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BRIEF COMMUNICATION

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Development of an artificial synapse using an electrochemical micropump

Abstract Improving the resolution of artificial sensory organs requires an interface that receives external information from electronic circuits and stimulates appropriate neurons individually in response to that information. The method of electric stimulation in available artificial sensory organs is fairly nonselective; therefore, we developed a method of chemical stimulation of neurons using a neurotransmitter containing an electrochemical micropump powered by the bubbling that occurs during water electrolysis. The micropump contains a glass nozzle with a tip 10 μm in diameter. Two blackened platinum electrodes for the electrolysis were inserted into the body of the pump, which was filled with neurotransmitter solution. The distance between a neuron of the gastropod *Aplysia* and the tip of the nozzle was adjusted to about 100 μm. A potential difference of 3.0 V was applied to the electrodes to propel the solution toward the neuron while its membrane potential was monitored. Administration of 1-mM acetylcholine to a resting neuron caused neural firing only when the voltage was applied for 0.5 s and without a time lag. During administration of 50-mM γ-aminobutyric acid to spontaneously firing neurons, the firing disappeared with a time lag of 1 s after application of 3.0 V. We concluded that an electrochemical micropump can be applied for rapid neurotransmitter administration to control the excitation and inhibition of neurons. This simple pump can be miniaturized to create "synapses" in artificial sensory organs.

Key words Neuron · Artificial synapse · Neurotransmitter · Electrolysis · Micropump

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Introduction

In prostheses such as a cochlear[1,2] or retinal[3,4] implants for damaged sense organs, a sensor captures information (e.g., vision or sound), a processor identifies features of the information, and an interface stimulates appropriate neurons that allow the recipient to sense the information.

The use of cochlear implants has increased over the past few decades[1,2] and the trial production of a retina implant for blind patients has been initiated.[3,4] The interfaces of these systems stimulate the neuron electrically. Using electrical stimulation for the neural interface in an artificial retina or cochlear implant has the advantage of operational simplicity; however, the mechanism of electrical stimulation is not so simple. Some neuroscientists have proposed that electrochemical stimulation causes the release of neurotransmitter by damaging the presynaptic neuron with the electric field instead of through a direct effect on the neural membrane.[5] Therefore, safe and selective stimulation of individual neurons using an electrical method is inherently difficult. Under physiological conditions, communication between neurons is accomplished by transport of neurotransmitters across synapses. Direct and highly selective administration of a neurotransmitter to neurons would improve the resolution of artificial sense organs and diminish damage to the neurons. Convectional transport of a neurotransmitter is more advantageous for selective administration than diffusive transport. Fishman et al. administered a neurotransmitter (bradykinin) using a commercial inkjet printhead by piezoelectric pumping,[5] which stimulated the neurons. The neurons could be selectively stimulated by the printhead, but the piezoelectric pump had the disadvantage of a complicated structure and the minimum width of a device incorporating the pump is 140 μm/device,[6] which is too large for central nervous system stimulation. Electrophoresis injection is the traditional method for applying chemicals to neurons for experiments in basic neurobiological research;[7–9] however this method cannot be applied to the administration of nonionic chemicals. Electroosmosis is widely used for microfluid pumping,[10–12] but requires

