Repetitive, daily tetanization of the corpus callosum induces lasting changes in sensorimotor cortex field potential responses, but the synaptic populations that mediate these responses and support long-term potentiation (LTP) have not been characterized. Current source density analyses of field responses were compared between control animals and those in which LTP was induced by 10 daily series of tetanizations. Tetanization and paired-pulse stimulation (100 ms interval) enhanced the duration of initial (~3 ms onset) deep-negative population spike activity generated by a current sink in layer V that peaked repeatedly at a frequency of ~400 Hz. The early (~10 ms to peak) surface-negative component of field responses was generated by a current sink in upper layer V and a source in layer VI. This monosynaptic component followed high stimulation frequencies, recovered quickly from the effects of anaesthesia, and was enhanced by both tetanization and paired-pulse stimulation. The late (~20 ms to peak) surface-negative component was generated by a sink in upper layer V and a source deep in layer V, and was greatly enhanced by tetanization and paired-pulse stimulation. The late component did not follow high-frequency stimulation and recovered slowly from anaesthesia, suggesting that it is driven polysynaptically. Potentiation of monosynaptic thalamic and cortico-cortical afferents probably mediates enhancements of the early component and population spikes, while potentiation of polysynaptic afferents to layer V may contribute to growth in the late component.

Introduction

Long-term potentiation (LTP) is an activation-induced, lasting enhancement of synaptic efficacy and is the most widely investigated model of cellular mechanisms of memory (Bliss and Lomo, 1973; Racine et al., 1983; Bliss and Collingridge, 1993; Singer, 1995). The neocortex is the putative site for long-term memory storage, but it is relatively resistant to LTP induction. Although neocortical LTP can be reliably observed in acute (Wilson and Racine, 1983; Iriki et al., 1991; Racine et al., 1994b; see also Baryani et al., 1991) and in vitro (Artola et al., 1990; Kirkwood et al., 1993; Aронiadou and Keller, 1995; Castro-Alamancos et al., 1995; Hess et al., 1996) preparations, it usually requires the use of young animals (Wilson and Racine, 1983; Baskys et al., 1990; Kato et al., 1991) or pharmacological reduction of GABAergic inhibition (Artola and Singer, 1987; Bindman et al., 1988; Hess and Donoghue, 1994; Kirkwood and Bear, 1994). The adult neocortex in chronic (unanaesthetized) preparations is even more resistant to LTP induction in response to standard tetanization parameters (Racine et al., 1994b). Chronically prepared preparation, however, to examine the properties of LTP within the normal milieu of the intact adult brain and to confirm that LTP effects are truly long-lasting.

We have recently determined that LTP of neocortical field responses can be reliably induced in chronic preparations when potentiating stimulation trains are spaced and repeated (Racine et al., 1995a,b). Field potentials in sensorimotor cortex of the awake rat evoked by corpus callosum stimulation show little or no change in response to tetanization on a single day (Racine et al., 1994b), but are gradually and markedly altered by high-frequency trains delivered over multiple days (Racine et al., 1995b; Trepel and Racine, 1998). This regimen causes the potentiation of an initial (~3 ms latency) deep-negative spike-like component. Increases in subsequent repetitive population spikes appear to cause a reduction in the amplitude of a second ‘early’ (~10 ms peak latency) surface-positive component. Repeated tetanization also markedly potentiates a ‘late’ (~20 ms peak latency) surface-negative field potential component. Experiments in this paper were designed to characterize the local circuits that generate these complex neocortical responses.

Sensorimotor cortex field responses evoked by stimulation of the corpus callosum in vivo resemble those recorded by others in related preparations. In the motor cortex in vitro, stimulation of deep layer V evokes current sinks in layers II–V, associated with a ~3 ms latency surface-positive deflection, and sinks in layers III–V associated with a polysynaptic 8–10 ms surface-negative deflection (Aroniadou and Keller, 1993). Layer III stimulation evokes short-latency sinks in layers II–III followed by sinks in layer V up to 1700 µm lateral to the stimulation electrode. Axon collaterals in layer III may therefore mediate longer-latency components evoked by white matter stimulation (Keller, 1993). Somewhat different patterns are observed in the sensorimotor cortex in vivo where ventrolateral thalamic nucleus stimulation evokes field responses generated by a layer V current sink, and ventroposterior lateral nucleus stimulation evokes a large layer IV sink that spreads to the supragranular layers (Castro-Alamancos and Connors, 1996a,b).

