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LONG-TERM POTENTIATION IN THE SENSORIMOTOR CORTEX OF THE AWAKE RAT IS EFFECTIVELY INDUCED BY THETA-PATTERNED STIMULATION

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ABSTRACT

Long-Term Potentiation in the Sensorimotor Cortex of the Awake Rat is Effectively Induced by Theta-Patterned Stimulation

Christine Werk

The neocortex of awake animals is resistant to the induction of long-term synaptic potentiation (LTP). To determine if intense stimulation patterned after the theta (4-12 Hz) electroencephalographic (EEG) rhythm may be effective in inducing synaptic plasticity in the sensorimotor cortex, LTP induction was examined *in vivo* using single 300-Hz trains and paired trains delivered at a 100-ms interval. Consistent with previous findings, single 300-Hz trains caused a reduction of the early monosynaptic field potential component, reflecting LTP of inputs to layer V, and a facilitation of the late polysnaptic component, reflecting the spread of activation across layer V. In response to paired trains, however, there was minimal change in the early component and a much larger increase in the late component as compared to single 300-Hz trains. To investigate the mechanisms mediating the effectiveness of paired trains in inducing LTP, an NMDA receptor antagonist was administered and effects on responses to LTP induction stimuli and short-pulse trains at theta-frequency were assessed. NMDA-receptor antagonism reduced the early and late component amplitudes and the amount of short-term facilitation observed during repetitive stimulation. This indicates that theta-patterned stimulation induces short-term potentiation effects that involve substantial NMDA-receptor mediated synaptic currents. Therefore, the enhanced induction of LTP in the neocortex induced by paired trains *versus* 300-Hz trains is likely due to enhanced NMDA receptor activation. These results suggest that learning-related plasticity in the sensorimotor neocortex may be promoted by rhythmic, population activity at theta-frequency.

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List of Abbreviations

AMPA	x-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
ANOVA	Analysis of variance
Ca^{2+}	Calcium
GABA	Gamma aminobutyric acid
I/O	Input/output
i.p.	Intra-peritoneal
K^{+}	Potassium
LTP	Long-term synaptic potentiation
Mg^{2+}	Magnesium
Na^{+}	Sodium
NMDA	N-methyl-*D*-aspartate

INTRODUCTION

Long-term potentiation (LTP) is a long-lasting enhancement of synaptic efficacy resulting from intense pre-synaptic stimulation and is a widely studied cellular model of memory formation (Bliss and Lomo, 1973; Bliss and Collingridge, 1993; McNaughton, 1993). The requirement of intense synaptic activation for LTP induction is related to the properties of the N-methyl-*D*-aspartate (NMDA) receptor which is activated only in the presence of glutamate and postsynaptic depolarization. Intense synaptic activation is required to induce the postsynaptic depolarization needed to activate the NMDA receptor. NMDA receptor activation provides the trigger for LTP induction because, while AMPA (x-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) glutamate receptors are permeable to Na^+ and K^+, the NMDA receptor is also permeable to Ca^{2+}. Calcium influx can induce synaptic strengthening by acting on calcium dependant protein kinases including calmodulin, and CaMKII that can lead to long-term increases in AMPA-mediated synaptic responses (Madison, Malenka, and Nicoll, 1991).

Because of the sensitivity of the NMDA receptor to both glutamate release and postsynaptic depolarization, the NMDA receptor is only activated during periods of intense pre- and post-synaptic activity. Thus, synapses that undergo NMDA receptor-dependent LTP are consistent with what is known as the "Hebbian synapse". In 1949, Donald Hebb proposed that a new memory trace was formed through the development or elaboration of a "cell assembly", by the strengthening of synaptic connections between neurons in the cell assembly. One of his main contributions was to propose a mechanism through which cell assemblies could be formed. He proposed that the connections

between neurons would be strengthened if the presynaptic cell consistently took part in causing firing of the postsynaptic cell. Thus, intense pre- and post-synaptic activity is a property of both LTP induction and the Hebbian synapse.

LTP is attractive as a cellular model for memory due to properties of associativity and input specificity (Bliss and Collingridge, 1993). Associativity occurs when weak synaptic inputs, that are not potentiated when stimulated alone, are potentiated in the presence of a strong tetanus (stimulation train) delivered to other synaptic inputs. This feature of LTP is a cellular analogue of classical conditioning and is consistent with the properties of the Hebbian synapse. Input specificity, where inputs that are not active during the tetanus are not potentiated, is also important because it means that the strengthening of tetanized pathways does not reflect an overall increase in postsynaptic excitability or a strengthening of all synaptic inputs to the cell. Input specificity makes LTP computationally more powerful because the role of a given neuron in multiple cell assemblies can be modified more selectively.

Although LTP in the hippocampus is thought to reflect mechanisms involved in the rapid acquisition of memory, LTP in the neocortex may reflect mechanisms involved in the consolidation of memory (Bear and Kirkwood, 1993; Brown, Chapman, Kairiss and Keenan, 1988; Chapman, Trepel, Ivanco, Froc, Wilson and Racine, 1998; Rioult-Pedotti, Friedman and Donoghue, 2000). It is believed that memories are initially processed by the hippocampus and that long-term memories are consolidated in the cortex. In this way, the hippocampus serves to integrate new sensory information, and to contribute to the reactivation of memories contained in the neocortex until intra-cortical

connections that support the memory become strengthened to the point that they can sustain the reactivation of the memory independently of the hippocampus (Teyler and DiScenna, 1987).

LTP is more difficult to induce in the neocortex than in the hippocampus, and preparations that reliably show neocortical LTP *in vivo* have generally used juvenile animals or acute preparations (in which the animal is anaesthisized) such that the synaptic plasticity observed may reflect mechanisms of development or be affected by the anesthetic (Wilson and Racine, 1983; Komatsu, 1994; Kimura, Nishigori, Shirokawa and Tsumoto, 1989; Crair and Malenka, 1995; Heynen and Bear, 2001). In the cortical slice preparation, the routine addition of bicuculline (blocker of inhibitory, $GABA_A$ receptors) to the bathing medium is also often necessary to obtain LTP (Kirkwood and Bear, 1994; Hess, Aizenman and Donoghue, 1996). Bicuculline promotes LTP induction by blocking $GABA_A$ receptors and enhancing neuronal excitability.

A marked potentiation can be observed in sensorimotor cortex field potentials evoked by stimulation of the corpus callosum in the awake, adult rat (Racine, Chapman, Trepel, Teskey and Milgram, 1995; Chapman et al., 1998), and the trains that are used to induce the potentiation must be delivered daily over a period of at least 4-5 days, and maximal LTP effects are observed after 10 to 20 days (Trepel and Racine, 1998). The length of time and the temporal spacing of stimulation that is required for the induction of this form of cortical LTP, compared to hippocampal LTP, is consistent with the idea that cortical LTP may reflect mechanisms similar to those involved in the slow consolidation of long-term memories.

Rhythmic patterns of neuronal population activity, such as the theta rhythm, are thought to contribute to the control of naturally-occurring plasticity in the neocortex (Singer, 1993; Başar, Başar-Eroğlu, Karakaş, and Schürmann, 1999; Wu, Guan and Tsau, 1999). Theta activity is a quasi-sinusoidal rhythm of between 4 to 12 Hz in the electroencephalogram. In the hippocampus, theta activity is observed most commonly during voluntary movement (Vanderwolf , 1969), and it is also thought to contribute to processes mediating learning and memory (Komisaruk, 1970; Semba and Komisaruk, 1978). Theta activity is also observed in the neocortex (von Stein, and Sarnthein, 2000; Kahana, Seelig, and Madsen, 2001) where it is thought to contribute to memory function (Jensen, Idiart, and Lisman, 1996; Cape, Manns, Alonso, Beaudet, and Jones, 2000). Theta activity is thought to contribute to learning and memory in both the hippocampus and neocortex because of synchronous activation of large numbers of neurons during each phase of the rhythm. Synchronization of the resulting synaptic inputs could play an important role in learning-related synaptic plasticity by inducing postsynaptic depolarization (Singer, 1993).

Intense stimulation delivered at the frequency of the endogenous theta rhythm is effective at inducing LTP in excitatory (Larson and Lynch, 1996; Staubli and Lynch, 1987; Morgan and Teyler, 2001) and inhibitory circuits (Chapman, Perez and Lacaille, 1998; Perez, Chapman, Woodhall, Robitaille and Lacaille, 1999) in the hippocampus but it has also been found to be effective in experiments examining potentiation effects in a variety of neocortical pathways. In white-matter inputs to layer III of the visual cortex, 100 Hz stimulation is usually ineffective at inducing LTP unless bicuculline is applied

concurrently, whereas theta-burst stimulation induces a reliable LTP in the absence of bicuculline (Kirkwood and Bear, 1994). Stimulation patterned after the theta rhythm is also effective at inducing LTP in horizontal layer II/III connections of the sensorimotor (Rioult-Pedotti, et al., 2000) and motor (Hess, et al., 1996) cortices. The effectiveness of theta-patterned stimulation in inducing LTP in the sensorimotor cortex *in vivo* has not, however, been assessed.

The effectiveness of theta-patterned stimulation in inducing LTP may be due to enhanced NMDA receptor activation resulting from the induction of short-term facilitation effects. Short-term facilitation effects occur primarily through a mechanism in which repetitive stimulation leads to increases in residual pre-synaptic Ca^{2+} which enhances release of neurotransmitter vesicles (Zucker, 1989). Short term potentiation can lead to enhanced LTP induction because the increased transmitter release leads to larger excitatory postsynaptic potentials, and greater postsynaptic Ca^{2+} influx due to enhanced NMDA receptor activation.

Long-term synaptic potentiation in the hippocampus induced by primed-burst stimulation is an example of the enhancement of LTP induction by a short-term facilitation effect. Primed-burst stimulation patterns consisting of a priming pulse and a short train delivered 100 ms later (that would otherwise be ineffective at inducing LTP) are effective because the priming pulse enhances activation of NMDA glutamate receptors during the train (Diamond, Dunwiddie and Rose, 1988). The priming pulse is effective because the short-term potentiation that it induces is active during the train, and enhances the amount of postsynaptic depolarization induced by the train. In the cortex, a

striking form of short-term facilitation is the thalamocortical augmenting response in which there is a progressive enhancement of evoked potentials recorded in the sensorimotor cortex that occurs during repeated low-frequency (approximately 5-10 Hz) stimulation (Dempsey and Morison, 1943; Spencer and Brookhart, 1961; Castro-Alamancos and Connors, 1996a,c). LTP induction in the sensorimotor cortex *in vivo* is due primarily to potentiation of synapses in layer V (Chapman et al., 1998) and layer V pyramidal neurons are also known to mediate the augmenting response in the sensorimotor cortex (Castro-Alamancos and Connors, 1996c). This suggests that the augmenting response could contribute to LTP induction in this area because it may enhance NMDA receptor activation during the train delivery by causing substantial post-synaptic depolarization. Although the computational purpose of the augmenting response is not known, one possibility is that the augmenting response may reflect mechanisms mediating naturally occurring synaptic plasticity.