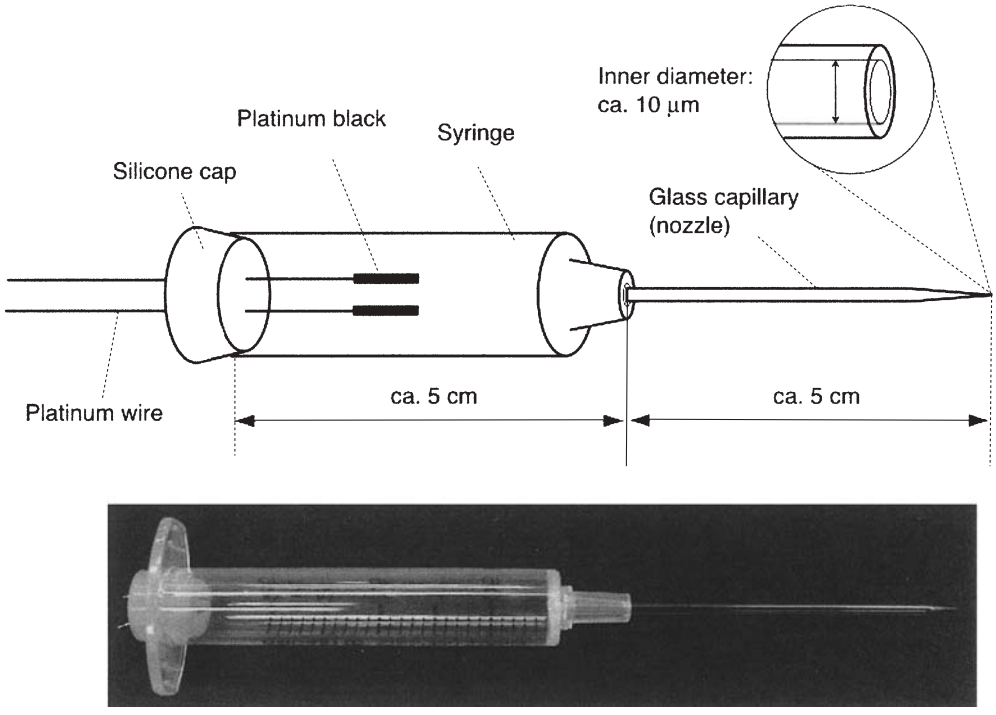

Fig. 1. Structure of the electrochemical micropump

voltages of the order of kilovolts, and this is too dangerous for direct use on cells.

We have designed a new interface based on administering a neurotransmitter to neurons using the electrochemical microscale pumping method developed by Suzuki et al.[13] The pump is powered by the bubbling resulting from electrolysis of water using blackened platinum electrodes. The fluid is forced out of a reservoir by the pressure created by gas build-up during the voltage application for the electrolysis. The bubbles generated by the electrolysis disappear rapidly through catalysis by the blackened platinum when the voltage application ceases.[13] Thus, the jet of the neurotrans-mitter solution is rapidly controllable through voltage application during electrolysis. The simple pump structure is advantageous for miniaturization to sizes of the order of 10 μm, which is similar to the size of a human neuron.

This study reports the fabrication of an electrochemical micropump used for the chemical stimulation of neurons to determine its feasibility as an interface for artificial sense organs. The sea snail (*Aplysia*) was used as an experimental animal because of its large nerve cells (100–1000 μm diameter).[14]

Materials and methods

Chemicals

Artificial seawater (Marine Art BR for invertebrates: 1.00 w/v% Na^+, 0.046 w/v% K^+, 0.122 w/v% Mg^{2+}, 0.038 w/v% Ca^{2+}, 1.83 w/v% Cl^-, 0.20 w/v%, SO_4^{2-}, 0.006 w/v% Br^-) was purchased from Senju Pharmaceuticals (Osaka, Japan). L-15 medium containing L-glutamate was purchased from Dainippon Pharmaceuticals (Osaka, Japan). 4-(2-Hydroxyethyl)-l-piperazine ethane sulfonic acid (HEPES) buffer was purchased from Dojindo Laboratories (Kumamoto, Japan). Neutral protease (Dispase II) was obtained from Boehringer Mannheim (Indianapolis, IN, USA). All other chemicals were purchased from Wako Pure Chemical (Osaka, Japan).

Preparation of electrochemical micropump

The structure of the electrochemical micropump is illustrated in Fig. 1. The blackened platinum electrodes for electrolysis of water were prepared by applying a voltage of 3.23 V to platinum wires in a mixed aqueous solution of 81-mM hexachloroplatinic (IV) acid, 0.75-mM lead acetate, and 0.1-M sodium chloride for 2 min. Glass capillary tubing containing an internal glass fiber GD-1.5 (Narishige, Tokyo, Japan) was pulled by a PN-3 heating puller (Narishige) to form the nozzle of the pump and the glass electrode for monitoring the membrane potential of nerve cells. The inner diameter of the tip was about 10 μm. The pulled capillary was fixed onto the tip of a 2.5-ml syringe (SS-02S, Terumo, Tokyo, Japan). The syringe was filled with a solution of acetylcholine, dopamine, or γ-aminobutyric acid (GABA) in artificial sea water. Two blackened platinum wires were placed in the syringe and the end of the syringe was plugged with silicone rubber. The jetting velocity of the pump was estimated by propelling artificial seawater containing 1 wt% toluidine blue and observing the flow with a 3CCD DXC-C33 video camera (Sony, Tokyo, Japan) and an SZ-60 stereomicroscope (Olympus, Tokyo, Japan).

