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Holographic photolysis of caged neurotransmitters

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Abstract

Stimulation of light-sensitive chemical probes has become a powerful tool for the study of dynamic signaling processes in living tissue. Classically, this approach has been constrained by limitations of lens-based and point-scanning illumination systems. Here we describe a novel microscope configuration that incorporates a nematic liquid crystal spatial light modulator (LC-SLM) to generate holographic patterns of illumination. This microscope can produce illumination spots of variable size and number and patterns shaped to precisely match user-defined elements in a specimen. Using holographic illumination to photolyse caged glutamate in brain slices, we demonstrate that shaped excitation on segments of neuronal dendrites and simultaneous, multi-spot excitation of different dendrites enables precise spatial and rapid temporal control of glutamate receptor activation. By allowing the excitation volume shape to be tailored precisely, the holographic microscope provides an extremely flexible method for activation of various photosensitive proteins and small molecules.

INTRODUCTION

With the aid of a growing list of photosensitive probes, light microscopic methods are increasingly being used to make quantitative measurements of biochemical processes in single cells, to manipulate intracellular biochemistry, and to mimic intercellular signaling in living tissue. Due to the complicated nature of neuronal tissues, these new optical methods have had a notable impact in neuroscience. A commonly used approach in such experiments involves light activation (photolysis) of chemically-caged neurotransmitters¹. Caged versions of glutamate, the primary excitatory neurotransmitter in the brain, have been used in combination with a variety of optical configurations. Single² or 2-photon^{3,4} photolysis within diffraction limited illumination volumes can generate localized and brief transients of glutamate similar to those underlying unitary synaptic events. To create more diffuse and slow actions of glutamate over entire neurons or groups of neurons, large cylindrical excitation volumes have been created using lens based systems and laser-, or lamp-based illumination^{2,5}. However, these methods can not be adjusted to form spot sizes over a wide range (from diffraction-limited to wide-field) in real-time and are limited to single, uniform, and usually circular illumination areas. To mimic physiological synaptic activation required to reach threshold for neuronal spiking, it is necessary to simultaneously activate many synapses. Laser-based systems can be integrated into scanning devices which can be used to illuminate arbitrary shapes or multiple spots^{6,7}. However, residence and traveling time dictate limits on the number of positions that can be ‘simultaneously’ activated on a biological timescale⁸.

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LC-SLMs have recently been recognized as ideal devices to modulate the distribution of light with applications ranging from optical manipulation^{9–13} to adaptive optics¹⁴. Here we extend their use to produce complex photo-activation patterns in brain tissues. We show that one can easily switch between different excitation configurations. Holographic illumination is used to tune the size of individual spots, shape illumination to match fine neuronal structures, or project multiple spots of various sizes. As a proof of principle for this new experimental method, we applied holographic illumination to uncage glutamate on different types of neuronal cells in brain slices while measuring the responses with patch clamp electrophysiology. We used the system to target AMPAR activation on single dendritic branches and then, by controlling the size and shape of the illumination, were able to modulate the temporal and spatial distribution of receptor activation. We also show that holographic illumination can elicit truly simultaneous activation of multiple synaptic contacts.

RESULTS

Principles of holographic illumination

The scheme of the holographic microscope is shown in Figure 1a. Light from a 405 nm diode laser, was converted into a *holographic beam* by the use of a parallel-aligned, nematic LC-SLM. The device was controlled by custom-designed software that, given a target intensity distribution at the focal plane of the microscope objective, calculates the corresponding phase-hologram and addresses the pattern to the LC-SLM. The program uses the spherical wave approximation (SWA) method¹⁵ for multi spot generation and an iterative Fourier transform algorithm (IFTA)¹⁶ for adjusting the size and shape of illumination volumes (**Supplementary Note** online).

Different illumination shapes were generated by using the LC-SLM (Fig. 1). A near diffraction-limited spot (Fig. 1b) was generated by addressing a phase grating (Fig. 1c) to the LC-SLM. Modifications in the grating such as the lines spatial frequency and orientation allow control of the spot position. Multiple spots were simultaneously generated (Fig. 1d) by using a phase pattern which is a combination of diffraction gratings and Fresnel lenses (circular structures) (Fig. 1e). Moreover, unlike fast scanning methods, the holographic illumination approach permits a fast modulation of the excitation spot size and the possibility to simultaneously generate multiple spots of different diameters (Fig. 1f). The x-y intensity profile of the spots are characterized by sharp edges and show weak speckle-like variations in the intensity of an amplitude $B = 15\%$ around a constant mean value, A (Fig. 1g). However, the impact of speckling for many applications is negligible (**Supplementary Note** online). The phase-hologram used to generate the spots is presented in Figure 1h.

