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Short communication

High-throughput study of synaptic transmission at the neuromuscular junction enabled by optogenetics and microfluidics

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ABSTRACT

Over the past several years, optogenetic techniques have become widely used to help elucidate a variety of neuroscience problems. The unique optical control of neurons within a variety of organisms provided by optogenetics allows researchers to probe neural circuits and investigate neuronal function in a highly specific and controllable fashion. Recently, optogenetic techniques have been introduced to investigate synaptic transmission in the nematode *Caenorhabditis elegans.* For synaptic transmission studies, although quantitative, this technique is manual and very low-throughput. As it is, it is difficult to apply this technique to genetic studies. In this paper, we enhance this new tool by combining it with microfluidics technology and computer automation. This allows us to increase the assay throughput by several orders of magnitude as compared to the current standard approach, as well as improving standardization and consistency in data gathering. We also demonstrate the ability to infuse drugs to worms during optogenetic experiments using microfluidics. Together, these technologies will enable high-throughput genetic studies such as those of synaptic function.

1. Introduction

In the last few years, a large number of researchers have begun to use the light-activated channelrhodopsin-2 (ChR2) as a tool for neural stimulation. This research includes mapping of synaptic connectivity in mice (Wang et al., 2007), dissecting neuronal circuitry in brains with Parkinsons disease (Gradinaru et al., 2009), examining the escape response in zebrafish (Douglass et al., 2008), and examining mechanosensory and avoidance circuits in *Caenorhabditis elegans* (Guo et al., 2009; Nagel et al., 2005). Recently, we have used ChR2 to examine synaptic transmission in *C. elegans*—a technique termed optogenetic investigation of neurotransmission (OptIoN) (Liewald et al., 2008). In this technique, behavioural changes in *C. elegans* (relative changes in body length) are induced by muscle contractions or relaxation caused by excitatory or inhibitory signals from either cholinergic or GABAergic motoneurons expressing ChR2, respectively, which are stimulated by blue light illumination. Defects in synaptic transmission alter this behavioural output and can therefore be observed and quantified. To do this, individual worms (wild-type or mutant) are picked onto plates. A video of the worm is then recorded under no illumination and blue light illumination, which is manually moved to track the worm as it moves. Finally the videos are analyzed only semi-automatically (identifying the centre-line of the animal and measuring the length) to compare mutants to wild-type worms to infer functions of genes. Besides being manual, an additional problem with this approach is that photo-stimulation of cholinergic cells causes animals to coil, due to co-stimulation of GABAergic neurons via cholinergic input. The coiling causes significant problems in image processing and length determination. Although already a powerful new technique, OptIoN's current drawbacks of low-throughput, manual manipulation of animals, variation in animal analysis and long data processing time need to be overcome to make it a widely applicable tool in neurogenetics.

Here, we combine the ChR2 technology with microfluidics and automated image processing for high-throughput, non-biased, and well-controlled experimentation to study synaptic transmission in large numbers of animals. Microfluidics when combined with automated image processing has been shown to greatly increase the speed and efficiency of handling, imaging, analysis, and sorting of cells and small organisms like *C. elegans* (Chung et al., 2008; Crane et al., 2009; Hulme et al., 2010; Hung et al., 2005; Rowat et al., 2009).