Isolation and culture of neurons

Nerve cells were obtained from *Aplysia* sea snails collected along the seashore in Hayama (Japan) or purchased from the Rosenstiel School of Marine and Atmospheric Sciences at the University of Miami (USA). Institutional guidelines for the care and use of laboratory animals were observed. The animals were bred in artificial seawater and fed dried *Undaria* seaweed. The medium for cultivation of the nerve cells was prepared by mixing the three solutions (A, B, and C) listed in Table 1. The pH of the medium was adjusted to 8.0 using concentrated HCl or NaOH. The bottoms of Petri dishes were coated with Silgard 184 silicone resin (Dow Corning, Midland, MI, USA) and then filled with a 1-wt% aqueous solution of polyethyleneimine buffered by 0.1-M borate saline at pH 9.0 for 1 h to positively charge the surface of the resin. The dish was washed with artificial seawater and autoclaved for incubation of the nerve cells.

The *Aplysia* were anesthetized by injection of a 0.4-M aqueous solution of magnesium chloride. (The amount of fluid injected was 40% of the animal's weight.) Pedal and buccal ganglia were extracted from the head of the animal. The ganglia were soaked in a medium containing 1-wt% Dispase II to soften the fibrous sheath of the ganglia. The sheath was opened and the nerve cells were isolated individually using 15000-00 micro-scissors (Fine Science, North Vancouver, BC, Canada). The cells were soaked in medium in a Petri dish prepared for the incubation. The cells were incubated overnight at room temperature to promote their adherence onto the silicone-coated bottom of the dish.

A glass capillary electrode for monitoring the membrane potential of the neuron was prepared by the same procedure used for preparing the nozzle of the electrochemical pump. The membrane potential of the cell was monitored by inserting the glass capillary electrode containing 3-M potassium acetate solution and an Ag/AgCl wire. The wire was connected to an MEZ-7200 microelectrode amplifier (Nihon Kohden, Tokyo, Japan). Each incubated neuron was identified as a resting neuron or a spontaneously firing neuron by monitoring the membrane potential. A resting neuron was used as the target for administration of an excitatory neurotransmitter (acetylcholine or dopamine); a spontaneously firing neuron was used for studies involving the inhibitory neurotransmitter γ-aminobutyric acid (GABA).

Table 1. Composition of solutions for nerve cell culture medium

Solution	Solute	Concentration (g/l)	Volume (ml)
A	L-15	13.76	700
B	NaCl	15.37	200
	KCl	0.34	
	$MgSO_4 \cdot 7H_2O$	6.26	
	$MgCl_2 \cdot 6H_2O$	5.49	
	$NaHCO_3$	0.17	
	D-glucose	6.00	
	HEPES buffer	3.74	
C	$CaCl_2 \cdot 2H_2O$	1.43	50
	Streptomycin	0.20	
	Benzyl penicillin potassium	0.06	

HEPES, 4-(2-hydroxyethyl)-l-piperazine ethane sulfonic acid

Monitoring neuronal response to neurotransmitter administration

A system for monitoring the neural response to the electrochemical pumping of neurotransmitter is illustrated in Fig. 2. The neurotransmitter was applied while monitoring membrane potential with the glass capillary electrode. Membrane potential was recorded using an operational amplifier and a PowerLab/4SP interface (ADInstruments, Colorado Springs, CO, USA) with Chart software version 3.5 (ADInstruments).

The distance between the cell and tip of the nozzle was adjusted to about 100 μm using an MM-3 micromanipulator