The major synaptic populations that mediate sensorimotor cortex field responses evoked by corpus callosum stimulation were localized here in the awake animal using current source density (CSD) analysis techniques (Haberly and Shepherd, 1973; Nicholson and Freeman, 1975; Mitzdorf, 1985). Depth profiles for CSD analysis were obtained from control and tetanized animals. Because paired-pulse stimulation with a 100 ms interval causes a facilitation that resembles potentiated responses (Racine et al., 1995b; Castro-Alamancos and Connors, 1996a), pairs of stimulation pulses were delivered during depth recordings. The ability of field potential components to follow high-frequency stimulation, and to recover from anaesthesia were monitored in subsequent experiments to discriminate monosynaptic and polysynaptic components. Evoked multi-unit discharges were also recorded to localize cells generating population spike activity and to characterize discharge patterns with respect to field responses.

Materials and Methods

Surgery

Male Long–Evans hooded rats (260–350 g) were anaesthetized with 0.9

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mg/kg ketamine and 0.05 mg/kg xylazine i.p., and placed in a stereotaxic frame with the skull surface horizontal. When required for later depth profile recordings, a 1.5 mm diameter hole was drilled 2.0 mm anterior and 4.0 mm lateral to Bregma. Care was taken not to damage the dura mater and the opening was filled with soft sterile wax.

Chronic stimulating and recording electrodes were then implanted. Bipolar, Teflon-coated, stainless-steel twisted-wire electrodes (125 µm exposed tip diameter) were implanted in the right corpus callosum (A 2.0 mm, L 2.0 mm and V 2.8-3.4 mm from Bregma), and sensorimotor cortex (A 2.0 mm, L 4.0 mm and V 1.8 mm to the pial surface). Tip separation was 0.5 mm for stimulating electrodes and 1.0 mm for recording electrodes. Vertical placements of stimulating electrodes were adjusted to minimize current intensity thresholds, and vertical placements of recording electrodes were adjusted to maximize the amplitude of the early field potential component. Stainless-steel screws for ground and reference electrodes were placed in the left frontal bone and posterior parietal bone respectively. Electrode leads were connected to gold-plated pins mounted in a connector. The assembly was embedded in dental cement anchored to the skull.

When later depth profile recordings were required, a thin plastic sheet was embedded in the cement to protect the hole in the right frontal bone. Four indentations in the cement were then made using movable earbar attachments that each carried two pointed horizontal rods. 12.0 mm apart, 15.0 mm above the interaural line. The drying cement formed closely around the tip of each rod so that the attachments could be used to painlessly immobilize the rat's head during depth recordings (see also Bassant et al., 1990).

Animals were housed individually in 18 × 20 × 35 cm stainless-steel hanging cages on a 12 h on, 12 h off, light–dark cycle. Animals were handled and monitored for wound inclusion during a 2-week recovery period and all subsequent testing was conducted during the lights-on period.

**Stimulation and Recording**

Animals were tested in a quiet, resting state after habituation to a 30 × 40 × 30 cm wooden chamber with Plexiglas front and wire-grid floor. Electrical stimuli were generated with a Grass S88 stimulator and photoelectric stimulus isolation units (Grass SIU6B) were used to deliver 0.1 ms biphasic constant current pulses. Both monopolar and differentially recorded field potentials were filtered (0.3–3 kHz), amplified (Grass Model 12) and digitized (10 kHz; 12-bit A/D board) for storage on computer hard disk.

**Input/Output Tests**

Field potentials evoked by a range of test-pulse intensities were recorded during input/output (I/O) tests to monitor the stability of baseline field potentials and changes following tetanization. Ten evoked potentials were recorded and averaged at each of ten logarithmically spaced intensities (range 40–1260 µA). There was a 10 s intertrial interval. Mean I/O curves were obtained by averaging results standardized to baseline responses evoked by the highest stimulus intensity.

Baseline I/O tests were recorded once every 2 days over a 5-day period. Animals for depth profile experiments were split into two groups with similar responses, and one group was randomly assigned to receive tetanization. Input/output tests were conducted once every 2 days, and during head restraint prior to depth profile recordings (see below). Changes in response amplitudes were assessed using repeated measures ANOVAs.

**Paired-pulse Tests**

Paired-pulse tests were conducted on the last baseline day and one day after LTP induction. Two pulses were delivered to the corpus callosum at 10 interpulse intervals (50, 70, 100, 150, 200, 300, 500, 700, 1000 and 2000 ms). Pulse intensity was set to evoke an early component ~75% of maximum amplitude. Response amplitudes for the second pulse were expressed as a percentage of responses to the first pulse (Racine and Milgram, 1985).

**LTP Induction**

Ten daily sets of 30 trains were delivered to induce LTP (Racine et al., 1995b; Trepel and Racine, 1998). Stimulation trains consisted of eight biphasic square-wave pulses delivered at 300 Hz. Pulse duration was 0.1 ms and pulse intensity was 1000 µA. There was a 10 s intertrain interval. Control animals were handled the same way but received no stimulation.

