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Properties and application of a multichannel integrated circuit for low-artifact, patterned electrical stimulation of neural tissue

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Abstract

Objective—Modern multielectrode array (MEA) systems can record the neuronal activity from thousands of electrodes, but their ability to provide spatio-temporal patterns of electrical stimulation is very limited. Furthermore, the stimulus-related artifacts significantly limit the ability to record the neuronal responses to the stimulation. To address these issues, we designed a multichannel integrated circuit for patterned MEA-based electrical stimulation and evaluated its performance in experiments with isolated mouse and rat retina.

Approach—The Stimchip includes 64 independent stimulation channels. Each channel comprises an internal digital-to-analog converter that can be configured as a current or voltage source. The shape of the stimulation waveform is defined independently for each channel by the real-time data stream. In addition, each channel is equipped with circuitry for reduction of the stimulus artifact.

Main results—Using a high-density MEA stimulation/recording system, we effectively stimulated individual retinal ganglion cells (RGCs) and recorded the neuronal responses with minimal distortion, even on the stimulating electrodes. We independently stimulated a population of RGCs in rat retina and, using a complex spatio-temporal pattern of electrical stimulation pulses, we replicated visually-evoked spiking activity of a subset of these cells with high fidelity.

Significance—Compared with current state-of-the-art MEA systems, the Stimchip is able to stimulate neuronal cells with much more complex sequences of electrical pulses and with significantly reduced artifacts. This opens up new possibilities for studies of neuronal responses to electrical stimulation, both in the context of neuroscience research and in the development of neuroprosthetic devices.

1. Introduction

Multielectrode array (MEA) systems for electrical interfacing with networks of living neurons have proven to be very attractive tools for neuroscience research and biosensing applications. These systems can record the activity of a large number of neurons simultaneously, both in-vitro and in-vivo, as well as stimulate this activity by injecting electrical currents into the extracellular medium. The current state-of-the-art systems use large-scale MEAs with electrode diameters and interelectrode spacings approaching the size

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and spacing of neurons (Csicsvari *et al* 2003, Eversmann *et al* 2003, Litke *et al* 2004, Berdondini *et al* 2005, Blanche *et al* 2005, Frey *et al* 2010). Since the signal amplitudes sensed by extracellular electrodes are in general on the order of a few hundred μV or lower, these systems require a dedicated low-noise, high-gain readout circuitry. The readout electronics is usually designed in the form of a dedicated multichannel integrated circuit that is bonded to the array (D browski *et al* 2005, Charvet *et al* 2010; for review, see Jochum *et al* 2009) or integrated with the MEA on a single chip (Eversmann *et al* 2003, Berdondini *et al* 2005, Frey *et al* 2010). Such systems are able to record the activities of a large number of closely spaced neurons in parallel and they open up new possibilities for the investigation of information processing in local neural networks (Petrusca *et al* 2007, Fujisawa *et al* 2008, Pillow *et al* 2008, Tang *et al* 2008, Field *et al* 2010).

However, in contrast to the capability of modern MEA-based systems to record complex spatio-temporal neural network activity patterns, the functionality of these systems with respect to electrical stimulation of complex neuronal activity is very limited. Stimulation is usually restricted to a single electrode, or several electrodes following the same stimulation protocol (D browski *et al* 2004). More sophisticated approaches include the definition of several independent stimulation signals that can be repeated by groups of electrodes (Charvet *et al* 2010, Frey *et al* 2010) or the fast switching of single-channel stimulation circuitry between electrodes (Wagenaar *et al* 2004a). Although these systems can generate patterns of stimulation signals that are distributed in space and time, none of them exploits the potential of modern MEAs to elicit complex, arbitrarily defined activity patterns in a large number of neurons.

An additional problem common to all systems aiming at simultaneous electrical stimulation and recording is the stimulation artifact. The electrical signals applied to activate neurons are sensed by all electrodes of the array as stimulus-related artifacts, with amplitudes several orders of magnitude larger than the amplitudes of the recorded action potentials. This can result in saturation of the recording amplifier and makes detection of the neuron response very difficult. Although the artifact can be vastly reduced by optimization of the stimulation circuitry and experimental protocol (Jimbo *et al* 2003, Brown *et al* 2008, Frey *et al* 2010), as well as by additional signal post processing during data analysis (Wichmann 2000, Wagenaar *et al* 2002, Gnadt *et al* 2003, Sekirnjak *et al* 2006), current MEA systems are unable to record neuronal responses on the stimulating electrode for at least two milliseconds following the stimulus, and for at least half a millisecond on the near-by non-stimulating electrodes. In comparison, the delay of the elicited action potential in response to the stimulation pulse can be as short as 100 μs (Sekirnjak *et al* 2008) and the duration of the recorded pulse is on the order of a millisecond. Therefore, detection and proper identification of fast neuronal responses with such systems are extremely difficult.

In this paper we describe the design and application of the Stimchip - an Application Specific Integrated Circuit (ASIC) designed for low-artifact stimulation of a population of neurons with complex patterns of stimulation signals, defined arbitrarily in space and time. We present an application of the chip to stimulation of rodent retinal ganglion cells using a high-density MEA and an optimized stimulation pulse waveform. Finally, we demonstrate that the Stimchip allows for the elicitation of arbitrarily defined sequences of spikes in a population of neurons and makes possible the recording of the elicited short-latency spikes with minimal artifact-related distortions, even on the electrodes used for stimulation.

2. Materials and methods

2.1. Design of the stimulation circuitry

A functional schematic diagram of a single Stimchip channel is shown in figure 1A. The channel comprises a programmable bipolar current source to generate current stimulation signals of either polarity, a current-to-voltage converter that allows the generation of the stimulation signals in the voltage mode, and switching circuitry for artifact suppression. The basic specification parameters are summarized in figure 1C.

The current source is controlled by an internal 7-bit digital-to-analog converter (DAC). The DAC design is based on binary weighted current sources and provides unipolar current from 0 to 250 μA . The DAC output current waveform is defined by a stream of real time data received continuously from the off-chip controller. The data stream specifies, independently for each ASIC channel, the stimulation signal level for each 50 μs sampling period, with 7 bits to specify the amplitude and 1 bit to specify the polarity. It is thus possible to generate complex patterns of stimulation pulses, with both the stimulation signal waveforms and spatio-temporal distribution of the pulses defined arbitrarily by the user. The DAC output signal is scaled in one of eight selectable output current buffers to match the required current range (see figure 1C). The bipolar current pulse from the selected buffer is finally sent to the electrode, either directly - if the current stimulation mode is selected - or via the current-to-voltage converter. In the latter case, the stimulation signal amplitude is additionally corrected for the electrode offset held on the 10 pF hold capacitor (Jimbo *et al* 2003). The amplitude range and the mode of stimulation (current vs. voltage) are defined prior to the experiment, independently for each channel, by sending appropriate commands to the chip. For details of the design of the DAC, the output current buffers and the current-to-voltage converter, see Hottowy *et al* (2008a).

The artifact suppression circuit follows the idea proposed by Jimbo *et al* (2003) and consists of a hold capacitor, a tracking amplifier of gain equal to 1 and three switches controlled by three logic signals: *record*, *discharge*, and *hold*. The *record* switch is used to disconnect the recording amplifier from the electrode while the stimulation pulse is generated. When this switch is open, the electrode pre-stimulation potential is stored on the hold capacitor and connected to the input of the recording amplifier.

For cancellation of the charge left at the electrode-electrolyte interface by the stimulation pulse, the electrode is connected to the tracking amplifier output (*discharge*=ON) that reproduces the electrode pre-stimulation potential. During the discharging phase the amplifier is disconnected from the electrode (*record*=OFF). The discharging period can be adjusted in 50 μs steps to find the optimal compromise between minimization of the remaining residual artifact, and reduction of the delay between the stimulation pulse and the moment when the amplifier is reconnected to the electrode. The transistors used in the *discharge* switch must be relatively large to ensure low impedance of the closed switch and therefore a fast discharge time. In order to avoid parasitic charge injection from the *discharge* switch to the recording amplifier input through the feedback loop and input capacitance of the voltage follower, the *hold* switch can be used to separate the input of the recording amplifier from the tracking amplifier during the discharging phase; in such a configuration the input voltage for the recording amplifier is stored only at the input capacitance of the tracking amplifier. Details of the artifact cancellation circuitry design are described in (Hottowy *et al* 2008a).

2.2. Stimulation protocol

When a given stimulation channel is not active, the electrode is connected to the recording amplifier (signal *record*=ON) and both the stimulation signal source and the artifact

suppression circuitry are disabled (*stimulate=OFF*, *discharge=OFF*, *hold=ON*). In this configuration, the signal sensed by the electrode is continuously recorded by the amplifier.

