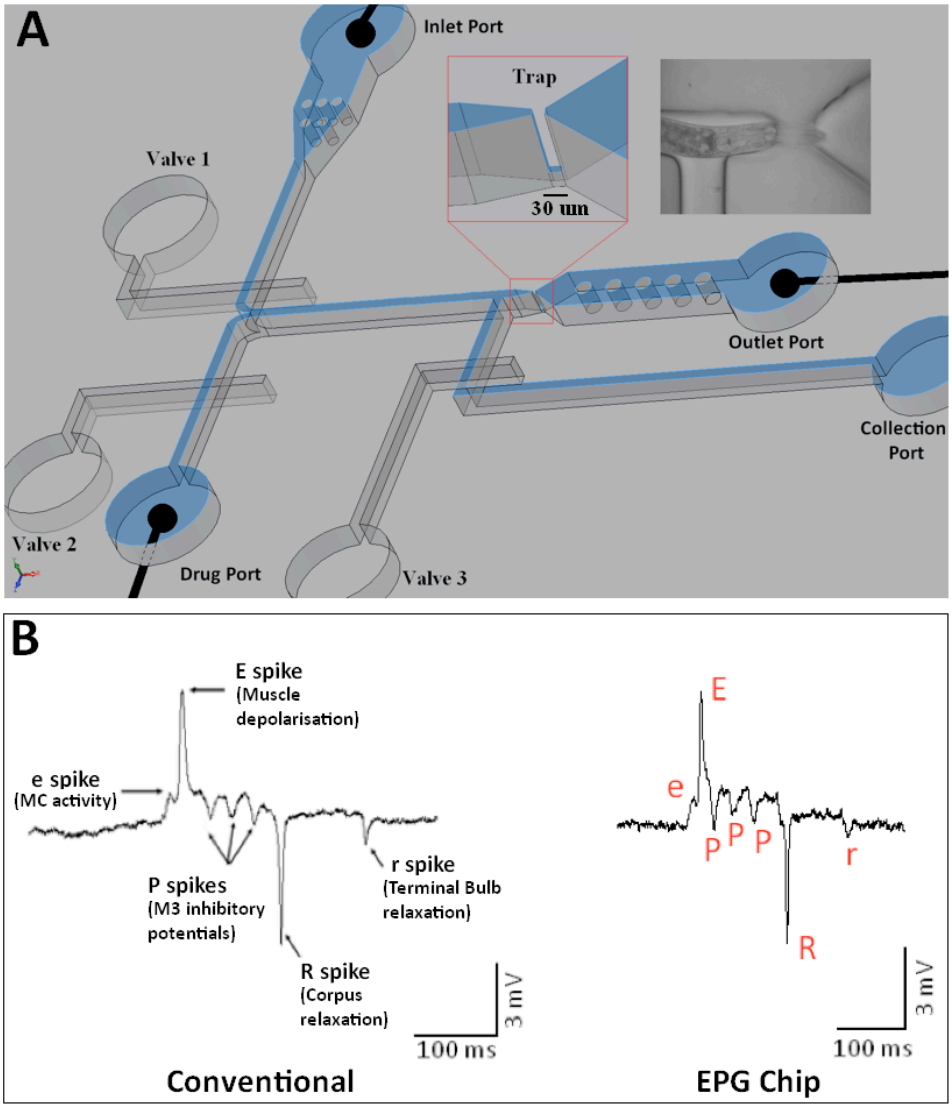

Figure 1: A. Schematic of the microfluidic device for EPG recording. The blue layer is the flow layer, containing a trapping channel (red square) and several bypass channels. The grey layer is the control layer consisting of three micro-valves. A real image of a trapped worm is shown to the right of the trapping channel. B. Comparison of EPG signals obtained from the conventional method and EPG chip. They have similar amplitude and pump duration. Every functional spike can be observed and detected from the signal collected from the EPG chip. (Worms were synchronised for age, 1 day old adult was used in both EPG experiments).