**Depth Recordings**

Pilot tests showed that anaesthesia with urethane (1.5 g/kg), somnotol (60 mg/kg) or a ketamine/xylazine mixture (0.9 and 0.05 mg/kg) attenuated or eliminated field potential components. Because depth profiles reflecting all response components could not be obtained under anaesthesia, tests were conducted in unanaesthetized preparations. Each animal was wrapped in light cotton fabric and placed in a stereotaxic apparatus with the head immobilized via anchor points in the electrode assembly. One or two other rats were placed within sight of the animal to reduce stress (Parry and McElligot, 1993). Animals were adapted to restraint during three 50 min periods, 2, 3 and 4 days prior to depth recordings. Restraint was tolerated well and animals typically settled within 5 min while remaining alert. The plastic sheet above the right neocortex was removed, and the exposed dural surface was cleared with physiological saline. An I/O test was conducted after 10 min to assess the effect of restraint on field potentials.

Monopolar depth recordings were obtained using insulated 5 MΩ tungsten electrodes (A/M Systems) placed in a Narishige mechanical micromanipulator. Electrodes were aimed at the center of the exposed cortex (A 2.0 mm, L 4.0 mm), and field potentials were sampled perpendicular to the cortical surface by advancing the electrodes at an angle 15° off the sagittal plane. The electrode was advanced and retracted twice to reduce cortical dimpling, and was then lowered to a point 2.3 mm from the surface. Warmed mineral oil was applied to prevent drying, and a 20 min period preceded subsequent testing.

The recording electrode was retracted in 50 µm steps, and 10 evoked responses were recorded for averaging at each depth. A pair of pulses separated by 100 ms was delivered on each trial with a 10 s intertrial interval. Pulse intensity was set to reflect the largest potentiation of the late component recorded from the fixed electrode. The same intensity was used in matched control animals (range 400–630 µA; mean 560 µA).

**Frequency of Following Tests**

Monosynaptic responses ‘follow’ (or are capable of being repeatedly evoked by) much higher frequencies of stimulation than polysynaptic responses. Polysynaptic components usually fail at frequencies <40–50 Hz, whereas monosynaptic components should follow frequencies near 100 Hz (Berry and Pentreath, 1976; Laroche et al., 1990; Liu and Bilkey, 1996; Chapman and Racine, 1997). Responses following at 500 Hz typically reflect fibre volleys and/or antidromic invasion. Frequency of following tests were conducted after baseline I/O tests and 1 day after the induction of LTP (n = 4). Trains of 10 pulses at five frequencies (20, 40, 80, 160 and 320 Hz) were delivered to the right corpus callosum, and responses were recorded from ipsilateral neocortex. Three trains at each frequency were applied at an intensity of 250 µA, and tests were then repeated with 500 µA pulses. The intertrain interval was 5 min.

**Recovery from Anaesthesia**

Anaesthesia attenuated field responses in pilot experiments, and the late components were particularly affected. The differential susceptibility of field components to anaesthesia suggested that monitoring the recovery of components from deep anaesthesia could help resolve monosynaptic and polysynaptic components (see Barry and Pentreath, 1976). Somnotol (65 mg/kg, i.p.) was administered to a group of five animals following baseline I/O testing. Once a level of general anaesthesia was reached (absence of reflexive blinking), field responses to test pulses (160 µA) were monitored every 30 s for 4 h. Recovery of field responses from anaesthesia was monitored again following LTP induction.

**Multi-unit Recordings**

Six animals were surgically prepared and habituated to head-restraint as described for CSD experiments. LTP was induced in three animals following baseline I/O tests, and multi-unit recordings were collected from all animals 1 day after the final trains were delivered. After exposing the right neocortex, a 5 MΩ sharpened tungsten electrode was...
advanced at an angle of 15° off the sagittal plane. Warmed mineral oil was applied to prevent drying. Ten samples of evoked field potentials and multi-unit activity were recorded as the electrode was lowered in 150 µm steps to 3.0 mm ventral to the surface. Pulse intensity was 1000 µA. Multi-unit recordings were high-pass analogue filtered at 300 Hz, and digitally filtered at 900 Hz using the inverse Fourier transform of the Blackman window. Smoothing at this frequency did not mask or shift the high-frequency noise, a preliminary spatial smoothing was performed.

### Field Responses Recorded via Chronically Implanted Electrodes

Monopolar and bipolar field responses from chronically implanted electrodes were used to monitor LTP induction prior to depth profile recordings. Recordings were obtained in the left neocortex, contralateral to the stimulating electrode, to keep the ipsilateral cortex clear for depth profile recordings.