More complex excitation volumes can be produced by interactively designing shapes based on wide-field epi-fluorescence images (Fig. 2a–f). Such patterns can precisely reproduce original fine structures as is demonstrated by the overlays of the original fluorescence pictures with the shaped excitation spots (Figs. 2d and 2f).

Axial propagation of a holographic beam

A precise characterization of the excitation volume requires the knowledge of the holographic beam propagation along the optical axis. Since this distribution can be calculated directly, we implemented in the software the possibility of determining, for each output phase-hologram, the axial distribution of the corresponding *holographic beam* around the objective focal plane. Briefly, given an input phase-hologram at the SLM, $\Phi(x,y)$, and an incident beam of intensity I_0 , we calculated the beam irradiance around the objective focal plane after the diffracted electromagnetic field at the SLM plane ($U_{in}(x,y) = \sqrt{I_0} \times \exp(i\Phi(x,y))$) is propagated through the 4f telescope and the objective lens by using the thin element approximation (TEA) in the

angular spectrum approach of plane waves (ASPW)¹⁷. Figure 3a shows the calculated y-z intensity cross section for the case of a *holographic beam* that produces a focal spot with lateral diameter, s , of 9 μm . If we define a range of focus, b , as the full width at half maximum (FWHM) of the axial intensity distribution, we found for b a value of $18.5 \pm 0.5 \mu\text{m}$ and, for s varying between 3 μm and 25 μm , a linear relationship between b and s .

In order to experimentally verify these results, we used the double microscope setup described in the Methods (Fig. 3b). From the optical sectioning performed by scanning the excitation objective through a fluorescent thin layer of Coumarin we measured the y-z intensity cross section (Fig. 3b) and the range of focus, b , of holographic beams. The latter was extracted by measuring, for the different planes of the optical sectioning, the integrated intensity over a region of interest equal to the contour-line of the excitation shape in the focal plane (in this case a spot of 9 μm in diameter). From the resulting curve (Fig. 3c, dots) we extracted for b a value = $18.0 \pm 0.5 \mu\text{m}$, in good agreement with the value estimated from the theoretical curve (Fig. 3c, continuous line). By repeating the experiment for different values of s , we confirmed the linear relationship between b and s predicted by the theoretical simulation (Fig. 3c). These findings indicated that, for large spots, one can achieve a better axial optical confinement with respect to the case of a conventional Gaussian beam (**Note 3** and Supplementary Fig. 1 online). This result can be intuitively explained by considering that the SLM-based illumination takes advantage of a majority of the objective's numerical aperture, unlike the underfilling Gaussian beam method. This effect is even more pronounced for shaped illumination (Fig. 3d): from the z-y intensity cross section of the spot of Fig. 2f we measured a depth of focus, b , of 9 μm (Fig. 3e). Interestingly, to cover the same region of dendrite using a circular spot, one would need a holographic excitation spot of $\sim 25 \mu\text{m}$, such a spot would give rise to a larger depth of focus, b , of $\sim 37 \mu\text{m}$.

Holographic activation of glutamate receptors within a brain slice

Varying the density of synaptic input to a neuron can have a strong influence on the time course of synaptic currents in the CNS^{18, 19}. Holographic photolysis offers the opportunity to vary the volume of photolysis to control the spatiotemporal profile of glutamate. Whole-cell patch clamp recordings from cerebellar stellate cells were used to measure ionic currents in response to glutamate photolysis in brain slices. Individual stellate cells were loaded with Alexa 594 so that their dendritic morphology could be visualized. Local perfusion of 20 mM MNI-glutamate was provided by a nearby perfusion pipette. The LC-SLM-based system was used to modify the uncaging beam such that photolysis illumination was localized on a segment of primary dendrite situated 10–50 μm from the cell body. We created two different spot sizes in the image plane, either a small spot of $< 0.5 \mu\text{m}$ or a large spot of $\sim 6 \mu\text{m}$ (Fig. 4a–b). In addition, we tested a third shape designed to conform to the dendritic area underlying the large spot (Fig. 4c). We found that under conditions in which the illumination power density and number of activated receptors were similar, the resulting photolysis-evoked currents (pECs) exhibited different peak amplitudes and decay time courses (Fig. 4d). These currents were recorded in the presence of pharmacological blockers to isolate currents mediated solely by AMPA receptors¹⁹. Small spot illumination produced rapidly rising ($< 1\text{ms}$) and decaying pECs as would be expected for localized activation of dendritic AMPARs with small illumination volumes²⁰. We quantified the decay by normalizing the current peak amplitudes to one and then calculating the integral over the entire pEC. We found that for small spot the normalized decay integral (NDI) was $19 \pm 3.7 \text{ ms}$ ($n = 5$). Large spot illumination with matched power density yielded much larger responses, and often exhibited a plateau phase before decaying, indicative of slowed glutamate clearance; such responses resemble synaptic currents elicited by large numbers of presynaptic fibers¹⁹. By comparison, responses to shaped illumination (along a dendritic length similar to the diameter of the large spot) decayed much more rapidly (NDI: 236 ± 59 for large spots, $n = 5$; 51 ± 17 for shaped illumination, $n = 3$). Therefore, by

