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Achieving synaptically relevant pulses of neurotransmitter using PDMS microfluidics

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Abstract

Fast synaptic transmission is mediated by post-synaptic ligand-gated ion channels (LGICs) transiently activated by neurotransmitter released from pre-synaptic vesicles. Although disruption of synaptic transmission has been implicated in numerous neurological and psychiatric disorders, effective and practical methods for studying LGICs *in vitro* under synaptically relevant conditions are unavailable. Here, we describe a novel microfluidic approach to solution switching that allows for precise temporal control over the neurotransmitter transient while substantially increasing experimental throughput, flexibility, reproducibility, and cost-effectiveness. When this system was used to apply ultra-brief (~400 μ s) GABA pulses to recombinant GABA_A receptors, members of the cys-loop family of LGICs, the resulting currents resembled hippocampal inhibitory post-synaptic currents (IPSCs) and differed from currents evoked by longer, conventional pulses, illustrating the importance of evaluating LGICs on a synaptic timescale. This methodology should therefore allow the effects of disease-causing mutations and allosteric modulators to be evaluated *in vitro* under physiologically relevant conditions.

Keywords

Patch clamp; GABA_A receptor; Electrophysiology; Solution exchange; Solution switching; Kinetics; Photolithography; Cys-loop; Pharmacology; Ligand-gated; Ion channel

1. Introduction

Ligand-gated ion channels (LGICs) mediate fast synaptic transmission in the central and peripheral nervous systems. They are targeted by numerous clinically important drugs and have been implicated in a variety of neurological and psychiatric disorders (Kullmann and Hanna, 2002; Macdonald et al., 2004; Vincent et al., 2006). However, despite decades of research, effective and practical methods for studying LGICs *in vitro* under physiologically relevant conditions remain unavailable (Mozrzymas, 2008), largely reflecting the short timescale on which synaptic transmission occurs. Increasing evidence suggests that neurotransmitter levels in the synaptic cleft rise extremely rapidly following release from pre-synaptic vesicles, only to be cleared after hundreds of microseconds by a combination of diffusion, reuptake, and for some neurotransmitters, enzymatic hydrolysis (Holmes, 1995; Clements, 1996; Glavinovic, 1999; Ventriglia and Di, 2003). Mimicking the synaptic transient in the experimental setting thus requires not only that neurotransmitter be applied rapidly, but also that it be applied briefly.

Failure to activate LGICs under synaptically relevant conditions, however, can cause the effects of disease-causing mutations and potential therapeutic compounds to be obscured or even missed entirely (Mozrzymas et al., 2007). Indeed, LGIC currents are known to be exquisitely sensitive to both the rate and duration of neurotransmitter exposure. For example, because most LGICs activate in the millisecond time domain before undergoing rapid and extensive desensitization, slowly changing the concentration of neurotransmitter can lead to underestimation of current amplitudes (Jones and Westbrook, 1996; Bianchi and Macdonald, 2002). Conversely, prolonged neurotransmitter applications can lead to overestimation of current amplitudes, particularly when intrinsic current rise times are much longer than the synaptic transient (Mozrzymas, 2004; Lagrange et al., 2007; Rula et al., 2008). Prolonged applications can also artificially prolong deactivation (the process by which currents return to baseline), the result of receptor accumulation in long-lived “desensitized” states from which neurotransmitter cannot directly unbind (Jones and Westbrook, 1995; Lagrange et al., 2007; Rula et al., 2008).

Currently, solution switching has proven the most effective technique for generating brief neurotransmitter pulses (Franke et al., 1987; Jonas, 1995; Clements, 1997; Hinkle et al., 2003). In contrast to photoactivation of “caged” neurotransmitter (Niu et al., 1996), this method does not require use of expensive reagents or radiation sources, is not constrained by the existing library of photoactivatable compounds, and most importantly, provides better control over the rate of neurotransmitter application and washout. Solution switching is typically accomplished by reversibly translating parallel control and neurotransmitter-containing solution streams generated from an array of glass capillary tubing across stationary cells or excised membrane patches. This approach allows neurotransmitter to be applied extremely rapidly to experimental preparations, with solution exchange times less than 100 μ s having been reported (Mozrzymas et al., 2007). Terminating the neurotransmitter pulse after synaptically relevant durations (i.e., 300–600 μ s), however, has yet to be performed reliably (Jonas, 1995; Clements, 1997; Hinkle et al., 2003; Mozrzymas et al., 2007; Mozrzymas, 2008).

