Imaging spatio-temporal patterns of long-term potentiation in mouse hippocampus

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Spatio-temporal patterns of neuronal activity before and after the induction of long-term potentiation in mouse hippocampal slices were studied using a real-time high-resolution optical recording system. After staining the slices with voltage-sensitive dye, transmitted light images and extracellular field potentials were recorded in response to stimuli applied to CA1 stratum radiatum. Optical and electrical signals in response to single test pulses were enhanced for at least 30 minutes after brief high-frequency stimulation at the same site. In two-pathway experiments, potentiation was restricted to the tetanized pathway. The optical signals demonstrated that both the amplitude and area of the synaptic response were increased, in patterns not predictable from the initial, pretetanus, pattern of activation. Optical signals will be useful for investigating spatio-temporal patterns of synaptic enhancement underlying information storage in the brain.

Keywords: long-term potentiation; voltage-sensitive dye; optical recording; synaptic plasticity

1. INTRODUCTION

It is commonly supposed that memories are laid down in the brain as distributed patterns of altered synaptic strength. Testing this hypothesis, however, has been extremely difficult, largely because of the lack of experimental methods for visualizing spatial and temporal patterns of synaptic plasticity. LTP of synaptic transmission, wherein high-frequency stimulation of excitatory pathways results in a persistent increase in synaptic strength (Bliss & Lømo 1973), is the most widely studied model of memory formation in the mammalian brain (Bliss & Collingridge 1993). Typically LTP is monitored at one or a few sites by means of intracellular or extracellular electrodes, with excellent temporal resolution but little ability to resolve spatial patterns of activation. Multisite optical recording, using voltage-sensitive dyes, provides an alternative approach with greatly increased spatial resolution (Orbach *et al.* 1985). Here, we use a fast digital imaging system in conjunction with a voltage-sensitive absorbance dye to monitor evoked synaptic responses in acutely prepared hippocampal slices. By comparing responses to identical stimuli before and after induction of LTP we are able to extract the spatiotemporal pattern by which synaptic enhancement is expressed in this network.

2. MATERIAL AND METHODS

(a) Hippocampal slice preparation

Male *ddy* mice ($n = 11$; Hokudo, Sapporo, Japan), 4–5 weeks of age, were decapitated under ether anaesthesia. The brain was rapidly removed into ice-cold physiological saline, then mounted onto the stage of a vibroslicer, and 300–320 μm thick horizontal slices were cut through the hippocampus. The slices were stored for at least 1 h in a holding chamber filled with ACSF containing (in mM): NaCl, 120; KCl, 3; NaHCO₃, 23; NaH₂PO₄, 1.2; glucose, 11; CaCl₂, 2.4; MgSO₄, 1.2; bubbled with a 95% O₂/5% CO₂ gas mixture yielding pH 7.3.

(b) Electrophysiological recording

After staining (see § 2c), the slice was transferred to a recording chamber on the stage of an inverted microscope (TMD-300; Nikon) and was continuously superfused with oxygenated ACSF at 33 °C at a rate of 2–3 ml min⁻¹. Electrical test pulses (up to 40 V, constant voltage, 50 μs duration; Master-8 stimulator with Iso-flex isolator; A.M.P.I., Israel) were applied at a rate of 0.033 Hz via a bipolar tungsten electrode (5 μm tip, 5 M Ω ; A-M systems, Inc., WA, USA). One electrode was placed in CA1 stratum radiatum to stimulate Schaffer collateral inputs; a second was placed some distance from the first to stimulate a different set of afferent fibres, either in stratum radiatum or stratum oriens. A test stimulus was applied via this second electrode 100 ms after each test stimulus through the first electrode. The evoked PS or fEPSP in response to the test stimuli was recorded through a glass extracellular electrode (filled with 2 M NaCl and 5% brilliant blue) placed in CA1 stratum pyramidale or stratum radiatum. Responses were amplified (MEG-1251, Nihon Koden, Tokyo, Japan), digitized (ITC-16; InstruTech, NY, USA) and input to a Macintosh computer. Experimental control and analysis were carried out via A/DVANCE software (McKellar Designs, Vancouver, Canada). Stimulus intensity was adjusted