If the stimulation channel is enabled, the *record*, *stimulate*, *hold* and *discharge* switches, as well as the DAC and polarity settings, are controlled in real-time in 50 μ s time steps by a stream of external data. For measurements employing the same electrode for stimulation and recording the standard protocol is as follows:

1. Before stimulation, the amplifier is connected to the electrode and the neuronal signals are recorded continuously.
2. In the first stage of stimulation the amplifier gets disconnected from the electrode (by setting *record=OFF*) and the amplifier input potential is stored across the 10 pF hold capacitor.
3. Once the amplifier is disconnected, the stimulation signal can be applied to the electrode. In this phase the stimulation circuitry is connected to the electrode (*stimulate=ON*) and both the *DAC data* and *polarity* control signals are updated every 50 μ s, following the required shape of the stimulation pulse.
4. After the stimulation pulse the active discharging can be applied (*stimulate=OFF*, *discharge=ON*, *hold=OFF*). The duration time of the discharging phase is defined in 50 μ s time steps.
5. After the discharging period the amplifier is connected back to the electrode (*discharge=OFF*, *record=ON*, *hold=ON*) and recording is continued.

A simplified version of the protocol, including only disconnection of the recording amplifier, can be applied simultaneously to the non-stimulating channels. To achieve maximum flexibility of the protocol, 12 bits of control data (7 amplitude bits, 1 polarity bit and 4 switch state bits) are updated in each sampling period and in each channel independently according to the external data stream.

To minimize the delay between the stimulation pulse and the start of the recording, one should not only optimize the shape of the stimulation pulse and the discharging duration time, but also minimize the delay between the end of the stimulation pulse and the start of the discharging period (steps 3–4) as well as between the end of discharging and the moment when the amplifier gets reconnected to the electrode (steps 4–5). In our design, the amplitude of the stimulation signal, as well as the states of the *stimulate*, *record*, *hold* and *discharge* switches, are refreshed synchronously with four separate trigger signals with appropriate delays to ensure non-overlapping switching of the switches in the channel. The triggers are generated in the chip based on a 5 MHz clock signal from the external controller, with relative timing set by the user with steps of 0.2 μ s.

2.3. Stimchip architecture and control

The chip comprises 64 independent stimulation channels, programmable voltage and current bias generators, internal bandgap reference and control logic. The chip status is controlled by digital commands sent to the chip through the command line. A single command line can control independently up to 32 Stimchips. The commands are decoded by the logic block and the control signals are sent to logic circuits in selected channels. The channel logic controls the channel stimulation status (*enabled/disabled*), stimulation mode (*current* or *voltage*) and the range of the output signal (current or voltage depending on the stimulation mode). In addition, commands are used to define the relative timing of the internal trigger signals (section 2.2). All these parameters must be defined prior to the experiment and cannot be changed in real time.

The stimulation waveforms and states of the artifact suppression circuits in all the active channels are controlled by a stream of real-time data received from an external PC through the 4-bit, 5 MHz data bus. To achieve a 50 μ s period for refreshing the control data in all channels, 12 bits of information must be sent to each channel within this time frame. Using a 4-bit data bus one has to send 192 words to load all 64 channels. The data are loaded into memory cells in the channels and then the states of the DACs are refreshed synchronously with an internal trigger generated by the on-chip logic block. Following this, the logic block generates triggers for refreshing the control signals (*stimulate*, *discharge*, *record* and *hold*) for the artifact suppression circuits. At a clock frequency of 5 MHz one can execute the described protocol in 50 μ s, with 38.4 μ s used for transmitting the data and 11.6 μ s for updating the parameters of the stimulation signals and the states of the switches in all channels. Within the 11.6 μ s time window, the times to refresh the individual switches are defined by the user before the experiment in 0.2 μ s steps (section 2.2). For details of the real-time data protocol see Hottowy *et al* (2008a).

The Stimchip has been designed and manufactured in the 0.35 μ m, four-metal, two-poly CMOS process from Austria Microsystems. Since the ASIC architecture imposes cross-linking of the analog and digital functionality in each channel and across the whole chip, careful design of the mask layout was required to minimize injection of parasitic signals from the digital switching circuits to the high-precision analog circuits. The mask layout has been designed using full-custom technique, except for the logic block, which has been synthesized automatically. Particular attention has been paid to the symmetry of the layout and to the distribution of the power to ensure the best possible matching of parameters across the 64 channels (Hottowy *et al* 2008a). The chip power consumption is 30 mW when stimulation is disabled on all channels, and increases by 1.1 to 1.7 mW for each enabled channel depending on the defined stimulation mode and the range of the output signal. The modulation of power consumption by the specific stimulus shape is negligible.

A photo of the Stimchip is shown in figure 1B. The total die area is 6400 $\times$ 2800 μ m². The pitch of the input and output bonding pads is 100 μ m so that the stimulation ASIC can be wire bonded directly to the recording ASIC (Grybos *et al* 2006).

2.4 Experimental setup

The Stimchip has been implemented in a set-up based on a custom-made high-density MEA comprising 61 microelectrodes of 5 μ m diameter, arranged in a hexagonal pattern with 60 μ m inter-electrode spacing (Litke 1999). The circular platinum return electrode is integrated with a plastic chamber that contains the neural tissue perfused with physiological solution (figure 1D). Prior to an experiment the electrodes are routinely electroplated with platinum black for impedance reduction using the current generators of the Stimchip, which can be configured to deliver the electroplating DC current. Arrays of the same type have been used previously for electrical stimulation of retinal ganglion cells (RGCs) in rodents and primates (Sekirnjak *et al* 2006, 2008, 2009). However, those experiments were carried out using a stimulation/recording system based on an older stimulation integrated circuit ('Platchip'; see D browski *et al* 2004) with a functionality that is very much limited compared to the Stimchip.

The connections between the traces on the glass MEA substrate and the printed circuit board are made with an elastomeric connector. Due to the geometry of the leads, two Stimchip ASICs are used for generation of the stimulation signals (figure 1D), with 32 channels of each Stimchip connected to the MEA; as the MEA includes 61 electrodes, three channels of the electronics are not used. The configuration commands and the stream of real time data for the chips are sent from a control PC equipped with a digital I/O card (National

Instruments PCI-6534) and running a Labview application (the PC uses the standard 32-bit Windows XP operating system).

The neuronal signals from all the electrodes are recorded by two Neuroplat ASICs (Grybo *et al* 2006), with each Neuroplat chip wire-bonded to a Stimchip (figure 1E). The Neuroplat ASIC comprises 64 independent AC-coupled recording channels with tunable gain and frequency pass band, and an analog multiplexer. The available ranges of the recording channel parameters are: 160–840 for the amplifier gain, 12–110 Hz for the lower cut-off frequency of the passband filter, and 50–4500 Hz for the higher cut-off frequency. In addition, it is possible to set the input resistance value (within the range of 4 M Ω –1 G Ω) which along with the coupling capacitor (190 pF) controls the cutoff frequency of the input AC-coupling stage. By changing the input resistance value and the lower cut-off frequency of the pass-band filter, it is possible to shape the noise characteristic of the recording channel (see D browski *et al* 2005 for details). In our experiments the input resistance was set to 70 M Ω , which corresponds to an AC coupling cut off frequency of 12 Hz, and the pass band was set from 45 Hz to 2000 Hz. The input-referred noise of the complete system, with these settings and with platinized electrodes immersed in a 0.9% NaCl solution, was 4.5–5 μ V. We used gain values from 270 to 800, depending on the range of the stimulation currents used in a given experiment. The output signals from all the channels were sampled simultaneously with a frequency of 20 kHz, multiplexed and sent to the control PC for digitization and data storage. The digitization was carried out with a National Instruments PCI-6110 card equipped with 12-bit analog-to-digital converters. The system design has been described in more detail by Hottowy *et al* (2008b).

2.5. Pilot experiments

2.5.1. Experimental procedure—In the pilot experiments we stimulated and recorded from retinal ganglion cells (RGCs) of adult mice and rats. Isolated pieces of retina were placed flat, ganglion cell layer down, on top of the array and superfused with oxygenated Ames' solution. The temperature of the bath was kept at 32° C during the experiment (experimental details were the same as described in Sekirnjak *et al* 2006). For classification of the RGC types, we stimulated the photoreceptor layer of the retina with a flickering white-noise checkerboard visual image and simultaneously recorded the spiking activity of 30–40 RGCs in a single preparation (Litke *et al* 2004). The identification of the individual neurons and their corresponding spike times were carried out with custom-designed software in Java. As one example, in a rat retina preparation that provided the data described in subsections 3.3 and 3.4, thirteen RGCs of type 'OFF-1' were identified. These cells are OFF cells with large visual receptive fields. (For discussion on the functional classification of rat RGCs, see Anishchenko *et al* 2010.)

2.5.2. Electrical stimulation protocol—In all the experiments we used a triphasic electrical stimulation signal waveform (positive-negative-positive sequence, as shown in figure 2) generated in the current mode. The relative amplitudes of the three phases were 2:–3:1 and the duration of each phase was set to 100 μ s. In the following text the term 'pulse amplitude' refers to the second (negative) phase of the pulse (for example, a pulse amplitude of 1.0 μ A corresponds to a 0.67/–1.0/0.33 μ A current phase sequence). All 61 electrodes of the array were routinely disconnected from the recording circuits for the duration of the pulse generated by any electrode to avoid saturation of the amplifiers. All the non-stimulating electrodes were reconnected to the amplifiers 5 μ s after the end of the last phase of the stimulation waveform. In the case of the electrode used for stimulation, a delay of 55 μ s was sufficient to obtain low artifact-related distortions and this value was used in all measurements.

