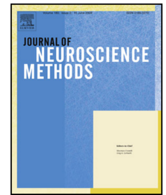
Journal of Neuroscience Methods 219 (2013) 292–296

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth



Basic Neuroscience
Short communication

A multifunctional pipette for localized drug administration to brain slices

Aikeremu Ahemaiti[a], Alar Ainla[a], Gavin D.M. Jeffries[a], Holger Wigström[b], Owe Orwar[a], Aldo Jesorka[a], Kent Jardemark[a,c,*]

[a] Department of Chemical and Biological Engineering, Chalmers University of Technology, Kemivägen 10, SE-41296 Göteborg, Sweden
[b] Institute of Neuroscience and Physiology, The Sahlgrenska Academy at Göteborg University, Box 430, SE-405 30 Göteborg, Sweden
[c] Department of Physiology and Pharmacology, Karolinska Institutet, SE-17177 Stockholm, Sweden

HIGHLIGHTS

- A microfluidic device for perfusion of drugs to brain slices is presented.
- This device administers drugs to the slices with high spatio-temporal resolution.
- The tool delivers solution to cells 20× faster than conventional perfusion systems.
- The device is able to store and deliver up to 4 solutions in series.
- The tool is free standing and can be used in combination with other probes.

ARTICLE INFO

Article history:
Received 15 March 2013
Received in revised form 11 July 2013
Accepted 10 August 2013

Keywords:
Microfluidic technology
Perfusion
Brain slices
Prefrontal cortex
Hippocampus
Glutamate
Electrophysiological techniques

ABSTRACT

We have developed a superfusion method utilizing an open-volume microfluidic device for administration of pharmacologically active substances to selected areas in brain slices with high spatio-temporal resolution. The method consists of a hydrodynamically confined flow of the active chemical compound, which locally stimulates neurons in brain slices, applied in conjunction with electrophysiological recording techniques to analyze the response. The microfluidic device, which is a novel free-standing multifunctional pipette, allows diverse superfusion experiments, such as testing the effects of different concentrations of drugs or drug candidates on neurons in different cell layers with high positional accuracy, affecting only a small number of cells. We demonstrate herein the use of the method with electrophysiological recordings of pyramidal cells in hippocampal and prefrontal cortex brain slices from rats, determine the dependence of electric responses on the distance of the superfusion device from the recording site, document a multifold gain in solution exchange time as compared to whole slice perfusion, and show that the device is able to store and deliver up to four solutions in a series. Localized solution delivery by means of open-volume microfluidic technology also reduces reagent consumption and tissue culture expenses significantly, while allowing more data to be collected from a single tissue slice, thus reducing the number of laboratory animals to be sacrificed for a study.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

In vitro brain slices constitute a valuable experimental model system for studying communication between, or the effects of drugs on, neurons and astrocytes in an environment which preserves the cellular network, i.e. the organization in cell layers, processes, and synapses. Unlike *in vivo* studies on brain using live anesthetized animals, experiments on brain slice preparations have the advantage that the influence of anesthetics on cellular functions is eliminated. Single cells are more easily accessible by probes and imaging techniques, which enables precise physiological and pharmacological studies of the functions and properties of the neuronal networks present in these slices.

There are several examples of micro-perfusion devices designed for extracellular delivery of biologically active substances to brain slices. For example, glass micropipettes, with the capability of applying substances via a laminar flow through a micro scale aperture, have been widely used (for review see Huang et al., 2012).

* Corresponding author at: Department of Physiology and Pharmacology, Karolinska Institutet, SE-17177, Stockholm, Sweden. Tel.: +46 8 52487918; fax: +46 8 308424.
E-mail address: Kent.Jardemark@ki.se (K. Jardemark).

0165-0270/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneumeth.2013.08.012

However, control of flow dynamics is quite limited due to diffusion, limiting solution confinement in addition to challenging device fabrication and complex construction.

Microfluidic devices are emerging as powerful tools for neuroscientists (Huang et al., 2012), allowing spatiotemporal control over solution delivery to the extracellular environment around neurons and astrocytes in brain tissue preparations. The major ambitions of microfluidics in the context of brain tissue slices are to provide efficient nutrient and oxygen delivery, in addition to waste removal, while allowing spatio-temporal control over the local chemical environment, and enabling diverse imaging and probing techniques. Solutions to one or more of these problems have emerged, utilizing microfluidic flow chambers which support interstitial flow for better gas penetration of thick tissue slices (Rambani et al., 2009), or focal perfusion within conventional slice chambers for improved control over the solution environment in a selected slice region (Blake et al., 2007). A new promising approach is the use of hydrodynamically confined flow (HCF) technology for localized microperfusion (Queval et al., 2010). By continuous uptake of the delivered solution, HCF devices produce a confined liquid volume outside the device, i.e., a virtual flow chamber which merely touches a selected area of the tissue slice. This principle is an extension of the many variants of the push-pull arrangement of glass capillaries (Veselovsky et al., 1996). The use of microfabricated devices eliminates the disadvantages associated with glass needles, and adds additional benefits such as facilitated interfacing and a broader choice of materials.