To study LGICs under physiologically relevant conditions, we took a microfluidic approach to solution switching. In contrast to existing systems, we fabricated drug application devices from polydimethylsiloxane (PDMS), an inexpensive, durable, and bio-compatible polymer, using photolithography and replica molding. This allowed for the miniaturization and customization of device features, which dramatically reduced the width of individual channels and their septa while increasing experimental flexibility and throughput. By translating ultra-thin fluid streams generated by these devices across stationary excised

membrane patches with a stepper motor, solution exchange times as brief as ~100 μ s and application durations as brief as ~400 μ s were achieved reproducibly. When applied to recombinant GABA_A receptors, members of the cys-loop family of LGICs, these ultra-brief GABA pulses yielded currents with kinetic properties similar to those of inhibitory post-synaptic currents (IPSCs) and different from currents evoked by conventional, longer pulses. We thus anticipate that this novel approach to solution switching will provide new insights into the role of different receptor isoforms, allosteric modulators, and disease-causing mutations in synaptic physiology.

2. Materials and methods

2.1. Fabrication of microfluidic device molds from SU-8 using photolithography

Device molds were fabricated in a class-100 clean room at the Vanderbilt Institute for Integrative Biosystems Research and Education at Vanderbilt University. SU-8 2050 (MicroChem, Newton, MA), a negative-tone photoepoxy, was dispensed onto a polished silicon wafer (mechanical grade) on a spinner. To uniformly coat the wafer, the rotational speed was ramped at 100 rpm/s to 500 rpm and held for 5 s. To achieve a final film thickness of 100 μ m, the speed was then increased to 1750 rpm at 300 rpm/s and held for 30 s. Next, the coated wafer was soft baked for 5 min at 65 °C and then for an additional 15 min at 95 °C. The coated wafer was subsequently patterned with a customized chromium mask by contact photolithography using 230 mJ/cm² of UV radiation (Fig. 1a). The patterned wafer was subjected to a post-exposure bake of 5 min at 65 °C followed by 10 min at 95 °C. The unexposed SU-8 was removed from the wafer by incubation in SU-8 Developer (MicroChem, Newton, MA) for 30 min, leaving behind an SU-8 complementary replica of the microfluidic device (Fig. 1b). For prolonged stability, the resulting SU-8 mold was hard baked by temperature-ramping from 25 to 200 °C over 30 min and allowed to cool at room temperature overnight.

2.2. Fabrication of microfluidic devices from PDMS using replica molding

Microfluidic devices were fabricated from the SU-8 masters. Each device consisted of two layers of Sylgard 184 PDMS (Dow Corning Corp., Midland, MI), a silicone-based organic polymer widely used for microfluidic applications. Although PDMS is hydrophobic, unreactive to most reagents, non-toxic, and stable over a wide temperature range (McDonald and Whitesides, 2002; Mata et al., 2005), it should be noted that the hydrophobic nature of PDMS renders it incompatible with most organic solvents, which cause the material to swell. One layer was “imprinted” with a replica of the device design (i.e., the fluidic channels), and the other was left “unimprinted”, serving as a cover for the fluidic channels. To generate the imprinted layer, PDMS was prepared with a 5:1 ratio of pre-polymer to catalyst and poured over the SU-8 molds to a thickness of 5 mm (Fig. 1c). The PDMS was degassed in a vacuum chamber for 15–30 min and allowed to fully cure by incubation at 65 °C for 4 h. Once cured, the imprinted PDMS layer was peeled from the SU-8 molds (Fig. 1d), and the circular solution input ports were cored using a blunt 18-gauge syringe needle. To generate the unimprinted layer, PDMS was prepared with a 20:1 ratio of pre-polymer to catalyst and poured to a final thickness of 2 mm in a 10 cm culture dish (Corning Glassworks). The unimprinted PDMS layer was also degassed in a vacuum chamber for 15–30 min, but allowed to only partially cure for 30–45 min at 65°. The imprinted layer was then placed on the unimprinted layer (Fig. 1e), and the two were incubated at 65 °C for 3 h to fully cure and form a monolithic sealed device. Completed microfluidic devices were cut out using a surgical scalpel, and polyethylene tubing (Becton Dickinson, Sparks, MD) was connected to the solution input ports.