2.5.3. Stimulation scan—To evaluate the responses of the RGCs to electrical stimulation, we generated stimulation pulses on all the 61 electrodes, with currents ranging from 0.15 μA to 1.6 μA and with 10% amplitude increments. We always pulsed one electrode at a time, with 15 ms delay between pulses on consecutive electrodes. This delay between stimulation pulses was sufficient for the neural network response to return to a steady state after the stimulation. Within one second a single pulse was applied to each electrode. By repeating this procedure 100 times for each amplitude, in less than 45 minutes we recorded the responses of a population of RGCs as a function of the stimulating electrode location (61 electrodes) and current amplitude (26 values).

2.5.4. Analysis of stimulation efficacy—The response to electrical stimulation of a given cell, with the cell identified as described in the subsection 2.5.1, was analyzed based on signals recorded for up to 2 ms after the beginning of the stimulation pulse. We analyzed the signals recorded by the electrode that showed the largest signal recorded from this neuron (named the ‘primary recording electrode’) and the six neighboring electrodes. To separate the signals coming from possibly several neurons plus the stimulation artifacts, the spike waveforms from the primary electrode and the neighboring electrodes were projected onto Principal Component Analysis (PCA) space and classified manually based on the three most significant PCA variables. The stimulation efficacy was defined as the probability for initiation of an action potential in a cell stimulated by a specific electrode. The experimental data of efficacy ϵ versus the stimulation current I was fit to the sigmoidal function $\epsilon = 1 / (1 + \exp(-aI + b))$. The stimulation current threshold was then defined from this fit as the current necessary for stimulation of the given cell with an efficacy of 50%. The ‘primary stimulation electrode’ for the given cell was the electrode that provided the lowest stimulation threshold for this cell.

2.5.5. Stimulation with spatio-temporal patterns—In one experiment we aimed at replication of the light-evoked activity in the population of thirteen ‘OFF-1’ RGCs that were described in subsection 2.5.1. Based on the results of the stimulation scan we identified online the primary stimulation electrodes and the stimulation thresholds for ten out of the thirteen ‘OFF-1’ cells identified in this preparation. (We note that the offline analysis performed after completion of the experiment indicated that twelve ‘OFF-1’ cells were effectively stimulated during the scan. The thirteenth cell was not stimulated by currents up to the maximum value of 1.6 μA employed in the scan.) To replicate the visually-evoked spiking activity generated by a single RGC, we defined a sequence of stimulation pulses that matched the time series of action potentials recorded previously as this RGC was stimulated with a one-second long spatio-temporal white noise visual stimulus. The current pulses were generated on the primary stimulation electrode of this cell, with the minimal amplitude necessary to stimulate the cell with 100% efficacy.

To replicate the activity of the population of RGCs, we combined the pulse sequences for each of the ten OFF-1 cells into one spatio-temporal pattern of stimulation pulses. All 61 electrodes were routinely disconnected from the recording amplifiers synchronously with the stimulation pulse generated on any of the electrodes. All the pulse timings were binned with a step of 2 ms to ensure that the recording following the stimulation pulse on one electrode was not disturbed by the stimulation pulse on another electrode.

Since the sensitivity of the RGCs to electrical stimulation can be reduced for high frequency stimulation (Sekirnjak *et al* 2006), the stimulation pulses were generated with an additional scaling of the pulse amplitudes. All the amplitudes were multiplied by the same factor, incremented from 0.8 to 2.0 with a 10% increase for each consecutive step. For each scaling factor value, we applied the complete pattern of stimulation pulses 20 times with a repetition period of 3 seconds. The complete procedure took ~11 minutes.

After the recording, we suppressed the spiking activity by adding the sodium channel blocker TTX (1 μ M) to the perfusion solution and repeated the stimulation protocol to record the stimulation artifacts. These artifacts were subtracted from the original data in the off-line analysis to reconstruct the RGC responses to the stimulation pulses (Sekirnjak *et al* 2006).

To estimate the timing of an evoked spike with respect to the stimulation pulse, we used the recorded response on the primary recording electrode after artifact subtraction (as explained above). Since the first (negative-going) edge of the neuronal spike can be slightly distorted by the artifact, we used the second (raising) edge of the (negative) spike waveform to estimate the spike times. For each spike, we found the time when this spike edge crossed the level equal to one-half of the negative spike amplitude, and this time minus the average spike width at the half-amplitude (estimated based on light-evoked activity of this cell) was defined as the spike latency. It should be noted that while in engineering papers the stimulation response time is typically defined in reference to the end of the stimulation pulse – as in this study – some neuroscientists prefer to define the latency in reference to the pulse onset (Sekirnjak *et al* 2006). For direct comparison of our results with some studies, the spike latencies reported here must be increased by the value of pulse duration (300 μ s).

The latency uncertainty was calculated as the standard deviation of the spike time distribution.

3. Results

3.1. Low-artifact stimulation of individual neurons

Figure 2A shows 100 overlaid responses of a mouse RGC to 0.43 μ A stimulation pulses, recorded on the stimulating electrode. One can easily notice two classes of the recorded waveforms. We classified 86 of the recorded waveforms as the superposition of stimulation artifacts and the elicited neuronal spikes (red traces), and 14 waveforms as the artifacts only (black traces).

The three-phase stimulation pulse was initiated at $t = -0.3$ ms and terminated at $t = 0.0$ ms. The amplifier input was disconnected from the stimulating electrode for the period from $t = -0.35$ ms to $t = 0.05$ ms for each stimulation waveform, and the artifacts recorded during that period (figure 2B) are due to crosstalk between the electrode and the amplifier input through the parasitic capacitances of the open CMOS switch (the *record* switch in figure 1A). The optimized shape of the stimulation current waveform results in a minimal residual charge left on the electrode-electrolyte interface and we observe only a low-amplitude artifact after reconnecting the amplifier input to the electrode.

The amplitudes of the recorded artifacts are within the same range as the typical amplitudes of the neuronal spikes and well within the amplifier linear range (± 1 mV input signal for the gain set to 800). Thus, we can assume that the system can recover the elicited spikes, after artifact subtraction, with a quality similar to that of the spontaneously generated spikes. To verify this, we averaged the waveforms within each of the two classes shown in figure 2A and subtracted the obtained average waveforms to find the average shape of the recorded elicited spikes (figure 2C). The same procedure was applied also to the electrodes adjacent to the one used for stimulation. As shown in figure 2D, the averaged elicited spikes match very well the averaged spikes generated by the same neuron spiking spontaneously. Therefore, with a simple artifact subtraction, it is possible to reconstruct the shapes of the elicited spikes with minimal distortion, even on the electrodes used for stimulation and for spikes with latencies as short as ~ 0.1 ms relative to the end of the stimulation pulse.

The method presented for spike shape reconstruction requires that the signals recorded after the stimulation pulse, that are combinations of stimulus artifacts and neuronal responses, do not exceed the linear range of the recording circuitry. As the artifact level changes with stimulation amplitude, this requirement may not be met for larger stimulation amplitudes. Based on results of the stimulation scan procedure (section 2.5.3) we analyzed for each channel the responses to stimulation pulses generated on the given channel. For the stimulation amplitudes up to 1.6 μA and the gain of the amplifiers set to 270, only 3 channels show saturation following the stimulation pulse. The remaining 58 channels (>95%) were able to start recording of neuronal responses immediately after they were connected back to the electrode.

3.2. Multineuron stimulation with a single electrode

By increasing the amplitude of the stimulation current, we could stimulate several neurons with the same electrode. In such a case the responses to the stimulation pulses can include different combinations of spikes from these cells. In figure 3A we show overlaid responses (recorded on the stimulating electrode and one of the neighboring sites) to 0.65 μA stimulation pulses that activated three independent mouse RGCs (#1–3) with efficacies of 100%, 23% and 38%, respectively (the stimulus was repeated only 98 times in this experiment). The signal from neuron #1 contributes equally to each of the displayed responses and it cannot be separated from the stimulus artifact - we identified this neuron based on the analysis of responses to lower stimulation amplitudes (data not shown).

The displayed waveforms split well into four separate clusters in the Principal Component Analysis space (figure 3B), based on the correlated waveforms recorded on the two indicated electrodes. We associate the four PCA clusters with the following groups of signals: (1) the artifact combined with the signals from neuron #1 – 45 red traces; (2) the artifact superimposed with the signals from neurons #1 and #2 – 16 black traces; (3) the artifact and the signals from neurons #1 and #3 – 30 green traces; and (4) - the artifact combined with signals from neurons #1, #2 and #3 – 7 blue traces. The average signal waveforms associated with each cluster, on each of the two electrodes, are shown in figure 3C.