### Baseline Field Responses

Field responses had three major components (Figs 2 and 3A). The earliest component was a deep-negative, surface-positive, spike-like deflection with a peak latency of 2.9 ± 0.1 ms and a differentially recorded amplitude of 1.41 ± 0.15 mV (n = 14). Negative deflections were then observed in both surface and deep recordings (Fig. 2). The peak of the negativity was earlier in deep sites (5.3 ± 0.2 ms) than in superficial sites (8.1 ± 0.5 ms). Responses in deep sites then reversed into a large, positive deflection that peaked shortly after the surface negativity at a latency of 12.5 ± 0.3 ms. The resulting voltage dipole produced large differential recordings of an early component with a peak latency of 10.3 ± 0.4 ms (peak 2.85 ± 0.21 mV). The third major field component was a surface-negative, deep-positive deflection that peaked at latencies near 20 ms. The late component was clearest at the highest stimulus intensities (mean amplitude = 2.09 ± 0.22 mV) but was not always distinct from the decaying phase of the early component.

### Long-term Potentiation at Fixed Recording Sites

Field responses were markedly altered after 10 daily tetanizations of the corpus callosum (Figs 2 and 3, dashed traces). Spike amplitude at the highest stimulus intensity increased to 128 ± 10% of baseline values in tetanized animals, and declined to 87 ± 5% of baseline levels in controls. There was a significant interaction between group, session and intensity for the differentially recorded spike amplitude [F(9,108) = 4.52; P < 0.0001].

The amplitude of the differentially recorded early component was stable in control animals (91 ± 6% of baseline) but was reduced in tetanized animals (77 ± 3% of baseline) due to a large increase (144 ± 15%) in the amplitude of the early negative deflection in deep sites [F(9,108) = 4.35; P < 0.0001]. Tetanization also enhanced the late surface-negative component, which peaked at a mean latency of 21.0 ± 0.6 ms. In contrast to the spike and the early component, relative increases in the late component were greatest at low to mid-range test-pulse intensities (Fig. 3B).

### Paired-pulse Facilitation

Paired-pulse responses recorded from fixed electrodes (n = 14, data not shown) were used to select an optimal interpulse interval for depth profile recordings. Paired-pulse stimulation...
affected all field components at intervals <500 ms, and the largest changes occurred at an interval of 100 ms. The spike component was facilitated to 113 ± 3% of single-pulse levels, and effects were much larger for the early (18 ± 8%) and late (261 ± 57%) components. No significant effects of LTP induction on paired-pulse facilitation were observed (n = 7). Because paired-pulse effects at an interval of 100 ms resembled the effects of LTP induction, this interval was used in depth profile recordings to determine if paired-pulse facilitation and LTP induction affect membrane currents in the same cortical layers.

Depth Profile Recordings and CSD Analysis
Head restraint did not affect the first two field potential components recorded from fixed electrodes, and produced a small, non-significant reduction in the late component. The restraint procedure therefore allowed field potentials unaffected by anaesthesia to be recorded for CSD analysis. Field response amplitudes and CSD results were similar across animals. Results of CSD analysis in control animals were standardized to the amplitude of the layer V current sink associated with the early component (see below). Between-groups comparisons were made by standardizing CSD data from tetanized animals to the mean value of the current sink in controls.

Initial Spike Component
The initial deep-negative spike component peaked at latencies near 2.5 ms and reversed to a surface positivity at depths near 700 µm in layer IV (Fig. 4). CSD analysis showed that the spike was generated by a current sink in the lower third of layer V.
which was flanked by smaller current sources in layer VI and upper layer V (Fig. 5). The sink in both control and tetanized animals contained small subpeaks that repeated over at least three cycles at a frequency of $\sim 400$ Hz. The mean amplitude of the initial deep-negative spike was similar for control ($-1.23 \pm 0.32$ mV) and tetanized ($-1.24 \pm 0.14$ mV; $t_{9} = 0.03$, $P = 0.98$) groups, and there was also no difference in CSD results at this latency ($t_{9} = 0.06$, $P = 0.95$). The duration of the layer V sink, however, was roughly twice as long in tetanized animals relative to controls ($13.0 \pm 1.7$ versus $6.2 \pm 0.8$ ms; $t_{9} = 3.11$, $P < 0.05$).

Paired-pulse facilitation of the spike component tended to be greater in control animals than in tetanized animals ($215 \pm 43$ versus $146 \pm 7$%; $t_{7} = 1.56$, $P = 0.16$). Although the amplitude of the layer V current sink showed no significantly greater facilitation in controls than in tetanized animals ($165 \pm 19$ versus $150 \pm 14$%), the duration of the current sink was significantly facilitated in control animals ($10.5 \pm 1.2$ versus $6.2 \pm 0.8$ ms; $t_{5} = 3.1$, $P < 0.05$) but not in tetanized animals ($14.8 \pm 1.8$ versus $13.0 \pm 1.7$ ms; $t_{5} = 2.0$, $P = 0.14$; Figs 5 and 6).