2.3. Cell culture and expression of recombinant GABA_A receptors

Human GABA_A receptor $\alpha 1$, $\alpha 3$, and $\alpha 2S$ subunits were individually sub-cloned into the pcDNA3.1+ mammalian expression vector (Invitrogen, Grand Island, NY). The coding region of each vector was sequenced by the Vanderbilt University Medical Center DNA Sequencing Facility and verified against published sequences (accession numbers NM 000806, NM 000814, and NM 000816 for the $\alpha 1$, $\alpha 3$, and $\alpha 2S$ subunits, respectively). HEK293T cells (American Type Culture Collection, Manassas, VA) were maintained at 37 °C in humidified 5% CO₂/95% air using Dulbecco's Modified Eagle Medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), 100 i.u./ml penicillin (Invitrogen), and 100 µg/ml streptomycin (Invitrogen). Cells were plated at a density of $\sim 10^6$ cells per 10 cm culture dish (Corning Glassworks, Corning, NY) and passaged every 2–4 days using trypsin–EDTA (Invitrogen). For electrophysiological recordings, cells were plated at a density of 4×10^5 cells per 6 cm culture dish (Corning Glassworks) and trans-fected ~ 24 h later with equal amounts (1 µg/subunit) of $\alpha 1$, $\alpha 3$, and $\alpha 2S$ subunit cDNA. One µg of pHoek-1 cDNA (encoding the cell surface antibody sFv) was included so positively transfected cells could be selected 24 h later by immunomagnetic bead separation. All transfections were performed using FuGene6 (Roche Diagnostics, Indianapolis, IN) per manufacturer recommendations. The day after transfection, cells were selected and re-plated at low density on collagen-coated 35 mm dishes for electrophysiological recording the next day.

2.4. Electrophysiology

Patch clamp recordings were performed at room temperature from excised outside-out membrane patches. Cells were maintained during recordings in a bath solution consisting of (in mM): 142 NaCl, 8 KCl, 6 MgCl₂, 1 CaCl₂, 10 glucose, and 10 HEPES (pH adjusted to 7.4; 325–330 mOsm). All chemicals used for solution preparation were purchased from Sigma–Aldrich (St. Louis, MO). Recording pipettes were pulled from thin-walled borosilicate capillary glass (Fisher, Pittsburgh, PA) on a Sutter P-2000 micropipette electrode puller (Sutter Instruments, San Rafael, CA) and fire polished with a microforge (Narishige, East Meadow, NY). When filled with a pipette solution consisting of (in mM) 153 KCl, 1 MgCl₂, 5 EGTA, 10 HEPES, and 2 MgATP (pH adjusted to 7.3; 300–310 mOsm) and submerged in the bath solution, this yielded open tip resistances of ~ 2 M Ω and a chloride equilibrium potential (E_{Cl}) of ~ 0 mV. Currents were recorded at a holding potential of -20 mV using an Axopatch 200A amplifier (Molecular Devices, Foster City, CA), low-pass filtered at 2 kHz (5 kHz for open-tip experiments) using a 4-Pole Bessel filter, digitized at 10 kHz (20 kHz for open-tip experiments) using the Digidata 1322A (Molecular Devices), and stored offline for analysis. All results shown are from a minimum of 2 days of experiments, each from a different batch of transfected cells. GABA was prepared as a stock solution. Working solutions were made on the day of the experiment by diluting stock solutions with the bath solution. All solutions were brought to room temperature prior to the experiments to minimize formation of bubbles in the microfluidic devices.

2.5. Data analysis

Current kinetic properties were analyzed using Clampfit 9 (Molecular Devices). Rise time was defined as the time required for currents to increase from 10 to 90% of their peak. The time course of deactivation was fit using the Levenberg–Marquardt least squares method to the form $a_n e^{-(t/\tau)^n} + C$, where t is time, n is the number of components, a is the relative amplitude, τ is the time constant, and C is the fraction of current remaining, with $a_n = 1$. Additional components were accepted only if they significantly improved the fit, as determined by an F -test automatically performed by the analysis software on the sum of squared residuals. Deactivation was typically biphasic, though as many as four components could be resolved with larger amplitude currents. To facilitate comparison, the time course