The average signal shape for neuron #2 can be estimated as the difference between the averaged waveforms associated with clusters (2) and (1), or as the difference between the waveforms for clusters (4) and (3). Similarly, the signal shape for neuron #3 can be obtained either by subtracting cluster (1) from cluster (3) or cluster (2) from cluster (4). The estimated signal shapes are shown in figure 3D. One can see that for both neurons #2 and #3 the two estimation methods give nearly identical results, which confirms our hypothesis regarding the origins of the recorded signals. These results indicate that at least in some cases the signal recorded from multiple neurons spiking simultaneously is a linear superposition of the signals generated by these neurons and that the low-artifact stimulation provided by the Stimchip allows for proper identification of the responses from individual stimulated neurons, even if more than one cell is activated with the same electrode and stimulation amplitude.

3.3. Spatial map of stimulation threshold

We used the automated ‘scan’ procedure (section 2.5) to find how RGCs in the rat retina responded to stimulation pulses applied to various electrodes with different amplitudes. Typical results for one specific type of RGC are shown in figure 4. These RGCs are of type ‘OFF-1’ as has been described in subsection 2.5.1.

Figure 4A shows the “electrophysiological image (EI)” (Litke *et al* 2004) of an individual OFF-1 cell, based on the average spike waveforms recorded from this RGC, during visual stimulation, on the 30 indicated electrodes, including the 7 electrodes that effectively electrically stimulated the cell. Three of these electrodes (labeled 7, 14 and 15) recorded the biphasic somatic signals and the four remaining electrodes (1 and 53–55) recorded the triphasic, propagating axonal signals of the cell (Litke *et al* 2004).

The stimulation efficacies as a function of the current amplitudes are shown in figure 4B for each of the seven electrodes. The non-local, axonal stimulation by electrodes 1 and 53–55 are examples of antidromic stimulation whereby stimulation of the neuron’s axon is followed by backward propagation of the evoked impulse towards the soma and subsequent action potential generation by the cell. The efficacy curves in this case are very steep, with the response rate switching from 0 to 100% for a current increase of about 10%, while for cell body stimulation (electrodes 7, 14, 15) the efficacy vs. amplitude dependence is much more gradual (this effect was typical for all analyzed cells). This cell was stimulated with the lowest threshold by electrode #7 that also recorded the largest signal from this neuron. When stimulating with this electrode, the initiation of an action potential with a probability of 50% required a current of 0.25 μA , while for all the other electrodes the required current value was at least 0.7 μA .

For the thirteen ‘OFF-1’ RGCs identified in this preparation, based on the white noise visual stimulation procedure (subsection 2.5.1), twelve cells were effectively stimulated by at least one electrode. (As noted in section 2.5.5, the thirteenth cell could not be stimulated by currents as high as 1.6 μA , the highest current employed in the scan.) For ten of these cells the primary recording electrode, which always indicated a cell body signal, was the same as the primary stimulating electrode (section 2.5.4), consistent with the example shown in figure 4A. For the two remaining cells, one was stimulated most effectively with an electrode recording a somatic signal and adjacent to the primary recording electrode. For the other cell, the soma was located near the array corner (probably outside the active MEA area) and this neuron was stimulated most effectively with an electrode located close to its axon.

In figure 4C we show the distribution of the stimulation thresholds for the twelve cells and the 61 electrodes of the MEA. The minimal stimulation amplitudes required for stimulation of a given cell with 100% efficacy, using the ‘primary stimulation electrode’ for this cell, are shown with full symbols. The minimal stimulation current, leading to activation of a cell with an efficacy of 10% or more, was marked with an open symbol at the corresponding electrode ID. By inspection of the plot, one can identify for each of these RGCs an electrode and stimulation amplitude which provides stimulation of the given RGC with 100% efficacy without stimulating activity in the other identified cells of the same type.

One should note the following factors that made independent stimulation of individual RGCs within the ‘OFF-1’ population possible, namely: (1) the ‘OFF-1’ RGCs form a mosaic (Field and Chichilnisky 2007) thereby providing spacing ($\sim 100\text{--}150\text{ }\mu\text{m}$) between the RGC cell bodies; (2) the interelectrode spacing of the MEA is 60 μm , approximately one-half of the ‘OFF-1’ spacing; (3) by stimulating a specific RGC with an electrode located in the vicinity of the cell body, one gets a significantly lower stimulation threshold than for different electrode locations and (4) the Stimchip can provide independent stimulation pulses, with tunable current amplitudes, to each electrode. Those conditions make possible independent stimulation of the individual cells. However, there remain three important related issues that will be discussed in section 4.4: (1) the independent stimulation of RGCs of different cell types; (2) non-local axonal stimulation; and (3) optimization of the

stimulation currents, taking account of changes in thresholds due to recent past stimulations and/or spiking activity.

3.4. Stimulation with spatiotemporal patterns

According to the results presented in subsection 3.3 and shown in figure 4C, for twelve of the thirteen identified 'OFF-1' RGCs the primary stimulation electrode can stimulate a given neuron at a current level that makes simultaneous activation of the other 'OFF-1' cells unlikely. This suggests that it should be possible to independently stimulate individual 'OFF-1' RGCs and finally to generate a precisely defined sequence of spikes in a population of these neurons.

To verify this we first measured the spiking activity, evoked by one second of white noise visual stimulus, in the population of 'OFF-1' RGCs. We then defined a spatio-temporal pattern of electrical stimulation pulses to replicate this activity in ten of these cells and applied this stimulation pattern through the corresponding primary stimulation electrodes. The ten cells included in the patterned stimulation are the same cells as in figure 4C, except for neurons #7 and #9 for which the stimulation thresholds were not identified during the online analysis. (These thresholds were identified offline after the experiment; see subsection 2.5.5).

The complete pattern included 208 stimulation pulses distributed over the ten electrodes during one second. This pattern was repeated 20 times with a period of 3 seconds for each value of the scaling factor (subsection 2.5.5). The best compromise between an efficient activation of the targeted cells, and avoidance of activation of non-targeted cells within the population of 'OFF-1' RGCs was obtained for the scaling factor value of 1.56. For seven of the stimulated neurons we found the desired response: each of these cells was reliably activated by its primary stimulating electrode, and showed no activity synchronized with the stimulation pulses applied to any of the other electrodes (these were neurons 2, 3, 4, 5, 6, 10 and 11, as indicated in figure 4C). An eighth cell was clearly activated by both its primary stimulation electrode and one of the other nine electrodes. This RGC is indicated as neuron #1 in figure 4C; this is the same neuron that is shown in figures 4A and B. Finally, for the two remaining cells (8 and 12) the results of the crosstalk analysis were not clear. We noted that the current pulses generated at two of the ten stimulating electrodes initiated large, axon-like signals recorded on many electrodes of the MEA (data not shown). Analysis of the spatial distribution of these signals suggests that they were associated with stimulation of axonal bundles, however, more analysis is needed to understand this phenomena completely. Since these signals were also detected at the primary recording electrodes for neurons 8 and 12 and they could potentially mask the action potentials from these cells, making the analysis of the crosstalk for these cells problematic, we excluded these neurons from further analysis.

The EIs of the seven cells are shown in figure 5A; the red dot indicates the electrode used for stimulation of the given cell. The corresponding stimulation pulse times are shown as red lines in figure 5B. The 20 responses of each of the seven neurons to the 20 applied sequences of stimulation pulses are shown as blue diamonds in the same figure. The neuron spiking activity follows the applied stimulus sequences with excellent reproducibility, with an efficacy of ~99%, an average spike latency of ~50 μ s, and a spread of the spike latency (SD) of ~50 μ s (see figure 5C). We conclude that with careful optimization of the stimulation pulse amplitudes, the Stimchip-based system can generate a complex, precisely defined pattern of activity in a population of selected RGCs.

In figure 5D we show the EI of the eighth OFF-1 RGC (neuron #1 in figure 4C) that responded well to stimulation on its primary stimulation electrode (#7, indicated by the red

dot) but was also antidromically activated by electrode #55 (indicated by the green diamond) that was located close to the cell's axon, as shown in figure 4A. The axonal stimulation of neuron #1 has, as a consequence, crosstalk in the patterned stimulation. We see in figure 5E that in addition to the desired spiking responses of neuron #1 (blue diamonds) by electrode #7 (red lines), mimicking the visually evoked activity, there are extra (undesired) responses (green diamonds) due to crosstalk from the stimulation of neuron #11 by electrode #55 (red lines for the bottom cell in figure 5B). Figure 5F shows the spike latency distributions for the primary (blue) and axonal (green) stimulations.

In addition, we analyzed the responses to the pattern stimulation of the 15 RGCs of other than 'OFF-1' types identified in this preparation. We found antidromic axonal stimulation in two of these cells and somatic stimulation in three cells. As none of these 15 cells were targeted for stimulation, all these responses must be considered as crosstalk effects.