**Early Surface-negative Component**

The early surface-negative field component peaked at latencies near 6 ms, was strongest at intermediate recording depths of 900 $\mu$m in upper layer V, and reversed into a positivity at depths around 1500 $\mu$m near the layer V/VI border (Fig. 4). This (depth 1400 $\mu$m) which was flanked by smaller current sources in layer VI and upper layer V (Fig. 5). The sink in both control and tetanized animals contained small subpeaks that repeated over at least three cycles at a frequency of $\sim 400$ Hz. The mean amplitude of the initial deep-negative spike was similar for control ($-1.23 \pm 0.32$ mV) and tetanized ($-1.24 \pm 0.14$ mV; $t_{9} = 0.03$, $P = 0.98$) groups, and there was also no difference in CSD results at this latency ($t_{9} = 0.06$, $P = 0.95$). The duration of the layer V sink, however, was roughly twice as long in tetanized animals relative to controls ($13.0 \pm 1.7$ versus $6.2 \pm 0.8$ ms; $t_{9} = 3.11$, $P < 0.05$).

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positivity peaked in layer VI at latencies near 9 ms. The peak amplitude of the early component did not differ between control and potentiated animals, but the surface negativity was more prolonged in tetanized animals, which may reflect longer-lasting synaptic potentials or the recruitment of polysynaptic activity. At latencies near 10 ms, therefore, the amplitude of the negative component was greater in tetanized animals (−1.05 ± 0.23 mV) than in controls (−0.57 ± 0.27 mV). In addition, the enhanced repetitive spiking observed in tetanized animals was associated with a shift in the deep layer VI positivity to more negative values (1.38 ± 0.31 versus 2.12 ± 0.58 mV).

The early surface-negative component was associated with a
large current sink that peaked at latencies near 6 ms in upper layer V, 1100 µm below the surface (Fig. 5). The peak of the sink was centred at about the same depth in tetanized and control animals but spanned somewhat deeper sites in control animals and was briefly continuous with the deeper sink associated with population spikes. These two sinks remained distinct throughout in tetanized animals (Figs 5 and 6). The amplitude of the upper layer V sink in tetanized animals was 117 ± 15% of that observed in control animals but this difference was not significant ($t_9 = 0.95, P = 0.37$).

The separation between the sink associated with the early component and that associated with population spikes was clear at a latency of 9 ms in both groups. Tetanized animals, which had a more prolonged surface-negative component, showed an upper layer V sink that was 24% larger than in controls at this latency (0.95 ± 0.12 versus 0.77 ± 0.11; $t_9 = 1.00, P = 0.34$). The

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**Figure 6.** The mean CSD results for the control ($n = 5$) and tetanized ($n = 6$) groups indicating the locations of membrane current sources and sinks in the neocortex following stimulation of the corpus callosum. The mean result for the first 35 ms following the first (Conditioning Response) and second (Test Response) stimulation pulses are shown. Cortical divisions indicated are the mean of depths obtained from individual animals. Conventions are as in Figure 5.
current source associated with the early component was located in layers VI, 1700–2100 µm below the surface, in both groups. The source was more prolonged in tetanized animals and was 40% larger than in controls during the peak of the deep-positive field component (0.98 ± 0.12 versus 0.70 ± 0.06; \( t_9 = 3.14, P < 0.05 \)).

Paired-pulse stimulation produced similar effects on the early component in control and tetanized animals (Figs 4–6). The amplitude and duration of the surface-negative component were enhanced, and there was a shift in the deep-positive component towards more negative values. These changes account for the reduction in the amplitude of the differentially recorded early component in chronic paired-pulse recordings. The increased negativity shifted reversal points from layer IV to deeper sites in layer V without affecting the locations of current sinks. Tetanized animals tended to show greater paired-pulse facilitation of the negative deflection (229 ± 50% versus 168 ± 24%) and the layer V current sink at latencies of 6 ms (125 ± 12% versus 119 ± 10%), but these differences were not significant (\( t_9 = 0.90, P < 0.40 \)). There was also a non-significant tendency towards greater paired-pulse depression of the deep-positive potential in tetanized (49 ± 12%) versus control (89 ± 24%) animals (\( t_9 = 1.41, P = 0.20 \)), but the current source showed no depression in either group (control, 102 ± 8%; tetanized, 107 ± 15%).

CSD analysis of paired-pulse responses revealed more clearly a current source in layers I–III (depth 200–600 µm) at latencies near 4 ms. The peak latency of the source did not match with deeper sinks, suggesting it is not generated by passive current flow. The source was large relative to layer V currents in both control (0.58 ± 0.18) and tetanized (0.77 ± 0.11) animals, and was greatly facilitated by paired-pulse stimulation (control, 210 ± 23%; tetanized, 163 ± 21%). The facilitation of the source was associated with a reduction of the slope of the surface-negative potential in layers I–III (Fig. 4) which may contribute to reductions in the rising portion of the early component.

