Rapid visual stimulation induces N-methyl-D-aspartate receptor-dependent sensory long-term potentiation in the rat cortex

Wesley C. Clapp\textsuperscript{a}, Michael J. Eckert\textsuperscript{b}, Tim J. Teyler\textsuperscript{c,d} and Wickliffe C. Abraham\textsuperscript{b}

\textsuperscript{a}Research Center for Cognitive Neurosciences, University of Auckland, Auckland, \textsuperscript{b}Department of Psychology, University of Otago, Dunedin, New Zealand, \textsuperscript{c}Medical Education Program, University of Idaho, Moscow, Idaho and \textsuperscript{d}Department of Veterinary and Comparative Anatomy, Pharmacology, and Physiology, Washington State University, Pullman, Washington, USA

Correspondence and requests for reprints to Mr Wesley Clapp, Psychology Department, City Campus, University of Auckland, Private Bag 92019, Auckland, New Zealand

Tel: + 64 9 373 7599 ext 84249; fax: + 64 9 373 7450; e-mail: w.clapp@auckland.ac.nz

All procedures were approved by the University of Otago Ethics Committee.

Sponsorship: NIH grant RO1 MH064508 and NZ Marsden Fund.

Received 5 January 2006; revised 23 January 2006; accepted 24 January 2006

Previously we have demonstrated that rapidly presented sensory stimulation (visual or auditory) can induce long-lasting increases in sensory evoked potentials recorded from the human cortex. Long-term potentiation was suggested as the underlying mechanism of these increases. In the present experiment, we applied the same visual paradigm to anesthetized rats to investigate the properties and mechanisms of this effect. Our results indicated that visual evoked responses were significantly enhanced for at least 1 h and, when followed, up to 5 h after the presentation of a 'photic tetanus'. Furthermore, the potentiation was N-methyl-D-aspartate receptor-dependent and cortically generated. This type of sensory long-term potentiation may underlie perceptual learning, and serves as a model system for investigating sensory-evoked plasticity.

\textbf{Keywords:} long-term potentiation, N-methyl-D-aspartate receptor, rat, sensory stimulation, visual cortex

\section*{Introduction}

Long-term potentiation (LTP) is an enduring, activity-dependent increase in synaptic efficacy that is the principal candidate synaptic mechanism underlying learning and memory formation [1,2]. LTP is typically induced by repeated electrical stimulation of an afferent pathway, and in many systems it is mediated by activation of postsynaptic N-methyl-D-aspartate receptors (NMDAR) [3]. LTP occurs widely throughout the brain including in the neocortex, where particular emphasis has been given to the somatosensory [4], auditory [5,6] and visual cortices [7–11]. Cortical LTP occurs throughout development and adulthood [8], and there is considerable evidence that NMDAR-dependent LTP-like mechanisms contribute to the development of the visual system [9,12]. LTP, or LTP-like changes, have also been inferred as the basis of perceptual learning [13–15].

As most studies use electrical stimulation to induce and test for LTP, it remains debated whether LTP is a phenomenon of significance for real world events. The case that sensory cortex LTP is behaviorally relevant would be buttressed if it could be shown that sensory evoked responses can themselves undergo potentiation. This has been suggested by the demonstration in visual cortex that a neuron’s preferred orientation and ocularity can be shaped by the pairing of visual stimuli with intracellularly applied currents [16]. Furthermore, electrical tetanic stimulation of the dorsal lateral geniculate nucleus (LGN) can induce an NMDAR-dependent increase in the LGN-evoked field potential in the primary visual cortex that is associated with a potentiation of the cortical responses to full field flashes (visual evoked potentials, VEPs) [8].

Given these findings, it is reasonable to ask whether repetitive sensory stimulation by itself is sufficient to elicit LTP. Indeed, repetitive visual stimulation elicits an NMDAR-dependent LTP in selective retinotectal synapses of the tadpole [17]. Moreover, Eyding \textit{et al.} [18] found that repetitive visual stimulation can lead to long-term receptive field plasticity in the cat primary visual cortex. In humans, high- or low-frequency electrical stimulation of the skin can induce long-lasting increases or decreases, respectively, in perceived pain [19].

