Biomed Microdevices (2012) 14:799–809
DOI 10.1007/s10544-012-9659-2

An intra-cerebral drug delivery system for freely moving animals

Sven Spieth · Axel Schumacher · Tahl Holtzman · P. Dylan Rich · David E. Theobald · Jeffrey W. Dalley · Rachid Nouna · Stephan Messner · Roland Zengerle

Published online: 24 May 2012

Abstract Microinfusions of drugs directly into the central nervous system of awake animals represent a widely used means of unravelling brain functions related to behaviour. However, current approaches generally use tethered liquid infusion systems and a syringe pump to deliver drugs into the brain, which often interfere with behaviour. We address this shortfall with a miniaturised electronically-controlled drug delivery system ($20\times17.5\times5$ mm^3) designed to be skull-mounted in rats. The device features a micropump connected to two 8-mm-long silicon microprobes with a cross section of 250×250 μm^2 and integrated fluid microchannels. Using an external electronic control unit, the device allows infusion of 16 metered doses (0.25 μL each, 8 per silicon shaft). Each dosage requires 3.375 Ws of electrical power making the device additionally compatible with state-of-the-art wireless headstages. A dosage precision of 0.25 ± 0.01 μL was determined *in vitro* before *in vivo* tests were carried out in awake rats. No passive leakage from the loaded devices into the brain could be detected using methylene blue dye. Finally, the device was used to investigate the effects of the NMDA-receptor antagonist 3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid, (R)-CPP, administered directly into the prefrontal cortex of rats during performance on a task to assess visual attention and impulsivity. In agreement with previous findings using conventional tethered infusion systems, acute (R)-CPP administration produced a marked increase in impulsivity.

Keywords Drug delivery · Micropump · Silicon microprobes · Microfluidics · Five-choice serial reaction time task · Impulsivity · (R)-CPP · Infralimbic cortex · Neuroscience

S. Spieth (✉) · A. Schumacher · R. Nouna · S. Messner · R. Zengerle
Institut für Mikro-und Informationstechnik der Hahn-Schickard-Gesellschaft e.V. (HSG-IMIT), Wilhelm-Schickard-Str. 10, 78052 Villingen-Schwenningen, Germany
e-mail: sven.spieth@hsg-imit.de

T. Holtzman · P. D. Rich · D. E. Theobald · J. W. Dalley
Behavioural and Clinical Neuroscience Institute and Department of Experimental Psychology, University of Cambridge, Downing St, Cambridge CB2 3EB, UK

P. D. Rich
Janelia Farm Research Campus, Howard Hughes Medical Institute, 19700 Helix Drive, Ashburn, VA 20147, USA

J. W. Dalley
Department of Psychiatry, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK

R. Zengerle
Laboratory for MEMS Applications, Department of Microsystems Engineering (IMTEK), University of Freiburg, Georges-Koehler-Allee 106, 79110 Freiburg, Germany

R. Zengerle
BIOSS - Centre for Biological Signalling Studies, University of Freiburg, Hebelstr. 25, 79104 Freiburg, Germany

1 Introduction

Elucidation of the neurobiological basis of behaviour is a mandatory first step to decipher how the brain orchestrates cognitive processes such as learning, memory and attention. There are two general research methods applied in behavioural neuroscience: (i) recording of selected electrophysiological or neurochemical parameters during behaviour and (ii) pharmacological modification of the nervous system to alter behaviour during an experiment. The majority of behavioural studies are performed in rodents, placing particular constraints on the 'payload' that can be carried by these small animals. Combining these approaches in awake, freely-moving animals is technically demanding and constrained by the need for tethered based systems which restrict behavioural output.

Over the last three decades, the fabrication processes developed for silicon-based microelectromechanical systems (MEMS) have opened new perspectives with respect to system integration of neural microprobes (Wise et al. 2008; Kipke et al. 2008; HajjHassan et al. 2008). For instance, recording of electrophysiological or neurochemical signals is now possible with microprobes integrated with electrodes (Hoogerwerf and Wise 1994; Norlin et al. 2002; Ruther et al. 2010) and biosensors (Johnson et al. 2008; Frey et al. 2010), respectively. Additionally, microprobes with electrodes for electrical stimulation (Motta and Judy 2005; Musa et al. 2009), optical waveguides for optogenetics (Royer et al. 2010; Zorzos et al. 2010), and integrated fluidic channels for drug delivery (Chen et al. 1997; Seidl et al. 2010) have been presented and allow temporally-controlled manipulation of neural activity. Furthermore, fluidic probes for drug delivery combined with electrophysiological or optogenetic capabilities would open a variety of new avenues for manipulating brain function.

Amongst the methods for altering behaviour during an experiment, electrical stimulation is non-specific and only increases neuronal activity. On the other hand, application of optogenetics enables the control of specific, genetically modified cells by using light. However, optogenetics in behaving animals is still an evolving approach. Therefore, intra-cerebral pharmacological intervention to manipulate cells represents the present state-of-the-art in behavioural neuroscience.

Traditionally, pharmacological manipulations employ either microiontophoresis or pressure ejection (Lacey 1997; Lalley 1999) in order to deliver drugs in the brain. Microiontophoresis is limited to drug compounds that can be electrically polarised and consequently this method is not suitable for drugs with low charge-to-mass ratios. Additionally, the effective drug flow cannot be calculated from the applied current and is influenced by several additional factors, e.g. geometry and material of the micropipette, solvent, and the medium into which it is iontophoresed (Lalley 1999). This makes it impossible to determine accurately the amount of drug injected. Pressure-driven liquid infusion systems are more commonly used and are independent of the charge states of the drug molecules. However, diffusion-based leakage of drug from the probe, channel blockages, and physical displacement of the surrounding tissue during injection can be disadvantageous.

Typically, microprobes require external connections, e.g. electrical connections or fluidic tubing, and it is important that these interfaces do not interfere with the behaviour of animals during experiments. This issue has been optimally solved for microprobe electrophysiology using animal-mounted miniature wireless transmitters (Fan et al. 2011; Szuts et al. 2011; Bonfanti et al. 2011). Ideally, modified wireless devices could be used to control 'on-demand' drug delivery from fluidic microprobes, alongside conventional electrophysiology. Early approaches to address these issues used electrolysis to generate the flow required for infusion whilst accepting possible degradation of the drug itself (Criswell 1977; Bozarth and Wise 1980). Similarly, miniaturised mechanical pumps coupled to steel capillaries have been suggested for intracranial infusions (Ikemoto and Sharpe 2001). Alternatively, small-sized implantable drug delivery systems for general use with rodents are commercially available although they are not specifically tailored for neural experiments or microprobes, including the pre-programmable iPRECIO® micro infusion pump (Primetech Corporation, Tokyo, Japan) and the wireless controlled ithetis™ drug delivery device (Antlia SA, Lausanne, Switzerland).