**Late Surface-negative Component**

Both surface-negative and deep-positive responses contributed to the late component (Fig. 4), as they did for the early field potential component. The surface negativity peaked at latencies near 14 ms and reversed in upper layer V. The deep positivity peaked ~6 ms later at latencies near 20 ms. The late component is reliably enhanced by tetanization (Figs 2 and 3), but between-group depth profile comparisons did not reflect this potentiation. Paired-pulse stimulation strongly facilitated both the negative (control, −1.99 ± 0.31 mV; tetanized, −1.93 ± 0.26 mV) and positive (control, 1.63 ± 0.56 mV; tetanized, 2.51 ± 0.63 mV) field potential components.

The late component was associated with an upper layer V current sink and a deep layer V current source, with peak latencies corresponding to the surface-negative and deep-positive field components respectively. The sink peaked at a latency near 14 ms in upper layer V (depth 800–900 µm) and the source peaked at a latency near 19 ms deep in layer V (depth 1300 µm). A smaller source in layers II–III, which peaked at the same time as the upper layer V sink, was also observed following paired-pulse stimulation. The upper layer V sink formed a smooth extension of the sink associated with early field potential component, but the layer V source associated with the late component was located more superficially than the early layer VI source.

The amplitude of the late layer V sink evoked by the first stimulation pulse was similar in control (0.73 ± 0.09) and tetanized (0.70 ± 0.09) animals. The deep layer V source, however, was smaller in tetanized animals (0.49 ± 0.04 versus 0.76 ± 0.04; \( t_9 = 4.34, P < 0.01 \)) due to a more linear increase in the deep positivity as a function of recording depth. Tetanized animals showed larger paired-pulse facilitation of the deep layer V source than controls (258 ± 30 versus 123 ± 10%; \( t_9 = 3.43, P < 0.05 \)), but facilitation of the upper layer V sink was not significantly different between groups (244 ± 50 versus 216 ± 29%; \( t = 0.4, P = 0.70 \)).

**Frequency of Following Tests**

Frequency of following results were the same for both pulse intensities (250 and 500 µA). The initial spike component followed trains of 10 pulses at frequencies up to 320 Hz in all but one animal (\( n = 4 \)), suggesting this component is generated partly by the fibre volley and antidromic activation. The initial spike and subsequent repetitive spiking were attenuated considerably at frequencies ≥80 Hz, however, suggesting that monosynaptic inputs also drive a large part of these responses. The initial spike, for example, was reduced to between 9 and 22% of single pulse levels when following 320 Hz stimulation.

The early component of field responses followed at 80 Hz with little decrement, suggesting that this component is driven monosynaptically (Fig. 7). Amplitudes of responses evoked by the last pulse in 80 Hz trains ranged from 60 to 125% of the first responses. The facilitation of some responses (e.g. Fig. 7) appeared to result from attenuation of repetitive population spikes that compete with field currents underlying the early component. The early component was blocked or greatly attenuated during 160 Hz stimulation (<10% of single pulse levels) and was eliminated during 320 Hz stimulation.

The late field potential component was attenuated by stimulation at only 40 Hz and was eliminated during 80 Hz stimulation (e.g. Fig. 7). Failure to follow these frequencies suggests that the late component is generated polysynaptically.

Results of frequency of following tests were similar following
LTP induction. The initial spike followed frequencies of 320 Hz (three of four animals), the early component followed frequencies of 80 Hz or more, and the late component failed at frequencies <80 Hz. In two out of four animals, attenuation of repetitive population spikes during 80 Hz stimulation was associated with a growth in the early component to levels greater than observed in baseline recordings (Fig. 7). This potentiation of the early component was further examined in the next experiment in which barbiturate anaesthesia also preferentially attenuated cell discharge.

Recovery from Anaesthesia
Pilot tests showed that anaesthetics attenuated repetitive population spikes and the late component more than the early component. The recovery of responses from anaesthesia was therefore monitored to differentiate individual field potential components. Further, because anaesthesia attenuates repetitive population spikes that reduce the voltage dipole associated with the early component, anaesthesia before and after LTP induction allowed an examination of potentiation-induced changes in the early component. Anaesthesia attenuated all field potential components, with the late component most strongly affected. The initial spike was attenuated by 9 ± 1% and the early component was attenuated by 50 ± 1%. The strong attenuation of the late component (average decrement 73%), and the ~30 min delay in the onset and rate of recovery of the late component relative to the early component (Fig. 8), is consistent with the polysynaptic generation of the late component. Responses returned to unanaesthetized levels after about 3.5 h.