A distinct problem with the initially introduced HCF devices is the difficulty to use conventional light microscopes, as the vertical architecture with perpendicular apertures blocks the optical path. Moreover, it is impossible to use auxiliary probes, such as electrophysiological recording electrodes, in conjunction with this superfusion architecture. We have recently developed a HCF based free-standing multifunctional pipette in polydimethylsiloxane (PDMS) which largely overcomes these disadvantages (Ainla et al., 2010, 2012). Briefly, the test solution is selected from a set of local reservoirs by an internal fast-acting liquid switch zone, and fed to a delivery channel at the tip of the pipette, close to the biological specimen. Vacuum channels located on either side of the delivery channel enable the formation of a virtual flow chamber of the test solution. The device is applied at an angle to the microscope table, allowing additional probes to be used in tandem, addressing the same environment at the tip of the pipette.

Here, we demonstrate the capabilities and benefits of the multifunctional pipette to administer the glutamate receptor agonist α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and its antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) to electrophysiologically recorded pyramidal cells within rat hippocampal and cortical brain slices.

2. Materials and methods

2.1. Preparation of brain slices

2.1.1. Hippocampus

Hippocampal slices were prepared (see Abbas et al., 2011) from 2- to 4-week-old Sprague-Dawley rats (Scanbur BK, Sollentuna, Sweden). The procedures conformed to the guidelines of the Swedish Council for Laboratory Animals and were approved by the Gothenburg Ethical Committee for Animal Experimentation. The animals were sacrificed by decapitation after initial isoflurane anesthesia, and the hippocampi were quickly dissected. Transverse slices (400 μm thick) were prepared by an in-house designed McIlwain-type tissue chopper and placed in a holding chamber containing an oxygenated Ringer's solution at room temperature, composed of (in mM): NaCl 119, KCl 2.5, $CaCl_2$ 2.0, $MgCl_2$ 2.0, $NaHCO_3$ 26, NaH_2PO_4 1.0, and D-glucose 10, gassed with 95% O_2/5% CO_2. After storage for at least 90 min, the slices were transferred as needed to a recording chamber where they were submerged by a continuously superfusing solution saturated with 95% O_2 and 5% CO_2. The composition of the perfusion solution was the same as in the holding chamber except for using 2.5 mM $CaCl_2$ and 1.3 mM $MgCl_2$, with the pH balanced to 7.4. A peristaltic pump (Ismatec, Labinett Lab AB, Sweden) was used to recirculate the solution (1.5–2.0 mL/min), keeping the flow rate constant and avoiding any flow artifacts. Experiments were performed at 30–31 °C.

2.1.2. Medial prefrontal cortex

Male Sprague-Dawley rats (Charles River, Germany) were used (80–250 g) for all the experiments. The animals were housed under standard laboratory conditions and maintained on a 12 h light/dark cycle (lights on at 06:00) with ad libitum access to food and water. All experiments at Karolinska Institutet were approved by, and conducted in accordance with, the Stockholm North Committee on Ethics of Animal Experimentation. Procedures for the preparation of rat medial prefrontal cortex (mPFC) slices have been described previously (Arvanov et al., 1997; Arvanov and Wang, 1998; Konradsson et al., 2006). Briefly, the rats were decapitated under halothane anesthesia. The brains were then rapidly removed and cooled in ice-cold Ringer's solution (pH 7.4) consisting of (in mM): NaCl 126, KCl 2.5, $CaCl_2$ 2.4, $MgCl_2$ 1.3, NaH_2PO_4 1.2, D-glucose 10, $NaHCO_3$ 18, and oxygenated with 95% O_2/5% CO_2. The brains were then cut coronally, using a Vibroslice (Campden model MA 752, World Precision Instruments, Sarasota, FL, USA) instrument, in order to produce 450 μm slices. The brain slices were removed from the instrument and kept submerged in oxygenated Ringer's solution at room temperature for at least 1 h to allow for recovery.

2.2. Electrophysiological recordings

2.2.1. Extracellular recording of field excitatory postsynaptic potentials (fEPSPs) in the hippocampus

The commissural–Schaffer collateral pathway of the hippocampal CA1 area was stimulated using an in-house developed programmable pulse generator. Stimuli consisted of 100 μs, 20–50 μA, negative constant-current pulses, delivered via an insulated sharpened tungsten wire (type TM33B01, World Precision Instr., FL, USA) at a rate of 0.1–0.2 Hz. Extracellular field potentials were recorded in the middle of the pyramidal cell dendritic layer (stratum radiatum), 200–500 μm away from the stimulating electrode, via a glass micropipette filled with 1 M NaCl (resistance 3–5 MΩ; made from Kwik-Fil borosilicate glass capillaries, World Precision Instr., FL, USA; pulled by a P-97, Sutter Instruments, Novato, CA, USA). Signals were amplified, filtered, digitized and transferred to a computer for analysis, using electronic equipment based on an Eagle Technology (RSA) multifunction board. Off-line data analysis was performed using pCLAMP-Clampfit software (Molecular Devices, CA, USA). The size of the recorded fEPSP, which was dominated by the contribution from AMPA-type glutamate receptors, was estimated as the negative peak amplitude relative to the pre-stimulus baseline. This measurement provides an index of the efficacy of AMPA receptor-mediated synaptic transmission (Muller et al., 1988, 1989; Shahi and Baudry, 1992).

2.2.2. Intracellular recording of AMPA-induced currents in medial prefrontal cortex

A single slice containing the mPFC was transferred to a recording chamber (32 °C) and was held submerged between two nylon nets. The chamber was continuously perfused with oxygenated Ringer's solution at a flow rate of 1–2 mL/min. A standard intracellular single-electrode technique was used to record from pyramidal

Download English Version:

https://daneshyari.com/en/article/6268971

Download Persian Version:

https://daneshyari.com/article/6268971

Daneshyari.com