4. Discussion

4.1. Stimchip functionality and compatibility

In table 1 we compare the functionality and performance of the Stimchip with other reported stimulators designed for MEA-based two-way electrical communication with neural networks. Compared with these designs, the Stimchip allows for the generation of much more complex stimulation patterns than any other system. The chip generates 64 independent stimulation signals, while the other reported systems allow for the generation of one to eight independent signals. Furthermore, thanks to the VLSI technology used for the design of the stimulator, it is possible to build a compact system comprising many Stimchips working in parallel and providing independent stimulation signals to hundreds of electrodes on a large-scale MEA. Indeed, we have developed a completely functional 512-electrode MEA system with 512 independent stimulation and recording channels. For a description of this system and preliminary test results with retinal and cultured brain slice preparations see (Hottowy *et al* 2010).

An important feature of the Stimchip is its compatibility with various applications and MEA designs. This implies the ability to generate the stimulation signals over a wide range of current and voltage amplitudes. For example, extracellular stimulation of individual cells typically requires current amplitudes on the order of microamps (Sekirnjak *et al* 2006, Houweling and Brecht 2008), but values as low as 40 nA have been reported for specific electrode-neuron configurations (Buitengeweg *et al* 2002). On the other hand, for eliciting behavioral responses stimulation amplitudes of several hundred microamps may be necessary (Tehovnik 1996). In addition, some researchers prefer generation of the stimulation signals in a voltage mode. In such a case the injected current amplitude and waveform are difficult to control, as the impedance of the electrode-electrolyte interface can vary from electrode to electrode, but direct control of the electrode voltage helps avoid water electrolysis, which can be dangerous for the tissue (Merrill *et al* 2005). Therefore, the final choice between the current and the voltage stimulation modes depends on the priorities of the specific experiment.

The integrated circuit presented in this paper can generate stimulation signals in both the current and the voltage mode, with amplitudes ranging from 0.5 nA to 1 mA and from 12 μ V to 1.5 V, respectively. The total dynamic range in the current stimulation mode is equal to 22 bits, including 8 bits controlled in real time by switching the DAC value and polarity, and an additional 14 bits of variable gain in the output buffers. In the voltage mode, the dynamic range is equal to 18 bits (8+10). These ranges are sufficient for a wide range of experiments based on electrical stimulation; however, the maximum output voltage of 1.5 V

(which applies to both the current and the voltage mode) can be a limiting factor in some applications.

The Stimchip's ability to generate current signals with extremely low amplitudes (down to 0.5 nA peak-to-peak) and arbitrary waveform shapes opens the possibility for fast measurement of the electrode impedance as a function of frequency. Since the Neuroplat chip can record signals with amplitudes up to 10 mV peak-to-peak (for minimum available gain = 160) the measurement of impedances up to 20 M Ω is feasible. Such measurement can be used to estimate the range of stimulation currents available in each channel without exceeding the maximum output voltage of the Stimchip circuitry. Since the impedance of the electrode-electrolyte interface decreases in general with an increase of the cross-interface voltage (McAdams *et al* 1995), the estimation of the maximum available current based on the impedance measured with low currents would be in fact fairly conservative. Based on the impedance model for the microelectrodes used in this study (Mathieson *et al* 2004) we estimate the maximum amplitude for the triphasic pulse (2:–3:1 relative amplitudes, 100 μ s duration per phase) in our system to be on the level of 11 μ A.

It should be noted that the implementation of the real-time control of the stimulation signal waveform and artifact cancellation circuitry in each channel, although offering uncompromised flexibility of the stimulation protocol, requires a massive data stream of ~15 Mb/s from the off-chip controller. Although this data transmission rate is not a problem for most applications, the chip design may not be optimal for a bandwidth-limited application, such as a wireless system. In such a case, predefinition of the stimulation current waveform (presumably for each channel independently) combined with real-time transmission of trigger signals could be a more appropriate solution.

4.2. Low-artifact stimulation

Another advantage of the Stimchip, compared with other reported systems, is its ability to provide electrical stimulation with exceptionally low artifacts (see table 1). In our design the artifact minimization protocol is controlled independently for each channel, with high temporal resolution, whether or not the given channel generates stimulation current. This flexibility, combined with an optimized shape of the stimulation pulse, allows for very efficient artifact minimization. In our experiments we were able to record the neuronal responses as soon as 55 μ s after the stimulation pulse on the stimulating channel and almost immediately (5 μ s after the pulse) on the other channels. In comparison, the other reported systems cannot record neuronal responses for at least 2 ms on the stimulating channel and 0.4 ms on the other electrodes (table 1). One exception is the system used for stimulation of retinal ganglion cells (Sekirnjak *et al* 2006, 2008, 2009) that allowed for detection of some of the neuronal responses on non-stimulating electrodes with delays similar to those reported in our study. However, using a microelectrode array identical to ours, they were able to identify responses from only 5–7 cells in a single preparation, while our system allowed for recording of the elicited activity from virtually each cell targeted for stimulation (sections 3.1 and 3.3) including signals from the stimulating electrode. In the context of data presented in figure 2, we conclude that the Stimchip-based system presented here offers very significant improvement in the quality of recording of neuronal responses to electrical stimulation, especially in the case of low-latency responses from directly activated cells.

We emphasize the necessity of reduction of both the artifact level recorded during the stimulation pulse, as well as the post-stimulus artifact related to charge accumulation at the double layer of the stimulating electrode. The electrode voltage waveform during the stimulation pulse can be very large compared to the amplitudes of the recorded spikes and the linear input range of the recording circuitry – specifically at the stimulating electrode, for which the electrode voltage is practically limited by the output voltage limit of the

stimulation circuitry. Significant reduction of this part of the artifact is critical to avoid saturation of the recording amplifier, as the recovery of the recording circuitry may take up to hundreds of milliseconds (for discussion see Jochum *et al* 2009) making detection of the neuronal response to the stimulation impossible. This requires careful scaling of the transistors forming the blanking switch (the *record* switch in figure 1A). These transistors need to have very high impedance in the open state to assure low crosstalk of the input artifact to the input resistance of the amplifier and, at the same time, must provide low-resistance connectivity in the closed state (preferably, much lower than the spread resistance of the electrode) to avoid a noticeable noise contribution during recording. In our design the resistance of the record switch in the closed state is ~ 1 k Ω , reasonably low compared to ~ 60 k Ω spread resistance of our microelectrodes, or even ~ 10 k Ω of spread resistance in the case of larger electrodes with a diameter of 30 μ m. We also note that we did not notice any saturation problems caused by the crosstalk-related artifact during our experiments.

By comparison of the modeled electrode voltage during the stimulation pulse, predicted using the linear electrode impedance model of Mathieson *et al* (2004), with the amplitude of the artifact visible in our data during the pulse on the stimulating channel, we conclude that use of the *record* switch allowed us to reduce the artifact level by a factor of approximately 200. This artifact reduction factor was fairly constant for the current values used in our scan procedure (0.1 to 1.6 μ A). This constancy is an expected result, as both the impedance of the open CMOS switch and the input resistance of the Neuroplat chip - the two impedances that form the voltage divider for the electrode voltage waveform - are independent of the electrode voltage. For the maximum output voltage of the Stimchip stimulation circuitry (± 1.5 V) the amplitude of the artifact resulting from the crosstalk effect should not exceed ± 7.5 mV. In comparison, the linear input range of the Neuroplat chip with the gain set to 160 (lowest available setting) is ± 5 mV. However, it should be noted that the artifact reduction factor is expected to be a function of the input resistance of the recording amplifier and, due to the capacitive impedance of the open *record* switch, also of the shape and duration of the stimulation pulse. With the ability to control the stimulation waveform as well as the amplifier gain and the input resistance of our system, it should be possible to avoid the crosstalk-related saturation effect even for larger stimulation amplitudes and for different electrode designs than that used in our study.

The problem of the reduction of the post-stimulus artifact is unique to the stimulating electrode. Due to the complex impedance of the electrodes, the stimulation pulse results in charge accumulation at the electrode surface that in turn leads to an artifact visible in the recordings after the stimulation pulse is finished and the recording amplifier is reconnected to the electrode. We note the importance of an optimized stimulation pulse shape for the effective minimization of this part of the stimulation artifact. For example, the detection of short-latency neuronal responses in our experiments was not possible when we used a standard biphasic stimulation pulse followed by active discharging of the electrode-electrolyte interface (data not shown). The short latency of the elicited responses allows only a very short time for the discharge, on the order of 100 μ s, while significant artifact reduction requires an active discharging time on the order of milliseconds (Jimbo *et al* 2003, Brown *et al* 2008). Since the time constant of the discharging process is determined by the impedance of the electrode-electrolyte interface and the spread resistance of the extracellular medium, it is very difficult to shorten this time for a given electrode technology and stimulation amplitude. However, by adding an additional phase to the stimulation waveform, as in the experiments discussed in this paper, the charge required for significant reduction of the electrode resting potential can be injected quickly. In our experiments such a triphasic stimulation pulse allowed us to record the neuronal responses at the stimulating electrodes without any post-stimulus saturation problems on $> 95\%$ of the channels (section 3.1). However, even if the shape used in this study allowed us to record the responses from retinal

ganglion cells with very low distortions, the generalization of this method to other systems and applications will require more systematic studies, including modeling of the artifact phenomena based on realistic models of the electrode-electrolyte interface impedance. For best results, the pulse shape should be optimized for the specific electrode design and stimulation amplitude. The optimization procedure should also take into account the intrinsic delay of the neuronal response to an electrical pulse, which limits the acceptable latency between the stimulation pulse and the time for signal recording recovery. It should be noted that the results presented here do not allow the precise identification of the moment when the membrane depolarization exceeds the threshold for action potential initiation. It is then difficult to predict, based on these data, the timing of the stimulated action potential in relation to the stimulation pulse for different pulse shapes and durations. Thus, despite minimal delays between the end of the stimulation pulse and the beginning of the response recording reported in this study, the fact that the recording functionality is turned off during the pulse duration can in principle be limiting. For example, if the action potentials were generated by the first pulse phase, our recording would effectively start $\sim 200 \mu\text{s}$ after the pulse initiation (at the non-stimulating electrode, assuming $100 \mu\text{s}$ per phase duration). One of the interesting future applications of the system presented here would be to investigate the dynamics of neuronal responses to electrical stimulation by examination of the effects of different waveform types, including triphasic pulses of different polarities (cathodal-anodal-cathodal vs anodal-cathodal-anodal sequences).