of deactivation was summarized as a weighted time constant in the form $a_n \tau_n$ with $a_n = 1$. The time course of peak current decay during repetitive stimulation was also fit to a sum of exponential functions, though typically, only a single component was required. Solution exchange time was defined as the time for an open-tip liquid-junction current to increase from 10 to 90% of its maximum value. Pulse duration was defined as the time an open-tip liquid-junction current spent at 90% of its maximum value. Data were reported as mean $\pm$ S.E.M. A paired Student's *t* test (one-tailed) was performed to compare the responses of an individual excised patch to different experimental protocols. Statistical significance was taken as $p < 0.05$.

3. Results

3.1. Custom microfluidic devices capable of generating synaptically relevant neurotransmitter pulses were fabricated from PDMS

Generating synaptic neurotransmitter pulses with conventional approaches to solution switching remains technically challenging, as most commercially available translators cannot reverse their motion to terminate the application (i.e., switch back to the control solution stream) after only hundreds of microseconds. High-velocity piezoelectric translators are currently used to overcome this limitation; however, they often suffer from resonant “ringing” after very brief excursions, which produces unwanted fluidic oscillations (Jonas, 1995; Clements, 1997; Mozrzymas et al., 2007), they require the use of high voltages, which requires electrical shielding to prevent additional noise contributions (Jonas, 1995; Clements, 1997; Heckmann and Pawlu, 2002), and they have a limited range of motion (typically 20–80 μm), which substantially limits the potential complexity of experimental protocols. Thus, if LGICs are to be studied *in vitro* under physiologically relevant conditions, an alternate approach is needed. One proposed method involves simultaneously delivering three, as opposed to two, parallel solution streams to the experimental preparation (Tang, 2001). Indeed, if only the central stream is loaded with neurotransmitter, then switching directly from the first to the third stream allows both application and washout of neurotransmitter to occur in a single motion. Ultra-brief pulses could therefore be generated simply by decreasing the width of the central solution stream. However, achieving solution channels less than $\sim 100 \mu\text{m}$ wide is not practical with glass capillary tubing, as heating and pulling the glass to achieve the necessary dimensions is not only labor intensive, but also causes application devices to be inherently fragile and rarely reproducible.

To overcome this obstacle, custom microfluidic devices were fabricated from PDMS using photolithography and replica molding (see Section 2). This approach allowed for micro-miniaturization of device features, while substantially improving device durability and reproducibility. The standard design, intended to mimic existing glass devices (Hinkle et al., 2003), employed three identical channels, each being 3 cm long and 100 μm wide (Fig. 2a). To generate ultra-brief pulses, the standard design was modified such that the central channel tapered to a final width of 50, 25, or 10 μm (Fig. 2b). All channels originated from circular solution access ports 300 μm in diameter (for connecting reagent reservoirs via polyethylene tubing) and were separated at their exits by 10 μm septa (to minimize the width of solution interfaces, allowing for rapid solution exchange; Supplemental Figs. 1 and 2). Due to current aspect ratio limitations, decreasing the widths of channels or their septa below 10 μm was not possible without decreasing channel heights, which needed to be at least 50–100 μm to facilitate channel visualization and electrode positioning. To rapidly translate the microfluidic devices during electrophysiological recordings, they were secured on an acrylic platform and connected to a Warner Instruments SF-77B stepper motor (Fig. 2c). Since this system has a reported translational velocity of $\sim 35 \mu\text{m}/\text{ms}$ (<http://www.warneronline.com>), we predicted the microfluidic devices with the narrowest central

channels (i.e., 10 and 25 μm) would be capable of generating synaptically relevant neurotransmitter pulses.