To address the shortfall in small devices for behavioural neuroscience which offer (i) implantable multifunctional microprobes, (ii) multiple independently triggered drug infusions into both brain hemispheres, and (iii) a power consumption low enough to be covered by state-of-the-art wireless headstages, we developed the *NeuroMedicator* device (Spieth et al. 2011; Spieth et al. 2012) in the framework of the EU-project *NeuroProbes* (Neves et al. 2007; Ruther et al. 2008). The *NeuroMedicator* is a compact drug delivery system with silicon microprobes and an electronic interface offering the possibility of wireless control. Operation of this device is based on thermally expandable microspheres introduced by the Stemme group as an actuation material in microfluidics (Griss et al. 2002; Roxhed et al. 2006; Samel et al. 2007).

2 Material and methods

2.1 NeuroMedicator design

The operational concept of the *NeuroMedicator* is illustrated in the cross-sectional views shown in Fig. 1. Individual liquid reservoirs are connected in a pearl-chain-like serial

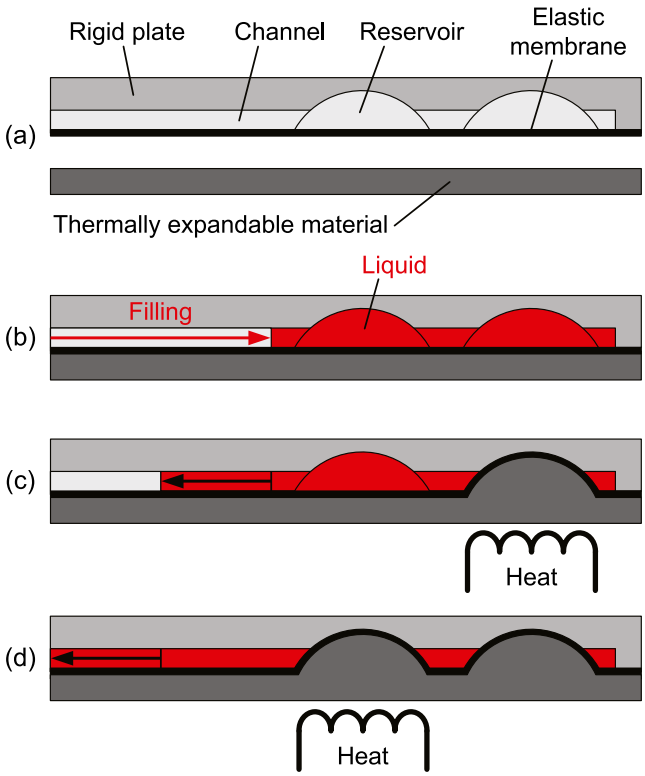

Fig. 1 Principle of operation of the *NeuroMedicator*: (**a**) Device components, (**b**) filling with liquid by using vacuum, (**c**) actuation of first reservoir, (**d**) actuation of second reservoir

arrangement with a blind end. Thereby, each reservoir is machined to have a nominal volume of 0.3 μL. As illustrated in Fig. 1(a), the liquid reservoirs as well as the connecting microfluidic channels are implemented into a rigid cyclic olefin polymer (COP) plate which is sealed on one side by an elastic thermoplastic elastomer (TPE) membrane. A thermally expandable layer underneath the reservoirs is used for actuation. The layer incorporates thermally expandable microspheres embedded into an elastic silicone matrix made from polydimethylsiloxane (PDMS) (Samel et al. 2007). Thereby, each microsphere can expand irreversibly up to 60 times in volume after heat treatment. After filling the reservoirs with liquid (Fig. 1(b)), the expandable material beneath the reservoirs is locally heated starting with the reservoir next to the blind end. The resulting expansion process inflates the membrane and displaces the corresponding volume for infusion (Fig. 1(c)–(d)). Hence, each liquid reservoir together with the underlying expandable material allows the delivery of a discrete volume of liquid having a nominal volume of 0.3 μL in the present case (see section 3).

The first device implementation described by Spieth et al. (2012) offered stability of the stored liquid only for a limited time frame due to evaporation and absorption issues. Therefore, the use of materials with higher barrier properties and less absorption was enforced, i.e. water permeable PDMS was replaced with COP and TPE materials where possible. The membrane is a key element required to be a barrier as well as a highly elastic element. Generally, this is a contradictory requirement for polymers as barrier properties decrease with increasing elasticity due to weaker cross linking (Domininghaus et al. 2008).

Illustrations of the *NeuroMedicator* are shown in Fig. 2. The geometrical design of the device is optimised to minimise the required contact area to the skull of a rat, thereby offering mechanical robustness and simplifying implantation as well as fixation. Together with the constraints of the fabrication technologies, this results in a T-shaped device design of $20\times17.5\times5$ mm^3. The narrow base of the 'T' with the silicon microprobes rests directly on the exposed skull of the rat. This reduces the contact area to 6×4 mm^2 being comparable to the 5×5 mm^2 required for a conventional double guide of a cannula system, e.g. the C232G (Plastics One Inc., Roanoke, VA, USA).

The device features two 8-mm-long silicon microprobes described elsewhere (Seidl et al. 2010) with a spacing of 1.5 mm tailored to access the prefrontal cortices in both hemispheres of the rat brain. Each of the two microprobe shafts has a cross section of 250×250 μm^2 and incorporates a fluidic channel with an outlet of 25 μm in diameter at a distance of 6.5 mm from the base. In each case, the fluidic channels are connected to eight liquid reservoirs.

The rear side of the housing features a centred anchor pin (Fig. 2(b)). The pin serves a dual purpose being used to attach the device to a stereotaxic manipulator during surgical insertion into the brain and providing extra purchase for the skull cement compound required to securely affix the device in place.

An exploded view of an assembled *NeuroMedicator* is shown in Fig. 2(c). The assembly is composed of four main components: (i) housing, (ii) actuators, (iii) insert with liquid reservoirs, and (iv) microprobes. The robust poly(methyl methacrylate) (PMMA) polymer housing serves as the common platform of the device incorporating the actuators, the insert with reservoirs, and the microprobes. The actuators are based on an array of microheaters on a printed circuit board (PCB) having a layer of thermally expandable material on top. Thereby, electrical connection to the microheaters is realised by a micro connector from the rear side of the PCB. The liquid reservoirs as well as the connecting microchannels are implemented in a rigid plate and sealed on one side with an elastic TPE membrane to form a self-contained insert. The silicon microprobes are directly bonded on top of the insert to enable fluidic connection to the liquid reservoirs. The calculated total liquid volume required to fill all reservoirs, channels, and microprobes is determined to be 7.4 μL of which 4.8 μL corresponding to 65 % can be used for infusions.