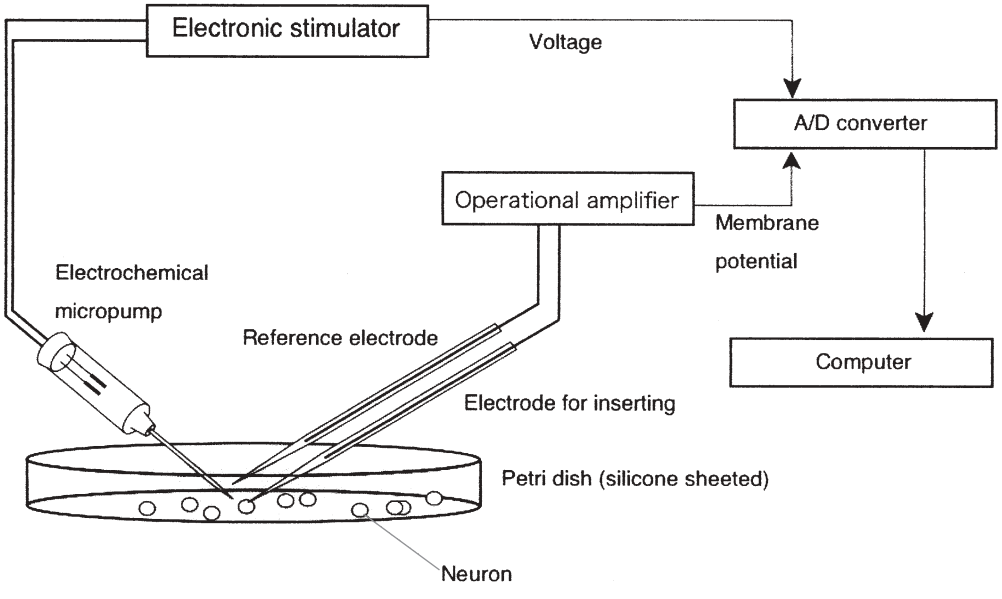

Fig. 2. Schematic of the apparatus for monitoring neural response against electrochemical pumping

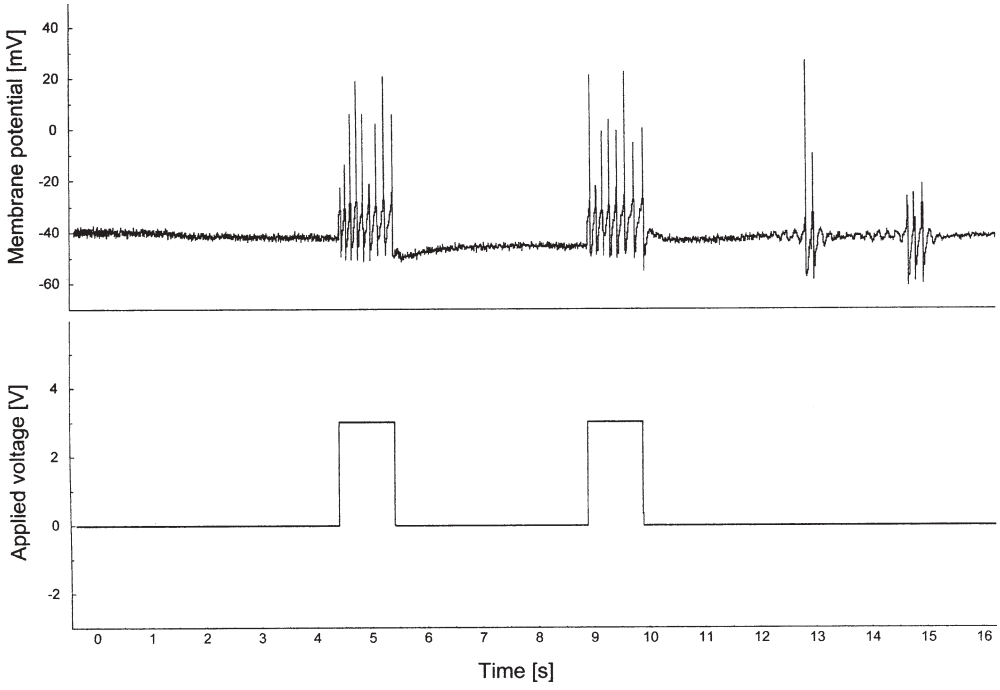

Fig. 3. Response of the membrane potential of a resting neuron in a buccal ganglion against voltage application to the electrode in a micropump containing 1-mM acetylcholine

(Narishige). The neurotransmitter solution was allowed to jet toward the cell by applying a voltage of 3.0 V with a SEN-3301 electric stimulator (Nihon Kohden) and the response of the membrane potential to the voltage application was observed.

Results and discussion

The jetting velocity of the pump was estimated to be about 700–1000 μm/s, which is much larger than that of the piezoelectric printhead used by Fishman et al. (100 μm/s).[5]

Administration of artificial seawater without additional neurotransmitter did not affect the membrane potential of either resting or spontaneously firing neurons. The time courses of the membrane potential of resting *Aplysia* neurons and the applied voltage to the electrodes in pumps containing acetylcholine or dopamine are illustrated in Figs. 3 and 4, respectively. The time course of the membrane potential of a spontaneously firing *Aplysia* neuron and the applied voltage to the electrode in a pump containing GABA is illustrated in Fig. 5.