using the LC-SLM to restrict illumination to the dendrite, receptor currents could be accelerated by nearly 5-fold.

To further examine whether it is possible to uncage glutamate uniformly over a large surface, we used 'shaped' illumination to uncage glutamate onto proximal segments of the apical dendrites of CA1 hippocampal pyramidal cells. The first 50–80 μm of CA1 dendrites are devoid of asymmetrical synapses²¹ and have uniform distributions of AMPARs²². We observed that increasing the illumination spot area along the dendrite (Fig. 5a) under conditions of constant power density leads to a linear increase in pECs. For example, a recording in which spot illumination area was increased by 1.44-, 1.72- and 2.47-fold led to respective increases in currents of 1.55-, 1.9-, and 2.57-fold (Fig. 5b–c). In seven cells in which the illumination area increased by a mean factor of ~ 2.5 (2.48 ± 0.47), the response amplitude increased by ~ 2.7 (from 0.038 ± 0.01 to 0.105 ± 0.02 nA). Despite these changes in amplitude, NDIs of pECs were unaffected by the change in illumination area (31.1 ± 3.3 ms versus 28.6 ± 2.9 ms, $p = 0.37$, Wilcoxon paired test). These results demonstrate that LC-SLM-mediated shaping of the excitation volume can be used to control the number of activated receptors without changing the response time course.

Functional mapping of AMPARs on cerebellar granule cells

Cerebellar granule neurons have a stereotyped morphology with 4 to 5 short dendrites, each terminating in a claw-like ending, emanating from a spherical cell body. Prior studies of mature granule neurons suggest that AMPA receptors are tightly concentrated on claw-like endings within glomeruli but excluded from cell bodies and dendritic shafts^{23–25}. Using the LC-SLM system we mapped responsiveness to caged glutamate by positioning small spots on cell bodies, dendritic shafts, and claw-like endings (Fig. 6a). A spot size of 3.6 μm was chosen to increase the likelihood of activating a cluster of AMPARs² while maintaining independent activation of single glomeruli as well as other neuronal compartments. Large and rapidly-rising currents were elicited by spots positioned on dendritic endings (Fig. 6b, curves 1 and 2) and small, slowly-rising responses when the spot was positioned in between endings (Fig. 6b, curve 3). No pECs were observed when the spot was located on the cell body (Fig. 6b, curves 4) or on the dendritic shaft (Fig. 6b, curve 5). On average, responses on shafts and in between or adjacent to endings were (13.6 ± 0.9) % ($n = 3$) and (26 ± 1.5) % ($n = 3$) of the peak response on an ending, respectively. These results, in agreement with previous suggestions^{2, 26} show that there are few functional AMPARs on the soma or along the primary dendrites.

In addition to changing spot size in a straightforward and graded manner, LC-SLM-based approaches offer another advantage: multiple positions can be illuminated simultaneously. To demonstrate this principle we illuminated with two spots on two different dendritic endings (Fig. 6a) and compared the arithmetic sum of responses from single endings to those elicited with simultaneous uncaging on both endings (Fig. 6c). In four similar recordings, the mean ratio of the peak amplitude in response to simultaneous stimulation divided by the summed peak amplitudes of individual responses was 0.96 ± 0.02 .