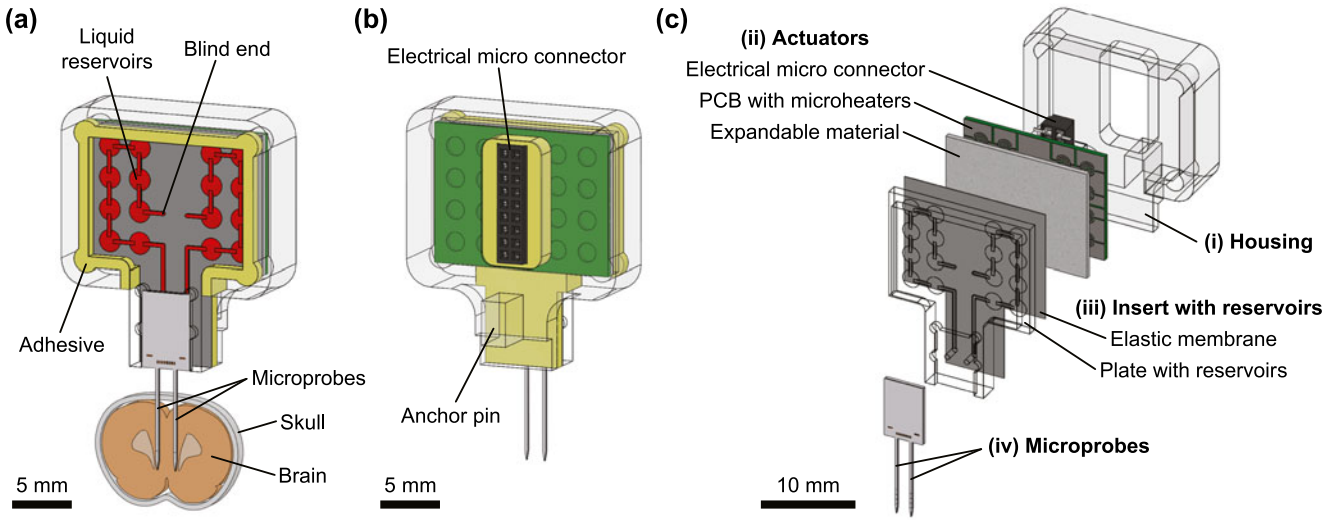

Fig. 2 Illustration of (**a**) the front side and (**b**) the rear side of the *NeuroMedicator* as well as (**c**) an exploded view highlighting the different components

2.2 Fabrication and assembly

The actuators, the insert with the liquid reservoirs, and the microprobes are first fabricated and pre-assembled as individual components. This is followed by final assembly into the device housing.

2.2.1 Actuators

The actuators are based on a 500-μm-thick double-sided PCB with an array of 16 micromachined titanium heaters on the front side. Detailed information on the fabrication of the microheaters is provided elsewhere (Spieth et al. 2012). To enable electrical contact to the microheaters, an electrical micro connector (CLM-109-02-L-D, Samtec, Germering, Germany) is attached to the rear side by using reflow soldering.

A layer of thermally expandable material is deposited on the front side of the PCB. To promote adhesion of the layer on the microstructured surface, an adhesion promoter is first applied. Afterwards, the expandable material is prepared by mixing PDMS RTV615 (Momentive Performance Materials, Inc., Albany, NY, USA) with thermally expandable microspheres Expancel® 820 DU 40 (Expancel, Sundsvall, Sweden) at a weight ratio of 2:1. A 500-μm-thick expandable layer is then spincoated and cured at 60°C. Thereby, the curing temperature is maintained low enough to prevent thermal expansion of the microspheres.

2.2.2 Insert with liquid reservoirs and microprobes

The insert with the liquid reservoirs is based on a 2-mm-thick COP plate (Zeonor 1420R, Zeon Corporation, Tokyo, Japan). The plate contains two separate microfluidic structures for storing and providing the liquid. Each structure is connected by a through-hole (diameter of 500 μm) to the averted side and couples there to the holder for the silicon microprobe chip. An individual structure includes eight reservoirs having each a nominal volume of 0.3 μL as well as microfluidic channels with a cross section of 170×300 μm^2 (depth×width) connecting them. To ensure complete depletion of the reservoirs after actuation, they are designed as spherical caps. The spherical structures are directly machined into the COP plate by milling, using standard ball nose cutters with a suitable diameter.

Sealing of the microfluidic reservoirs and channel structures is realised by laser-welding of a highly elastic TPE membrane made of a styrene-ethylene-butylene-styrene block copolymer (TPS-SEBS). The TPS-SEBS material (HTF 9467/44, KRAIBURG TPE GmbH & Co. KG, Waldkraiburg, Germany) having a thickness of 100 μm and a hardness of 54 Shore A offers a good compromise between barrier properties and elasticity. To realise laser welding, the laser beam has to be absorbed at the joining interface of the two materials. Typically, this is implemented by selection of black/transparent material combinations. In the present case both, the COP plate as well as the TPE membrane, are transparent. Black COP could be selected in order to achieve the required absorption. However, from the application point of view, it is desirable to keep the COP transparent in order to be able to inspect the content of the liquid reservoirs later on. Therefore, a biocompatible absorption coating (Clearweld® LD120C, Gentex Corporation, Zeeland, MI, USA) was applied to the side of the COP plate which contains the microfluidic structures by using a felt tip marking pen. After coating, the plate and the membrane

material which is not yet cut to size are pressed together by applying a pressure of 2 bar and welded using a diode-laser (OTF60-30-IP, Optotools, Heilbronn, Germany). Thereby, the welding trajectory is chosen to generously circumscribe the two microfluidic structures but not the individual components of the structures. The distance to the milled microfluidic structures ensures that no local overheating is generated by the laser beam at the edges possibly damaging the very thin membrane. In addition, it is ensured during assembly that the membrane is always pressed against the rigid plate. After welding, the excess material of the membrane is cut away.

The fluidic silicon microprobes are fabricated in a two-wafer bond process applying standard 300-μm-thick 4-inch silicon (100) wafers, deep reactive ion etching (DRIE), wafer grinding, and thin film processing as described by Seidl et al. (2010). Each microprobe is checked for potential blockages of the fluidic channels before assembly. The through-holes of the insert are designed to match exactly the two fluidic inlet ports with a diameter of 300 μm on the rear side of the 6×4 mm^2 common microprobe platform. The platform is then bonded into the holder by using adhesive (EPO-TEK® 353ND, Polytec PT GmbH, Waldbronn, Germany) and is cured at 85°C for 1 h. Finally, a 500-nm-thin layer of aluminium (Al) is optionally sputtered on top of the membrane to further improve the barrier properties of the TPE membrane with respect to air and water. The silicon microprobes are protected during the sputtering process to prevent any deposition of aluminium.