Device performance was characterized by loading outer and central channels with high and low (1:10 dilution) osmolarity solutions, respectively. This allowed electrode positioning relative to the channels to be monitored as a function of time, as solutions with different osmolarities yield different liquid-junction currents. Recordings began with an “open-tip” electrode positioned in one of the outer high osmolarity solution streams 25–50 μm from the channel exits (Fig. 2d). Maintaining a minimum of this distance from the channel exits prevented the electrode from passing through the solution “dead space” immediately distal to the septa, and instead, allowed it to pass through a narrower and more laminar solution interface (Sachs, 1999). To generate the brief pulse, the microfluidic device was translated orthogonally to its long axis with a single motion such that after being exposed to the first high osmolarity stream, the electrode was then exposed to the low osmolarity stream, and then to the second high osmolarity stream (Fig. 3a). With a fluid velocity of ~ 25 cm/s (the maximum sustainable by excised membrane patches; data not shown), solution exchange times were consistently ~ 100 μs (Supplemental Fig. 1) and exposure durations to the low osmolarity solution stream were 391 ± 47 , 799 ± 49 , 1783 ± 159 , and 3082 ± 259 ms when central channel widths were 10, 25, 50, and 100 μm , respectively ($n = 5$ devices) (Fig. 3b and c). These values were unaffected by using different osmolarity solutions or by repeatedly translating the devices at rates up to 80 Hz (data not shown). Pulse duration was linearly related to the width of the central channel ($r^2 = 0.99$), with a slope of 30.2 ± 1.9 $\mu\text{s}/\mu\text{m}$ (Fig. 3c). This corresponded to an average translational velocity of ~ 33 $\mu\text{m}/\text{ms}$, a value only slightly lower than that reported by the manufacturer (likely reflecting the larger mass of the acrylic platform and PDMS device compared to glass capillary tubing).

3.2. Currents evoked from GABA_A receptors were exquisitely sensitive to the duration of GABA application

Although synaptic LGICs are thought to be exposed to neuro-transmitter for only 300–600 μs , pulse lengths of 1–10 ms have historically been used to mimic synaptic transmission (Colquhoun et al., 1992; Jones and Westbrook, 1995; Lagrange et al., 2007; Bianchi et al., 2007; Rula et al., 2008). To determine if this difference in pulse length could alter the kinetic properties of LGICs, we compared the peak amplitude, deactivation time course, and sensitivity to high-frequency stimulation of GABA_A receptor currents evoked by either a “synaptic” 400 μs or a more “conventional” 10 ms pulse of 1 mM GABA (a synaptically relevant concentration). The 400 μs pulse was generated by translating the modified 3-channel device (Fig. 2b) containing the 10 μm central channel using the protocol described in the previous section (Fig. 3a), except that high and low osmolarity solutions were replaced by control and GABA-containing bath solutions, respectively. The 10 ms pulse was generated by translating the standard 3-channel device (Fig. 2a), again with control and GABA-containing bath solutions in place of high and low osmolarity solutions, respectively, using the traditional approach to solution switching (i.e., after exposing the recording electrode to the GABA-containing stream, it was then re-exposed to the original control stream by translating the microfluidic device in the opposite direction). Experiments were performed on outside-out membrane patches excised from HEK293T cells expressing the $\alpha 1 \beta 3 \gamma 2\text{S}$ receptor, an isoform expressed in adult hippocampal synapses (Pirker et al., 2000; Herd et al., 2008). To facilitate application of both pulse lengths to the same excised patch, a combination device containing both the standard and modified channel designs was used (Supplemental Fig. 3). Note that solution exchange times and application durations are known to be similar for “open-tip” and “patched” recording electrodes (Colquhoun et al., 1992; Mozrzymas et al., 2003).

Compared to currents evoked by conventional 10 ms pulses, those evoked by the more synaptically relevant 400 μ s pulses had smaller peak amplitudes (169.9 ± 55.3 pA vs. 225.4 ± 56.4 pA; $n = 10$; $p < 0.01$) (Fig. 4a and b). This difference could not be attributed to differences in the rates of GABA application, as solution exchange times were consistently ~ 100 μ s for all application protocols at a fluid velocity of 25 cm/s (Fig. 3b; Supplemental Fig. 1b) and were routinely checked before and after recording sessions. In addition, this difference could not be attributed to current “rundown” between applications, as 10 ms pulses were applied before and after the 400 μ s pulse, and the average of those responses was used for comparison. Instead, the smaller peak amplitude of currents evoked by synaptic pulses reflected the fact that $\alpha 1 \beta 3 \gamma 2$ S receptors were relatively slow to activate (Fig. 4c and d). In other words, while 10 ms pulses provided ample time for currents to reach their peak amplitude, 400 μ s pulses terminated receptor activation, causing currents to be truncated. Indeed, while maximal rise slopes were similar for 400 μ s and 10 ms pulses (Fig. 4c), indicating that the kinetics of receptor activation were similar following both application protocols, current rise time was substantially shorter following the 400 μ s pulse (0.61 ± 0.05 vs. 1.33 ± 0.14 ms; $n = 10$; $p < 0.001$) (Fig. 4c and d). In fact, the rise time of currents evoked by 400 μ s GABA pulses was approximately equal to the sum of the pulse duration (~ 0.4 ms) and the solution exchange time (~ 0.2 ms; i.e., ~ 0.1 ms $\times 2$, since the electrode must enter and exit the GABA stream), consistent with the idea that activation of this particular receptor isoform was limited by the duration of GABA exposure.