There was an interesting difference between recovery
patterns for the early component before and after LTP induction. Baseline recordings showed a rapid initial recovery of the early component. Responses following tetanization showed a similar initial recovery, but the recovery was then reversed due to concurrent recovery of potentiated repetitive population spikes (Fig. 8). The group means shown in Fig. 8 (upper panels) were taken at our usual latency. For measures taken slightly earlier, the entire post-LTP recovery curve grows in the reverse direction compared to pre-LTP responses. For measures taken slightly later, growth in the early component initially exceeded that of pre-LTP measures before reversing and growing in the opposite direction. This suggests that short-latency population spikes recover earlier than longer-latency population spikes as seen in Figure 8 (lower panels). The post-LTP early component in this animal recovered to a potentiated level (relative to baseline tests) before being reduced by the recovery of repetitive population spikes. In another animal (Fig. 9), anaesthesia largely eliminated repetitive spiking both before and after LTP induction and revealed a clear potentiation of the early component. These results support the conclusion that the reduced amplitude of the differentially recorded early component following tetanization is due to a potentiation of repetitive population spike activity and also support the conclusion that the early component is potentiated by repeated tetanization (Fig. 9; see also Fig. 7).

**Multi-unit Recordings**

Potentiation of population spikes can reduce increases in the amplitude of field EPSPs following tetanization of hippocampal afferents (Bliss and Lomo, 1973). In contrast to neocortical responses, however, changes in hippocampal field EPSPs can be visualized clearly due to the delayed and synchronous nature of evoked cell discharge. Neocortical field responses are affected over a wider range of latencies by potentiated population spike activity. Previous neocortical unit recordings demonstrated prolonged bursts of evoked cell discharge in acute preparations (Racine et al., 1975; Vanderwolf, 1990; Dringenberg and Vanderwolf, 1994), but data from the conscious animal are needed to characterize discharge patterns underlying population spikes and to verify that active cells are located near the layer V current sink identified in CSD experiments (Figs 5 and 6).

Strong evoked multi-unit activity was observed at depths greater than −1.2 mm below the surface (layer V; n = 5). Multi-unit discharge in layers I–IV was surprisingly sparse, supporting the interpretation that the early layer II–III source (Fig. 6) reflects active inhibition. Multi-unit activity increased as recording depth increased to 1.4 mm (layer V; Fig. 10) and was observed throughout layers V and VI until the electrode entered the corpus callosum. There was a much higher density of evoked multi-unit activity in the two tetanized animals than in the control animals (Fig. 10). Further, although samples were too small for statistical comparison, the mean duration of repetitive discharge in layer V was longer in tetanized animals (25.0 ms) than in control animals (20.4 ms).

**Discussion**

Evoked field potential measures in chronic preparations allow potentiation phenomena to be monitored for long periods in the presence of normal levels of inhibition and neuromodulatory activity. Field responses can be difficult to interpret due to low spatial resolution, however, and this problem is compounded in the neocortex, which contains multiple cell types activated by multiple mono- and polysynaptic pathways. We have used CSD analysis, multi-unit recordings, frequency of following tests and anaesthesia to characterize the substrates that generate components of sensorimotor cortex field responses. Early population spike activity is generated by repetitive spiking of neurons in layer V. The early surface-negative field response (peak latency 8–10 ms) is generated by large inward currents in upper layer V. Short-latency (~4 ms) outward currents, which may be mediated by local inhibitory neurons, are also observed in layers I–III. The late surface-negative field component (peak latency ~20 ms) is most markedly potentiated by tetanization and paired-pulse stimulation, and is generated by continued inward currents in upper layer V. The late component does not follow high-frequency stimulation, is greatly attenuated by anesthetics, and is therefore probably mediated polysynaptically (see Fig. 11). Enhancements in the late component may be largely secondary to strengthened monosynaptic inputs and increased

Figure 9. Comparison of neocortical field responses recorded at the peak of the anaesthesia effect, before (PRE-LTP) and after (POST-LTP) LTP induction. Anaesthesia almost completely blocked repetitive population spike activity in this animal, resulting in the unmasking of a potentiated early component (●). Vertical calibration 0.5 mV; horizontal calibration 10 ms.

Figure 10. A comparison of multi-unit activity and field responses (dashed lines) in layer II–III (0.4 mm below the cortical surface) and in layer V (1.4 mm below the surface) in a control and a potentiated animal. Multi-unit responses were reliably recorded deeper than 1.2 mm below the cortical surface, and increased in amplitude until around 1.4 mm. The responses were easily detectable until reaching the white matter. Vertical calibration 0.05 and 1.0 mV; horizontal calibration 10 ms.
of the layer V sink, its duration was increased in tetanized animals (Fig. 6). Potentiation effects in motor cortex bursting cells following deep layer stimulation have also been reported by Aroniadou and Keller (1995). Bursting cells in layers II–IV of the visual cortex are also potentiated by white matter stimulation in the absence of bicuculline (Artola and Singer, 1987).