2.2.3 NeuroMedicator assembly

The housing of the *NeuroMedicator* is designed for robustness and machined from PMMA. During assembly, the PCB with the actuators is first bonded into the housing using adhesive (EPO-TEK® 353ND-T, Polytec PT GmbH, Waldbronn, Germany) and cured at 60°C for 3 h. This is followed by assembly of the insert with the attached microprobes. To ensure good mechanical contact between the expandable material and the membrane, the expandable layer is pre-stressed by applying a weight of 100 g on top of the insert while securing the assembly with adhesive (EPO-TEK® 353ND-T, Polytec PT GmbH, Waldbronn, Germany) and curing at 60°C for 3 h. Finally, the assembly is completed by filling the remaining gaps with the same adhesive and curing scheme used in the previous step. A photograph of assembled *NeuroMedicators* is shown in Fig. 3.

2.3 Filling with liquid

In general, all liquids are 0.45-μm-filtered to prevent blockages by particles. As the device is implemented as an enclosed channel structure only open towards the microprobes, filling is realised directly through the microprobes by applying a vacuum in a desiccator. After cleaning the microprobes with alcohol, the probes as well as the liquid to be filled in are placed into the desiccator. The filling process can be divided into three phases: (a) evacuation of the device, (b) immersion of the microprobes into the liquid, and (c) reestablishment of atmospheric conditions. The separation of evacuation and immersion into liquid is required to ensure optimal vacuum inside the microfluidic channel structure. In addition, the lowest vacuum level is adjusted by a pressure controller (IRV10, SMC Corporation, Tokyo, Japan) to prevent unintended boiling of the liquid. After the vacuum is established and the microprobes are immersed into the liquid, reestablishment of atmospheric pressure pushes the liquid through the microprobes into the channel structures and liquid reservoirs. As the device is filled through the smallest fluidic feature, i.e. the outlet, the risk of blockages due to particles during operation is additionally reduced. Directly after filling, the microprobe shafts are again carefully wiped with alcohol to remove any residues from the liquid on the outside of the shafts, e.g. drug or dye.

2.4 Electronic control unit

As a precursor for a future wireless control, a miniature electronic control unit for wire-based operation of the *NeuroMedicator* was developed. The controller comprises a microcontroller (MSP430F169, Texas Instruments, Dallas, TX, USA), which is connected to the Universal Serial Bus (USB) port of a standard computer. Wire connection to the *NeuroMedicator* is realised by a set of flexible wires each having a conductor cross section of 0.022 mm^2 (STC-34T-1, Vishay Measurements Group GmbH, Heilbronn, Germany). A software program offering a graphical user interface (GUI) allows the specification of the reservoir to be actuated as well as the corresponding heating power and time.

Since the resistance of a microheater increases with temperature and might additionally vary slightly from one to the other, the microprocessor regulates the voltage during the heating period to keep the heating power at the predefined level. The power-controlled heating ensures that the same energy is dissipated for identical heating periods. In addition, the heater resistance over time is monitored to detect any malfunction.

Alternatively to a constant heating power, individual heating profiles can also be specified. For the current set of experiments, individual infusion of reservoirs was realised by power-controlled operation of the heaters with 225 mW over a time period of 15 s.

2.5 *In vitro* device characterisation

The volumes of the spherical caps representing the reservoirs were determined before assembly. For this purpose,

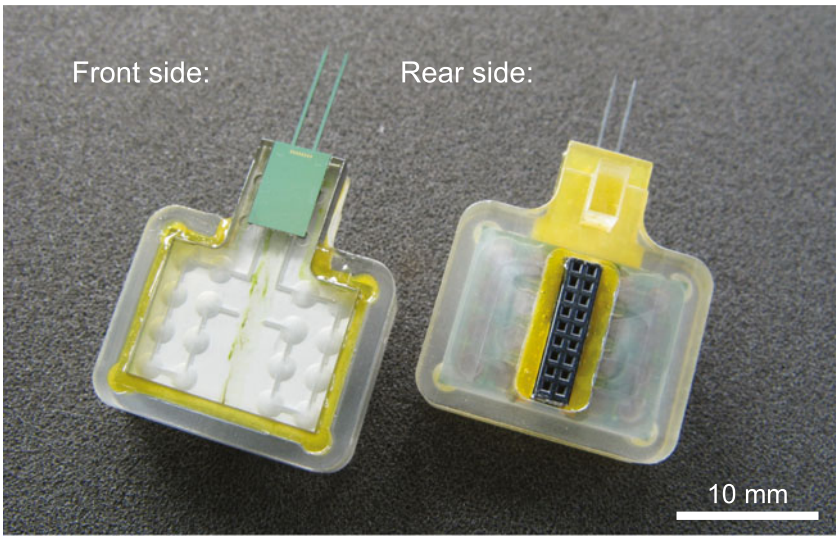

Fig. 3 Photograph showing the front and the rear side of assembled *NeuroMedicators*

the base diameters of the caps were optically measured and the volumes calculated by applying the diameter of the ball nose cutter used for milling. In order to be able to measure the delivery preciseness of the micropump, the microprobes of selected devices were replaced with fused silica capillaries of equivalent fluidic resistances enabling connection to a highly dynamic thermal flow sensor (HSG-IMIT, Villingen-Schwenningen, Germany).

The transient delivery characteristics and the delivered liquid volumes were determined. After filling the device with liquid and connection to the electronic control unit, the reservoirs were sequentially displaced as described in section 2.4 and the flow rates measured. Integration of the flow rates over time was then used to determine the delivered volumes.

2.6 *In vivo* experiments

The operation of the *NeuroMedicator* devices was verified by chronic *in vivo* experiments in rats. All animal related procedures complied with the requirements of the UK Animals (Scientific Procedures) Act 1986 and in accordance with the local institutional guidelines at Cambridge University, UK.

Two experiments were carried out: First, devices prepared with methylene blue dissolved in 0.9 % saline were implanted in rats and assessed over 14 days to monitor storage stability and passive fluid leakage when the device is exposed to the animal's home environment. In some cases, methylene blue dye was also delivered to characterise fluid spread within the brain.

Secondly, a device was implanted into the medial prefrontal cortex (mPFC) of a rat trained to perform a behavioural test of visual attention and impulsivity - the 5-choice serial reaction time task (5-CSRTT) (Robbins 2002; Bari et al. 2008). The device was filled with the NMDA receptor antagonist 3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid, (R)-CPP. Local infusion of this compound in the ventromedial prefrontal cortex (or infralimbic cortex) increases the likelihood of rats to respond impulsively (i.e. to anticipate the onset of a visual stimulus predictive of food reward as described later in section 2.6.3) (Murphy et al. 2005; Carli et al. 2006).