Upon applying 3.0 V, neurons were stimulated with acetylcholine and dopamine, which are excitatory neurotransmitters; however, spontaneous firing could be terminated by application of GABA, which is an inhibitory neurotransmitter. The spontaneous firing lasted for several hours unless GABA was administered. These results indicate that voltage application can force the neurotransmitter solution in the pump out through the tip to stimulate a neuron, as designed.

Neural firing occurred instantly upon acetylcholine administration and disappeared when acetylcholine administration ended, likely due to the high jetting velocity of the pump and the reversibility of seawater electrolysis on the blackened platinum electrodes. Acetylcholine results indicated that rapid administration could be accomplished successfully by the pump. Acetylcholine administration using the electrochemical micropump may be applicable to real-time control of neuronal signals. However, unexpected firing occurred after several applications, as shown in Fig. 3, probably due to leakage of the neurotransmitter from the pump. These results indicate that the pump must be improved for more precise control of jetting. A time lag of approximately 1 s existed between dopamine administration and neural stimulation; neuron firing lasted longer than 30 s after termination of dopamine administration. The slow response and long-lasting neural signal were similar to those observed by Fishman while using a printhead for bradykinin administration to PC12 cells.[5]

The differences in response time between the acetylcholine and dopamine administrations may result from differences in the receptors. Two types of neurotransmitter receptors exist: one couples with the ion channel directly and the other couples indirectly (with protein reaction steps occurring between neurotransmitter reception of the indirectly-coupling receptor and gating of the ion channel). The response of the indirectly coupled receptor is slower toward chemical stimulation and its response lasts longer than that of the directly coupled receptor.[15] Thus, the neurons treated with acetylcholine could be utilizing directly coupled receptors, whereas neurons treated with dopamine could be utilizing indirectly coupled receptors. If this conjecture is true, the slow response by dopamine is not a serious disadvantage because such neurons do not require a rapid response, even in natural systems.

Spontaneous firing of active neurons was inhibited by GABA administration with a time lag of approximately 1 s. The duration of the inhibition was approximately 10 times

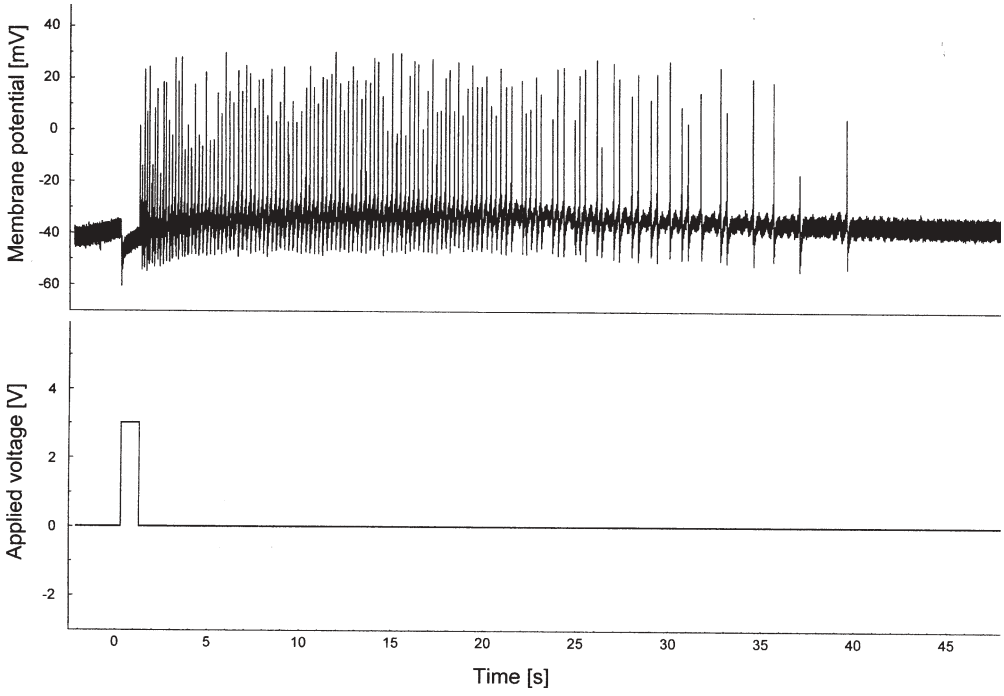

Fig. 4. Response of the membrane potential of a resting neuron in a pedal ganglion against voltage application to the electrode in a micropump containing 1-mM dopamine

