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Spatial characterization of a multifunctional pipette for drug delivery in hippocampal brain slices

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HIGHLIGHTS

- A microfluidic device for localized drug perfusion to brain slices is presented.
- The tool utilized hydrodynamically confined flow for localized drug delivery.
- The drug delivery efficiency of the tool is comparable with whole slice perfusion.
- The tool requires orders of magnitude less drugs than whole slice perfusion.
- The tool can be used in combination with other probing and imaging techniques.

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ABSTRACT

Background: Among the various fluidic control technologies, microfluidic devices are becoming powerful tools for pharmacological studies using brain slices, since these devices overcome traditional limitations of conventional submerged slice chambers, leading to better spatiotemporal control over delivery of drugs to specific regions in the slices. However, microfluidic devices are not yet fully optimized for such studies.

New method: We have recently developed a multifunctional pipette (MFP), a free standing hydrodynamically confined microfluidic device, which provides improved spatiotemporal control over drug delivery to biological tissues.

Results: We demonstrate herein the ability of the MFP to selectively perfuse one dendritic layer in the CA1 region of hippocampus with CNQX, an AMPA receptor antagonist, while not affecting the other layers in this region. Our experiments also illustrate the essential role of hydrodynamic confinement in sharpening the spatial selectivity in brain slice experiments. Concentration-response measurements revealed that the ability of the MFP to control local drug concentration is comparable with that of whole slice perfusion, while in comparison the required amounts of active compounds can be reduced by several orders of magnitude.

Comparison with existing method: The multifunctional pipette is applied with an angle, which, compared to other hydrodynamically confined microfluidic devices, provides more accessible space for other probing and imaging techniques.

Conclusions: Using the MFP it will be possible to study selected regions of brain slices, integrated with various imaging and probing techniques, without affecting the other parts of the slices.
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1. Introduction

Pharmacological tests *in situ* on single cells or groups of cells in living tissue, such as brain slices and tissue cultures, generally require precise control of the chemical environment. Well-controlled chemical stimulation protocols, above all the

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determination of concentration-response curves, are typically required in pharmacological studies on cellular networks in situ, where they significantly contribute to increase the understanding of complex mechanisms of action of drugs and other bioactive compounds. The currently available tools are, however, limited in their temporal and spatial specificity, not to mention that they often do not minimize the amount of drug used. To date, there are a number of micro-perfusion tools, e.g., glass micropipettes (Ling and Gerard, 1949; Neher and Sakmann, 1976; Huang et al., 2012), and microfluidic perfusion chambers (Blake et al., 2007), for delivery of chemical stimulus to the vicinity of cells in tissue slices or cultures, allowing substantial savings of expensive drugs. However, these devices are not optimal in the sense that they are unable to maintain a localized perfusion without causing diffusion of the bioactive compound from the target region, e.g. a cell layer or an isolated cell (Huang et al., 2012), to nearby areas that may or may not be part of the cellular network being studied.

The optimal device for perfusion of cells in tissues or cultures should allow for contamination-free, repetitive administrations of different concentrations of the bioactive compound without the need of exchanging or even moving the delivery device, such as a pipette needle, while addressing a specific individual cell or group of cells in a biological preparation. In order to accomplish these requirements, we have developed a free-standing microfluidic device, the multifunctional pipette (MFP) in polydimethylsiloxane (Ainla et al., 2010, 2012a). This pipette utilizes an advanced fluid recirculation principle which provides a hydrodynamically confined flow (HCF), i.e., a closely confined virtual flow cell that enables high-resolution spatial control of the distribution of a biological active substance(s) administered to a specific cell layer or an isolated cell. Previously, we have shown applications of this MFP in different experimental setups. Using, for example, an uptake assay, we have shown that the MFP can be used to generate concentration-response curves *in situ* of proton-activated human transient receptor potential vanilloid (*h*TRPV1) receptors expressed in cultured and adherent Chinese hamster ovary cells (Ainla et al., 2010). Moreover, practical use of the MFP in conjunction with electrophysiological recordings of these cells by the patch clamp technique was also demonstrated (Ainla et al., 2010). Recently, we have examined the compatibility of the multifunctional pipette with electrophysiological recordings of pyramidal cells in hippocampal and prefrontal cortex brain slices from rats (Ahemaiti et al., 2013). In that work, we determined the dependence of the responses of these recordings on the distance of the MFP from the recording site, documented a multifold gain in solution exchange time as compared to whole slice perfusion, and showed that the device is able to store and deliver up to four solutions in a series (Ahemaiti et al., 2013). Thus, we have demonstrated the use of the MFP for localized perfusion of bioactive compounds, showing that the microfluidic device is compatible with other probing devices, such as electrophysiological recording equipment.