In addition, activating GABA_A receptors with ultra-brief as opposed to conventional pulses decreased the weighted time constant of deactivation (10.67 ± 2.44 ms vs. 17.99 ± 4.58 ms; $n = 10$; $p < 0.01$) (Fig. 4e and f), suggesting that limiting the duration of GABA exposure decreased receptor accumulation in long-lived conformations, which serve to delay GABA unbinding (Jones and Westbrook, 1995; Bianchi et al., 2007). Deactivation was typically bi-phasic (although as many as four components could be resolved with larger currents), and interestingly, while the relative amplitudes of fast and slow phases were similar for synaptic and conventional pulses ($A1$: 0.71 ± 0.05 vs. 0.83 ± 0.04 ; $A2$: 0.29 ± 0.05 vs. 0.17 ± 0.04 ; $p > 0.05$ in both cases), synaptic pulses were associated with faster time constants of deactivation (τ_1 : 1.83 ± 0.38 ms vs. 3.32 ± 0.47 ms; τ_2 : 20.88 ± 5.04 ms vs. 55.24 ± 8.66 ms; $p < 0.01$ in both cases). Synaptic pulses also decreased the sensitivity of GABA_A receptors to repetitive stimulation (Fig. 5), which has been demonstrated to progressively decrease peak current amplitudes (Jones and Westbrook, 1995; Lagrange et al., 2007; Rula et al., 2008). While this was not apparent at low stimulation frequencies (1 Hz; Fig. 5a and b), where the inter-pulse interval was sufficiently long to allow currents evoked by both synaptic and conventional pulses to fully deactivate, a substantial reduction was noted in the rate at which peak current amplitudes decayed when evoked by high frequency synaptic, as opposed to conventional, pulses (20 Hz; Fig. 5c and d). Indeed, when the peak amplitude of the last current was compared to that of the first current in a 20 Hz pulse train, $62 \pm 11\%$ of the current amplitude remained when synaptic pulses were used, while only $27 \pm 8\%$ remained when conventional pulses were used ($n = 3$; $p < 0.05$) (Fig. 5e and f). As with the effect on peak current, these effects of pulse length on deactivation and repetitive stimulation could not be attributed to differences in the rate of GABA washout, as solution exchange times were similar for all experimental protocols (data not shown).

4. Discussion

The fact that synaptic transmission occurs on a sub-millisecond timescale has made studying LGICs *in vitro* under physiologically relevant conditions technically challenging. Indeed, mimicking the synaptic transient requires that neurotransmitter be applied not only extremely rapidly, but also extremely briefly. Here, we describe the design, fabrication, optimization, and implementation of a novel microfluidic solution-switching system that

allows for solution exchange times as brief as $\sim 100 \mu\text{s}$ and pulse durations as brief as $\sim 400 \mu\text{s}$, thus fulfilling both of these requirements. When applied to recombinant $\alpha 1 \beta 2 \gamma 2$ GABA_A receptors, an isoform expressed highly in adult hippocampal synapses (Pirker et al., 2000; Herd et al., 2008), currents evoked by ultra-brief pulses of GABA had kinetic properties similar to those of inhibitory post-synaptic currents (IPSCs) recorded from adult hippocampal CA1 pyramidal neurons, including sub-millisecond rise times and biphasic deactivation time courses with weighted time constants of $\sim 10 \text{ ms}$ (Cohen et al., 2000). These kinetic properties, however, were substantially altered for currents evoked by longer, conventional pulses of GABA, consistent with the predictions of several theoretical modeling studies (Glavinovic, 1999; Mozrzymas, 2004; Lagrange et al., 2007). This raises the possibility that approximating synaptic conditions with longer neurotransmitter pulses could obscure the effects of disease-causing mutations or potential therapeutic compounds on LGIC currents, particularly when there are large differences between the length of application durations and intrinsic current rise times (i.e., the time required for currents to reach peak during continuous neurotransmitter exposure) (Mozrzymas, 2004; Mozrzymas et al., 2007).