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Figure 1. Optical imaging of synaptic responses and potentiation in a hippocampal slice. (a) Optical signals in response to two stimuli at 100 ms intervals, just prior to high-frequency stimulation. Images were obtained at 0.6 ms intervals; every third image is shown in this montage, i.e. at intervals of 1.8 ms, reading left to right and top to bottom. (An ordinary image of the slice is visible in the last element of the montage, and at higher magnification in part (e), where the positions of the stimulating and recording electrodes are indicated.) The false-colour scale indicates the fractional change in intensity in per cent, $\% \Delta I/I$. (b) Optical signals recorded as in (a), 20 min after high-frequency stimulation applied via the second stimulating electrode. The response to the second stimulus is increased. (c) Electrical responses to two-pathway stimulation, recorded prior to, and after, potentiation of the second stimulated pathway. On the left is plotted the fEPSP slope in response to 0.033 Hz stimuli applied to electrode 1 (blue circles) and electrode 2 (red squares). At time 0, a brief high-frequency stimulus was applied via electrode 2, resulting in potentiation of the synaptic response. The traces on the right were obtained at the times labelled (i) and (ii) on the left; the dashed regions correspond to the intervals over which the EPSP slopes were measured. (d) Optical image of synaptic potentiation, obtained by frame-by-frame digital subtraction of the series shown in (a) from the series in (b). The false-colour scale shows the percentage change in the optical signal, $\%LTP$. (e) The spatial extent of significant LTP (post-tetanus optical signal more than two standard deviations different from the pretetanus level) is indicated by coloured areas, superimposed on the grey-scale reference image of the slice. False colours correspond to the scale in (d). Black and white stars indicate positions of S1 and S2 stimulating electrodes, respectively; the white cross indicates the position of the recording electrode. The filamentous shadows are cast by lens paper on which the slice is mounted in the perfusion chamber. The field of view is 1.7 mm on each side.

Figure 2. The spatio-temporal pattern of potentiation is not predictable from the pattern of the baseline synaptic response. (a) All frames, at 0.6 ms intervals, are now shown of the portion of the optical recording ($\% \Delta I/I$) in figure 1a covering the onset of the response to S2. The colour scale is as in figure 1a. (b) The equivalent frames at 0.6 ms intervals from the $\%LTP$ series of figure 1(d). The potentiation appears as an expanding wavefront. The colour scale is as in figure 1(d).

to evoke a half-maximal fEPSP. LTP was induced in either pathway ('potentiated pathway') by the application of a tetanus consisting of four 1 s bursts of 100 Hz stimulation (50 μ s duration and same strength as the test stimuli) with 3 s intervals between bursts, to one stimulating electrode ('S2, potentiated pathway'). No stimulus was applied to the other stimulating electrode ('S1, reference pathway') during the tetanus. After the tetanus, test stimuli were applied at rate of 0.033 Hz to both pathways as before. PS amplitude or fEPSP slope was measured and plotted with ADVANCE, with LTP expressed as per cent change from the pretetanus baseline.

(c) Optical recording

Immediately before transfer to the recording chamber, slices were stained for 10 min with voltage-sensitive oxonol dye, NK-3630 (Nihon Kanko Shikiso Kenkyusho, Okayama, Japan) (Momose-Sato *et al.* 1999) diluted in ACSF at a final concentration of 0.25–0.5 mg ml⁻¹. A Deltaron (Fujifilm, Tokyo, Japan) imager with a 128 × 128 MOS photodetector array and 0.6 ms frame interval was attached to the camera port of the microscope for optical recording. Details of the Deltaron system have been described elsewhere (Kita *et al.* 1995). The image of the hippocampal slice was focused on the photodetector array by means of a magnification ×4, 0.20 N.A. objective (Nikon). With this arrangement, the array detected an area of 1.7 mm × 1.7 mm in the plane of the hippocampal slice (i.e. each pixel covered *ca.* 13 μ m × 13 μ m in the specimen plane). The slice was transilluminated for 1 s in each trial by a stabilized high-intensity tungsten filament lamp via a 700/30 nm interference filter, corresponding to the major absorption peak of the dye NK-3630.

At the beginning of each optical recording trial, a reference image was taken 600 ms before application of the test stimuli and stored in the Deltaron's memory. Each of the subsequent frames was subtracted from this reference, amplified 400 times, and stored in the memory as a difference image. The frame rate of the system was 0.6 ms, and 512 frames (i.e. total sequence *ca.* 300 ms) were recorded for each trial. The timing of stimulus test pulses with respect to the start of the optical recording was constant from trial to trial.

(d) Image processing and data analysis

From the reference and difference images, absorbance changes at each pixel at each time point were calculated as fractional change in light intensity, $\% \Delta I/I = 100((d/400)/I)$, where d is the value of the pixel in the difference image, and I is the pixel's intensity in the reference image. Calculated $\% \Delta I/I$ image series were viewed using IMAGEJ (W. Rasband, NIH). For each experiment, the optical recordings were carried out twice, once

just prior to the application of tetanus and a second time 20 min after the tetanus. For optical analysis of synaptic plasticity, after 3 × 3 median filtering of each image, the percentage of potentiation ($\%LTP$) was calculated as $100(\text{post-tetanus image} - \text{pretetanus image})/(\text{pretetanus image})$ for each corresponding image pair in the two series. Pixels from each frame with $\%LTP$ greater than twice the standard deviation were projected to a single plane with IMAGEJ, and superimposed on the reference image using Adobe PHOTOSHOP to visualize the total spatial extent of plasticity.