DISCUSSION

Traditional optical systems modify excitation light patterns through the use of lenses, diaphragms, curved mirrors, gratings, or optical fibers. They are typically limited to simple patterns (generally circles) and are relatively difficult to change during the course of an experiment. In contrast, *active* optical elements, such as digital micro-mirrors-, acousto-optical- and liquid-crystal- based spatial light modulators, permit laser light to be spatially redistributed by modulation of its phase and/or amplitude. Such approaches permit the formation of arbitrary shapes and their active nature allows illumination patterns to be more rapidly and conveniently adapted to experimental needs. Here, we have incorporated an LC-

SLM in a standard microscope, enabling both simple and elaborate patterns of illumination to be produced using holographic approaches. We demonstrated that holographic illumination allows rapid modification of the size of an excitation spot during the relatively brief time of electrophysiological experiments.

We also used the microscope to create excitation patterns that closely match the fluorescence profile of fine cellular processes. Patterned illumination has several advantages over large circular illumination, with the primary advantage being to target the illumination to specific parts of the cell while minimizing 'unwanted' photo-activation of other regions. This is true in all directions due to the increased axial confinement of shaped regions in comparison with circular patterns (Fig. 3). Such approaches may allow for more effective photo-stimulation as they offer an alternative to lengthening pulse durations or increasing laser intensity. The improved spatial control should also prove advantageous for photoactivation of intracellular probes and cages.

Finally, we have shown that LC-SLM devices allow simultaneous generation of multiple spots with sizes ranging from diffraction limited to several microns. Diffraction limited spots can be positioned with nanometric precision in lateral dimensions²⁷, can be generated in two- and three-dimensional patterns^{11–13}, and can be placed in a large number of locations⁹ with intensities limited by the total available laser power.

Although patterned photo-activation can be achieved with alternative methods, each of these has advantages and drawbacks. Digital micro mirror-based devices²⁸ have the advantage of a fast refresh rate (kHz vs 60Hz for LC-SLM) allowing different intensity patterns to be sequentially generated with sub-millisecond temporal resolution. However, a large fraction of laser power is lost because the intensity patterning is created by redirecting unwanted light out of the excitation field. For example, for an illumination field of $50 \times 50 \mu\text{m}^2$ using a digital micromirror would allow only a fraction of ~0.008%, ~0.2% and ~9% of the incident light to be delivered in the three patterns of Fig. 1b,d,f while by phase modulation with an SLM we measured a value of 63%, 46% and 50%, respectively. Moreover, spot generation is restricted to the objective focal plane. Fast scanning devices such as acousto-optical deflectors (AOD) and galvanometric devices can be used for multiple spot photolysis^{6, 7} and recent advances with these elements offer the possibility of scanning in 3D volumes^{29, 30}. However, these systems are challenged under conditions in which a large number of spot locations (arranged in discrete points or to uniformly fill a region of interest) must be visited with sub-millisecond resolution. The point to point traveling time is ~40 μs in the case of AODs⁷, but is further increased by the time needed to uncage at each location. This can be as brief as 20 μs for single photon activation of MNI-Glut², but increases up to ~200 μs using two photon excitation (2PE) (due to its lower two photon-cross section). Thus, considering multiple excitation spots over 15 locations, a total time of ~0.9ms (or 3ms in 2PE) would be required. This value rapidly increases when one has to fill a defined contour: to generate a spot of 10 μm in diameter with a 500 nm spot one would need a time of ~25 ms (~80ms), or in the case of the shaped illumination in Fig. 3h, a time of ~1s (~3s). An additional drawback of AODs is that scanning 2PE beams requires compensation for pulse broadening; this effect is negligible for the case of a beam reflected by a LC-SLM (unpub.data).

Despite the reduced photolysis efficiency at 405 nm (~ 10 % of the nUV peak assuming that uncaging efficiency changes linearly throughout the absorption spectra³¹), the use of a diode lasers has the following advantages: 1) they are more convenient and compact than large frame argon-based UV lasers, 2) their built-in electronic control of illumination time and intensity obviate the need for external shuttering devices (in contrast to nUV and DPSS lasers), and 3) they have high output power (up to 100 mW for 405 nm).