Field potentials recorded in the sensorimotor cortex following stimulation of the corpus callosum are usually recorded using a bipolar electrode with one tip located relatively superficially in layer III/IV, and a second tip located deep in layer V or VI (Chapman et al., 1998). In bipolar recordings, an initial spike component has a short latency of about 2-3 ms and is largely due to antidromic activation of axons projecting out of layer V. The spike is most prominent in monopolar recordings as a negative deflection in deep recording sites. The initial spike component can be followed by two to eight other repetitive, small-amplitude spikes that suggest synchronous, repetitive firing in a large number of layer V pyramidal neurons. The intensity and duration of repetitive spiking is

often enhanced following LTP. A second, early latency (6-8 ms) component is largely due to monosynaptic activation of layer V neurons. The bipolarly recorded early component is associated with superficial-negative and a deep-positive components in monopolar recordings. Although the early component primarily reflects monosynaptic activation, it can also be affected by spiking activity that sometimes continues for latencies of up to ≈ 10 ms which are coincident with the peak of the early component. The late component has a longer latency of about 17 to 20 ms and is largely due to polysynaptic activation of layer V neurons. The polysynaptic activation is initiated following the monosynaptic activation of layer V neurons reflected in the early component. In monopolar recordings, superficial-negative and deep-positive responses are observed during the late component.

To investigate the effectiveness of stimulation patterned after the naturally occurring theta rhythm in inducing LTP in the sensorimotor cortex, high-frequency stimulation trains (8-pulse, 300-Hz trains) or pairs of 4-pulse, 300-Hz trains separated by 100 ms (which corresponds to 10 Hz or theta-frequency) were compared for their ability to induce LTP. Due to the effectiveness of theta-patterned stimulation in inducing LTP in other preparations, the paired trains were predicted to be more effective at inducing LTP than 300-Hz trains. The recruitment of NMDA receptor activation during short-term potentiation effects induced by various patterns of stimulation was also investigated by challenging the short-term increases in field potential component amplitudes with an NMDA receptor antagonist. The effects of NMDA antagonists were assessed by monitoring responses to trains used for LTP induction, single pulses, and short trains of

pulses at 10 Hz.

MATERIALS AND METHODS

Surgery. Forty-one male Long-Evans hooded rats (300-400g) were pre-treated with atropine methyl nitrate (0.1 mg/kg) and anaesthetized with either sodium pentobarbital (70 mg/kg) or ketamine (85 mg/kg) and xylzene (15 mg/kg) and placed in a stereotaxic frame with the skull surface on the horizontal plane. Bipolar, Teflon-coated stainless-steel twisted-wire electrodes (125 μm exposed tips) 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 the stimulating electrodes and 1.2 mm for recording electrodes. Vertical placements of electrodes were adjusted, by examining test pulses, to minimize current thresholds, and to maximize the amplitude of the early component of field potentials by spanning the voltage dipole. A stainless-steel screw in the posterior parietal bone served as the ground electrode. Electrode leads were connected to gold-plated pins and mounted in a connector. The assembly was embedded in dental cement and anchored to the skull with stainless-steel jeweller's screws.

Animals were housed individually on a 12 hr on/off, light-dark cycle with all experimental testing conducted during the lights on period. Animals were handled every 2 to 3 days during a $\geq$ 2 week recovery period following surgery.

Stimulation and Recording. Animals were habituated to a 30 x 40 x 30 cm Plexiglas

testing chamber with a wire-grid floor, and recordings were collected while animals were in a quiet, resting state. Biphasic constant current stimulation pulses (0.1 msec duration) were delivered to the corpus callosum using a linear stimulus isolation unit (WPI Model A395, or A-M Systems Model 2200) regulated by a computer DAC channel (20 kHz). Evoked field potentials were filtered (0.1 Hz to 5 kHz, passband), amplified (A-M Systems, Model 1700), and digitized at 20 kHz (16 bit) for storage on computer hard disk using Experimenters Workbench software (Datawave Technologies). Both monopolar and bipolar responses were recorded from the sensorimotor cortex, and routine analyses focussed on bipolar responses (Froc, Chapman, Trepel and Racine, 2000; Chapman et al., 1998).

Input/Output Testing. Input/output (I/O) tests were used to monitor changes in field potential responses evoked by a range of stimulus intensities. I/O tests were conducted every second day during a one-week baseline period and during the 20-day LTP induction period (see below). Following LTP induction, I/O tests were conducted every second day for one week and then weekly for 4 weeks. During each I/O test, 10 evoked potentials were recorded and averaged at each of 8 test-pulse intensities (100, 200, 300, 400, 500, 600, 800 and 1000 μA). The inter-pulse interval was 10 sec and pulses were delivered in ascending order with respect to intensity. Mean I/O curves for each group were obtained by expressing the peak amplitude of responses as a percentage of the average response to the highest stimulus intensity during the four baseline I/O tests.

LTP Induction. Groups of animals matched for response amplitude and morphology were randomly assigned to receive pairs of trains (n = 10), high-frequency trains (n = 8), or no trains (n = 8). High-frequency stimulation trains consisted of 8 pulses delivered at 300-Hz and paired trains consisted of two 4-pulse, 300-Hz trains separated by 100 ms. Pulse intensity was 800 μA and the inter-train interval was 10 seconds. Ten trains (or pairs of trains) were delivered daily for 20 days to induce LTP. Control animals were handled the same way, but did not receive stimulation trains.

Short-Term Facilitation Effects. The effects of NMDA receptor antagonists on evoked responses and short-term potentiation effects were assessed by comparing a variety of tests administered both before and after an injection of either ketamine (30 mg/kg, i.p.; n = 10) or MK-801 (0.1 mg/kg, i.p.; n = 5). Animals that received ketamine also received a 10% supplemental dose after 30 minutes. Doses of both of these non-competitive NMDA receptor antagonists were chosen based on past studies showing that they block LTP induction in brain areas other than the sensorimotor cortex (Zhang and Levy, 1992; Morgan and Teyler, 1999; Salami, Fathollahi, Esteky, Motamedi and Atapour, 2000). Because there were no significant nor systematic differences in the changes in evoked responses induced by the two drugs during the tests conducted, the data obtained using both drugs were pooled (Figure 5). Input/output tests that were identical to those used to assess LTP induction were used to assess NMDA-receptor activation in response to single pulses of varying intensities. To assess NMDA receptor activation during delivery of trains similar to those used to induce LTP, a short series of stimulation trains was

administered. Trains were identical to those used for LTP induction except that more prolonged sets of theta-patterned trains were delivered, with each set consisting of six, 4-pulse trains with a 100-ms inter-train interval. Single 800 μA pulses were delivered every 10 sec for 2 minutes before, and 5 minutes following train delivery, to ensure that there were no lasting changes in field responses. LTP induced by these trains takes several days to develop, and although no short-term changes in responses to single pulses were observed following the trains, the order of train delivery was counterbalanced across subjects; theta-patterned trains were delivered first in half of the animals and high-frequency trains were delivered first in the other half.

To determine if substantial and prolonged NMDA receptor activation is induced during less intense theta-frequency stimulation of layer V of the sensorimotor cortex, single stimulation pulses were delivered at theta-frequency for a period of 2 sec (20 pulses at 10 Hz). Single 800 μA pulses were also delivered every 10 seconds for 2 minutes preceding, and 5 minutes following, the theta-patterned trains to characterize changes in field potentials that might outlast the trains.

Following pre-drug testing, the onset of the effects of the NMDA receptor antagonists on evoked responses was monitored by delivering single 800 μA pulses every 20 seconds for 10 minutes before, and 20 minutes following, injection of ketamine or MK-801. Post-drug tests were presented in the same order as pre-drug tests with the exception of the high-frequency and intense theta-frequency trains which were administered after all other tests.

Histology. Animals were deeply anaesthetised and brains were removed. Brains were stored in 10% formal-saline and frozen 40 μm-thick coronal sections were sliced with a cryostat and stained with cresyl violet. Electrode placements were determined using a Bausch and Lomb microprojector.

Data Analysis. Peak amplitudes of field potential components were measured at latencies corresponding to the spike (2 ms), early (7 ms) and late (17-18 ms) components of field potentials in superficial, deep, and bipolar recordings. Mixed Design Factorial ANOVAs and Dependent samples t-tests were used to analyze changes in peak amplitudes of field potential components. Significant effects were investigated by analyzing the simple effects of individual factors and by using Tukey's honest significant difference (HSD) test where appropriate. The induction of LTP of the spike and late component were assessed by determining if there were significant increases in the amplitude of responses to test-pulses of the same intensity. Significant changes in the amplitude of the early component were further analyzed by examining changes in the superficial negative response, and by examining repetitive spiking activity in the deep recordings.

RESULTS

Histology and Baseline Responses. Histological analysis showed that electrode placements were close to targets in the corpus callosum and sensorimotor cortex, $\approx$ 1.6 to 2.2 mm anterior to Bregma. The deepest tips of the recording electrodes were located 3.3

to 4.2 mm lateral to Bregma at a depth of 1.5 to 3.2 mm below pia matter in Layer V-VI (Figure 1). Stimulating electrodes were located in or near the corpus callosum, 1.5 to 2.3 mm lateral to Bregma, and 2.5 to 3.8 mm below pia.

Baseline responses were similar to those observed previously (Figure 2; e.g., Froc et al., 2000) and recordings showed an early deep-negative spike-like component (0.99 ±0.18 mean peak amplitude), an early surface-negative component (1.62 ±0.22 mV), and a later surface-negative component (Figure 3; 1.23 ±0.19 mV). These components have been shown previously to reflect an antidromically activated spike, early monosynaptic activation in layer V, and later, polysynaptic activation of layer V (Chapman et al., 1998).

LTP Induction. Similar to other studies (Chapman, et al., 1998; Trepel and Racine, 1998), changes in evoked responses during the 20-day LTP induction period were largest at intermediate test pulse intensities (Figure 3). Changes in evoked field potential components were slow to develop and were maximal after about 15 days of tetanization.

A 3 (Group) X 8 (Stimulation intensity) X 22 (Day) factorial ANOVA was conducted for each of the three components in bipolar recordings(the spike, early and late components). Changes in the amplitude of the initial spike were not significantly different following LTP in either of the tetanized groups. Spike amplitude changed from 1.06 ±0.26 to 0.95 ±0.19 mV following paired trains, and from 0.44 ±0.13 to 0.46 ±0.11 following 300-Hz trains at 600 μA; [$F(2, 23) = 0.92$, $p = 0.41$, ns.]. Significant group by day by intensity interactions were found for both the early [$F(294, 2940) = 1.15$, $p < 0.05$] and late [$F(294, 3234) = 1.48$, $p < 0.01$] field potential components.

Analysis of Partial interaction comparisons revealed a day by intensity interaction for both early [$F(147, 882) = 2.02$, $p < 0.01$] and late [$F(147, 1029) = 1.67$, $p < 0.01$] field potential components in the 300-Hz train group. In animals that received 300-Hz stimulation, there was a large reduction in the amplitude of the early component during LTP induction (Figure 2; [$F(21, 126) = 4.02$, $p < 0.01$]), and this reduction was observed at supra-threshold intensities and was reflected in an intensity by day interaction, [Figure 3; $F(7, 42) = 15.55$, $p < 0.01$]). Tukeys HSD revealed a significant decrease in the early component at all intensities greater than 200 μA ($p < 0.01$). The reduced early component has been shown previously to be due to prolonged deep-negative spiking that reduces the voltage dipole in bipolar recordings (Chapman et al., 1998). Animals that received 300-Hz stimulation also showed a potentiation of the amplitude of the late component following LTP induction [$F(21,147) = 2.56$, $p < 0.01$]. This potentiation was observed at intermediate test-pulse intensities but there was minimal change in the late component at higher test-pulse intensities (Figures 2 and 3; [$F(7,49) = 12.73$, $p < 0.01$]). Tukey's HSD revealed significant increases following LTP at 300 μA ($p < 0.05$).