Recently, Teyler \textit{et al.} [20] reported that rapid presentation of checkerboard stimulation (a ‘photic tetanus’) can induce LTP-like changes [termed here as sensory long-term potentiation (sLTP)] in VEPs recorded noninvasively from human study participants. Additionally, Clapp \textit{et al.} [21] showed that a long-lasting enhancement of event-related desynchronization occurs after a photic tetanus, suggesting
that the rapid visual stimulation induces an increase in neuronal output. A supporting functional magnetic resonance imaging study demonstrated that the photic tetanus induced an enduring change in the cortical hemodynamic response localized to extrastriate areas [22]. It has also been found by this group that rapidly presented tone pips can induce a similar sensory potentiation in the human auditory cortex [23]. These findings reinforce the idea that endogenous neural activity, triggered by sensory experience, can induce synaptic potentiation. Noninvasive human studies, however, are limited in their ability to determine the mechanisms underlying the potentiation. Therefore, in the present study, we attempted to induce sLTP in the rat visual cortex using a photic tetanus to first confirm that this is a useful animal model for studying sLTP, and to begin to elucidate the mechanisms underpinning this effect.

Methods

Surgery

Fifteen Brown Norway rats (aged 4–6 weeks) were used in this study. The animals were anesthetized with urethane (3 g/kg, intraperitoneally) combined with atropine (50 mg/kg, intraperitoneally) to reduce respiratory distress. A rectal thermometer was used to monitor body temperature, which was maintained at 37.0°C throughout the experiment. The rat was mounted in a stereotaxic apparatus and small holes were drilled over the left visual cortex (from bregma, channel 1: 5.6 mm posterior, 2.0 mm lateral, channel 2: 6.1 posterior, 4.8 lateral) to allow small stainless steel screw electrodes to be inserted. Another skull screw positioned over the frontal cortex served as a reference electrode. The two recording electrodes were centered over V2MM (Ch 1) and that could be stably recorded during baseline stimulation.

Recording

Following surgery, the anesthetized animal remained in the stereotaxic apparatus and was enclosed within a thick, black curtain to attenuate ambient illumination. Within the curtain, a 17-inch flat-panel liquid crystal display was positioned 15 cm from the rat’s right eye (a small piece of electrical tape was used to close the left eye). Visual stimuli consisted of checkerboards subtending 27.8° of visual angle from the vertical and horizontal midline. Individual checks subtended 1.11° of visual angle. LGN stimulation consisted of constant current pulses of 0.15 ms duration, ranging in intensity from 10 to 150 μA. All evoked responses were band-pass filtered (0.3 Hz–3 kHz), amplified and digitized (10 kHz) using custom Labview software (National Instruments, Austin, Texas, USA).

Procedure

During the baseline period, the checkerboards were presented at an average rate of approximately 0.067 Hz (duration 33 ms) and VEPs were recorded from the two skull screw electrodes. A block of baseline testing consisted of 30 stimulus presentations (~8 min). Four baseline testing blocks were recorded over a 1-h period. After an hour of stable responses, the photic tetanus was delivered. The photic tetanus involved presentation of the checkerboard stimulus at a frequency of 9 Hz for ~120 s (1000 presentations). Subsequently, participants were given a 2-min break from stimulation. Following the block of tetanic stimulation, blocks of VEPs were again collected at the baseline presentation rate at four different times after the tetanus: 3–11, 19–27, 35–43, 51–59 min. Following this, a second photic tetanus was delivered, followed by a further 1 h of baseline testing.

In the saline/CPP experiment, 1 h of baseline testing was recorded, followed by the injection of either the competitive NMDAR antagonist 3-(2-carboxyppiperazin-4-yl)propyl-1-phosphonic acid (CPP; Tocris, Bristol, UK) or saline. Following the injection, baseline responses were recorded for an additional 2 h after which the first photic tetanus was delivered. After a further 1 h of baseline testing, a second photic tetanus was delivered to test for saturation of sLTP, and then a final hour of baseline testing was recorded.