4.3. Stimulation of individual retinal ganglion cells

The stimulation current thresholds for direct activation of individual ganglion cells presented in section 3.3 are consistent with published results from similar experiments (Jensen *et al* 2003, Sekirnjak *et al* 2006). Our result on the specifically high sensitivity of RGCs to stimulation currents injected in the vicinity of the cell body (section 3.3) was also reported before (Jensen *et al* 2003, Sekirnjak *et al* 2008) and attributed to the very high density of the sodium channels in the proximal axon region (Fried *et al* 2009). To our knowledge, our observation on the steeper efficacy-stimulation current curve for axonal stimulation, compared to stimulation of the cell body, has not been previously reported.

4.4. Stimulation of a population of retinal ganglion cells

Our result on the replication of the spatio-temporal spiking activity in a population of neurons is new in the literature. It was shown previously that epiretinal stimulation with microelectrodes can initiate action potentials in individual RGCs with high reliability and temporal precision, even at high stimulation frequencies (Fried *et al* 2006, Sekirnjak *et al* 2006). Fried *et al* (2006) used relatively large electrodes ($>5000 \mu\text{m}^2$) and stimulation currents ($>100 \mu\text{A}$) to replicate light-evoked activity in retinal ganglion cells, but the issue of independent stimulation of individual cells was not addressed. Sekirnjak *et al* (2008) used a microelectrode array identical to ours to selectively activate individual Parasol-ON and Parasol-OFF cells in the primate retina, with independent stimulation of even the closest-neighbors in RGC mosaics. However, the electronics used in that experiment did not allow for patterned stimulation. By using the Stimchip it was possible, for the first time, to replicate the natural activity in a selected population of retinal ganglion cells, and to record the evoked activity at the level of single spikes.

At the same time, our results bring up some important issues for future investigations. First, the issue of independent stimulation of RGCs of different types was not addressed in this study. Each RGC type is expected to form its own mosaic, but these mosaics will overlap and therefore the cell bodies of RGCs of different types may well be closely spaced, making independent stimulation by a single electrode a significant challenge. This is an area of

ongoing investigation, including the application of multielectrode stimulation to target individual RGCs (Jepson *et al* 2011).

Second, as noted in subsection 3.3, there can be non-local, antidromic stimulation of a neuron. One consequence of this non-local stimulation is the crosstalk discussed in subsection 3.4 and exhibited in figure 5, even in the case of stimulation of a mosaic of RGCs of a single type.

Third, although for the low-frequency stimulation used during the ‘scan’ procedure, we reliably and independently activated twelve out of thirteen ‘OFF-1’ cells (section 3.3), replicating the natural activity of these RGCs required scaling up the stimulation currents by more than 50%. The net result was unwanted initiation of action potentials in axonal fiber of at least one ‘OFF-1’ RGC (section 3.4). More generally, there is the issue of determining the optimal set of stimulation currents to replicate a desired pattern of spiking activity in a population of neurons with both high efficiency and minimal crosstalk. This is made even more challenging by the fact that the optimal stimulation currents may well depend on the recent past history of the applied stimulation pulses or the spiking activity of the neurons. We expect that the Stimchip, and the associated MEA system, will be an invaluable tool to investigate these and other issues related to the patterned stimulation of a neural population.

4.5. Future applications

For retinal prosthesis studies we believe that it will be especially interesting to combine the Stimchip with arrays of high spatial density (Mathieson *et al* 2004, Gunning *et al* 2007). With the ability of such systems to stimulate RGCs with combinations of pulses generated by several closely-spaced electrodes, and with the simultaneous detection of the neurons’ responses, it becomes possible to optimize techniques for the selective stimulation of the activity of small groups of neurons, or even individual cells. Furthermore, for an electrode array with high spatial density and high channel count it would be possible to stimulate hundreds of many closely spaced RGCs in parallel, to record the evoked spiking activity, and thereby investigate methods to replicate arbitrarily defined patterns of activity in a large population of cells. Such stimulation schemes could be of high interest for the development of high-resolution retinal prostheses. For preliminary results on the application of the Stimchip to studies on epiretinal prosthetic implants see (Jepson *et al* 2011).

For studies of neural networks, in brain tissue slices or intact brains, the stimulation of a population of neurons with complex spatiotemporal patterns of activity can be very useful in fundamental neuroscience research (Clarks *et al* 2011) as well as in biomedical applications, such as the development of multichannel neural prostheses (Fitzsimmons *et al* 2007) and deep brain stimulation techniques (Johnson *et al* 2008). It has been shown recently that the stimulation of activity of even a single neuronal cell in-vivo can lead to detectable behavioural responses (Houweling and Brecht 2008) or change of the global brain state (Li *et al* 2009). It is also known that the activity of neural networks can show a high level of complexity (Beggs and Plenz 2003) and that the precise relative timing of action potentials in different neurons may encode important information in some neural systems (Golisch and Meister 2008, Yang *et al* 2008). Consequently, one would like to stimulate the activity of a neural network with high spatial and temporal resolution and with a complexity and timing precision corresponding to the natural activity of the network. Unfortunately, the methods dedicated to the stimulation of individual neurons, using intracellular electrodes (Li *et al* 2009) or techniques based on juxtacellular labeling (Houweling and Brecht 2008) are hardly scalable to more than just a few neurons. On the other hand, it is still not well known how the brain circuits respond to electrical stimulation with extracellular microelectrodes (for discussion see Histed *et al* 2009). Systems based on large-scale, high-density arrays of extracellular electrodes and the stimulator presented in this paper can help improve our

understanding of the brain network's responses to complex microstimulation patterns, and can be unique and very useful tools for studying information processing in such networks, in both in-vitro and in-vivo applications.

It is to be noted that a new technique for neural stimulation using optogenetics is under rapid development (Deisseroth 2011, Fenno *et al* 2011). This powerful method has the ability to target genetically-tagged cell types, and to silence as well as stimulate neural activity with high spatial and temporal precision. It will be an important topic for future investigation and discussion to compare, and perhaps combine, optogenetic with electrical methods for patterned stimulation, in both space and time, of neural systems.

5. Conclusions

The multichannel stimulation ASIC presented in this paper is a major step forward in the development of large-scale MEA systems for two-way communication with networks of neuronal cells. The Stimchip can provide independent stimulation signals to 64 electrodes in parallel and allows for the simultaneous recording of the network response with very low artifacts. In the pilot experiments we were able, for the first time, to record short-latency spikes from stimulated neurons with minimal distortions even on the electrodes sending the stimulation currents, and to replicate the natural spiking activity in a selected population of living neuronal cells. The chip is also compatible with various MEA designs and is suitable for building systems with several hundred independent channels (Hottowy *et al* 2010). The performance and flexibility of the Stimchip make it a very powerful tool for a broad variety of applications, including studies for the development of the next generation of retinal and neural prostheses and research in the field of fundamental neuroscience.