Animals that received paired trains showed a different pattern of results than those that received 300-Hz trains. Partial interaction comparisons revealed a day by intensity interaction for late component [$F(147, 1176) = 1.56$, $p < 0.01$], but not the early component in the paired trains group . The reduction in the early component following delivery of paired trains was much smaller than following 300-Hz trains (Figures B_1 and A). The early component changed from 1.10 ±0.25 to 0.88 ±0.18 mV; [$F(21, 189) = 1.05$, $p = 0.40$, ns.] following paired trains, and from 0.88 ±0.28 to 0.44 ±0.16 mV at 400

μA following 300-Hz trains) and there was a much larger increase in the amplitude of the late component than was observed in the 300-Hz group (Figures 2B and 3B). The late component amplitude changed from 0.66 ±0.28 to 1.07 ±0.17 mV; [$F(21,168) = 4.68$, $p < 0.01$] following paired trains, and from 0.77 ±0.28 to 0.91 ±0.26 mV at 500 μA following 300-Hz trains). Further, the enhancement in the late component following paired trains was observed at both intermediate and high test-pulse intensities (Figure 3B; [$F(7,56) = 19.19$, $p < 0.01$]). Tukey's HSD revealed significant increases following LTP at intensities above 300 μA ($p < 0.05$). No significant effect of Day was found in the Control group [$F(21, 105) = 1.13$, $p > 0.05$, ns].

Monopolar recordings obtained during input-output tests were examined to investigate differences between groups in the potentiation of the bipolarly recorded early and late field potential components (Figure 4). Three (Group) X 8 (Stimulation intensity) X 22 (Day) Mixed Factorial ANOVAs were conducted for the early and late components for both the superficial and deep recordings. Reductions in the bipolarly recorded early component following 300-Hz stimulation are thought to be largely due to increases in prolonged deep-negative spiking that mask potentiation of monosynaptically activated superficial-negative currents (Chapman et al., 1998). In addition to changes in the amplitude of the initial spike, repetitive spiking activity was also enhanced in both groups following tetanization, and visual inspection of traces suggested that the amount of repetitive spiking was greater in the 300-Hz group *versus* the paired train group. Although repetitive spiking activity is difficult to quantify, there was a larger decrease in the amplitude of the early deep-positive component following 300-Hz stimulation *versus*

following the paired trains. The deep-positive early component was reduced from 0.75 ±0.25 to 0.33 ±0.16 mV following paired trains, and from 1.05 ±0.31 to 0.42 ±0.19 mV following 300-Hz stimulation delivered at 400 μA, which was reflected in a significant group by day by intensity interaction [$F(294, 1764) = 1.40$, $p < 0.01$]. The larger decrease in the early component following 300-Hz stimulation *versus* paired trains is therefore likely partly due to greater deep-negative spiking activity following 300-Hz stimulation that also reduced the amplitude of the deep positive field potential component (Figure $4A_2$, right panel).

Monopolar recordings of the early, superficial-negative component were also altered differently by the two tetanization patterns (Figure 4 B_1). Only animals that received paired trains showed a significant increase in the amplitude of the early superficial negative component. The amplitude of this component changed from 1.74 ±0.39 to 1.81 ±0.23 mV following paired trains, and from 1.75 ±0.40 to 1.46 ±0.45 mV at 600 μA following 300-Hz trains, and this was reflected by a Group by Day interaction; [$F(42, 462) = 1.60$, $p < .05$]). Smaller reductions in the bipolar early component following paired trains were therefore associated with larger increases in the superficial-negative component. These increases may have matched concurrent increases in deep-negative spiking, resulting in little net change in the bipolarly recorded early field potential component (Figure $4A_1$, left panel).

Analysis of monopolar recordings also showed that the greater potentiation of the bipolar late field potential component following paired trains was associated with much larger increases in the late, superficial-negative field potential component (Figure $4B_2$).

The amplitude of this component changed from 0.78 ±0.28 to 1.58 ±0.16 mV following paired trains, and from 1.11 ±0.39 to 1.19 ±0.27 mV at 400 μA following 300-Hz trains. These differences resulted in a significant group by day interaction [$F(42, 441) = 2.56$, $p < 0.01$]. Similar to results for the bipolar recordings, enhancements in the deep-positive late component in the 300-Hz group were restricted to intermediate test-pulse intensities (Figure 4), and the paired train group showed enhancements at all supra-threshold intensities, shown by a significant group by day by intensity interaction [$F(294, 3087) = 1.38$, $p < 0.01$].

Effects of NMDA Receptor Antagonists on Evoked Responses. The induction of LTP in this preparation is known to be dependent on NMDA glutamate receptor activation (Trepel and Racine, 1998), and it is possible that the greater LTP of the late component in animals that received paired trains is due to an increase in NMDA receptor activation induced by the paired trains. To investigate this idea, a number of tests were conducted to monitor short-term facilitation before and after i.p. administration of the NMDA receptor antagonists, MK-801 (0.1 mg/kg, n = 5) or ketamine (30 mg/kg, n = 10). Responses to single pulses showed that MK-801 had a slower onset than ketamine, but similar changes were observed in both the early and late components 25 to 30 minutes after drug administration (Figure 5A, B). There were also no differences between ketamine and MK-801 in any of the further tests, and the data obtained using both drugs were combined. A 2 (Drug-type) X 13 (Stimulation intensity) X 2 (Time) Repeated Measures ANOVA was conducted for the bipolar recordings of the spike, early and late components

recorded during I/O tests. Comparison of I/O tests before and after drug administration showed that the NMDA receptor antagonists had no significant effect on the amplitude of the spike [$F(1,13) = 3.68$; $p = 0.08$, ns.], produced a small reduction of the amplitude of the early component (Figure $5C_1$, 11.3% at 600 μA, [$F(1,13) = 21.06$; $p < 0.01$]), and a much larger reduction of the amplitude of the late component (Figure $5C_2$, 55.7% at 600 μA, [$F(1,13) = 11.17$; $p < 0.01$]).

To assess the role of NMDA receptors in mediating field potential responses to 300-Hz and theta-patterned trains, responses to the trains were recorded before and after administration of the NMDA receptor antagonists, and the amplitudes of train-evoked responses were measured at latencies corresponding to the early and late field potential components. Repeated measures t-tests were conducted on the amplitudes of the early and late components evoked by 300 Hz trains before and after drug administration. Following drug administration, reductions in responses to 300-Hz trains were found in both the early component (from 1.58 ±0.29 mV to 1.34 ±0.26 mV, [$t(13) = 3.08$; $p < 0.01$]) and the late component(from 0.98 ±0.16 mV to 0.76 ±0.14 mV, [$t(13) = 3.23$; $p < 0.01$]) relative to pre-drug tests (Figure 6A).

Because a series of six theta-patterned trains were delivered, it was also possible to track how NMDA receptor antagonism affected changes in early and late components during repeated delivery of trains (Figure 6B). A 2 (Drug) X 3 (Train number) ANOVA was conducted on the amplitudes of the early and late components to evaluate differences in responses across trains following drug administration. There was a main effect of Drug [$F(1,13) = 7.36$, $p < 0.05$], and Train number [$F(2, 26) = 28.59$, $p < 0.01$], for the early

component, however the interaction was not statistically significant [$F(2, 26) = 2.78$, $p = 0.08$]. The analysis of the late component revealed no main effect of drug [$F(1, 13) = .90$, $p = 0.36$], a main effect of Train number [$F(2, 26) = 12.81$, $p < 0.01$], and a significant interaction [$F(12, 26) = 9.79$, $p < 0.01$]. Repeated Measures t-tests were conducted to determine specific differences between trains. Drug-induced reductions in the early and late component responses to the first 4-pulse train (early: from 1.68 $\pm$0.23 to 1.5 $\pm$0.27 mV; late: from 1.69 $\pm$0.26 to 1.48 $\pm$0.26 mV) were similar to those observed for the single 300-Hz trains, but there were also marked reductions in the size of the facilitated responses to subsequent trains (Figure 6B). In pre-drug tests, the early component response to the second theta-patterned train was reduced by 29.6% relative to the first train-evoked response (from 1.68 $\pm$0.23 mV to 1.11 $\pm$0.20 mV, [$t(13) = 4.11$; $p < 0.01$]), and the late component showed a strong facilitation to 142.9% of the first train-evoked response (from 1.69 $\pm$0.26 mV to 2.28 $\pm$0.32 mV, [$t(13) = 4.24$; $p < 0.01$]) (Figure 6C$_1$, D). Responses to subsequent trains in these pre-drug tests showed a progressive decrease in the early component amplitude from 1.68 $\pm$0.23 in the first train, to 0.11 $\pm$0.15 mV during the sixth train (as spiking activity was increased), and a gradual decline in the facilitation of the late component, until it returned to levels observed during the first train (1.38 $\pm$0.19 mV; Figure 6B, D).

NMDA receptor antagonists reduced the amplitude of both the early and late field potential components during delivery of theta-patterned trains. The early component response to the second train was reduced to 46% of pre-drug levels (0.72 $\pm$0.17 mV) and the late component response was reduced to 90.7% of pre-drug levels (2.13 $\pm$0.33 mV;

Figure 6C, D). The reductions in the train-evoked responses induced by NMDA receptor antagonists were apparent during the first 4 to 5 trains, but pre- and post-drug responses to the sixth theta-patterned train did not differ significantly (early: pre, 1.11 ±0.20 mV; post, 0.72 ±0.17 mV; [$t(13) = 0.12$; $p = .92$]; late: pre, 1.38 ±0.19 mV; post, 1.53 ±0.25 mV, [$t(13) = 1.35$; $p = .14$]).

To determine if NMDA receptor activation may also be maintained during repetitive theta-patterned stimulation with single pulses, responses to 2 second duration, 10 Hz trains were also monitored. Two (Drug time) X 20 (Pulse number) Repeated Measures ANOVAs were conducted for the early and late components during train delivery. In pre-drug testing, there was a reduction in the early component (from 1.50 ±0.26 mV to 0.67 ±0.12 mV) and a facilitation of the late component (from 0.60 ±0.19 mV to 1.69 ±0.28 mV) during the trains (Figure 7A). In tests conducted after drug administration, responses to single pulses prior to the 10 Hz trains showed reductions in both early [$F(44,616) = 4.15$; $p < 0.01$] and late [$F(44,616) = 31.34$; $p < 0.01$] field potential components that were similar to the reductions observed in input-output tests (Figure $7A_1$, C). The reduction of the early component was maintained throughout the train, but the amount of facilitation of the late component declined gradually to 155% of baseline levels at the end of the train (Figure 7C; illustrated by a Drug by Pulse number interaction, [$F(1, 19) = 2.19$, $p < 0.01$]). Responses recorded during the 10 Hz trains showed that NMDA receptor antagonism did not affect the size of early component responses (Figure 7B, C_1, [$F(1, 14) = 0.02$ $p = 0.90$]), but did cause a reduction in the late component that was maintained throughout the 10 Hz trains (Figure 7B, C_2; [$F(1, 14) =$

16.91, $p < 0.01$].