In summary, holographic photo-activation can provide a wide variety of excitation patterns not accessible with existing devices. By combining a holographic photolysis system with a confocal or two photon microscope it will be possible to photostimulate in a different plane from the imaging plane¹³. Moreover, phase engineering of the optical wave front will allow simultaneous control of light patterning and correction for optical aberrations, as has been implemented for adaptive correction of optical traps efficiency¹⁴. Although we have demonstrated the flexibility and power of LC-SLM-based illumination for neurotransmitter photolysis, the same advantages hold for other photostimulation applications using different photoactivatable molecules such as intracellular caged compounds, photoactivatable ion channels^{32, 33} and receptors³⁴. In general, the patterned illumination could be useful for defining patterns of excitation for fluorescence correlation spectroscopy and fluorescence recovery after photobleaching, and other photoactivatable fluorescent proteins³⁵.

METHODS

Holographic photolysis and electrophysiology

The holographic microscope (Fig. 1a) was mounted around a commercial epi-fluorescence upright microscope (Olympus BX50WI). As a source for uncaging laser we used a 405nm diode laser (CUBE 405–50, Coherent).

The output beam was expanded (25x) to match the input window of a PALM-SLM (PPM X8267, Hamamatsu) which operates in reflection mode. A 4f telescope (L1 = 750mm, L2 = 300mm) was used to image via a dichroic mirror (DM) (Chroma Technology 425DCXR) the plane of the SLM to the rear aperture of the objective (LUMPLFL60XW/IR). The main source of power loss with an SLM device is related to the portion of un-diffracted light that forms an unwanted central spot (zero order) in the excitation field and to the light diffracted into higher orders (about 40% in total). The diffracted beam was spatially displaced from the zero order spot by introducing a phase grating in the hologram. The zero order spot, the higher orders and the ghost image were eliminated by placing a beam blocker and a diaphragm in the intermediate Fourier plane. This reduced the excitation field to a square of approximately $50 \times 50 \mu\text{m}^2$. Alexa 594 was excited with a 75-W Xenon arc source coupled to a monochromator (Optoscan, Cairn Research) (centre-wavelength at 540nm, slit width 30nm) and imaged using an emission filter Chroma Technology HQ 600/40M. The laser intensity and duration were controlled using a 1MHz Digital-to-Analog Converter (DAC) (National Instruments 6713), whose output clock was linked to the clock of the Analog-to-Digital Converter (ADC), in order to synchronize the illumination pulse and voltage-clamp acquisition. Sample fluorescence was captured on a CCD camera (CoolSNAP HQ2, Roper Scientific), at the upper port of the microscope. For the experiments on cerebellum slices a similar setup was integrated into a modified photolysis system (Prairie Technologies, WI, USA) on an upright microscope (Nikon) equipped with a 100x water immersion objective (Nikon, Plan 1.1 NA) and a 405 nm diode laser (Deep Star, Omicron, Germany). For a description of preparation of brain slices, recording conditions and data analysis see **Supplementary Method** online.

3D scanning of excitation volumes

Analysis of holographic beam propagation around the objective focal plane was done with a double microscope.

In this system the upper objective (LUMPLFL60xW/IR) was used to generate the holographic excitation volume. Different sections of the holographic beam were obtained by varying, with a piezo objective positioner (P-721.CDQ, Physik Instrumente), the position of the objective with respect to a Coumarin sample of $\sim 0.3 \mu\text{m}$ in thickness. The lower objective (UPLSAPO60xW) was used for collection of the fluorescence and kept at a fixed position. The

fluorescence was imaged by a collection lens (150mm) to a CCD camera (CoolSNAP HQ2, Roper Scientific). To reject the excitation light, an emission filter (Chroma Technology HQ 535/50M) and a dichroic filter (Chroma Technology 425DCXR) were placed in front of the CCD camera. Optical sectioning and image acquisition have been performed using the Metamorph software (version 7.1; Molecular Devices). Axial movement of the upper objective corresponds to a variation of the distance, d , between the focal plane of L2 and the rear aperture of the objective (Fig. 1a). This only affects a phase factor in the Fourier transform relation between the input field distribution and the field distribution at the object focal-plane ($U_f(x_f, y_f)$)¹⁷:

$$U_f(x_f, y_f) = \frac{\exp[i \frac{k}{2f}(1 - \frac{d}{f})(x_f^2 + y_f^2)]}{i\lambda f} \mathfrak{x}\mathfrak{z}(\frac{x_f}{\lambda_f}, \frac{y_f}{\lambda_f})$$

and induces a wave front curvature to $U_f(x_f, y_f)$. For an optical scanning of 100 μ m around the focal plane of an objective with $f = 3$ mm the value of d/f varied between 0.983 and 1.017. The effect on the axial propagation of the transmitted field, is therefore negligible.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Optical setup and spot generation in a layer of coumarin