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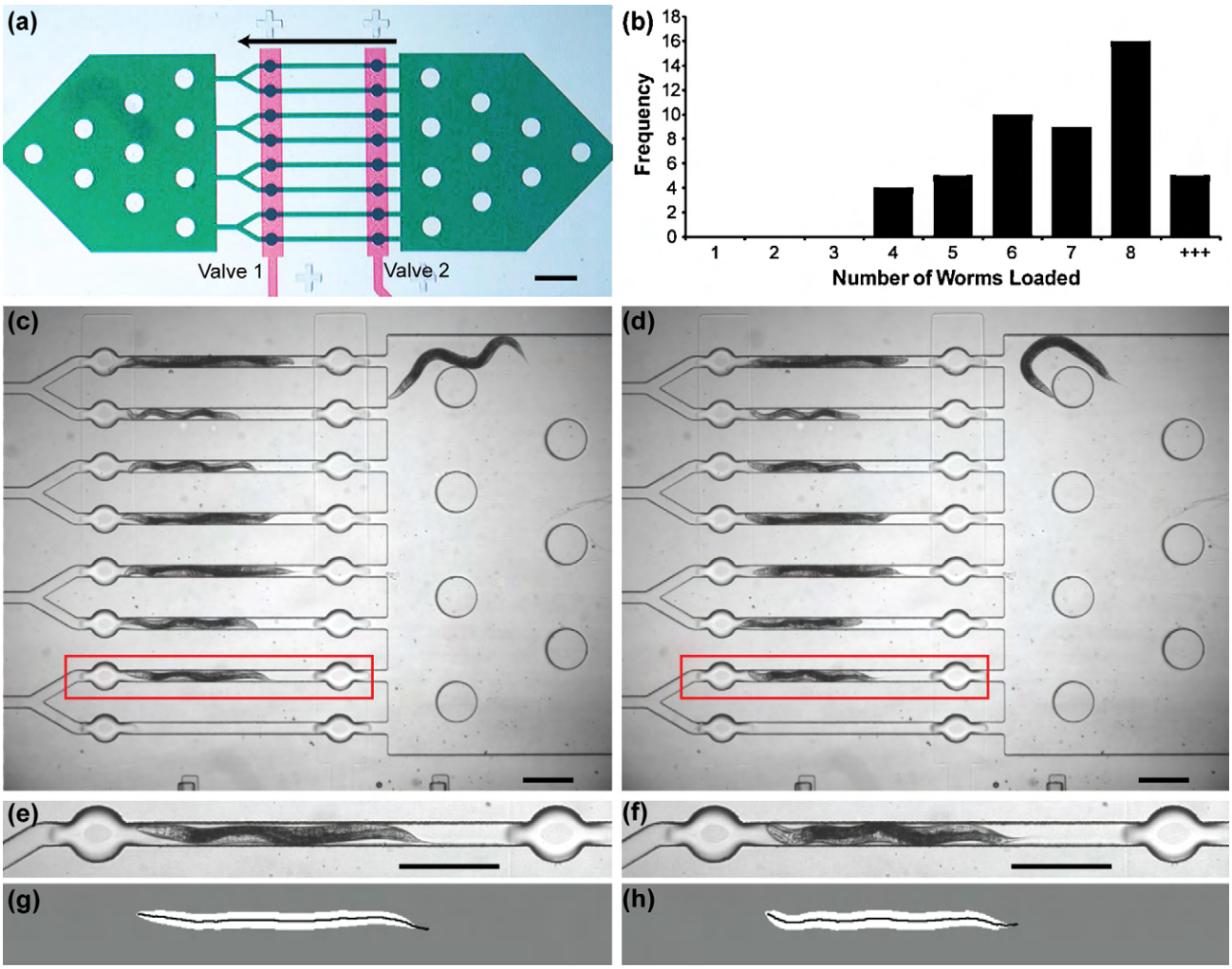

Fig. 1. Microfluidic chip greatly increases the speed of animal handling and sample processing. (a) Microfluidic device for parallel investigation of *C. elegans* responses to light stimulation. Green channels are in the flow layer (where *C. elegans* are located) and the red channels are in the valve control layer. Arrow indicates direction of worm loading. Scale bar is 500 μm. (b) Histogram of the worm loading efficiency. Channels with zero or multiple worms (column +++) were not analyzed. We found an average of ~6.6 worms could be analyzed per loading cycle. (c)–(h) Schematics of computer data processing. Worm strain in this image is ZX497, expressing ChR2 in the cholinergic neurons in an *unc-49(e407)* background. (c) Bright field image of loaded worms prior to blue light illumination. (d) Bright field image of loaded worms 2 s after turning on blue light illumination. These animals show body length shortening due to muscle contraction. The analysis program identifies each channel (red box) and separates it for further processing. (e) and (f) Zoom-in view of the areas selected by the red boxes. (g) and (h) For each channel section, the worm is first identified and separated from the rest of the image, and then thresholded (white). Then a curve (black) is fit to the midline of the animal and its length is measured. This process is done for every frame of the movie (8 fps). From this length data the curves in Fig. 2a are generated. Scale bars are 250 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2. Materials and methods

2.1. C. elegans culture

All worm strains used in this study were grown at 22 °C in the dark on standard nematode growth medium (NGM) plates with OP50 bacteria. All-*trans* retinal (ATR) is a required cofactor for channelrhodopsin and must be supplemented to *C. elegans* in order to have active channelrhodopsin. Those experiments using plates containing ATR (Sigma–Aldrich) were made by diluting a 50 mM stock ATR solution (in ethanol) in 300 μl OP50 to a final concentration of 100 μM and spreading on a 5.5 cm NGM plate. All animals tested were F1 progeny of P0 adults picked onto ATR or no-ATR plates 3.5 days prior to experiments. The strains used in this paper include ZX426: N2; *zxIs3[punc-47::chop-2(H134R)::yfp; lin-15+]*, ZX460: N2; *zxIs6[punc-17::chop-2(H134R)::yfp;lin-15+]* and ZX497: *unc-49(e407); zxIs6.*

2.2. Microfluidic device design, fabrication, and operation

To achieve the high-throughput in a controllable integrated system, we designed and fabricated a two-layer polydimethylsiloxane (PDMS, Dow-Corning) device using multi-layer soft lithography (Unger et al., 2000). The device is composed of eight parallel imaging channels connecting a loading and an unloading chamber. These channels can be isolated (trapping the worm) by actuating a set of two valves (Valve 1 and Valve 2) (Fig. 1a). The main imaging channels are 60 μm wide: slightly larger than the width of a young adult worm. The cross-sections of the valves are rectangular and are therefore only partially closed, allowing some fluid flow (as well as very young worms) while preventing larger animals to pass through the channels. We chose to use a single large valve, which covers all channels, instead of eight individual valves. This greatly simplifies the device construction, set-up, and operation. To aid in downstream image processing, we filled the valve channels