Figure 1. Histological results showing the locations of the deepest tips of stimulating (●) and recording (■) electrodes in the corpus callosum and sensorimotor cortex respectively. Resuits are shown on representative sections taken from the atlas of Paxinos and Watson (1998). There were no systematic differences in electrode placements between groups. Numbers indicate mm anterior to Bregma.

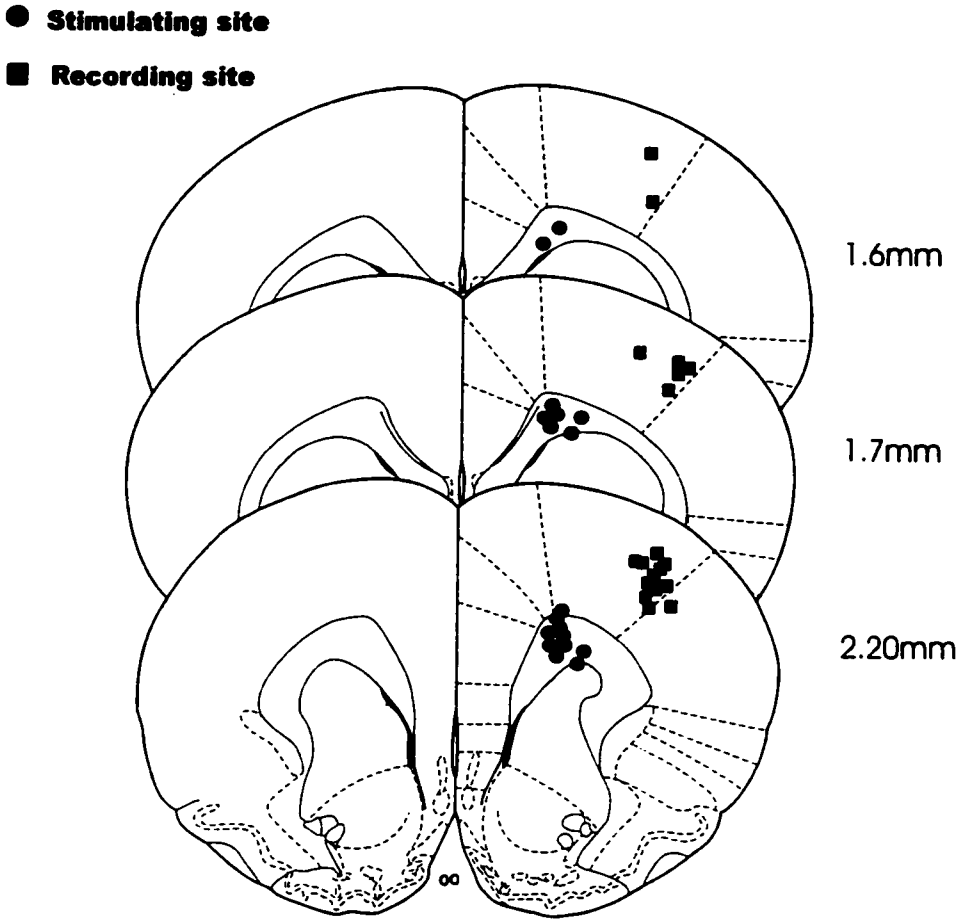

Stimulating site
Recording site
1.6mm
1.7mm
2.20mm

Figure 2. Changes in evoked field potentials in the sensorimotor cortex during tests for LTP induction using either 300-Hz trains, or pairs of trains patterned after the theta rhythm. **A.** Representative field potentials recorded before and after LTP induction are shown for animals that received either paired trains (A_1) or 300-Hz (A_2) stimulation. Symbols indicate the peak latencies of the spike (◆), early (●), and late (■) field potential components. **B.** Group means of response amplitudes at intermediate test-pulse intensities showed a large reduction in the early component following 300-Hz stimulation, and only a small reduction following paired-train stimulation (B_1). In contrast, the amplitude of the late component increased moderately following 300-Hz stimulation, and there was a much larger increase following stimulation with paired trains (B_2). Changes in both the early and late components peaked after approximately 15 days of tetanization and were maintained for days to weeks.

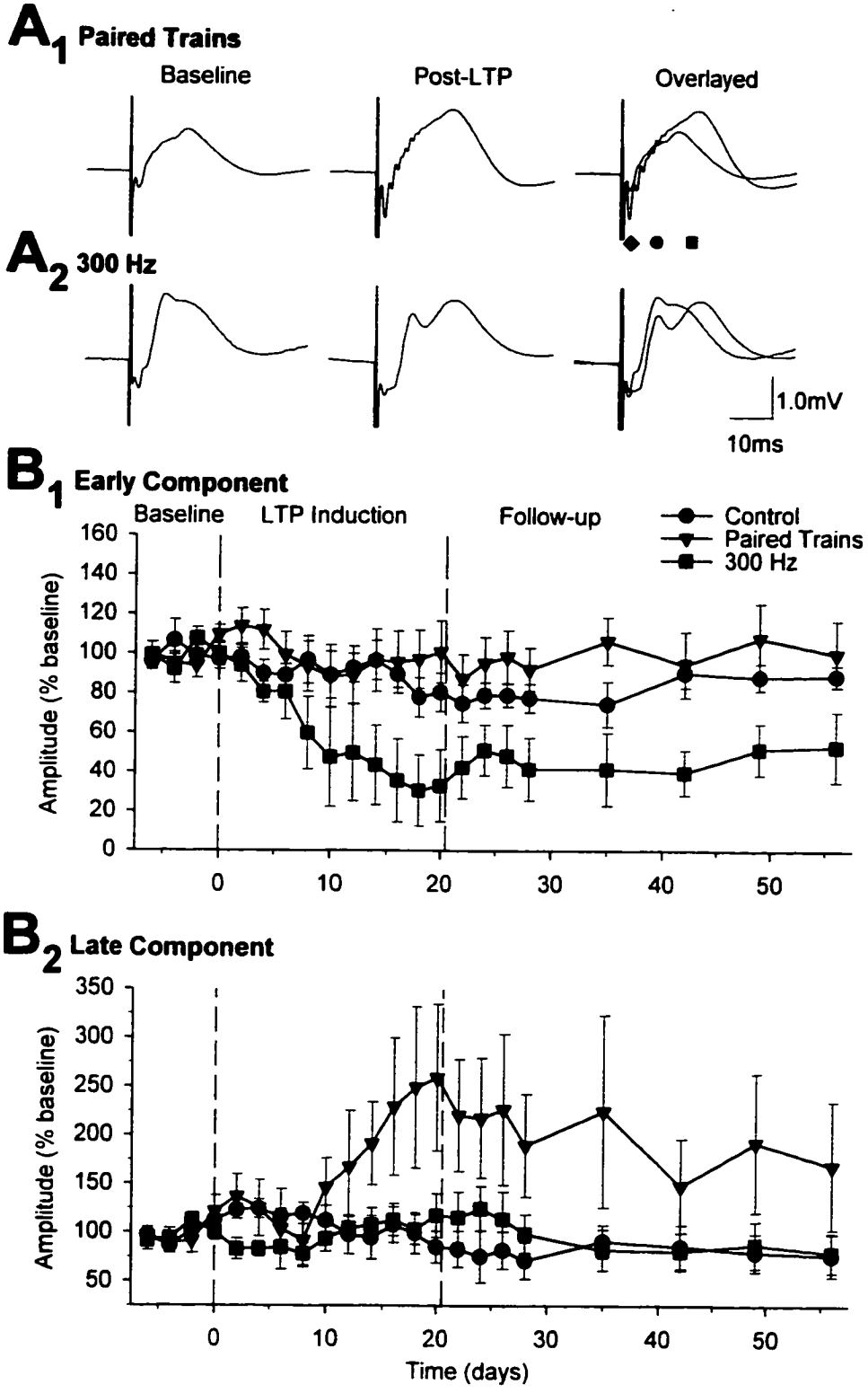

A1 Paired Trains
Baseline
Post-LTP
Overlayed
A2 300 Hz
1.0mV
10ms
B1 Early Component
Baseline
LTP Induction
Follow-up
Control
Paired Trains
300 Hz
Amplitude (% baseline)
160
140
120
100
80
60
40
20
0
0
10
20
30
40
50
B2 Late Component
Amplitude (% baseline)
350
300
250
200
150
100
50
0
10
20
30
40
50
Time (days)

Figure 3. Mean results of input/output tests recorded before, and one day after, the 20-day LTP induction period in which animals received either 300-Hz trains, paired trains, or no tetanization. **A.** There was minimal reduction in the mean amplitude of the early component following LTP induction using paired trains, and a much larger reduction in the early component, following 300-Hz stimulation (A). **B.** There was a large increase in the late component amplitude at all supra-threshold stimulation intensities following LTP induction with paired trains, and a moderate increase at stimulation intensities between 200 and 500μA following 300-Hz trains. There was no change in amplitude of the early or late field potential components in the control group.

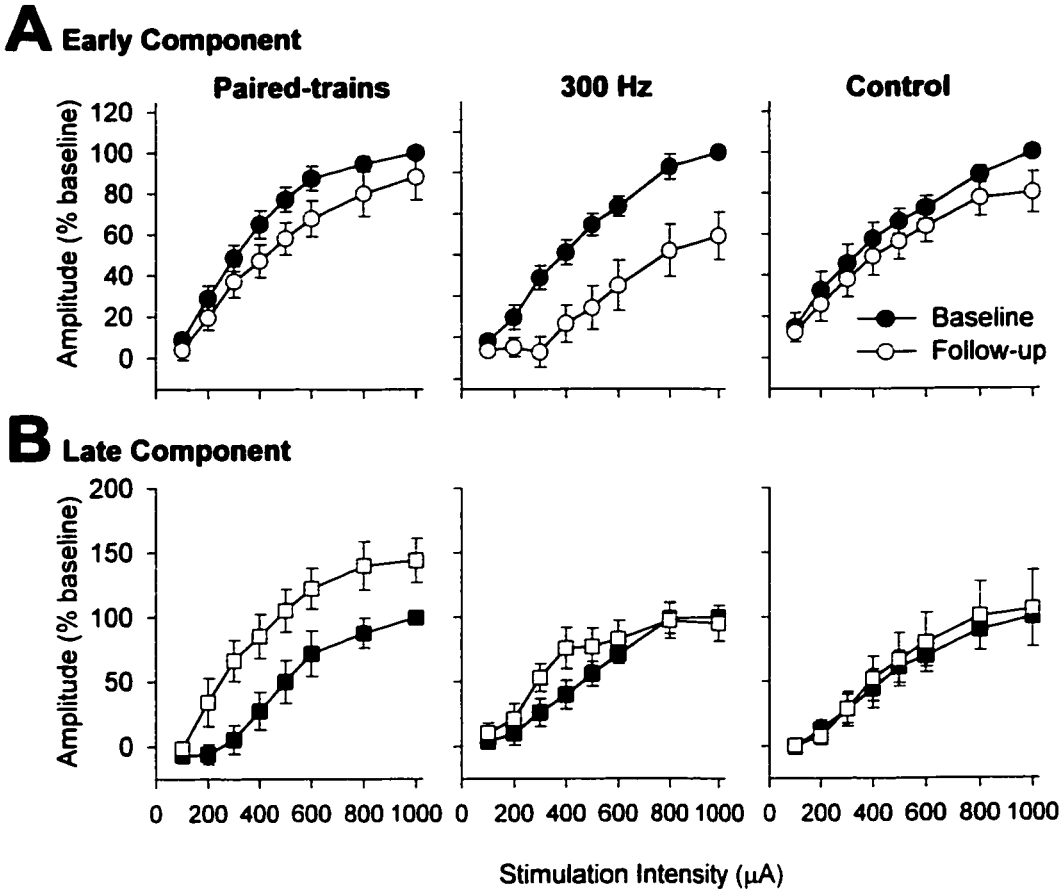

A Early Component
Paired-trains
300 Hz
Control
Amplitude (% baseline)
120
100
80
60
40
20
0
Baseline
Follow-up
B Late Component
Amplitude (% baseline)
200
150
100
50
0
0 200 400 600 800 1000
0 200 400 600 800 1000
0 200 400 600 800 1000
Stimulation Intensity (μA)

Figure 4. Changes in monopolarly and differentially-recorded field potentials following LTP induction using either paired trains or 300-Hz stimulation. **A.** Monopolar recordings from the superficial and deep tips of the recording electrode are shown with the differential bipolar recordings. Negativity is downward and stimulation intensity was 600 μA. **B.** The smaller decrease in the early component of bipolar recordings following paired trains was associated with a larger increase in the superficial negative component (B_1), and smaller increases in repetitive spiking in deep recordings as compared to animals receiving 300-Hz stimulation. The larger increase in the bipolarly recorded late component in animals receiving paired trains was associated with larger enhancements in the late superficial-negative component (B_2).

