INVESTIGATIONS OF NEURONAL NETWORK RESPONSES TO ELECTRICAL STIMULATION IN MURINE SPINAL CULTURES

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Spontaneous activity in neuronal networks *in vitro* is common and has been well documented. However, alteration of spontaneous activity in such networks via conditioning electrical stimulation has received much less experimental attention. Two different patterns of electrical stimulation were used to enhance or depress the level of spontaneous activity in spinal cord cultures. High-frequency stimulation (HFS), a method routinely shown to increase the efficacy of synaptic transmission, was employed to augment spontaneous activity. Low-frequency stimulation (LFS), the technique often applied to depress synaptic efficacy, was employed to decrease spontaneous activity. In addition, LFS was used to reverse the effect of HFS on spontaneous activity. Likewise, HFS was applied to counter the effect of LFS. Because these networks were grown on multi-microelectrode plates (MMEPs), this allowed the simultaneous stimulation of any combination of the 64 electrodes in the array. Thus, the possible differences in response to single versus multi-electrode stimulation were also addressed. Finally, test-pulses were delivered before and after the conditioning stimulation on the same stimulation electrode(s) in order to assess the change in mean evoked action potentials (MEAPs). Dissociated spinal tissue from embryonic mice was allowed to mature into self-organized

networks that exhibited spontaneous bursting activity after two weeks of incubation. Spontaneous activity was monitored from up to 14 recording channels simultaneously. Although uniform responses to stimulation across all recording electrodes were rarely observed, a large majority of the recording channels had similar responses. Spontaneous activity was increased in 52% of 89 HFS trials, whereas activity was decreased in 35% of 75 LFS trials. The duration of most of these increases was less than 5 minutes. When there were substantial and long-term (> 15 min) changes in spontaneous activity, the opposing stimulation pattern successfully reversed the effect of the previous stimulation. The percent change in MEAPs following conditioning stimulation suggested that synaptic modification had taken place in 75% of all test-pulse stimulation trials.

ABSTRACT

Spontaneous activity in neural networks *in vitro* is common and has been well documented. However, alteration of spontaneous activity in such networks via conditioning electrical stimulation has received much less experimental attention. Two different patterns of electrical stimulation were used to enhance or depress the level of spontaneous activity in spinal cord cultures. High-frequency stimulation (HFS), a method routinely used to increase the efficacy of synaptic transmission, was employed to augment spontaneous activity. Low-frequency stimulation (LFS), the technique often applied to depress synaptic efficacy, was employed to decrease spontaneous activity. In addition, LFS was used to reverse the effect of HFS on spontaneous activity. Likewise, HFS was utilized to reverse the effect of LFS. Because these networks were grown on multi-microelectrode plates (MMEPs), this allowed the simultaneous stimulation of any combination of the 64 electrodes in the array. Thus, the possible differences in response to single versus multi-electrode stimulation was also addressed. Finally, test-pulses were delivered before and after the conditioning stimulation on the same stimulation electrode(s) in order to assess the change in mean evoked action potentials (MEAPs). Dissociated spinal tissue from embryonic mice was allowed to mature into self-organized networks that exhibited spontaneous bursting activity after two weeks of incubation. Spontaneous activity was monitored from up to 14 recording channels simultaneously.

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CHAPTER I

INTRODUCTION

The nerve cell network may be the single most important organizational entity that can be studied to understand the basic phenomena underlying information processing and storage. Compared to the whole brain or brain subsystems, it is a relatively simple dynamic component that still expresses the summated effects of all plasticity mechanisms residing in the specific tissue selected for study. Hence, it should reflect functional dynamic changes in response to stimulation and may reveal basic organizational strategies involved in short-term and long-term alteration of network behavior. In addition, these systems provide a possibility of demonstrating emergent storage phenomena that may not be expressed on the single cell or single synapse level.