2.6.1 Device preparation and implantation

Immediately prior to implantation, the *NeuroMedicator* was filled with liquid according to the procedure described in section 2.3. Three Wistar Albino rats (350–400 g) and one Lister-Hooded rat (450 g) were anesthetised with isofluorane (3 % in oxygen) and placed into a standard stereotaxic frame. The bregma skull landmark was exposed and a small craniotomy was made rostral to this. Three custom-modified M1.6 stainless steel bolts were implanted into the skull as anchor points. Filled devices were mounted on to a stereotaxic manipulator with a custom-made clamp designed to attach to the anchor pin on the rear of the device. The microprobes were implanted in the rat's mPFC (bregma +2.8 mm, ~0.7 mm lateral, 2 to 5.5 mm deep) on both sides of the brain. The geometry of the microprobe comb was designed to record from the anterior cingulate, prelimbic, and infralimbic cortices. Devices were fixed in place using cold-cure glass ionomer cement (FujiCem, GC Europe N.V., Leuven, Belgium) to avoid any thermal stress on the loaded devices. The skin was gathered neatly and sutured around the cement and animals were allowed to recover.

A photograph of an implanted *NeuroMedicator* is shown in Fig. 4. This specific photograph shows the *NeuroMedicator* filled with (R)-CPP after use in the 5-CSRTT described later in section 2.6.3.

Fig. 4 Photograph of a *NeuroMedicator* implanted into the mPFC of a rat after infusion of one reservoir on each side during the 5-CSRTT

2.6.2 Methylene blue infusion

In order to assess storage stability, local spread of the infusions, and potential leakages from non-operated probes, devices with and without Al-barrier were prepared with methylene blue dissolved in 0.9 % saline. The devices were implanted in the three rats according to the procedure described in section 2.6.1. A device with an Al-barrier was used for infusions. In this case, the animal was allowed to recover for three days. On the third day, the content of two liquid reservoirs was consecutively infused into the left brain hemisphere whereas the right hemisphere remained without infusion. For this purpose, the *NeuroMedicator* was connected to the electronic control unit and operated as described in section 2.4. After 14 days of implantation, the rat was sacrificed by overdose of pentobarbitone, the device carefully explanted, and the brain processed for histology.

2.6.3 (R)-CPP infusion in the 5-CSRTT

A detailed description of the 5-CSRTT apparatus and training has been published previously by Bari et al. (2008). The apparatus measures 25×25×25 cm^3 and contains a magazine at the front where food rewards are delivered and a curved wall at the rear containing five equally spaced apertures (1.5×1.5×1.5 cm^3), each fitted with an infrared photobeam to monitor nose poke responses and an light-emitting diode (LED) at the back. Each daily session on the 5-CSRTT comprises 100 discrete trials, which are initiated by the rat entering the food magazine. Rats are trained over approximately 3 months to detect with a nose poke response the spatial location of a brief light stimulus presented on a pseudorandom basis in any one of the five apertures. A correct response is rewarded with a single food pellet delivered in the magazine. Responses in an adjacent aperture or omitted responses are signalled with a loss of food reward and the house light being extinguished for 5 s (time-out). Impulsivity is operationally defined on this task by responses made *before* the onset of the target stimulus; their occurrence is immediately punished by a 5 s time out period.

Before implanting the *NeuroMedicator* device, the drug solution was prepared by dissolving (R)-CPP (0.1 g/L) in saline followed by filling of the *NeuroMedicator* and implantation into the infralimbic cortex according to the procedure described in section 2.6.1. After implantation, impulsive responses in the 5-CSRTT were monitored on a daily basis to ensure a stable base-line. On the fourth day, the *NeuroMedicator* was connected to the electronic control unit and operated as described in section 2.4 to infuse the drug content of one reservoir on each side. Immediately after the infusion, performance in the 5-CSRTT was monitored.

3 Results

A photograph of assembled *NeuroMedicators* is shown in Fig. 3. Each device weighs 1.7 g. After assembly, the transient delivery characteristics and the delivered liquid volumes of the device were first determined *in vitro*. This was followed by *in vivo* assessment of storage stability, passive leakage, and fluid spread of implanted devices. Finally, the *NeuroMedicator* was evaluated as a tool for pharmacological manipulation of behaviour.

3.1 *In vitro* characterisation

A representative delivery characteristic for consecutive actuation of eight reservoirs, i.e. one half of a device, is shown in Fig. 5. The average geometrical volume of the reservoirs

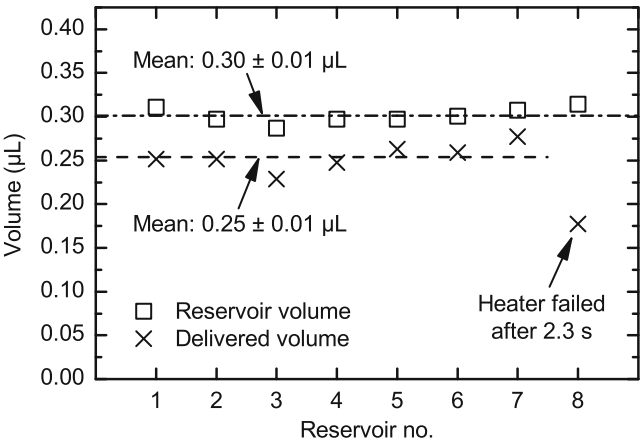

Fig. 5 Measured volumes of the liquid reservoirs before assembly and delivered volumes after consecutive actuation of eight reservoirs with 225 mW for 15 s. The heater of reservoir no. 8 failed after 2.3 s of operation

was optically determined to be 0.30±0.01 μL before assembly. Individual heating with 225 mW for 15 s as described in section 2.4 and integration of the resulting liquid flow rates was used to determine the delivered volume and resulted in a mean delivered volume of 0.25±0.01 μL. However, in this specific actuation sequence, the last heater failed after 2.3 s of operation resulting in incomplete displacement of the reservoir.

To analyse the discrepancy between the geometrical reservoir volume and the delivered volume, the average transient characteristic of the deliveries is shown in Fig. 6. The graph includes the mean cumulative delivered volume as well as the corresponding standard deviations. It can be deduced from the graph that most of the liquid is already delivered within the first few seconds of the 15 s heating period with a mean peak flow rate of 10 μL/min. At the end of the heating period, a mean delivered volume of 0.28± 0.02 μL is achieved. However, after heating is stopped, a relaxation effect occurs which soaks back a small amount of the delivered liquid and results in an effective delivered volume of 0.25±0.01 μL.

3.2 *In vivo* characterisation

3.2.1 Assessment of storage stability, passive leakage, and fluid spread

Devices having an Al-barrier on the TPE membrane show significantly better storage stability. If occurring at all, the liquid loss due to evaporation was observed to increase over time resulting in a loss of 0.37 μL after 14 days.