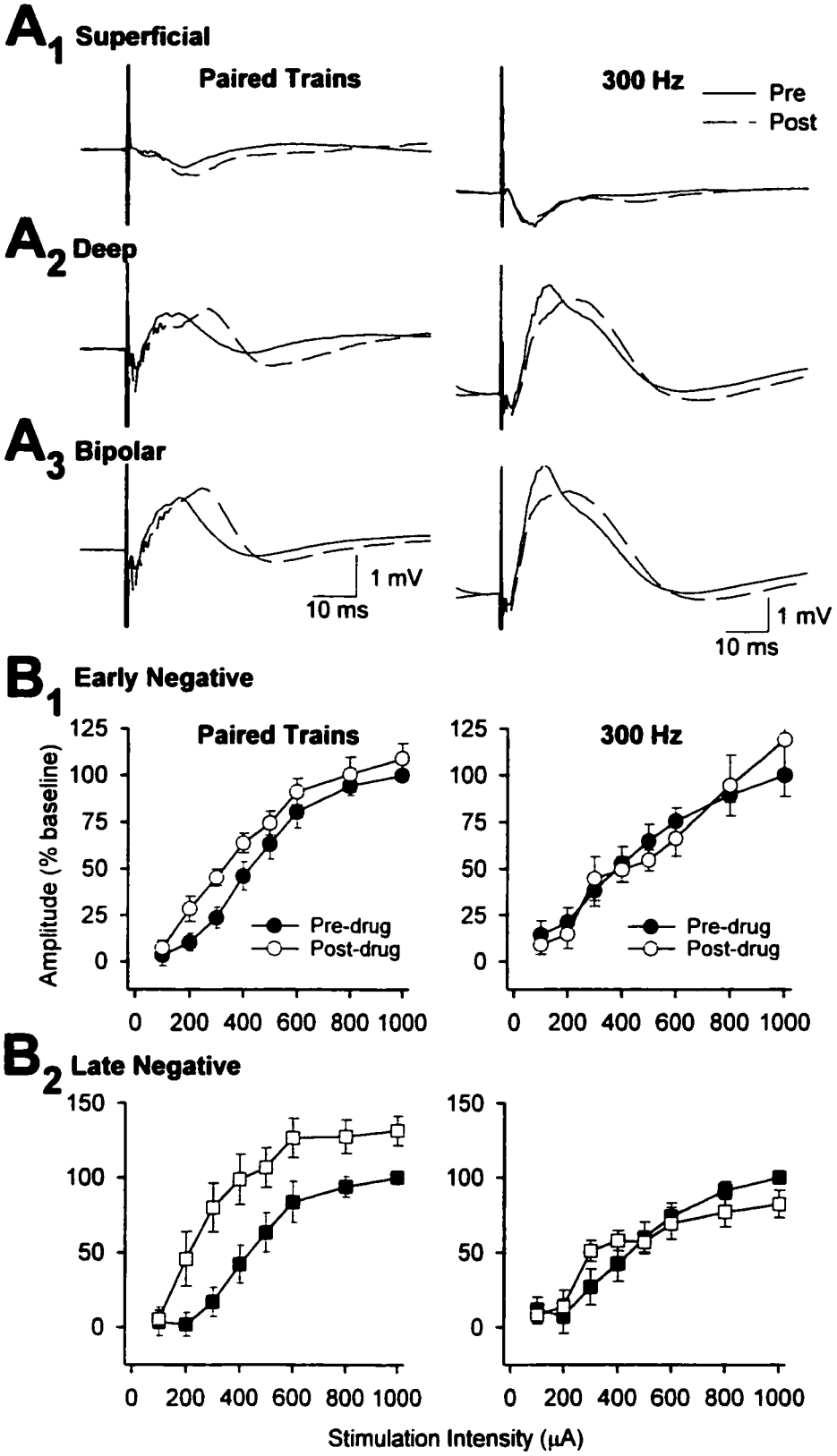

A1 Superficial
Paired Trains
300 Hz
Pre
Post
A2 Deep
A3 Bipolar
1 mV
10 ms
1 mV
10 ms
B1 Early Negative
Paired Trains
300 Hz
Amplitude (% baseline)
125
100
75
50
25
0
Pre-drug
Post-drug
0 200 400 600 800 1000
B2 Late Negative
150
100
50
0
Stimulation Intensity (μA)

Figure 5. Effects of NMDA receptor antagonists on field potential responses evoked by single stimulation pulses. **A.** Similar changes in evoked responses were observed 20 minutes following administration of either Ketamine (n = 10, 30 mg/kg; A_1) or MK-801 (n = 5, 0.1 mg/kg; A_2). **B.** While reductions in both the early and late components were slower to occur following MK-801 there was no difference between the drugs in the size of the reductions observed after 15 to 20 minutes. The amplitude of the initial spike component was not reduced. **C.** Results of input/output tests recorded before and after drug administration (n = 15) showed a moderate reduction in the amplitude of the early component (C_1), and a much larger reduction in late component (C_2). The arrow indicates the time of i.p. drug administration.

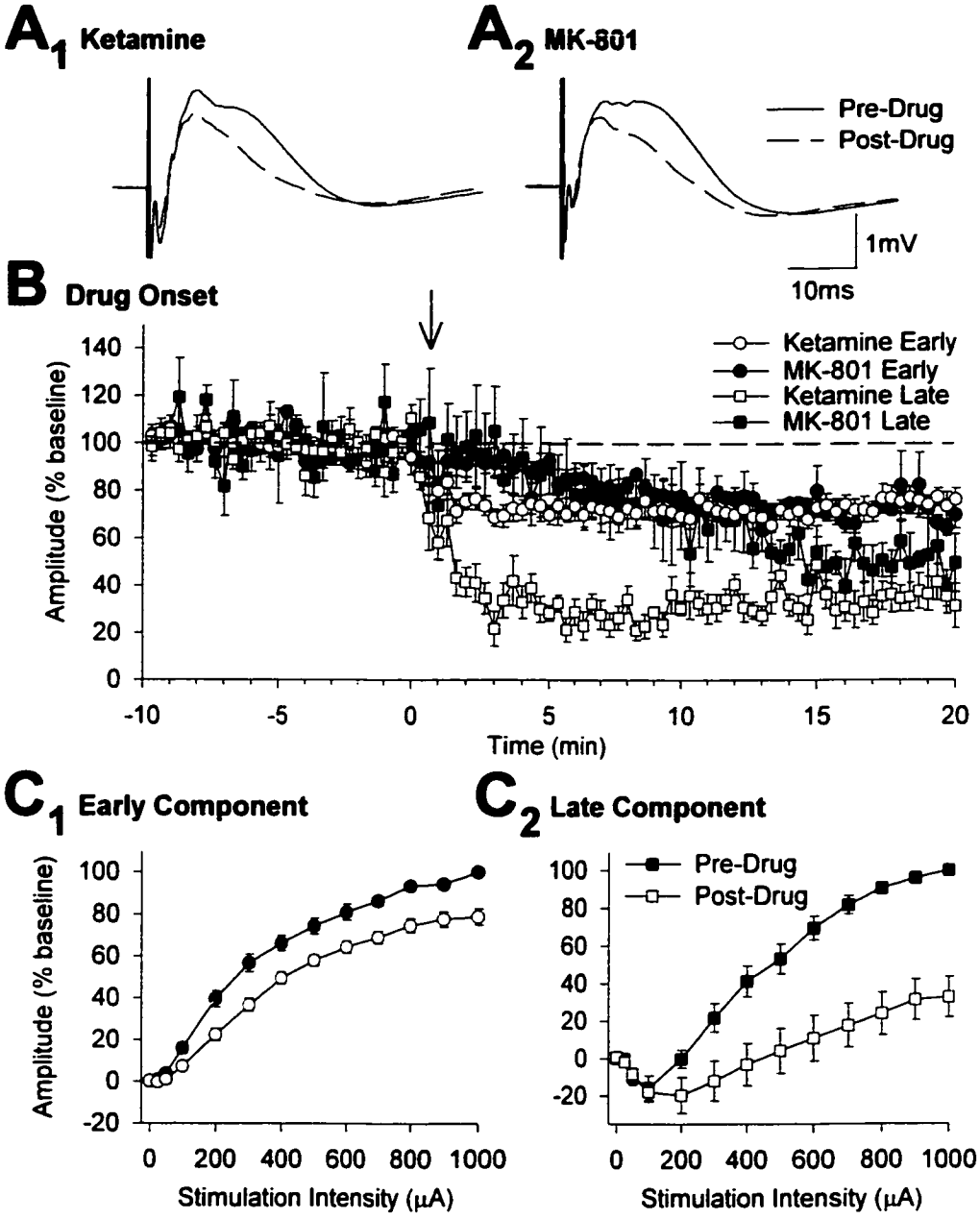

A1 Ketamine
A2 MK-801
Pre-Drug
Post-Drug
1mV
10ms
B Drug Onset
Ketamine Early
MK-801 Early
Ketamine Late
MK-801 Late
Amplitude (% baseline)
Time (min)
C1 Early Component
C2 Late Component
Pre-Drug
Post-Drug
Amplitude (% baseline)
Stimulation Intensity (μA)
Stimulation Intensity (μA)

Figure 6. Responses evoked by 300-Hz or paired train stimulation were reduced by NMDA receptor antagonists (Ketamine or MK-801; n = 15). **A and B.** Early (◆) and late(■) component responses were reduced by about 20% during 8-pulse, 300-Hz trains (A), and responses to the first 4-pulse train in the sets of 6 theta-patterned trains were also reduced by about 20% (B). The facilitation of responses during repetitive delivery of trains at theta-frequency was also reduced by NMDA receptor antagonists and the reductions were most marked during the first 4 or 5 trains (B). After the 6th train, however, similar amounts of facilitation were observed both before and after the drug. **C.** Comparison of representative responses to the first and second trains before and after drug administration shows a greater reduction in the early component and a smaller facilitation of the late component in the presence of NMDA receptor antagonists. Baselines of traces have been matched for comparison. **D.** Histograms show that before drug delivery, responses during repeated delivery of trains at theta-frequency showed a decline in the early component, and a facilitation of the late component. NMDA-receptor antagonists enhanced decline of the early component, and also reduced the amplitude of the late component during the first 4 to 5 trains.