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References

- Anishchenko A, Greschner M, Elstrott J, Sher A, Litke AM, Feller MB, Chichilnisky EJ. Receptive field mosaics of retinal ganglion cells are established without visual experience. *J Neurophysiol.* 2010; 103:1856–64. [PubMed: 20107116]
- Beggs JM, Plenz D. Neuronal avalanches in neocortical circuits. *J Neurosci.* 2003; 23:11167–77. [PubMed: 14657176]
- Berdondini L, van der Wal PD, Guenat O, de Rooij NF, Koudelka-Hep M, Seitz P, Kaufmann R, Metzler P, Blanc N, Rohr S. High-density electrode array for imaging in vitro electrophysiological activity. *Biosens Bioelectron.* 2005; 21:167–74. [PubMed: 15967365]
- Blanche TJ, Spacek MA, Hetke JF, Swindale NV. Polytrodes: High-density silicon electrode arrays for large-scale multiunit recording. *J Neurophysiol.* 2005; 93:2987–3000. [PubMed: 15548620]
- Brown EA, Ross JD, Blum RA, Nam Y, Wheeler BC, DeWeerth SP. Stimulus-artifact elimination in a multi-electrode system. *IEEE Trans Biomed Circuits Syst.* 2008; 2:10–21.
- Buitenweg JR, Rutten WLC, Marani E. Extracellular stimulation window explained by a geometry-based model of the neuron–electrode contact. *IEEE Trans Biomed Eng.* 2002; 29:1591–99. [PubMed: 12549741]
- Charvet G, et al. BioMEA: A versatile high-density 3D microelectrode array system using integrated electronics. *Biosens Bioelectron.* 2010; 25:1889–96. [PubMed: 20106652]

- Clark KL, Armstrong KM, Moore T. Probing neural circuitry and function with electrical microstimulation. *Proc R Soc B*. 2011; 278:1121–30.
- Csicsvari J, Henze DA, Jamieson B, Harris KD, Sirota A, Barthó P, Wise KD, Buzsáki G. Massively parallel recording of unit and local field potentials with silicon-based electrodes. *J Neurophysiol*. 2003; 90:1314–23. [PubMed: 12904510]
- D browski W, Grybo P, Hottowy P, Skocz A, Ćwientek K, Bezayiff N, Grillo AA, Kachiguine S, Litke AM, Sher A. Development of integrated circuits for readout of microelectrode arrays to image neuronal activity in live retinal tissue. *IEEE 2003 Nucl Sci Symposium Conference Record*. 2004; 2:956–60.
- D browski W, Grybo P, Hottowy P, Skocz A, Ćwientek K, Grillo AA, Kachiguine S, Litke AM, Sher A. Development of front-end ASICs for imaging neuronal activity in live tissue. *Nucl Instrum Meth Phys Res A*. 2005; 541:405–11.
- Deisseroth K. Optogenetics. *Nat Meth*. 2011; 8:26–9.
- Eversmann B, et al. A 128×128 CMOS biosensor array for extracellular recording of neural activity. *IEEE J Solid-State Circuits*. 2003; 38:2306–17.
- Fenno L, Yizhar O, Deisseroth K. The development and application of optogenetics. *Annu Rev Neurosci*. 2011; 34:389–412. [PubMed: 21692661]
- Field GD, Gauthier JL, Sher A, Greschner M, Machado TA, Jepson LH, Shlens J, Gunning DE, Mathieson K, Dabrowski W, Paninski L, Litke AM, Chichilnisky EJ. *Nature*. 2010; 467:673–7. [PubMed: 20930838]
- Field GD, Chichilnisky EJ. Information processing in the primate retina circuitry and coding. *Annu Rev Neurosci*. 2010; 30:1–30. [PubMed: 17335403]
- Fitzsimmons NA, Drake W, Hanson TL, Lebedev MA, Nicolelis MAL. Primate reaching cued by multichannel spatiotemporal cortical microstimulation. *J Neurosci*. 2007; 27:5593–602. [PubMed: 17522304]
- Frey U, et al. Switch-matrix-based high-density microelectrode array in CMOS technology. *IEEE J Solid-State Circuits*. 2010; 45:467–82.
- Fried SI, Hsueh HA, Werblin FS. A method for generating precise temporal patterns of retinal spiking using prosthetic stimulation. *J Neurophysiol*. 2006; 95:970–8. [PubMed: 16236780]
- Fried SI, Lasker ACW, Desai NJ, Eddington DK, Rizzo JF. Axonal sodium-channel bands shape the response to electric stimulation in retinal ganglion cells. *J Neurophysiol*. 2009; 101:1972–7. [PubMed: 19193771]
- Fujisawa S, Amarasingham A, Harrison MT, Buzsáki G. Behavior-dependent short-term assembly dynamics in the medial prefrontal cortex. *Nat Neurosci*. 2008; 11:823–33. [PubMed: 18516033]
- Gnadt JW, Echols SD, Yildirim A, Zhang H, Paul K. Spectral cancellation of microstimulation artifact for simultaneous neural recording in situ. *IEEE Trans Biomed Eng*. 2003; 50:1129–35. [PubMed: 14560765]
- Gollisch T, Meister M. Rapid neural coding in the retina with relative spike latencies. *Science*. 2008; 319:1108–11. [PubMed: 18292344]
- Grybo P, D browski W, Hottowy P, Fiutowski T, Bielewicz B. Neuroplat64 – low noise CMOS integrated circuit for neural recording applications. *Proc. 5th Int. Meeting on Substrate-Integrated Micro Electrode Arrays (Reutlingen)*; 2006. p. 208-9.
- Gunning DE, Chichilnisky EJ, Litke AM, O'Shea V, Smith KM, Mathieson K. Performance of ultra-high-density microelectrode arrays. *Nucl Instrum Methods Phys Res A*. 2007; 576:215–9.
- Heer F, Hafizovic S, Ugniwenko T, Frey U, Franks W, Perriard E, Perriard JC, Blau A, Ziegler C, Hierlemann A. Single-chip microelectronic system to interface with living cells. *Biosens Bioelectron*. 2007; 22:2546–53. [PubMed: 17097869]
- Histed MH, Bonin V, Reid RC. Direct activation of sparse, distributed populations of cortical neurons by electrical microstimulation. *Neuron*. 2009; 63:508–22. [PubMed: 19709632]
- Hottowy P, D browski W, Skocz A, Wi cek P. An integrated multichannel waveform generator for large-scale spatio-temporal stimulation of neural tissue. *Analog Integr Circuits Signal Process*. 2008a; 55:239–48.
- Hottowy P, D browski W, Kachiguine S, Skoczen A, Fiutowski T, Sher A, Rydygier P, Grillo AA, Litke AM. An MEA-based system for multichannel, low artifact stimulation and

- recording of neural activity. Proc. 6th Int. Meet. on Substrate-Integrated Micro Electrode Arrays (Reutlingen); 2008b. p. 259-62.
- Hottowy, P., et al. 512-electrode MEA system for spatio-temporal distributed stimulation and recording of neural activity. Proc. 7th Int. Meet. on Substrate-Integrated Micro Electrode Arrays; Reutlingen. 2010. p. 327-30.
- Houweling AR, Brecht M. Behavioural report of single neuron stimulation in somatosensory cortex. Nature. 2008; 451:65–8. [PubMed: 18094684]
- Jensen RJ, Rizzo JR, Ziv OR, Grumet A, Wyatt J. Thresholds for activation of rabbit retinal ganglion cells with an ultrafine, extracellular microelectrode. Invest Ophthalmol Vis Sci. 2003; 44:3533–43. [PubMed: 12882804]
- Jepson, LH.; Hottowy, P.; Gunning, DE.; Mathieson, K.; D browski, W.; Litke, AM.; Chichilnisky, EJ. Spatially patterned electrical stimulation of the retina for improved specificity. Proc. 5th Int. IEEE/EMBS Conf. on Neural Eng; 2011. p. 495-8.
- Jimbo Y, Kasai N, Torimitsu K, Tateno T, Robinson HPC. A system for MEA-based multisite stimulation. IEEE Trans Biomed Eng. 2003; 50:241–8. [PubMed: 12665038]
- Jochum T, Denison T, Wolf P. Integrated circuit amplifiers for multi-electrode intracortical recording. J Neural Eng. 2009; 6:012001. [PubMed: 19139560]
- Johnson MD, Miocinovic S, McIntyre CC, Vitek JL. Mechanisms and targets of deep brain stimulation in movement disorders. Neurotherapeutics. 2008; 5:294–308. [PubMed: 18394571]
- Li CT, Poo MM, Dan Y. Burst spiking of a single cortical neuron modifies global brain state. Science. 2009; 324:643–46. [PubMed: 19407203]
- Litke AM. The retinal readout system: a status report. Nucl Instrum Methods Phys Res A. 1999; 435:242–9.
- Litke AM, et al. What does the eye tell the brain? Development of a system for the large scale recording of retinal output activity. IEEE Trans Nucl Sci. 2004; 51:1434–40.
- Mathieson K, et al. Large-area microelectrode arrays for recording of neural signals. IEEE Trans Nucl Sci. 2004; 51:2027–31.
- McAdams ET, Lackermeier A, McLaughlin JA, Macken D. The linear and non-linear electrical properties of the electrode-electrolyte interface. Biosens Bioelectron. 1995; 10:67–74.
- Merrill DR, Bikson M, Jefferys JGR. Electrical stimulation of excitable tissue: design of efficacious and safe protocols. J Neurosci Meth. 2005; 141:171–98.
- Petrusca D, Grivich MI, Sher A, Field GD, Gauthier JL, Greschner M, Shlens J, Chichilnisky EJ, Litke AM. Identification and characterization of a Y-like primate retinal ganglion cell type. J Neurosci. 2007; 27:11019–27. [PubMed: 17928443]
- Pillow JW, Shlens J, Paninski L, Sher A, Litke AM, Chichilnisky EJ, Simoncelli EP. Spatio-temporal correlations and visual signaling in a complete neuronal population. Nature. 2008; 454:995–9. [PubMed: 18650810]
- Rolston JD, Gross RE, Potter SM. A low-cost multielectrode system for data acquisition enabling real-time closed-loop processing with rapid recovery from stimulation artifacts. Front Neuroeng. 2009; 210. 3389/neuro.16.012.2009
- Sekirnjak C, Hottowy P, Sher A, D browski W, Litke AM, Chichilnisky EJ. Electrical stimulation of mammalian retinal ganglion cells with multielectrode arrays. J Neurophysiol. 2006; 95:3311–27. [PubMed: 16436479]
- Sekirnjak C, Hottowy P, Sher A, D browski W, Litke AM, Chichilnisky EJ. High-resolution electrical stimulation of primate retina for epiretinal implant design. J Neurosci. 2008; 28:4446–56. [PubMed: 18434523]
- Sekirnjak C, Hulse C, Jepson LH, Hottowy P, Sher A, D browski W, Litke AM, Chichilnisky EJ. Loss of responses to visual but not electrical stimulation in ganglion cells of rats with severe photoreceptor degeneration. J Neurophysiol. 2009; 102:3260–9. [PubMed: 19726725]
- Tang A, et al. A maximum entropy model applied to spatial and temporal correlations from cortical networks in vitro. J Neurosci. 2008; 28:505–18. [PubMed: 18184793]
- Tehovnik EJ. Electrical stimulation of neural tissue to evoke behavioral responses. J Neurosci Meth. 1996; 65:1–17.