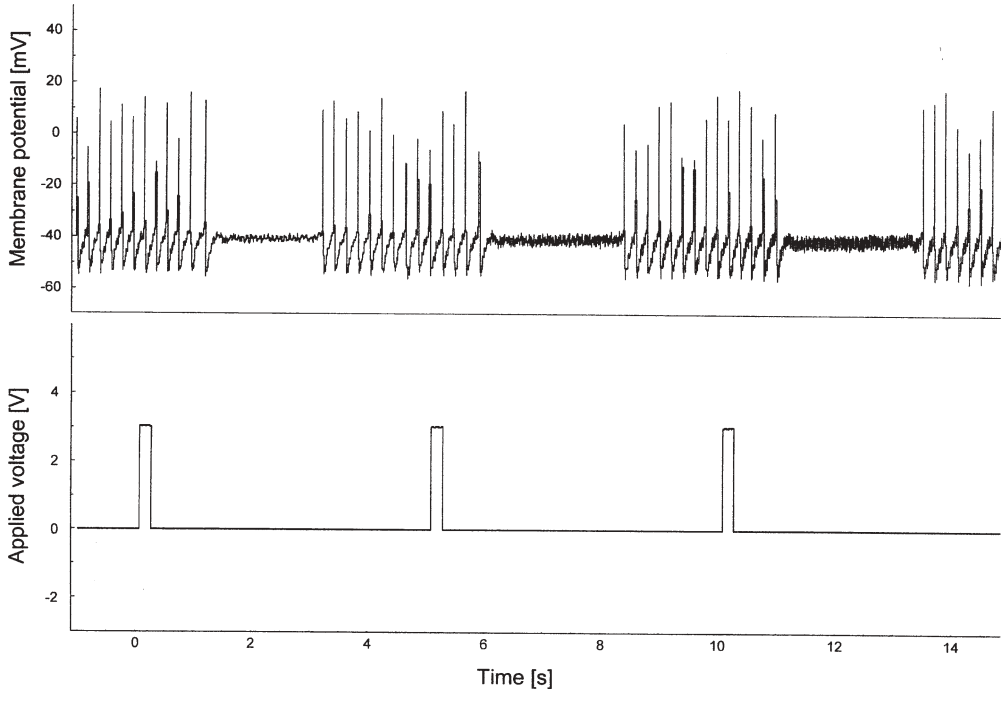

Fig. 5. Response of the membrane potential of a spontaneously firing neuron in a pedal ganglion against voltage application to the electrode in a micropump containing 50-mM γ-aminobutyric acid

greater than the duration of electrolysis voltage application. The GABA receptors in the treated neuron are thought to be an indirectly coupling type ($GABA_B$ receptor).[15] These results indicate that inhibition of neurons by GABA is possible using the electrochemical pump and that the electrochemical micropump electrodes allow control of the firing of nerve cells owing to the reversibility of the electrolysis of water on the blackened platinum electrodes. However, it did not confirm the ability of the pump to provide real-time inhibition.

For the informational interface of an electric circuit to stimulate the nerves in artificial sense organs, a large number of specific neurons must be controlled because the nervous system is based on very sophisticated parallel processing. Thus, integration of the device requires a simple structure. The electrochemical pump possesses simplicity in structure and fabrication compared to a piezoelectric pump. The minimum width of a device incorporating a piezoelectric pump is 140 μm/device as a result of its complicated structure,[6] whereas the size of the electrochemical

pump can be as small as 10μm/device using simple photolithography.

The products of electrochemical reactions, such as chlorine produced by electrolysis of artificial seawater, would not be negligible in a miniaturized pump and may damage neurons. Therefore, an appropriate electrolyte for the inner solution needs to be used. Leaks of the inner solution from the pump must be prevented and therefore design of a small-diameter nozzle needs to be optimized.

In conclusion, the electrochemical micropump that we developed is capable of rapid administration of neurotransmitters to neurons. However, hurdles remain that must be overcome in the design of informational interfaces (artificial synapses) in artificial sense organs. Administration of the neurotransmitter to the neurons, considering reaction conditions and transport issues, also must be optimized. (For example, the flow out of the pump must be laminar to jet the fluid within a strictly limited area.) These problems can be solved through chemical engineering approaches.

This pump also can be applied in the laboratory for basic research on neurons. Recent studies have indicated that interactions between different types of neurotransmitters (e.g., excitatory and inhibitory transmitters) generate multiple neural responses.[16] Use of the electrochemical pump can help clarify the mechanism of synaptic plasticity, which is one of the most important properties of neurons.

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