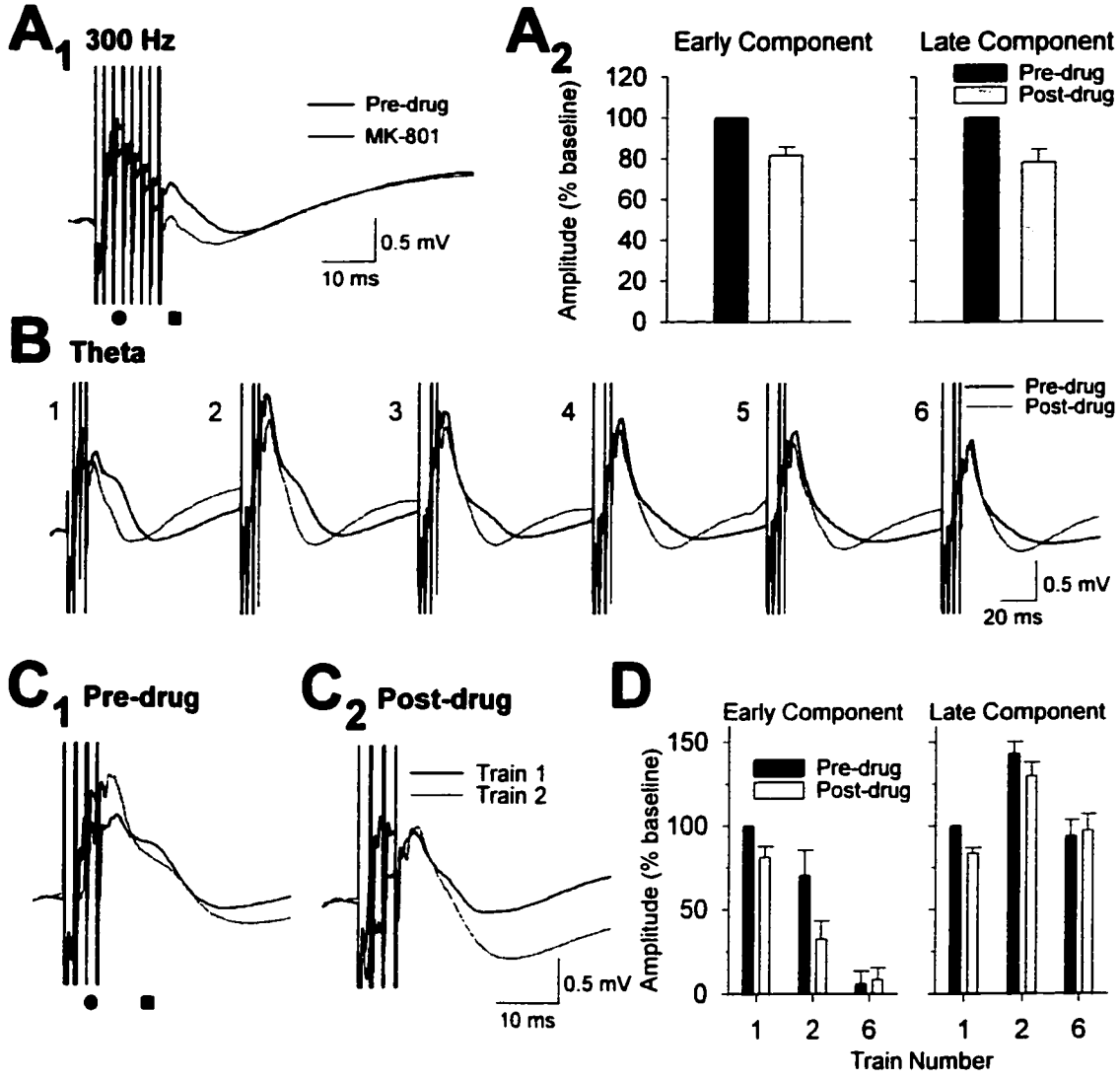
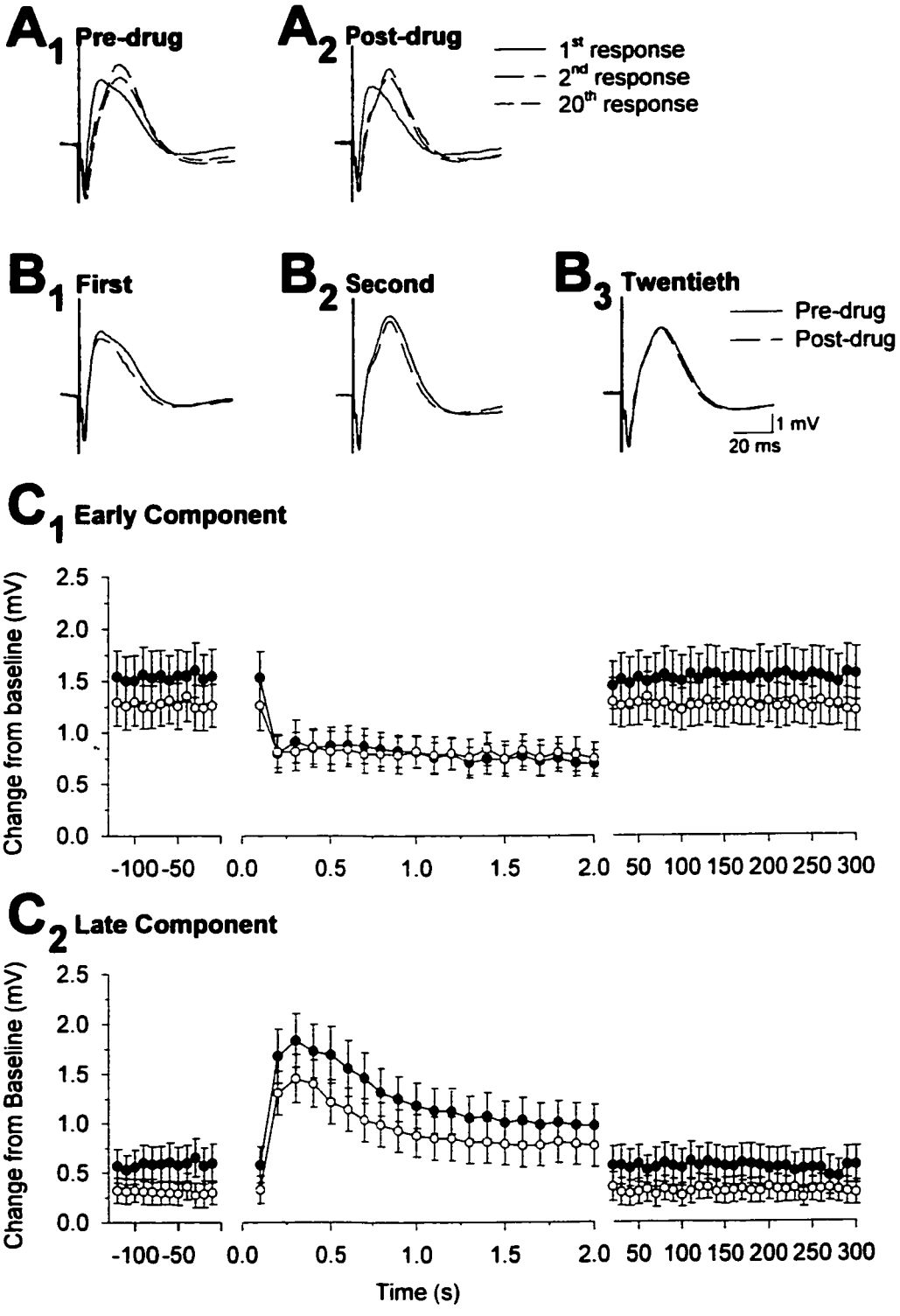

A1 300 Hz
Pre-drug
MK-801
0.5 mV
10 ms
A2
Early Component
Late Component
Pre-drug
Post-drug
Amplitude (% baseline)
120
100
80
60
40
20
0
B Theta
1
2
3
4
5
6
Pre-drug
Post-drug
0.5 mV
20 ms
C1 Pre-drug
C2 Post-drug
Train 1
Train 2
0.5 mV
10 ms
D
Early Component
Late Component
Pre-drug
Post-drug
Amplitude (% baseline)
150
100
50
0
1
2
6
1
2
6
Train Number

Figure 7. Responses to prolonged 10 Hz trains of single pulses were reduced by NMDA-receptor antagonists. **A.** A comparison of responses to the first, second, and twentieth pulses recorded before (A_1) and after (A_2) the administration of an NMDA antagonist shows that there was a decline in the early component, and enhancement in the late component during the trains both before and after drug administration. **B.** Traces recorded before and after drug delivery are superimposed. NMDA-receptor antagonists had a minimal effect on the decline of the early component, and caused a maintained reduction in the late component during the trains. **C.** Group means of response amplitudes showed that the reduction in the early component was stable throughout the trains. The large initial facilitation of the late component declined, but the late component amplitude remained facilitated above single-pulse levels throughout the trains.


A1 Pre-drug
A2 Post-drug
1st response
2nd response
20th response
B1 First
B2 Second
B3 Twentieth
Pre-drug
Post-drug
1 mV
20 ms
C1 Early Component
Change from baseline (mV)
2.5
2.0
1.5
1.0
0.5
0.0
-100 -50 0.0 0.5 1.0 1.5 2.0 50 100 150 200 250 300
C2 Late Component
Change from Baseline (mV)
Time (s)

DISCUSSION

The above experiments demonstrate that paired trains of stimulation delivered at an interval corresponding to the theta EEG rhythm are highly effective at inducing LTP in the sensorimotor cortex of the awake rat *in vivo*, and that paired trains preferentially enhance LTP of the late component as compared to single 300-Hz trains. Similar to previous findings (Chapman et al., 1998; Trepel and Racine, 1998), multiple days were required to induce LTP, and the LTP effects were maintained over days to weeks. The early *versus* late field potential components were affected differentially by paired trains and single 300-Hz trains, with paired trains causing much smaller changes in the early component, and a much larger potentiation of the late component than the single 300-Hz trains (Figures 2 & 3). This indicates that paired trains delivered at the interval of the endogenous theta rhythm cause sufficient activation to induce LTP in monosynaptic cortical inputs, and also suggests that theta-patterned stimulation induces much greater activation of polysynaptic horizontal pathways in layer V than the single 300-Hz trains.

Results of tests with NMDA receptor antagonists suggest that the effectiveness of theta-patterned stimulation in inducing LTP in horizontal connections is likely due to the induction of short-term facilitation effects that resemble the neocortical augmenting response. The augmenting response is usually observed in response to repeated delivery of single pulses (Castro-Alamancos and Connors, 1996c), and we have observed here a similar facilitation of train-evoked responses (Figure 6). NMDA receptor antagonists reduced train-evoked responses by about 20% during the first several trains, indicating that NMDA receptors are activated during LTP induction. Facilitation of responses

during prolonged, 2 sec trains of single pulses at theta-frequency were also reduced by NMDA receptor antagonists (Figure 7). This verified that synaptic facilitation effects involved activation of NMDA receptors during both the theta-patterned trains used for LTP induction, and also during repeated delivery of much less intense stimuli. This indicates that short-term facilitation effects that induce NMDA receptor activation in the neocortex can occur over prolonged periods in response to more physiologically realistic stimulation with single pulses.