Drugs are flowed across the worm's body using a separate channel. For proof of principle, 5-HT (serotonin; 10mM) was used. This drug regulates the activity of the M3 motor neurons and timing of pharyngeal muscle repolarization and thus shortens pump duration and increases frequency.[6] First the worm is pushed into the trap and stable recordings are made over 5minutes. The worm is perfused with the drug and the 5-HT response was observed for approximately 25 minutes. Fig. 2 shows that the pumping rate increases from 4.5 per minute to 208 per minute and the average pump duration decreases from 116.2 ms to 100.5 ms after perfusion with 5-HT (n=3). The average pump rate and pump duration were extracted using the software AutoEPG.[7] The delay time for the 5-HT response will be influenced by the time taken for the 5-HT to reach the worm in the microchannel and the time taken for it to cross the cuticle of the worm. The relative contribution of each is currently being determined by further experiments and improvements to chip design will be made to reduce the delay if necessary.

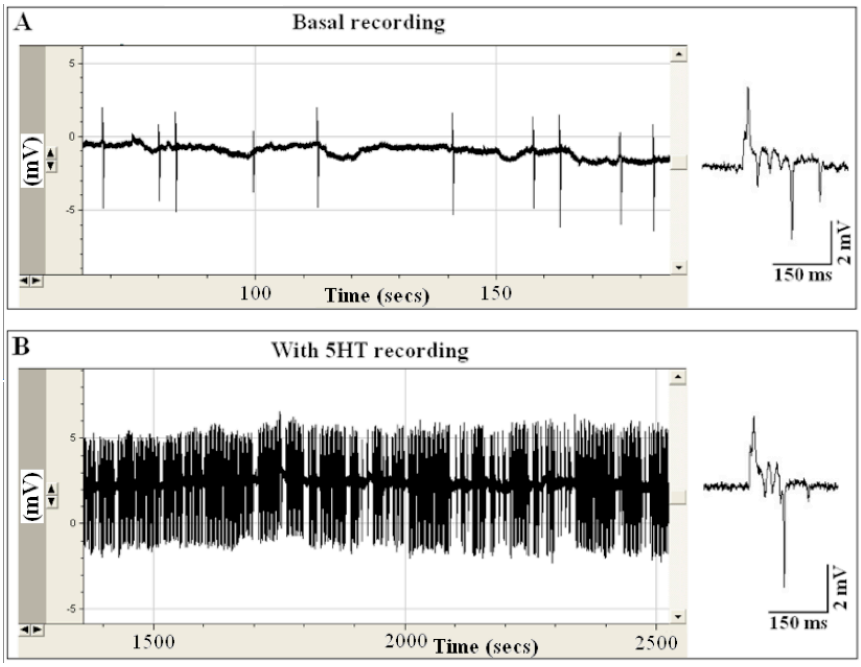

Figure 2: Change of EPG signals after treatment with 5HT. A. Basal recording from a wild type nematode. B. EPG recording 25 minutes after the worm being treated with 5HT for ~ 25 min, when the worm was fully affected. The pump rate increased from 0.075 per second to 3.46 per second. The pump duration decreased from 116.2 ms to 100.5 ms.

RESULTS AND DISCUSSION

A microfluidic device that allows for the trapping of single worms, delivery of stimuli with precise control and recording of EPG signals has been described. High quality recordings were collected from this device that have similar amplitude, pump duration and functional spikes to the conventional pipette method. Results from exposure to 5HT test were comparable to that from the conventional 5HT assay (data not shown).

In conventional EPG experiments, manipulation of the worm is complicated even for an expert operator. The microfluidic chip greatly simplifies this procedure and dramatically reduces drug usage. Our EPG chip can be used for EPG assays with or without the presence of 5HT; thus it improves on an earlier design [3] as it provides the capability for recording basal activity and thus can screen for excitatory or inhibitory drug actions or mutations. It provides an integration of structures for orienting the worm, soft trapping design, pneumatic control valves, and embedded microelectrodes electrodes which make operation more precise and high-throughput, providing an excellent microenvironment for high-quality neurogenetic and neuropharmacological analysis of nematodes.

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CONTACT

Lindy Holden-Dye, tel: +44 (0)23 8059 4295; L.M.Holden-Dye@soton.ac.uk
Hywel Morgan, tel: +44 (0)23 8059 3330; hm@ecs.soton.ac.uk