Here, we continue and extend the characterization of the MFP in conjunction with electrophysiological extracellular recordings of cells in rat hippocampal brain slices. Special emphasis is put on spatial specificity as determined from the drug effect on hippocampal electric activity and its dependence on distance between the site of activity and the MFP. The hippocampal formation plays an important role in establishing episodic and declarative memories (Tulving and Markowitsch, 1998; Eichenbaum, 1999; Rolls, 2010; Travis et al., 2014). With reference to its importance in normal functions as well as in several diseases (e.g. schizophrenia and depression) (Stockmeier et al., 2004; Tanti and Belzung, 2013), the hippocampal brain slice has been intensely used as a model for studying e.g. long-term potentiation, a mechanism underlying the generation of certain memories (see above), and for studies of mechanism of drugs actions in hippocampus-related diseases. We demonstrate herein the spatial capabilities of the MFP to specifically administer the amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) to electrophysiologically recorded pyramidal cells within different layers in the CA1 region of hippocampus.

2. Materials and methods

2.1. Hippocampus slice preparation

Sprague-Dawley rats (2- to 8-week old, Charles River, Germany) were used for preparing the hippocampus slices. The procedures were conducted following the guidelines of the Swedish Council for Laboratory Animals and were approved by the Gothenburg Ethical Committee for Animal Experimentation. The rats were anesthetized with isoflurane (Isoba®Vet) and the hippocampi were isolated immediately after decapitation. Hippocampi were then cut transversely into 400 μm thick slices by an in-home designed McIlwain-type tissue chopper and placed in a holding chamber with continuously oxygenated (95% O_2/5% CO_2) Ringer's solution (NaCl 120 mM; KCl 2.5 mM; NaH_2PO_4 1 mM; $NaHCO_3$ 26 mM, $CaCl_2$ 2 mM, $MgCl_2$ 6 mM) at room temperature for recovery. After at least 90 min of recovery, the slices were transferred into a recording chamber where they were fixed by a net in a continuously superfusing solution saturated with 95% O_2 and 5% CO_2, at 30 °C. The perfusion solution was similar to the Ringer's solution used in preparation of the slices, except that 2 mM $MgCl_2$ instead of 6 mM was used. Experiments were conducted after 15–30 min adaptation of the slices in the perfusion chamber.

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

Tungsten microelectrodes (type TM33B01, World precision Instruments, Inc.) were used to stimulate the Schaffer collateral pathway in the CA1 region. Negative constant-current pulses (−20 to −60 μA) with a duration of 100 μs were delivered via a stimulating microelectrode by an in-house developed adjustable pulse generator at a rate of 0.1–0.2 Hz. Extracellular field potentials were recorded in the middle of the pyramidal cell apical dendritic layer (stratum radiatum), 200–500 μm away from the stimulating electrode and at a depth of 100–150 μm below the surface of the slice, using a glass pipette filled with 1 mM NaCl (resistance 2–4 MΩ; made from borosilicate glass capillaries GC150F-10, Harvard apparatus, UK; pulled by a PP-83, Narishige Scientific Instrument Lab, Japan). The signals were amplified, filtered, digitized (16 bit multichannel A/D interface board) and transferred to an IBM-PC/AT compatible computer for monitoring and analysis, by Eagle Technology (RSA) based in-house designed electronic equipment, and software written in QuickBASIC (Microsoft Corporation).

Offline data analysis was conducted by means of the pCLAMP-Clampfit software (Molecular Devices, CA, USA). The slope of the recorded early fEPSP, which was mediated dominantly by AMPA-type glutamate receptors, was measured. This measurement represents the efficacy of AMPA receptor-mediated synaptic transmission.

2.3. Drugs and buffer

KCl, NaH_2PO_4, $CaCl_2$ and $NaHCO_3$ were all obtained from Merck (Darmstadt, Germany). $MgCl_2$ and glucose were purchased from VWR international (Leices-tershire, UK), NaCl from Riedel deHaen (Seelze, Germany). The competitive AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was obtained from Tocris Bioscience (Bristol, UK) or Ascent Scientific Ltd. (UK).

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