LTP of the Early, Monosynaptic Component. The LTP effects observed in animals that received single 300-Hz trains were similar to what has been reported previously for identical stimulation trains (Trepel and Racine, 1998). There was a reduction in the early component, and an increase in the late component at intermediate test-pulse intensities (Figures 2 and 3). The 300-Hz trains caused a larger reduction in the early component than the paired trains which suggests that there were larger changes in the monosynaptic cortical inputs. However, the net change in the early field potential component is affected by both increases in the superficial-negative component, which more directly reflects synaptic activation, and increases in repetitive spiking in deep recording sites. Previous work, in which spiking activity has been reduced by repetitive 80 Hz stimulation or anesthesia has shown that the reduction in the early component is caused by a growth in repetitive spiking activity which masks smaller increases in the amplitude of the early component caused by LTP of monosynaptic inputs to layer V (Chapman et al., 1998). In the 300-Hz group, there were large increases in repetitive spiking and little change in the

superficial-negative component, and the larger decrease in the early component in this group is therefore due mainly to increased deep-negative spiking activity that reduced the voltage dipole in bipolar recordings. The larger increase in repetitive spiking following single 300-Hz trains *versus* paired trains may be due to more intense antidromic activation during the longer 8-pulse trains (Lynch, Sayin, Golarai and Sutula, 2000).

In addition, the smaller reduction of the early component observed in animals that received paired trains may be due to greater LTP of monosynaptic inputs to layer V. Animals that received paired trains showed an increase in the amplitude of the early surface-negative component, whereas this was not observed in the 300-Hz group. This suggests that paired trains caused a larger potentiation of direct monosynaptic inputs to cells in layer V. This may have offset reductions in the early component due to increased deep-negative spiking and resulted in little net change in the early field potential component. Thus, although it is difficult to directly assess potentiation effects in the early field potential component because of concurrent changes in repetitive spiking, the smaller change in the early component following paired trains appears to reflect a greater potentiation of monosynaptic inputs to layer V paired with a more moderate increase in repetitive spiking.

LTP of the Late Polysynaptic Component. The potentiation of the late field potential component in the 300-Hz group was similar to that observed in previous studies, but a larger potentiation was observed in animals that received paired stimulation trains. Although single 300-Hz trains caused LTP of the late component restricted to

intermediate test-pulse intensities, it was enhanced at all supra-threshold stimulation intensities following LTP induction with paired trains (Figure 3). This indicates that paired trains induced a much greater strengthening of polysynaptic activation of neurons in layer V and/or that the spatial extent of this activation across layer V was increased.

The increase in the late component could be due to potentiation of synapses within horizontal layer V connections and/or direct potentiation of monosynaptic inputs which could lead to enhanced polysynaptic activation. Enhanced activation of layer V pyramidal cells due to potentiation of monosynaptic inputs could cause enhanced firing in these cells and therefore increase firing in their horizontal collaterals that mediate the late component. The idea that greater LTP of the late component in the paired train group is due to enhanced potentiation of monosynaptic inputs is consistent with the larger increase in the superficial negative component observed following paired trains (Figure 4). Previous research, has also demonstrated LTP in horizontal connections of the motor and sensorimotor cortices (Bindman, Murphy and Pockett, 1988; Baranyi, Szente and Woody, 1991; Aroniadou and Keller, 1993), and LTP of the horizontal connections is also likely to have contributed to greater LTP following the paired trains.

Effects of the NMDA receptor antagonists on I/O tests indicated that the late polysynaptic component is more susceptible to NMDA receptor blockade than the early monosynaptic component (Figure 5). The late component cannot be mediated exclusively by NMDA-receptor mediated currents, however, because it was not fully blocked by drug application, and it is strongly facilitated during paired trains and during 10 Hz pulse trains, even in the presence of NMDA receptor antagonists (Figures 6 and 7). Therefore,

although the late component involves activation of NMDA receptors, much of the late component evoked during repeated stimulation is likely mediated by non-NMDA glutamate receptor activation, and by enhanced spatial spread of activation in layer V.

Mechanisms Mediating Enhanced LTP by Theta-Patterned Stimulation. The large potentiation of the polysynaptic late component induced by paired-trains may have been partly due to greater LTP in monosynaptic cortical inputs, but it is also likely due to enhanced activation of horizontal connections during the trains. Although the single, 8-pulse 300-Hz trains were more intense than each 4-pulse train in the paired trains, the first 4-pulse train in each pair induced large responses, and the second train evoked responses were strongly facilitated (Figure 6B). Because LTP in this preparation is dependent on NMDA receptor activation (Trepel and Racine, 1998), the effectiveness of paired trains in inducing LTP is likely due to enhanced recruitment of NMDA receptor mediated currents during train delivery. NMDA receptor antagonists caused an overall reduction in train-evoked responses (Figure 6D) indicating that both early and late components of these responses involve NMDA receptor activation.

A number of mechanisms could have contributed to the facilitation of responses and enhanced activation of NMDA receptors during paired train delivery. The facilitation of the late component during paired trains indicates an enhancement of synaptic activation of horizontal connections, and/or the spatial extent of this activation, such that neurons that were not activated by the first train may have been activated by the second train. This may have resulted in an increased cooperativity among afferents in inducing post-synaptic

depolarization, and a much larger population of neurons being depolarized above threshold for the induction of NMDA-receptor dependent LTP.

NMDA receptor activation during paired trains may have also been affected by inhibitory mechanisms. In the hippocampal CA1 region, theta-patterned stimulation is effective at inducing LTP partly because activation of presynaptic $GABA_B$ autoreceptors for the first several hundred milliseconds following the first train, reduces inhibitory synaptic transmission during the second train, and results in increased levels of postsynaptic depolarization (Mott and Lewis, 1991; Davies and Collingridge, 1996). In the cortex, NMDA receptor mediated currents may also be enhanced because of activation of the hyperpolarization-activated, inward cationic current (Castro-Alamancos and Connors, 1996a). A major mechanism that mediates the augmenting response in ventroposterior thalamic inputs to sensorimotor cortex layer V is the presence of long-duration inhibitory postsynaptic potentials that last for approximately 300 ms following stimulation. The hyperpolarization activates the cationic current which strongly enhances the excitability of layer V and facilitates synaptic responses (Castro-Alamancos and Connors, 1996a; Castro-Alamancos, 1997). Thus, increased post-synaptic depolarization associated with the inward cationic current may have enhanced the LTP induced by the paired trains by promoting NMDA receptor activation.

During repeated delivery of theta-patterned trains, the facilitation of train-evoked responses was greatest during the first several trains, and then declined (Figure 6). The facilitation, however, was maintained throughout less intense, 10 Hz trains of single pulses. In response to single pulses, the augmenting response peaked after the second or

third stimulation pulse, and although the responses declined, the responses remained above single-pulse levels throughout the 2 sec train (Figure 7). This supports previous work indicating that the neocortex undergoes a sustained facilitation in response to rhythmic stimulation with single pulses (Castro-Alamancos, 1997).

The finding that the facilitation of the late component was not maintained during repetitive train delivery could be due to a number of factors including an accelerated reduction in the pool of releasable synaptic vesicles, and a faster growth of postsynaptic Ca^{2+} activated K conductances activated during train-evoked burst discharges (Kitagawa, Nishimura, Kumazawa, Akamine and Yamamoto, 2000; Empson and Jefferys, 2001). Increased inhibition of monosynaptically activated neurons may have contributed to the reductions in the early component, and also reduced the size of the volley mediating the polysynaptic late field potential component. These mechanisms which may counteract the augmenting response are likely to have much less impact during stimulation with single pulses, or during normal patterns of rhythmic activity in the cortex.

During trains of single pulses, NMDA receptor antagonists caused a significant reduction in the size of the facilitated response throughout the trains (Figure 7). The facilitation was not completely blocked by NMDA receptor antagonists, indicating that much of the facilitation is mediated by other mechanisms. Although NMDA receptors are not the primary mechanism mediating the augmenting response (Addae and Stone, 1987; Castro-Alamancos and Connors, 1996c), and other factors affecting network activation and postsynaptic excitability are known to play a more important role (Castro-Alamancos, 1997), results do indicate that a substantial component of the facilitated responses is

mediated, either by direct activation of NMDA receptors, or by NMDA-mediated activation of other synaptic responses. This indicates that rhythmic cortical activation at theta-frequency may induce NMDA receptor mediated currents that may contribute to synaptic plasticity mediating normal learning and memory.

The present results have shown that intense stimulation of the corpus callosum at an interval corresponding to the endogenous theta EEG rhythm results in a large and robust enhancement of horizontal connections in the sensorimotor cortex. This suggests that the sensorimotor cortex may be activated preferentially by thalamic and/or cortical inputs that are active at theta-frequency, and that theta-frequency activity in the cortex may contribute to learning-related long-term synaptic plasticity associated with the development of new motor programs, or the modification or consolidation of existing motor programs (Teskey, Monfils, VandeBerg and Kleim, 2002). The neocortex *in vivo* is particularly resistant to the induction of LTP, and requires several days of tetanization to result in lasting changes in field potentials. Theta-frequency activity, that may activate mechanisms that induce short-term facilitation effects such as the augmenting response, therefore, may be particularly important in generating sufficient levels of postsynaptic depolarization that lead to long-term changes in synaptic strength in the neocortex.

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Appendix 1

ANOVA tables

Table #1

Repeated-Measures Factorial Analysis of Variance for Bipolar Spike Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Group(A)	80.54	2	40.27	1008.09	23	43.83	0.92
Stimulation intensity(B)	276.29	7	39.47	143.29	161	0.89	44.59*
Day(C)	2.31	21	0.11	67.62	483	0.14	0.75
A X B	14.42	14	1.03	143.29	161	0.89	1.17
A X C	4.20	42	0.10	67.62	483	0.14	0.66
B X C	1.47	147	0.01	33.81	3381	0.01	1.15
A X B X C	2.94	294	0.01	33.81	3381	0.01	0.96

* $p < 0.05$

** $p < 0.01$

Table #2

Repeated-Measures Factorial Analysis of Variance for Bipolar Early Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Group(A)	36.70	2	18.35	1740.42	22	79.11	0.23
Stimulation intensity(B)	706.23	7	100.89	218.68	154	1.42	71.04**
Day(C)	61.32	21	2.92	226.38	462	0.49	5.99**
A X B	18.62	14	1.33	218.68	154	1.42	0.93
A X C	15.96	42	0.38	226.38	462	0.49	0.78
B X C	5.88	147	0.04	64.68	3234	0.02	2.09**
A X B X C	5.88	294	0.02	64.68	3234	0.02	1.19*

* $p < 0.05$

** $p < 0.01$

Simple Effects of Control Group for Bipolar Early Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	181.86	7	25.98	49.7	35	1.42	18.30**
Day(B)	12.18	21	0.58	55.65	105	0.53	1.09
A X B	1.47	147	0.01	28.35	135	0.21	.05

Simple Effects of Paired Train Group for Bipolar Early Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	414.89	7	59.27	89.46	63	1.42	41.73**
Day(B)	16.38	21	0.78	100.17	189	0.53	1.47
A X B	2.94	147	0.02	277.83	1323	0.21	0.10

Simple Effects of 300 Hz Group for Bipolar Early Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	180.74	7	25.82	69.72	42	1.66	15.55**
Day(B)	44.73	21	2.13	41.58	126	0.33	4.02**
A X B	7.35	147	0.05	17.64	882	0.02	2.02**