A photomicrograph of the coronal brain section in which the microprobes were inserted is shown in Fig. 7 after 14 days of implantation. Dark staining can be clearly seen on the right hemisphere due to infusion of 2×0.25 μL doses of methylene blue dye. In contrast, no apparent staining is visible on the non-dosed left hemisphere suggesting that passive leakage is negligible or is undetectable in this preparation.

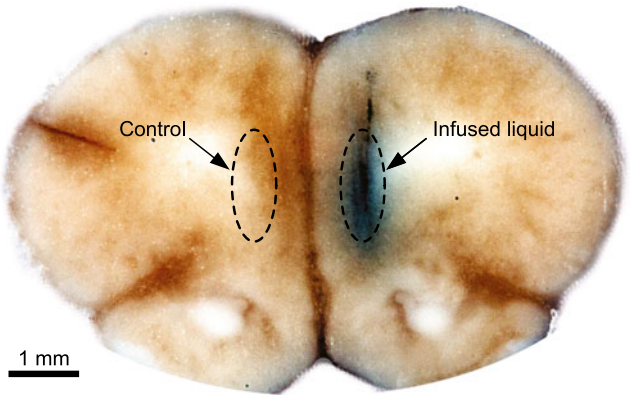

Fig. 7 Photomicrograph of the coronal brain section in which the microprobes were inserted showing the local deposition of methylene blue on the dosed side and no detectable dye-levels on the non-dosed (control) side

3.2.2 Behavioural modification using the NeuroMedicator

This experiment was set out to evaluate the utility of the *NeuroMedicator* as a tool for pharmacological manipulation of behaviour. A rat trained on the 5-CSRTT was used which allows the objective assessment of impulsivity. The frequency of impulsive responses made *before* the onset of the target stimulus for the subject rat are shown in Fig. 8. Impulsive responding was characterised on three days before and after surgery before a bilateral infusion of (R)-CPP targeted to the infralimbic cortices was performed. In the presence of (R)-CPP, impulsive responding was approximately three-fold higher (see Murphy et al. 2005), recovering to control

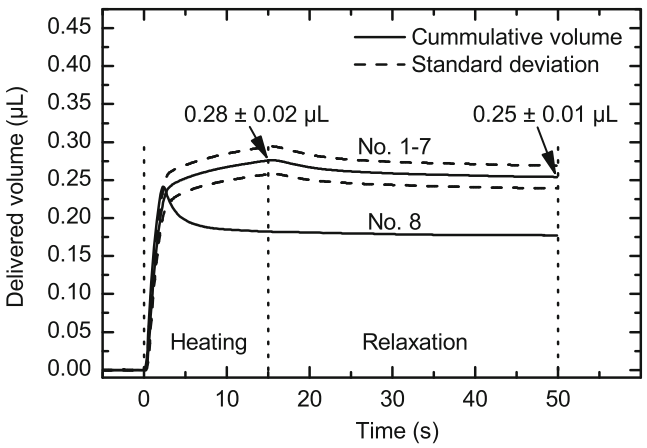

Fig. 6 Average transient delivery characteristics of eight consecutive actuations showing the heating as well as the relaxation phase. The heater of reservoir no. 8 failed after 2.3 s of operation

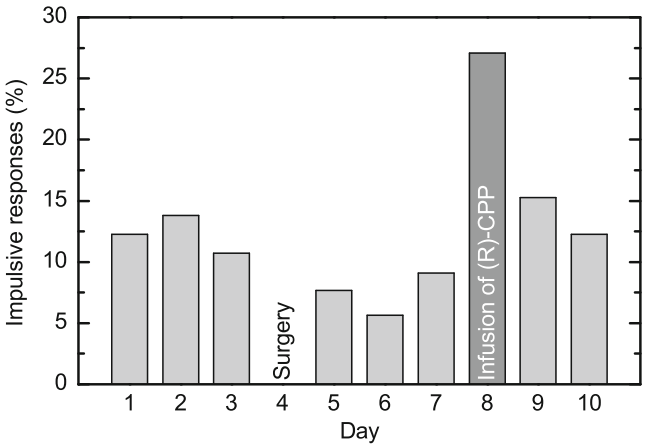

Fig. 8 Diagram showing the percentage of impulsive responses of an individual rat during the 5-CSRTT after infusion of (R)-CPP into the mPFC. Surgery was on day 4

levels on subsequent days. In addition, the stable base line after implantation suggests that passive drug leakage is negligible.

4 Discussion

The *NeuroMedicator* proved to provide discrete drug doses and was successfully applied for pharmacological manipulation of behaviour in freely behaving rats. The fabricated drug delivery system features liquid storage, fluidic silicon microprobes, and electrically controlled micropumping for infusion paving the way for wireless controlled drug infusions. The high degree of miniaturisation allows the microprobes to be rigidly attached to the liquid delivery mechanism eliminating the need for any fluidic interfaces or intermediate tubing and reducing the non-usable dead volumes.

In the presented design, the main focus was on mechanical robustness and ease of handling. Therefore, device size and weight can be considered as upper limits, subject to decrease after optimisation. Planar stacking of the device components facilitates assembly, but requires the narrow base of a T-shaped design to allow the animal's skin to knit around the device. Rotation of the reservoirs by 90° in the coronal plane would eliminate the requirement for a T-shape resulting in a lower-profile device.

Attachment of the TPE-membrane to the fluidic structure is currently realised by adhesive contact, local laser welding, and applying contact pressure during assembly. This is advantageous as no third materials need to be applied, i.e. adhesives or solvents. However, a bonding process irreversibly attaching the membrane over the whole surface area would further increase the device robustness and performance. In general, there are few processes capable of bonding a 100-μm-thin highly elastic membrane on a non-planar surface. Finally, the deposition of the Al-barrier is considered to be essential to maximise fluid stability. Unfortunately, the high Young's modulus of Al compared to the TPS-SEBS material reduces the elastic properties of the membrane. This demands additional power from the actuator.

Assuming ideal spherical caps, the average reservoir volume was measured to be 0.30 μL before assembly. However, the effective delivery volume was determined to be 0.25 μL during *in vitro* characterisation. This deficit can be attributed to a reduced reservoir volume as well as a backflow of liquid as depicted by the transient delivery characteristics (Fig. 6). During assembly, the reservoirs sealed with thin membranes are slightly pressed against the elastic expandable material to ensure good contact, although this somewhat reduces the effective volume. The backflow of liquid occurring after heating is switched off can be related to a structural relaxation. The expandable material relaxes due to the elastic restoring forces of the composite matrix surrounding the microspheres as well as the membrane with the thin Al-barrier. Furthermore, it is known that the microspheres can be compressed, especially in the expanded state (Roxhed et al. 2006; Akzo Nobel 2006). As it can be derived from the incomplete heating period of only 2.3 s in Fig. 6, most of the liquid delivery occurs within the first few seconds. However, the transient characteristic shows that extended heating is required to prevent high relaxation.