To determine whether the sLTP effect was occurring downstream of the LGN, we placed a stimulating electrode in the LGN, allowing us to evoke cortical potentials by both visual and LGN electrical stimulation. For this experiment, the animals received the same visual stimulation as described above, and additionally received test pulses to the LGN to monitor the LGN–cortex pathway. After a stable baseline of VEPs was achieved, an input/output (1/O) series was delivered to the LGN (15 intensities ranging from 10 to 150 μA). On the basis of this I/O series, an intensity that evoked a half-maximum response was used to monitor the LGN-evoked potential throughout the experiment. In these testing sessions, eight LGN samples were recorded (0.7 Hz) and averaged to obtain a value for each time point. Following the photic tetanus, another I/O series was conducted to monitor the sLTP effect across the range of stimulus intensities.

Analysis

The average of the last two blocks of testing (i.e. 0.5–1 h post photic tetanus 2) was used in all statistical analyses, and was either compared with the two blocks immediately preceding the photic tetanus using paired t-tests, or compared with other conditions using unpaired t-tests. To analyze the I/O data, a within-subject two-factor analysis of variance was performed.

Results

The checkerboard stimulus evoked a large positive potential in the contralateral visual cortex that peaked at ~100 ms and that could be stably recorded during baseline stimulation for both channels (Fig. 1). Following the initial photic tetanus, the VEP amplitude from channel 2 increased immediately (~134%) and showed no appreciable decline during the next hour of baseline recording (Fig. 1b). The channel 1 VEP also increased in amplitude, but to a lesser extent. Following the second tetanus, no further potentiation was apparent in either channel, suggesting that the first tetanus had saturated sLTP Statistical analysis revealed that the photic tetani significantly increased the VEP at channel 2 [(t(5) = 3.74, P = 0.013), but not at channel 1 [(t(3) = 1.48, P = 0.236)], suggesting that this effect was specific to V1b or neighboring areas.

We next investigated whether sLTP was dependent on NMDAR activation. In this experiment, CPP (10 mg/ml/kg, intraperitoneally) was administered to one group of animals
of the rats that received a saline injection for 5 h after the animal, taken at the times indicated. Scale bars: 0.1 mV, 100 ms.

mean increase in the VEP amplitude after the photic tetanus (Fig. 2a). To compare the CPP group with the combined control group and the CPP group revealed a significant difference [\(t(6)=3.76, P=0.002\)], suggesting that sLTP is dependent on NMDAR activation.

To verify the persistence of sLTP, we continued testing one of the rats that received a saline injection for 5 h after the second photic tetanus. This animal went through the normal procedure, but after the last baseline block the response continued to be monitored during the third and fifth hours after tetanus. The channel 2-evoked potential showed a clear sLTP effect that persisted stably over time (151% LTP at 1 h after tetanus, 145% LTP at 5 h; Fig. 2b).

To determine whether some aspect of the sensory LTP was downstream of the LGN, we recorded the LGN-induced cortical evoked potential in another experiment. Following the photic tetanus, the LGN-evoked potential

**Fig. 1** Induction of sensory long-term potentiation (LTP) in the rat visual cortex by rapidly presented checkerboards. Visual evoked potential (VEP) response amplitudes to slowly flashing checkerboards are expressed as a percentage of baseline. A photic tetanus (PT) was delivered at times marked by the arrows. (a) Data and waveforms for channel 1 (V2MM) showed a nonsignificant increase in the VEP amplitude after the tetanus (\(n=4\)). (b) Data and waveforms for channel 2 (V1b) revealed a significant increase in the VEP amplitude after the photic tetanus (\(n=6\)). Data are mean ± SEM. Waveforms are averages of 60 sweeps from a representative animal, taken at the times indicated. Scale bars: 0.1 mV, 100 ms.