Table #3

Repeated-Measures Factorial Analysis of Variance for Bipolar Late Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Group(A)	9.30	2	4.65	1570.36	22	71.38	0.07
Stimulation intensity(B)	842.38	7	120.34	397.32	154	2.58	46.61**
Day(C)	24.78	21	1.18	115.50	462	0.25	4.66**
A X B	6.58	14	0.47	397.32	154	2.58	0.18
A X C	21.84	42	0.52	115.50	462	0.25	2.06**
B X C	5.88	147	0.04	97.02	3234	0.03	1.46**
A X B X C	11.76	294	0.04	97.02	3234	0.03	1.48**

* $p < 0.05$

** $p < 0.01$

Simple Effects of Control Group for Bipolar Late Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	278.81	7	39.83	143.08	49	2.92	13.64**
Day(B)	5.04	21	0.24	36.75	147	0.25	0.96
A X B	2.94	147	0.02	30.87	1029	0.03	0.67

Simple Effects of Paired Train Group for Bipolar Late Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	346.5	7	49.50	144.48	56	2.58	19.19**
Day(B)	2751	21	1.31	47.04	168	0.28	4.68**
A X B	7.35	147	0.05	35.28	1176	0.03	1.67**

Simple Effects of 300-Hz Group for Bipolar Late Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	229.88	7	32.84	126.42	49	2.58	12.73**
Day(B)	15.33	21	0.73	36.75	147	0.25	2.92**
A X B	7.35	147	0.05	30.87	1029	0.03	1.67**

Table #4

Repeated-Measures Factorial Analysis of Variance for Superficial Early Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Group(A)	26.34	2	13.17	1957.78	22	88.99	0.15
Stimulation intensity(B)	1908.48	7	272.64	418.88	154	2.72	100.28**
Day(C)	15.33	21	0.73	244.86	462	0.53	1.36
A X B	14.28	14	1.02	418.88	154	2.72	0.37
A X C	35.7	42	0.85	244.86	462	0.53	1.60*
B X C	17.64	147	0.12	258.72	3234	0.08	1.37**
A X B X C	29.4	294	0.10	258.72	3234	0.08	1.13

* $p < 0.05$

** $p < 0.01$

Simple Effects of Control Group for Superficial Early Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	615.58	7	87.94	132.79	49	2.71	32.45**
Day(B)	14.7	21	0.70	77.91	147	0.53	1.32
A X B	11.76	147	0.08	82.32	1029	0.08	1.00

Simple Effects of Paired Train Group for Superficial Early Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	933.24	7	133.32	190.26	63	3.02	44.14**
Day(B)	19.11	21	0.91	124.74	189	0.66	1.39
A X B	20.58	147	0.14	145.53	1323	0.11	1.31**

Simple Effects of 300 Hz Group for Superficial Early Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	457.94	7	65.42	113.82	42	2.71	24.14**
Day(B)	16.38	21	0.78	66.78	126	0.53	1.47*
A X B	13.23	147	0.09	70.56	882	0.08	1.13*

Table #5

Repeated-Measures Factorial Analysis of Variance for Superficial Late Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Group(A)	15.82	2	7.91	1677.06	21	79.86	0.10
Stimulation intensity(B)	1656.69	7	236.67	438.06	147	2.98	79.45**
Day(C)	38.64	21	1.84	238.14	441	.54	3.42**
A X B	17.78	14	1.27	438.06	147	2.98	0.43
A X C	57.96	42	1.38	238.14	441	0.54	2.56**
B X C	294	147	0.20	308.7	3087	0.10	1.87**
A X B X C	41.16	294	0.14	308.7	3087	0.10	1.38**

* $p < 0.05$

** $p < 0.01$

Simple Effects of Control Group for Superficial Late Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	534.66	7	76.38	146.02	49	2.98	25.63**
Day(B)	11.97	21	0.57	79.38	147	0.54	1.06
A X B	13.23	147	0.09	102.90	1029	0.10	0.90

Simple Effects of Paired Train Group for Superficial Late Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	90.30	7	112.90	166.88	56	2.98	37.89**
Day(B)	66.57	21	3.17	120.96	168	0.72	4.40**
A X B	30.87	147	0.21	152.88	1176	0.13	1.62**

Simple Effects of 300-Hz Group for Superficial Late Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	397.81	7	56.83	145.74	42	3.47	16.39**
Day(B)	21.42	21	1.02	68.04	126	0.54	1.89*
A X B	27.93	147	0.19	88.2	882	0.10	1.9**

Table #6

Repeated-Measures Factorial Analysis of Variance for Deep Early Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Group(A)	229.08	2	114.54	433.44	12	36.12	3.17
Stimulation intensity(B)	276.29	7	39.47	97.44	84	1.16	33.99**
Day(C)	31.71	21	1.51	113.40	252	0.45	3.36**
A X B	7.00	14	0.50	97.44	84	1.16	0.43
A X C	21	42	0.50	113.40	252	0.45	1.11
B X C	4.41	147	0.03	35.28	1764	0.02	1.49**
A X B X C	8.82	294	0.03	35.28	1764	0.02	1.40**

* $p < 0.05$

** $p < 0.01$

Simple Effects of Control Group for Deep Early Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	101.78	7	14.54	36.54	21	1.74	8.36**
Day(B)	9.24	21	0.44	28.35	63	0.45	0.98
A X B	4.41	147	0.03	13.23	441	0.03	1.00

Simple Effects of Paired Train Group for Deep Early Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	101.15	7	14.45	48.72	42	1.16	12.46**
Day(B)	12.39	21	0.59	71.82	126	0.57	1.04
A X B	2.94	147	0.02	17.64	882	0.02	1.00

Simple Effects of 300 Hz Group for Deep Early Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	82.88	7	11.84	24.36	21	1.16	10.21**
Day(B)	30.45	21	1.45	28.35	63	0.45	3.22**
A X B	5.88	147	0.04	8.82	441	0.02	2.00**

Table #7

Repeated-Measures Factorial Analysis of Variance for Deep Late Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Group(A)	52.48	2	26.24	1145.04	12	95.42	0.28
Stimulation intensity(B)	517.51	7	73.93	260.40	84	3.10	23.86**
Day(C)	4.83	21	0.23	70.56	252	0.28	0.83
A X B	16.10	14	1.15	260.40	84	3.10	0.37
A X C	17.22	42	0.41	70.56	252	0.28	1.48*
B X C	4.41	147	0.03	52.92	1764	0.03	0.87
A X B X C	11.76	294	0.04	52.92	1764	0.03	1.36**

* $p < 0.05$

** $p < 0.01$

Simple Effects of Control Group for Deep Late Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	250.88	7	35.84	86.8	28	3.10	11.56**
Day(B)	7.35	21	0.35	23.52	84	0.28	1.25
A X B	4.41	147	0.03	17.64	588	0.03	1.00

Simple Effects of Paired Train Group for Deep Late Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	216.23	7	30.89	108.50	35	3.10	9.96**
Day(B)	10.92	21	0.52	29.4	105	0.28	1.86*
A X B	5.88	147	0.04	22.05	735	0.03	1.33*

Simple Effects of 300-Hz Group for Deep Late Component Amplitude during LTP Induction.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Stimulation intensity(A)	94.5	7	13.50	65.10	21	3.10	4.35**
Day(B)	3.78	21	0.18	17.64	63	0.28	0.64
A X B	5.88	147	0.04	13.23	441	0.03	1.33

Table #8

Repeated-Measures Factorial Analysis of Variance for Bipolar Spike Amplitude during I/O tests Pre- and Post an NMDA antagonist.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Drug type(A)	4.81	1	4.81	75.92	13	5.84	0.82
Stimulation intensity(B)	49.20	12	4.10	35.88	156	0.23	18.18**
Drug time(C)	1.39	1	1.39	4.94	13	0.38	3.68
A X B	1.44	12	0.12	35.88	156	0.23	0.54
A X C	0.69	1	0.69	4.94	13	0.38	1.82
B X C	0.36	12	0.03	1.56	156	0.01	2.12*
A X B X C	0.24	12	0.02	1.56	156	0.01	1.49

* $p < 0.05$

** $p < 0.01$

Table #9

Repeated-Measures Factorial Analysis of Variance for Bipolar Early Amplitude during I/O tests Pre- and Post an NMDA antagonist.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Drug type(A)	1.45	1	1.45	130.13	13	10.01	0.14
Stimulation intensity(B)	138.48	12	11.54	54.60	156	0.35	33.31**
Drug time(C)	7.51	1	7.51	4.68	13	0.36	21.06**
A X B	0.48	12	0.04	54.60	156	0.35	0.13
A X C	1.12	1	1.12	4.68	13	0.36	3.14
B X C	2.04	12	0.17	3.12	156	0.02	8.58**
A X B X C	0.36	12	0.03	3.12	156	0.02	1.61

* $p < 0.05$

** $p < 0.01$

Table #10

Repeated-Measures Factorial Analysis of Variance for Bipolar Late Component Amplitude during I/O tests Pre-and Post an NMDA antagonist.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Drug type(A)	3.97	1	3.97	36.14	13	2.78	1.43
Stimulation intensity(B)	32.16	12	2.68	23.4	156	0.15	17.67**
Drug time(C)	8.83	1	8.83	10.27	13	0.79	11.17**
A X B	1.08	12	0.09	23.4	156	0.15	0.63
A X C	0.35	1	0.35	10.27	13	0.79	0.44
B X C	4.8	12	0.40	0.64	156	0.04	11.12**
A X B X C	0.12	12	0.01	0.64	156	0.04	0.30

* $p < 0.05$

** $p < 0.01$

Table #11

Repeated-Measures Factorial Analysis of Variance for the Early Component of Single Pulses delivered Pre- and Post an NMDA Antagonist, before and after Single Theta-Frequency Trains.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Drug (A)	0.79	1	0.79	1.43	13	0.11	7.36*
Pulse # (B)	31.12	2	15.56	14.04	26	0.54	28.59**
A X B	0.50	2	0.25	2.34	26	0.09	2.78

* $p < 0.05$

** $p < 0.01$

Table #12

Repeated-Measures Factorial Analysis of Variance for the Late Component of Single Pulses delivered Pre- and Post an NMDA Antagonist, before and after Single Theta-Frequency Trains.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Drug (A)	0.10	1	0.10	1.43	13	0.11	0.90
Pulse # (B)	8.96	2	4.48	9.10	26	0.35	12.81**
A X B	0.50	2	0.25	0.78	26	0.03	9.79**

* $p < 0.05$

** $p < 0.01$

Table #13

Repeated-Measures Factorial Analysis of Variance for the Early Component of Single Theta-Frequency Trains delivered Pre- and Post an NMDA Antagonist.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Drug (A)	0.01	1	0.01	7.28	14	0.52	0.02
Train # (B)	11.40	19	0.60	10.64	266	0.04	13.82**
A X B	0.95	19	0.05	7.98	266	0.03	1.88*

* $p < 0.05$

** $p < 0.01$

Table #14

Repeated-Measures Factorial Analysis of Variance for the Late Component of Single Theta-Frequency Trains delivered Pre- and Post an NMDA Antagonist.

Source	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F
Drug (A)	13.29	1	13.29	11.06	14	0.79	16.91**
Train # (B)	49.02	19	2.58	23.94	266	0.09	30.14**
A X B	0.45	19	0.05	5.32	266	0.02	2.19**

* $p < 0.05$

** $p < 0.01$