Early Component

The early surface-negative field potential component was associated with inward currents in upper layer V and a source in deep layer V and layer VI (Fig. 6). The early component followed high-frequency stimulation and recovered quickly following anaesthesia, suggesting that it is mediated by monosynaptic afferents to layer V. Similar surface-negative potentials, peaking in layers II–III following white matter or deep layer stimulation, have been observed at similar latencies in visual (Berry et al., 1989; Teyler, 1989; Kirkwood and Bear, 1994) and motor cortices (Aroniadou and Keller, 1995). These in vitro responses were associated with more superficial current sinks in layers II–III (Bode-Greuel et al., 1987; Aroniadou and Keller, 1993; Aizenman et al., 1996). The deeper location of the layer V current sink associated with the early component examined here is more consistent with the location of sinks in sensorimotor cortex evoked by thalamic stimulation in vitro (Castro-Alamancos and Connors, 1996a,b). The relative absence of major layer II–III sinks in the present study may be due to minimal antidromic activation of these layers and/or relatively sparse activation of monosynaptic inputs.

Both repeated tetanization and paired-pulse stimulation reduced the differentially recorded amplitude of the early component. This reduction is due largely to enhanced deep-negative potentials associated with repetitive spiking in layer V, but facilitation of the putatively active short-latency source in layers II–III may also contribute. CSD analysis results showed, however, that both the deep layer V sink associated with spiking activity and the upper layer V sink associated with the early component were enhanced by tetanization and paired-pulse stimulation (Fig. 6). Tetanization-induced changes in the early component therefore reflect both enhanced monosynaptic excitation and repetitive spiking in layer V. Potentiation of the early component was also observed in frequency of following tests and during anaesthesia when repetitive spiking was reduced (Figs 7 and 9).

Previous experiments have demonstrated LTP effects in both superficial and deep layers, but the present results indicate that corpus callosum stimulation in vitro primarily affects layer V currents. White matter or deep layer stimulation potentiates layer III field responses in the cat (Chen et al., 1994) and rat (Aroniadou and Keller, 1995) motor cortex in vitro. Thalamic inputs to superficial layers of the motor cortex are also potentiated in the anaesthetized cat by combined tetanization of the ventrolateral nucleus and somatosensory cortex (Iriki et al., 1989, 1991). Potentiation of EPSPs in deep layer neurons has been observed following pairing of intracellular depolarization with either deep layer stimulation in rat sensorimotor cortex (Bindman et al., 1988) or thalamic stimulation in the conscious cat (Baryani et al., 1991). While potentiated monosynaptic inputs to layer V appear to be the main cause of alterations in the early component observed here (Figs 6 and 7), enhanced longer-latency components of monosynaptic EPSPs (Chen et al., 1994; Aroniadou and Keller, 1995) and fast polysynaptic activity (Keller and Asanuma, 1993; Thomson and Deuchars, 1994;

Spike Components

The initial spike-like component followed high-frequency stimulation (320 Hz) and the subsequent repetitive spikes failed at frequencies ≤80 Hz. Both were associated with multi-unit activity in layers V and VI and subpeaks superimposed on a middle layer V current sink (Figs 6 and 10). Therefore, the initial spike partly reflects the fibre volley and antidromic activation of layer V neurons (see Langdon and Sur, 1990; Lee et al., 1991; Chen et al., 1994), and repetitive spikes reflect synchronous discharges of layer V–VI neurons. Paired-pulse facilitation of the initial spike and its considerable attenuation at frequencies of ≥160 Hz indicate that much of this component is also synaptically driven. The high frequency of repetitive spiking (~400 Hz) is within the range reported for neocortical cells (e.g. Gray and McCormick, 1996).

Tetanization of the corpus callosum increased spike amplitude in chronic recordings (Fig. 5; Racine et al., 1995b). Although no significant between-groups differences were found in the peak neuronal discharge (Iriki et al., 1991; Kirkwood and Bear, 1994; Castro-Alamancos et al., 1995), but potentiation within horizontal projections and other polysynaptic pathways (Lee et al., 1991; Hess et al., 1996) may also contribute. The similarity between effects induced by LTP and paired-pulse stimulation suggests that synapses mediating changes in excitability during paired-pulse stimulation (Castro-Alamancos and Connors, 1996) are modified more permanently by repeated tetanization.
inputs to layer V may enhance the impact that activated afferents have upon motor output commands generated within restricted cortical regions. Polysynaptic potentiation effects within horizontal projections, modulated by local circuitry, are more likely to reflect mechanisms underlying associations between sensorimotor representations contained in neighbouring cortical regions. These potentiation phenomena may contribute to the complexity of motor acts, and/or to the formation of novel associations between sensory inputs and motor commands.

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