3. RESULTS

The voltage-sensitive dye used here had no overt pharmacological effects, with no conspicuous differences noted between the electrical responses in stained and unstained preparations. This is in agreement with previous results using the same dye (Momose-Sato *et al.* 1999). Bleaching was relatively slow under the conditions of these experiments, and was not corrected.

Montages of every third difference image in optical recordings of a representative preparation just before and 30 min after tetanic stimulation are shown in figure 1a,b, respectively. (The reference image of the slice can be seen in the last tile in the montage of figure 1a, and at larger magnification as the grey-scale component of figure 1e.) These $\% \Delta I/I$ images were calculated as described in § 2d, and false coloured according to the indicated scale. In the pretetanus trial (figure 1a), strong optical responses were elicited close to each stimulating electrode immediately after stimulation through each, with the expected 100 ms interval. In both cases the optical signals propagated orthodromically towards the subiculum, generally passing across stratum pyramidale to involve both stratum radiatum and stratum oriens. Qualitatively similar patterns of signal propagation were obtained in 10 out of 10 other preparations; in some of these, antidromic activation could also be detected with shorter latencies than the orthodromic signals. Orthodromic propagating signals, but not antidromic signals, were completely abolished in each of two separate experiments following the application of 20 μ M CNQX (data not shown), confirming that the propagating optical signals reflected excitatory glutamate-mediated postsynaptic responses.

In the post-tetanus optical recording (figure 1b), both stimuli evoked signals that propagated toward the subiculum as in figure 1a. However, the optical response to the second stimulus reached higher amplitudes and was more spatially extensive than before the tetanus.

Representative extracellularly recorded electrical responses are shown on the right-hand side of figure 1c; both stimuli evoked compound fEPSPs. The fEPSP slopes are plotted over time on the left-hand side of figure 1c. The tetanus resulted in a clear and stable potentiation of the EPSP in the potentiated pathway (S2), with no change in the response of the reference pathway (S1).

In the computed %LTP images (figure 1d), there is little difference between responses to the first stimulus (the small apparent reduction in this response may be an artefact of dye bleaching), but a robust potentiation of the response to the second stimulus is evident. Similar results were obtained in all other preparations following the induction of LTP. In several experiments, the magnitudes of the optically detected synaptic responses and/or LTP magnitude were larger at locations distant from, as compared to close to, the recording electrode.

A projection of all pixels in which the post-tetanus optical signal was more than two standard deviations beyond the pretetanus signal at any point during the recording is shown in figure 1e. Although this usefully summarizes the spatial extent and magnitude of potentiation, it masks the temporal component. Figure 2 shows all frames (at 0.6 ms intervals) beginning just prior to the second stimulus, during the pretetanus trial (figure 2a) and in the corresponding frames of the computed %LTP series (figure 2b). Whereas the synaptic activation (figure 2a) propagates as a coherent volume moving towards the subiculum, the potentiation signal moves as a roughly circular expanding wavefront (figure 2b).

4. DISCUSSION

Optical recordings of voltage-sensitive dye signals have been used previously to monitor spatio-temporal patterns of synaptic activation in the hippocampus (Grinvald *et al.* 1982; Bonhoeffer & Staiger 1988; Plenz & Aertsen 1993; Barish *et al.* 1996; Iijima *et al.* 1996; Jackson & Scharfman 1996; Nakagami *et al.* 1996; Sekino *et al.* 1997; Chesi *et al.* 1998; Antic *et al.* 1999; Otsu *et al.* 2000; Sato *et al.* 2000; Inoue *et al.* 2001). Such recordings have also detected the induction of LTP as an increase in the amplitude (Saggau *et al.* 1986; Bonhoeffer *et al.* 1989; Tominaga *et al.* 2000) and a reduction in latency (Nakagami *et al.* 1997) of the synaptically evoked voltage-sensitive dye signal. However, voltage-sensitive dyes have not previously been used to visualize the spatio-temporal pattern of potentiation.

The data presented here demonstrated that such patterns of potentiation can be monitored with high spatial and temporal resolution. Their shapes and trajectories are complex, reflecting the underlying connectivity of the network and possibly the history of activation. The ability to monitor these patterns in this relatively non-invasive manner may be of use in deciphering the encoding of natural memory in the brain.

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GLOSSARY

- ACSF: artificial cerebrospinal fluid
 CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione
 fEPSP: field excitatory postsynaptic potential
 LTP: long-term potentiation
 PS: population spike