The advantages of using PDMS microfluidic devices for studying LGIC currents, however, extend beyond their ability to effectively mimic the timescale of synaptic transmission. In contrast to existing rapid application systems, which typically allow for the delivery of only a few solution streams (Jonas, 1995; Clements, 1997; Hinkle et al., 2003), PDMS-based systems can, in principle, deliver tens to hundreds of solutions to experimental preparations. This tremendously increases the throughput of electrophysiological studies (see Fig. 6 for an example of a 12-channel microfluidic device used to determine the concentration response and pharmacological profiles of the $\alpha 4 \beta 3 \gamma 2$ GABA_A receptor isoform), and importantly, permits more complex experimental protocols to be performed. For example, different device designs can be included on the same microfluidic chip, thereby allowing neurotransmitter to be applied for different lengths of time (by varying central channel width; Supplemental Fig. 3) and/or at different rates (by varying septum width; Supplemental Fig. 2). Fabricating microfluidic devices from PDMS also makes them more durable and reproducible. Indeed, there is little risk of breaking PDMS devices even when they include micron-scale features, and should this occur, the replica molding process ensures that all replacement devices will be nearly identical to the original. Furthermore, taking a microfluidic approach to solution switching substantially reduces the cost of electro-physiological experiments, as PDMS-based devices are cheaper to fabricate, consume less reagent, and with short setup times, are less labor intensive.

The future for this microfluidic approach to solution switching thus appears highly promising, and new techniques are poised to further enhance its capabilities. Optimized methods for PDMS microfabrication allow for devices with higher aspect ratios (Liu et al., 2005), meaning that the widths of individual channels and their septa could be further decreased. This, combined with the availability of higher velocity stepper translators (Haydon Switch and Instrument Corp., Waterbury, CT), should allow for faster solution exchange times and briefer pulse durations. Moreover, given the recent development of microfluidic design elements that allow concentration gradients to be generated within individual channels (Chung et al., 2005), modulating the shape of the neurotransmitter transient should now be feasible. In other words, if the central neurotransmitter-containing solution stream is replaced with one that contains a neurotransmitter gradient, then passing this stream across the recording pipette will generate a non-square neurotransmitter waveform. This will allow the experimental neurotransmitter transient not only to be brief, but also to mimic the shape of the synaptic neurotransmitter transient, whose decay is likely better described by an exponential function (Clements, 1996). We thus anticipate that our

novel approach to solution switching will allow for LGIC currents to be evaluated *in vitro* under a wide range of physiologically relevant conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.jneumeth.2008.10.014.

Fig. 1.

Overview of microfluidic device fabrication using photolithography and replica molding. (a) The SU-8 photoepoxy is patterned with using the chrome mask by contact photolithography. (b) Unexposed SU-8 photoepoxy is removed, leaving behind the device mold. (c) PDMS is poured over the SU-8 mold to generate the “imprinted” layer. (d) After baking, the imprinted layer is peeled away from the SU-8 mold. (e) The imprinted layer of PDMS is bonded with an “unimprinted” layer to seal off the channels. Chrome mask, SU-8 photoepoxy, silicon wafer, and PDMS are indicated by the white, black, dark grey, and light grey layers, respectively. The pre-polymer to catalyst ratio is indicated within the PDMS layers.

Fig. 2. Microfluidic device design and integration for electrophysiological recording. (a) Standard 3-channel design (length not to scale). Individual channels (black) are 100 μm wide and separated by 50 μm septa (white) that taper to a final thickness of 10 μm . The circular enlargements at the origin of each channel (top) permit solution access via polyethylene tubing. (b) Modified 3-channel design for application of synaptically relevant agonist pulses. Dimensions are identical to those of the standard 3-channel device except for the width of the central channel, which tapers to a final width of 10 μm . (c) Schematic of the experimental setup. PDMS devices were connected to a stepper motor via an acrylic platform, allowing for translation of parallel solution streams across a stationary recording electrode. (d) Top-down view of a modified 3-channel microfluidic device (black arrows indicate device septa) and recording electrode (indicated by asterisk, *) submerged in bath solution, as typically arranged for electrophysiological recordings. Outer and central channels are shown delivering high and low osmolarity solution streams, respectively, allowing for visualization of solution interfaces (white arrows). Note that channels were ~ 80 μm high, though they appear smaller due to the image perspective.