**Fig. 2** N-methyl-D-aspartate receptor dependence and persistence of sensory long-term potentiation (LTP). (a) Baseline channel 2 responses were collected 1 h before injection of either 3-(2-carboxypiperazin-4-yl)-propyl-l-phosphonic acid (CPP) or saline. Photic tetani (PT) were delivered at the times marked by the arrows. In animals that received saline (\(n=5\)), a significant increase in evoked potential amplitude was recorded immediately after the delivery of a PT and remained increased throughout the testing. CPP (\(n=4\)) completely blocked the induction of sensory LTP. Inset, averaged evoked potentials from baseline checkerboard stimulation before and after tetanus for channel 2 in representative animals that received an injection of saline (left) or CPP (right) at the times indicated. Scale bars: 0.1 mV, 100 ms. (b) One animal that received saline was used to follow the persistence of sensory LTP over hours. The potentiated response was maintained for the entire 5 h after tetanus recording period (channel 2). Solid lines indicate periods when no stimulation or recording was undertaken. Inset, averaged evoked potentials from time points as indicated: pre-tetanus (black), 1 h after tetanus 2 (light gray) and 5 h after tetanus (dark gray). Scale bars: 0.1 mV, 100 ms.
Cortical localization of the sensory long-term potentiation (LTP) effect. (a) The lateral geniculate nucleus (LGN) was stimulated throughout the experiment to monitor the amplitude of the geniculocortical evoked potential. After the photic tetани (PT), the negative peak of the evoked field potential (FP) was significantly increased at channel 2, and remained so thereafter. Inset, average evoked potentials from the LGN probe stimulation before and after tetanization. The arrow marks the peak that was analyzed in both the probe and input/output (I/O) analyses. Scale bars: 0.1 mV, 50 ms. (b) Input/output series were recorded both before and after the photic tetanus. A significant potentiation across the linear portion of the rising phase of the I/O curve (35–90 μA; n=3) was observed.

Discussion

We have demonstrated a novel effect in animals whereby rapid presentation of visual stimulation, by itself, induces an immediate, long-lasting and robust plastic change. Thus, a block of rapidly presented checkerboards significantly and stably enhanced the amplitudes of checkerboard-evoked responses in the visual cortex for at least 1 h. This sLTP effect confirms previous studies done with humans [20–23]. In addition, we demonstrated for the first time that the sensory potentiation is NMDAR dependent, as it was blocked by CPP, which exerted no effect on the VEPs by itself. We also followed the potentiation for up to 5 h after tetanus and found no significant decay over this recording period, supporting the suggestion that sensory potentiation can entail long-lasting changes in the visual cortex. Taken together, these properties clearly link this phenomenon to other forms of sensory cortex plasticity, such as electrically evoked LTP [7,8].

The recordings from channel 2 (centered on V1b) showed significant potentiation, whereas those from channel 1 (centered on V2MM) did not, demonstrating that this was not an overall increase in excitability within the visual cortex. While relatively large skull screws cannot provide precise localization, these results indicate that sLTP was induced in or near the primary visual cortex, although we cannot rule out plasticity occurring at multiple sites in the retinorextrastriate pathway. Indeed, the sensory potentiation induced by a photic tetanus in humans was localized to the extrastriate visual cortex using both electroencephalogram source localization and functional magnetic resonance imaging techniques [20,22]. The differences between the rat and human visual systems, or the somewhat imprecise localization in the present study may account for the fact that the present results do not fully parallel the human findings in location, despite the overall effects being similar. Further localization tests are warranted in this rat model.

The sensory LTP evoked in the present study was rapidly and robustly induced, saturable, and NMDAR dependent. In all these regards, as well as the overall magnitude of the potentiation, the effect mimicked the cortical LTP induced by LGN tetanization [8]. In neither case, however, can it be certain that the LTP entails synaptic changes as opposed to some other form of plasticity, such as changes in cell excitability. Nonetheless, the net effect is one of enhanced throughput in the visual pathway, and therefore these studies pave the way for future tests of the behavioral correlates of sLTP, such as changes in visual acuity or detection thresholds. Alternatively, sLTP may provide a useful model for studying the mechanisms of pathological responses, such as seizures, to rapidly presented visual stimuli, or for screening the plasticity capabilities of people suffering from neurological diseases or memory disorders.

Conclusions

We have demonstrated that naturalistic sensory stimuli are sufficient to induce LTP in the adult brain. The sensory LTP serves as a model system for investigating synaptic plasticity noninvasively. As comparable sensory-induced potentiation effects have been found in the human sensory cortex with the use of similar paradigms [20–23], it is possible that sensory LTP may underlie phenomena such as perceptual learning.
References


7. Aroniadou VA, Teyler TJ. The role of NMDA receptors in long-term potentiation (LTP) and depression (LTD) in rat visual cortex. Brain Res 1991; 562:136–143.