Filling of the device is realised through the silicon microprobe outlets, i.e. the smallest microfluidic feature. This is an additional safety mechanism to filter potentially critical particle sizes from entering the microfluidic system. In addition, no further sealing of the devices is required. However, on the other hand the filling procedure demands a carefully controlled vacuum to prevent boiling of the liquid to be loaded. Moreover, the filling pressure, i.e. the pressure difference between the inside of the device and the atmosphere during filling, converges asymptotically to zero during filling sometimes resulting in a small slug at the end of the blind channel.

The T-shaped device design as well as the low weight allowed the device to be implanted directly on the skull of the animal. The basic *in vivo* operations of the device were verified with a custom-specific electronic control unit enabling tethered operation and simulating a future wireless control unit. Successful *in vivo* infusions were demonstrated using methylene blue dye, verified by histology. In addition, no significant leakage arising from passive diffusion from the silicon microprobe outlets was detected over a period of 14 days. In this regard, no detectable increase in impulsive behaviour was detected in the task-performing rat following surgery (assessed over 3 days). However, following the infusion of (R)-CPP, a drug known to increase impulsivity during the 5-CSRTT (Murphy et al. 2005), the impulsive behaviour of the task-performing rat was successfully modulated.

To address drug delivery combined with electrophysiology in the future, the silicon microprobes feature already recording electrodes which are not yet used in the current implementation. Connecting the electrodes to an electric connector will allow to access them, e.g. with a wireless headstage. Ideally, one headstage is used for both, electrophysiological recordings as well as control of drug delivery. Thereby, the power consumption required for the micropump of the *NeuroMedicator* is certainly the most critical parameter. However, operation of all sixteen reservoirs requires 16×3.375 Ws estimated to be less than 10 % of the available battery capacity of state-of-the-art wireless headstages such as the W-Series from Triangle BioSystems, Inc., Durham, NC, USA. Therefore, it appears to be feasible that a single wireless headstage is used for both, electrophysiological recording as well as controlled drug delivery with the *NeuroMedicator*.

5 Conclusion

Remote drug delivery by microprobes combined with wireless recording of neural activity is a desirable goal during behavioural experiments in neuroscience. Ideally, a single wireless headstage would allow controlled drug delivery and electrophysiological recording from nearby electrodes. Currently, small, lightweight headstages which can be attached directly to skull and close to the implanted electrodes are already available. To address the aspect of drug delivery, we developed the *NeuroMedicator*, a micropump with microprobes which is small and lightweight enough to be placed directly on the skull. Thereby, the electrical interface allows future connection to a wireless control unit.

The practical applicability of the device for drug infusions was successfully demonstrated in conjunction with the 5-CSRTT. Although not connected in the current implementation, the silicon microprobes of the *NeuroMedicator* feature electrodes (Seidl et al. 2010) which have already proven their ability to record neural signals under the same *in vivo* conditions (Spieth et al. 2009). Hence, a fully wireless system combining drug delivery with multichannel electrophysiological recordings will become feasible.

Acknowledgments This work was performed in the frame of the Information Society Technologies (IST) Integrated Project *Neuro-Probes* of the 6th Framework Program (FP6) of the European Commission (Project number IST-027017). The authors gratefully acknowledge funding support by the Wellcome Trust and MRC in the United Kingdom through support of the Behavioural and Clinical Neuroscience Institute (BCNI) at Cambridge University. We also acknowledge support from Karsten Seidl, Patrick Ruther, and the cleanroom facility of IMTEK, University of Freiburg, as well as the support from the cleanroom and the machine shop facilities at HSG-IMIT. The authors would like to thank Joachim Leicht, Bernd Ehrbrecht, Jürgen Merz, and Alexander Fabricius (all HSG-IMIT) for conception, assembly, and programming of the electronic control unit. Furthermore, the authors would like to thank Björn Samel and Göran Stemme of Royal Institute of Technology Stockholm for useful discussions and insights. The provision of microspheres from Expancel, Sundsvall, Sweden, TPE membranes from KRAIBURG TPE GmbH & Co. KG, Waldkraiburg, Germany, and COP plates from Zeon Corporation, Tokyo, Japan, is gratefully acknowledged.

References

Akzo Nobel, Eine technische Präsentation der Expancel® Mikrosphären, Technische Information Nr. 40 (2006)

A. Bari, J.W. Dalley, T.W. Robbins, The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. Nat. Protoc. **3**(5), 759–767 (2008)

A. Bonfanti, G. Zambra, G. Baranauskas, G.N. Angotzi, E. Maggiolini, M. Semprini, A. Vato, L. Fadiga, A.S. Spinelli, A.L. Lacaita, A wireless microsystem with digital data compression for neural spike recording. Microelectron. Eng. **88**(8), 1672–1675 (2011)

M.A. Bozarth, R.A. Wise, Electrolytic microinfusion transducer system: an alternative method of intracranial drug application. J. Neurosci. Methods **2**(3), 273–275 (1980)

M. Carli, M. Baviera, R.W. Invernizzi, C. Balducci, Dissociable contribution of 5-HT_{1A} and 5-HT_{2A} receptors in the medial prefrontal cortex to different aspects of executive control such as impulsivity and compulsive perseveration in rats. Neuropsychopharmacology **31**(4), 757–767 (2006)

J. Chen, K.D. Wise, J.F. Hetke, S.C. Bledsoe Jr., A multichannel neural probe for selective chemical delivery at the cellular level. IEEE Trans. Biomed. Eng. **44**(8), 760–769 (1997)

H.E. Criswell, A simple chronic microinjection system for use with chemitrodes. Pharmacol. Biochem. Behav. **6**(2), 237–238 (1977)

H. Domininghaus, P. Elsner, P. Eyerer, T. Hirth, *Kunststoffe: Eigenschaften und Anwendungen*, 7th edn. (Springer, Berlin, 2008)

D. Fan, D. Rich, T. Holtzman, P. Ruther, J.W. Dalley, A. Lopez, M.A. Rossi, J.W. Barter, D. Salas-Meza, S. Herwik, T. Holzhammer, J. Morizio, H.H. Yin, A wireless multi-channel recording system for freely behaving mice and rats. PLoS One **6**(7), e22033 (2011)

O. Frey, T. Holtzman, R.M. McNamara, D.E.H. Theobald, P.D. van der Wal, N.F. de Rooij, J.W. Dalley, M. Koudelka-Hep, Enzyme-based choline and l-glutamate biosensor electrodes on silicon microprobe arrays. Biosens. Bioelectron. **26**(2), 477–484 (2010)