Fig. 3.

Microfluidic device characterization. (a) Schematic depicting protocol for generating brief neurotransmitter pulses. A modified 3-channel device (not to scale) is shown in the starting position (left) with the recording pipette positioned in the high osmolarity (HIGH) fluid stream. With a single lateral movement of the device (black arrow), the recording pipette is exposed briefly to the low osmolarity (LOW) stream and then to the second high osmolarity stream (right). (b) Representative open-tip liquid junction currents obtained using the protocol in Panel A using devices with different central channel widths. Pulse durations for these currents were 410, 810, and 1620 μs (for the 10, 25, and 50 μm central channels, respectively). (c) Relationship between central channel width and pulse duration. The data were fit using a linear regression (see Section 3; $r^2 = 0.99$). Although not always visible, error bars representing the S.E.M. were included for all points.

Fig. 4.

Effect of pulse duration on $\alpha 1 \beta 3 \gamma 2S$ GABA_A receptor current kinetics. (a) Representative currents evoked by 400 μ s (left) or 10 ms (right) pulses of 1 mM GABA applied to the same outside-out patch. (b) The effect of pulse duration on peak current amplitude. (c) Comparison of current activation during 400 μ s (black) or 10 ms (grey) pulses of 1 mM GABA. (d) The effect of pulse duration on current rise time. (e) Comparison of current deactivation following 400 μ s (black) or 10 ms (grey) pulses of 1 mM GABA. Currents were normalized to their amplitudes at the start of GABA washout. (f) The effect of pulse duration on the weighted time constant of deactivation. Filled bars above currents indicate duration of GABA exposure. ** $p < 0.01$; *** $p < 0.001$.

Fig. 5.

Effect of pulse duration on 1-3 2S GABA_A receptor current responses during repetitive stimulation. (a–d) Representative current responses from 400 μ s (panels a and c) and 10 ms (panels b and d) pulses of 1 mM GABA applied at frequencies of 1 Hz (panels a and b) or 20 Hz (panels c and d). Note that there was no appreciable reduction of peak currents at 1 Hz for either pulse length, while there was a gradual reduction of peak current amplitudes at 20 Hz. To facilitate comparison of the rates of peak current reduction during repetitive stimulation, the maximal responses in panels a and c were normalized to those of panels b and d. (e) Representative currents evoked by the first (left) and last (right) pulses in the 20 Hz condition for either 400 μ s (black) or 10 ms (grey) pulses. Currents were normalized to the first pulse in the train. (f) Longer GABA pulses are associated with more pronounced loss of peak current amplitude during high frequency repetitive stimulation. * $p < 0.05$.

Fig. 6.

Schematic of a microfluidic device that allows for screening of LGIC concentration response and pharmacological profiles. (a) Channel exits in a custom 12-channel device are shown (numbered above device for clarity; not to scale). Even-numbered channels were loaded with GABA solutions (G) of increasing concentration (subscript denotes concentration in μM). Odd-numbered channels were loaded with external bath solution (E). Shown below the device are the corresponding currents (normalized to the 1000 μM trace) evoked from lifted whole cells expressing rat $\alpha 4 \beta 2$ GABA_A receptors. One-second GABA applications were separated by 45-s washes in external solution to allow for complete agonist unbinding (omitted traces indicated by //). Following exposure to the final GABA solution (channel 12), neurotransmitter washout was accomplished by stepping the device in reverse to channel 11 (indicated by light gray box; far right). (b) As above, except that even-numbered channels contained either low (10 μM ; lanes 2, 4, and 6) or high (100 μM ; lanes 8, 10, and 12) concentrations of GABA along with either a putative agonist or antagonist (PB = pentobarbital; TH = THDOC; PX = picrotoxin; BI = bicuculline; subscript denotes concentration in μM). Agonists (PB and TH) and antagonists (PX and BI) were co-applied with 10 and 100 μM GABA, respectively. Dashed lines indicate control peak current amplitudes for reference.