(a) Layout of the optical setup. (b) A near diffraction-limited spot generated at a chosen position. Scale bar, 1 μm . (c) Phase hologram used to generate the spot in (b), showing the typical structures of a diffraction grating. (d) Simultaneous generation of 15 spots. Scale bar, 10 μm . (e) Phase hologram used to create the multiple spots in (d). Fresnel lenses are visible. These were used to focalize the different spots, superimposed on grating-like structures to control their lateral positions. (f) Excitation with multiple spots of diameters $s = 12, 9.0, 6.5$ and 3.5 μm . Scale bar, 10 μm . (g) Intensity profile across the spot in (f) with diameter of 9 μm . A, mean intensity value; B, variation in intensity. (h) Phase hologram used to create the spots in (f). The diffraction efficiency, Z , defined as the integrated intensity of the first order over the integrated intensity of the zeroth order with SLM off, was 63%, 46% and 50% for the phase holograms in (c), (e) and (h), respectively.

Figure 2. Shaping of an excitation spot to match fine cellular processes

(a,b) Wide-field epifluorescence image of a cerebellar granule cell (a) in which a selected region of interest (dashed box) was used to obtain the phase hologram shown in b. (c) Illumination shape generated in coumarin at the objective focal plane with the phase hologram of b. (d) Overlay of a and c. (e) Similar to a but using a CA1 hippocampal pyramidal cell to calculate the phase hologram. (f) Similar to the image in d, overlay of the image in e, with the generated holographic spot (green). Scale bars, 10 μm .

Figure 3. Axial propagation of a holographic beam

(a) Calculated y-z and x-y intensity crosssections for a holographic beam generated to produce a circular spot at the focal plane with a lateral diameter, s , of $9\ \mu\text{m}$. (b) Experimental y-z and x-y intensity cross-sections for the same beam, measured with a double microscope where the excitation (upper) objective was scanned with a step size of $500\ \text{nm}$ through a coumarin layer and the lower objective was used for imaging, as in the schematic. (c) Integrated intensity profile of the calculated (continuous line) and measured (dotted line) y-z cross-sections in (a) and (b) (top). Range of focus, b , measured for different spot sizes, s , showing a linear dependence of the two parameters (bottom). (d) Experimental y-z crosssection of the holographic beam used for the excitation shape in Figure 2f, measured as in (b). (e) Axial profile of the intensity distribution in (d), showing a range of focus, b , of $9\ \mu\text{m}$. The intensity was integrated over a region of interest equal to the contour line of the excitation shape in the focal plane. Scale bars, $10\ \mu\text{m}$.

Figure 4. Varying the shape of illumination for photolysis influences the time course of glutamate clearance in cerebellar slices

(a–c) Wide-field epifluorescence images of a stellate cell in a cerebellar slice visualized using the fluorescent indicator Alexa 594 (top) and PEC elicited by exciting at 405 nm the region indicated by the green area on the image after local perfusion with MNI-glutamate (bottom). Current response evoked by a 20-ms pulse within a small illumination spot (a). Response evoked by photolysis within a large illumination spot (6 μm diameter, 12 μm focus depth) (b). Response evoked by illumination shaped to match a dendritic segment with length corresponding to the diameter of the large spot in b (calculated focus depth of 4 μm) (c). (d) Superimposed peak normalized responses from a–c showing the different decay time courses. Scale bars, 6 μm .

Figure 5. Using increasing lengths of shaped illumination to uncage glutamate along a dendrite preserves the time course of PECs in hippocampal slices
 (a) Illumination spots of increasing area located on the initial portion of the apical dendrite of a CA1 hippocampal pyramidal cell. (b) Eight superimposed traces of PECs from each of the illumination spots in a. (c) Averaged PECs from b (left). Averaged responses normalized to the excitation spot area showed similar amplitudes and constant decay time courses (right). Scale bars, 8 μ m.

Figure 6. Simultaneous uncaging of glutamate at two spots on granule-cell dendritic claws in acute cerebellar slices

(a) Wide-field epifluorescence picture of a granule cell loaded by patch-clamp with Alexa 594. Green circles indicate the five locations of the uncaging spot that produced the traces in b. Scale bar, 10 μm . (b) Photolysis-evoked single-spot responses obtained in the five different locations shown in a. (c) Superimposed traces depicting simultaneous photolysis at positions 1 and 2 (black) and the sum of individual responses at positions 1 and 2 (red).