- Wagenaar DA, Pine J, Potter SM. Effective parameters for stimulation of dissociated cultures using multi-electrode arrays. *J Neurosci Methods*. 2004a; 138:27–37. [PubMed: 15325108]
- Wagenaar DA, Potter SM. A versatile all-channel stimulator for electrode arrays, with real-time control. *J Neural Eng*. 2004b; 1:39–45. [PubMed: 15876621]
- Wagenaar DA, Potter SM. Real-time multi-channel stimulus artifact suppression by local curve fitting. *J Neurosci Methods*. 2002; 120:113–20. [PubMed: 12385761]
- Wichmann T. A digital averaging method for removal of stimulus artifact in neurophysiologic experiments. *J Neurosci Methods*. 2000; 98:57–62. [PubMed: 10837871]
- Yang Y, DeWeese MR, Otazu GH, Zador AM. Millisecond-scale differences in neural activity in auditory cortex can drive decisions. *Nat Neurosci*. 2008; 11:1262–3. [PubMed: 18849984]

Figure 1. Stimchip design. (A) Schematic functional diagram of a stimulation channel. The control signals are labeled in italic font. The signals controlled in real time are labelled in red. (B) Microphotograph of the chip. The die area is 6.4 x 2.8 mm². (C) Basic parameters of the chip. (D) The central section of the printed circuit board (PCB) that includes the recording chamber, the 61-electrode MEA, and two Stimchip/Neuroplat pairs with fan-ins. The inset shows the photograph of the MEA. The interelectrode distance is 60 μ m. (E) Magnified view of a fan-in (pitch adapter) and the Stimchip and Neuroplat chip assembled on the PCB. The wire-bond connections between the Stimchip, the fan-in and the Neuroplat chip are visible.

Figure 2.

Low-artifact stimulation and recording of a single mouse retinal ganglion cell. (A) 100 responses to $0.43 \mu\text{A}$ stimulation pulses recorded on the stimulating electrode. The artifact-only responses are shown in black and the responses including spikes are shown in red. The stimulation current waveform and the state of the *record* switch disconnecting the electrode from the amplifier are shown in blue. (B) The same signals shown in an expanded time scale. The correlation between artifact shape and the stimulation current waveform is visible. (C) The averaged signal shape from the stimulated neuron shown after subtraction of the averaged artifact shape. (D) Comparison of the signals generated on the primary electrode and the 6 neighboring electrodes by the same neuron spiking under visual stimulation (black traces) and in response to electrical stimulation (red traces, shown after artifact subtraction). The relative position of each plot in the figure follows the position of the corresponding electrode on the MEA layout. Both the vertical and horizontal scales are the same for each plot. The $0.43 \mu\text{A}$ stimulation pulse was applied to the central electrode.

Figure 3.

Detection and identification of signals from simultaneously stimulated neurons in the mouse retina. (A) 98 responses to $0.65 \mu\text{A}$ stimulation pulses recorded on the stimulating electrode (left panel) and one of the neighboring electrodes (right panel). The vertical scale range is the same for both panels. The four colour-coded clusters of the recorded responses originate from the combined activity of three stimulated neurons (see text). The MEA layout indicates the location of the stimulating electrode (on the left) and the neighboring electrode. The legend box shows the colour code, in A, B and C, for the four response combinations of the three neurons. (B) Scatter plot showing the four colour-coded clusters based on the two most significant Principal Component Analysis variables derived from the spike waveforms shown in A. (C) Averaged waveform shapes for the four clusters of recorded responses shown in A. (D) Reconstructed shapes of the signals recorded from neurons 2 and 3 on the two electrodes, based on the average waveforms shown in C (see text).

Figure 4.

Sensitivity of a rat RGC to stimulation applied to various electrodes. (A) The electrophysiological image for neuron #1 (as labeled in the legend for C), based on the averaged waveforms recorded from the cell on 30 electrodes during visual stimulation. The red traces mark the 7 electrodes (labeled with the electrode IDs as in C) that activated the cell during electrical stimulation with currents ranging from 0.15 to 1.6 μA . The relative position of each plot in the figure follows the position of the corresponding electrode on the MEA layout. (B) Stimulation efficacy as a function of the stimulation current for the 7 indicated electrodes. The blue diamonds show the experimental results and the red curves show the fits with a sigmoidal function. (C) The threshold currents, for 100% and 10% stimulation efficacies, as a function of electrode number for 12 'OFF-1' RGCs in a single retina. The symbols mark the minimal currents needed for stimulation of a given cell with 100% efficacy (full symbols – shown for the 'primary stimulation' electrode for each cell) or 10% efficacy (open symbols).

Figure 5.

Replication, in an isolated rat retina, of the visual stimulus-evoked spiking activity of a retinal ganglion cell (RGC) population with patterned electrical stimulation. (A) Averaged recorded visually-evoked signal amplitudes of 7 'OFF-1' RGCs, shown on all 61 electrodes (Electrophysiological Images, EIs). The circle diameter is proportional to the average amplitude and the circle center corresponds to the position of the given recording electrode. The red circle marks the electrode that was used for the electrical stimulation of the given cell. The number in the upper right corner of the array marks the neuron ID, as indicated in figure 4C. (B) RGC responses to the patterned electrical stimulation. For each cell, the red lines mark the stimulation pulse times and the blue diamonds mark the spike times. The spike times are presented in 20 rows that correspond to 20 repetitions of the one-second electrical stimulation pattern. The stimulation amplitude was adjusted for each cell independently. (C) Distributions of spike delays relative to the end of the stimulation pulse; τ – average delay; σ – variability (SD) of neuron response delay; ϵ – stimulation efficacy; p – probability of response within 2 ms after the stimulation pulse. (D,E,F) EI, stimulation

response and spike delay distribution for one RGC (neuron #1, as labeled in figure 4C) that was stimulated by both its primary electrode (#7) and another electrode (#55), as shown in fig. 4A. The electrode (#55) generating the crosstalk (D), the spikes resulting from the crosstalk (E) and the distribution of these spike delays, relative to the stimulation by this electrode, (F) are marked with the green color.

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Table 1

Comparison of the functionality and performance of the Stimchip-based system and other MEA systems for two-way communication with neuronal networks.

Reference	Technology of stimulation/ recording electronics	Number of stimulation channels	Number of independent stimulation signal sources	Electrode diameter/ spacing [μm]	Application	Artifact duration on stimulating electrode/ adjacent electrode [ns]
Jimbo <i>et al</i> 2003	discrete elements	64 voltage	1 external	10-50/ 50-250	Rat cortex	>2 / not reported
Wagenaar <i>et al</i> 2004b	discrete elements	64 current or voltage	1 internal (8 bit)	30/200	Rat cortex	>40 / >1
Sekirnjak <i>et al</i> 2006, 2008	CMOS	64 current	1 external	5/60	Rodent and primate RGCs	5 / 0.05
Heer <i>et al</i> 2007	CMOS (MEA integrated on chip)	128 voltage	1 internal (8-bit)	30/250	Rat hippocampus	>2 / >1
Brown <i>et al</i> 2008	CMOS	16 voltage	1 external	40/200	Hippocampus (mouse or rat)	3 / 0.5
Rolston <i>et al</i> 2009	discrete elements	64 current	1 external	33/175	Rat hippocampus	5 / 0.4
Frey <i>et al</i> 2010	CMOS (MEA integrated on chip)	126 voltage	2 internal (10-bit)	7/18	not reported	>10 / >50
Charvet <i>et al</i> 2010	CMOS	256 current	8 external	various	mouse hindbrain	not reported
This work	CMOS	64 current or voltage	64 internal (8-bit)	5/60	Rodent RGCs	0.055 / 0.005