P. Griss, H. Andersson, G. Stemme, Expandable microspheres for the handling of liquids. Lab Chip **2**(2), 117–120 (2002)

M. HajjHassan, V. Chodavarapu, S. Musallam, NeuroMEMS: neural probe microtechnologies. Sens. **8**(10), 6704–6726 (2008)

A.C. Hoogerwerf, K.D. Wise, A three-dimensional microelectrode array for chronic neural recording. IEEE Trans. Biomed. Eng. **41**(12), 1136–1146 (1994)

S. Ikemoto, L.G. Sharpe, A head-attachable device for injecting nanoliter volumes of drug solutions into brain sites of freely moving rats. J. Neurosci. Methods **110**(1–2), 135–140 (2001)

M.D. Johnson, R.K. Franklin, M.D. Gibson, R.B. Brown, D.R. Kipke, Implantable microelectrode arrays for simultaneous electrophysiological and neurochemical recordings. J. Neurosci. Methods **174**(1), 62–70 (2008)

D.R. Kipke, W. Shain, G. Buzsáki, E. Fetz, J.M. Henderson, J.F. Hetke, G. Schalk, Advanced neurotechnologies for chronic neural interfaces: new horizons and clinical opportunities. J. Neurosci. **28**(46), 11830–11838 (2008)

G. Lacey, Microelectrophoresis and Pressure Ejection Methods, in *Neuroscience Methods: A Guide for Advanced Students*, ed. by R. Martin (Harwood Academic Publishers, Amsterdam, 1997), pp. 80–84. Chap. 12

P.M. Lalley, Microiontophoresis and Pressure Ejection, in *Modern Techniques in Neuroscience Research*, ed. by U. Windhorst, H. Johansson (Springer, Berlin, 1999), pp. 193–212. Chap. 7

P.S. Motta, J.W. Judy, Multielectrode microprobes for deep-brain stimulation fabricated with a customizable 3-D electroplating process. IEEE Trans. Biomed. Eng. **52**(5), 923–933 (2005)

E.R. Murphy, J.W. Dalley, T.W. Robbins, Local glutamate receptor antagonism in the rat prefrontal cortex disrupts response inhibition in a visuospatial attentional task. Psychopharmacol. (Heidelberg, Ger.) **179**(1), 99–107 (2005)

S. Musa, M. Welkenhuysen, R. Huys, W. Eberle, K. Kuyck, C. Bartic, B. Nuttin, G. Borghs, Planar 2D-Array Neural Probe for Deep Brain Stimulation and Recording (DBSR), in *Proc. 4th Eur. Conf. of the IFMBE, IFMBE Proceedings*, vol. 22 (Springer, Berlin, 2009), pp. 2421–2425

H.P. Neves, G.A. Orban, M. Koudelka-Hep, T. Stieglitz, P. Ruther, Development of modular multifunctional probe arrays for cerebral applications, in *Proc. 3rd Int. IEEE EMBS Conf. on Neural Eng.*, pp. 104–109 (2007)

P. Norlin, M. Kindlundh, A. Mouroux, K. Yoshida, U.G. Hofmann, A 32-site neural recording probe fabricated by DRIE of SOI substrates. J. Micromech. Microeng. **12**(4), 414–419 (2002)

T.W. Robbins, The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. Psychopharmacol. (Heidelberg, Ger.) **163**(3–4), 362–380 (2002)

N. Roxhed, S. Rydholm, B. Samel, W. van der Wijngaart, P. Griss, G. Stemme, A compact, low-cost microliter-range liquid dispenser based on expandable microspheres. J. Micromech. Microeng. **16** (12), 2740–2746 (2006)

S. Royer, B.V. Zemelman, M. Barbic, A. Losonczy, G. Buzsáki, J.C. Magee, Multi-array silicon probes with integrated optical fibers: light-assisted perturbation and recording of local neural circuits in the behaving animal. Eur. J. Neurosci. **31**(12), 2279–2291 (2010)

P. Ruther, A. Aarts, O. Frey, S. Herwik, S. Kisban, K. Seidl, S. Spieth, A. Schumacher, M. Koudelka-Hep, O. Paul, T. Stieglitz, R. Zengerle, H. Neves, The NeuroProbes project – Multifunctional probe arrays for neural recording and stimulation. Biomed. Techn. **53**(Suppl. 1), 238–240 (2008)

P. Ruther, S. Herwik, S. Kisban, K. Seidl, O. Paul, Recent progress in neural probes using silicon MEMS technology. IEEJ Trans. Electr. Electron. Eng. **5**(5), 505–515 (2010)

B. Samel, P. Griss, G. Stemme, A thermally responsive PDMS composite and its microfluidic applications. J. Microelectromech. Syst. **16**(1), 50–57 (2007)

K. Seidl, S. Spieth, S. Herwik, J. Steigert, R. Zengerle, O. Paul, P. Ruther, In-plane silicon probes for simultaneous neural recording and drug delivery. J. Micromech. Microeng. **20**(10), 105006 (11pp) (2010).

S. Spieth, A. Schumacher, K. Seidl, K. Hiltmann, S. Haeberle, R. McNamara, J.W. Dalley, S.A. Edgley, P. Ruther, R. Zengerle, *Robust microprobe systems for simultaneous neural recording and drug delivery, in Proc. 4th Eur. Conf. of the IFMBE, IFMBE Proceedings*, vol. 22 (Springer, Berlin, 2009), pp. 2426–2430

S. Spieth, A. Schumacher, S. Messner, T. Holtzman, P.D. Rich, J.W. Dalley, R. Zengerle, A miniaturized on-demand drug delivery system for neural research, in *Proc. 6th Int. Conf. on Microtechn. in Med. and Biol.*, pp. 62–63 (2011)

S. Spieth, A. Schumacher, S. Messner, R. Zengerle, The NeuroMedicator - A micropump integrated with silicon microprobes for drug delivery in neural research. J. Micromech. Microeng., in press (2012)

T.A. Szuts, V. Fadeyev, S. Kachiguine, A. Sher, M.V. Grivich, M. Agrochão, P. Hottowy, W. Dabrowski, E.V. Lubenov, A.G. Siapas, N. Uchida, A.M. Litke, M. Meister, A wireless multi-channel neural amplifier for freely moving animals. Nat. Neurosci. **14**(2), 263–269 (2011)

K.D. Wise, A.M. Sodagar, Y. Yao, M.N. Gulari, G.E. Perlin, K. Najafi, Microelectrodes, microelectronics, and implantable neural microsystems. Proc. IEEE **96**(7), 1184–1202 (2008)

A.N. Zorzos, E.S. Boyden, C.G. Fonstad, Multiwaveguide implantable probe for light delivery to sets of distributed brain targets. Opt. Lett. **35**(24), 4133–4135 (2010)