This study involved the detection and analysis of changes in spontaneous activity in response to electrical stimulation and the development of an investigative protocol allowing long-range, multielectrode analyses of neural tissue in a constant and controlled environment. Although cultured networks have reduced synaptic density, seemingly random architecture, reduced glia cell number, and lack sensory input, these monolayer cultures should not prohibit statistical descriptions that focus on highly probable, gross behavioral features of macroscopically similar cultures with identical origin, similar neuronal densities, and controlled environmental parameters (Gross, 1994). In this study, emphasis was placed on the identification and characterization of evoked responses following electrical stimulation that can be observed repeatedly under the culture and recording conditions described in Methods. The characterization was based primarily on "coarse-grain analyses" of spatio-temporal burst patterns that represent a platform from which

specific and more quantitative questions can be approached in the near future. The main intent of this project was to determine whether such networks can generate reliable responses to electrical stimulation and maintain stimulus-induced "state changes" for a period of time after stimulation. Whenever possible, linkage to established plasticity mechanisms was attempted.

The two electrical stimulation protocols used in this study were HFS (high frequency stimulation) and LFS (low frequency stimulation). Both are commonly used to induce synaptic plasticity mechanisms in various preparations. Tetanic stimulation (also known as HFS) has been used extensively as a method to produce long-term potentiation (LTP) which is currently described as “a long-lasting enhancement of synaptic effectiveness that follows certain types of tetanic electrical stimulation” (Bear and Malenka, 1994).

Long-term potentiation dates back to the now classic experiments performed by Bliss and Lømo in 1973. They reported LTP in the dentate area of anesthetized rabbits following tetanic stimulation. They showed a reduction in the latency of the population spike, and an increase in the amplitude of the population excitatory post-synaptic potential (EPSP), as well as the population spike, following tetanic stimulation. Twenty years later, Randic and colleagues (1993) showed that a high frequency train (three tetani of 1 sec duration, at 100 Hz and 10 sec intervals) was sufficient to induce LTP in a spinal cord slice. In our system, the same stimulation parameters used by Randic were used to induce changes in spontaneous activity. Because tetanic stimulation is a common method used to induce potentiation, the intent was to link increases in spontaneous activity in our preparation with the possible expression of storage mechanisms.

Long-term depression (LTD) can typically be induced by repetitively stimulating at low frequency [0.5-5 Hz for ~15 min (900 pulses)] (Linden, 1994). Low frequency repetitive stimulation has been used extensively to induce LTD in several mammalian

systems. The same general method of repeated presentation of non-noxious stimuli is also used in producing the effect of habituation. An adaptation of the repetitive stimulation parameters utilized by Linden (and many others) was employed in attempts to induce depression of spontaneous network activity. The low frequency stimulation protocol used in most of the repetitive stimulation trials was 1 pulse per second for 15 min.

The terms LTP and its accepted counterpart long-term depression (LTD), refer to evoked responses to test pulses. In contrast, the following studies were focused on changes in spontaneous activity following electrical stimulation. That is primarily where this study deviates from the vast majority of the literature. The other departures from most of the past and ongoing research is the preparation itself and the delivery method of stimulation. Given these differences, it seemed justifiable, if not obligatory, to devise separate but parallel terminology to describe the responses to HFS and LFS under these conditions. Because the durations of the responses are variable, much like that of LTP and LTD, the terms are based not only on the type of stimulation, but also on the duration of the effect. Table 1 categorizes these responses and links the terms with common terms in the literature:

Table 1A. Responses to Tetanic Stimulation

Duration	My Term	Corresponding Term
Less than 15 min	Short-term spontaneous activity potentiation (STSAP)	Short-term potentiation (STP)
Greater than 15 min	Long-term spontaneous activity potentiation (LTSAP)	Long-term potentiation (LTP)

Table 1B. Responses to Repetitive Stimulation

Duration	My Term	Corresponding Term
Less than 15 min	Short-term spontaneous activity depression (STSAD)	Short-term depression (STD)
Greater than 15 min	Long-term spontaneous activity depression (LTSAD)	Long-term depression (LTD)

In an attempt to avoid a possible source of confusion, one should note that the terms are based on the conditioning stimulation pattern (i.e. tetanic or repetitive), and not on the direction of the change in activity. The expected response to tetanic stimulation was an overall increase in spontaneous activity, and the expected response to repetitive stimulation was a general decrease in spontaneous activity. Yet, because it has been widely reported that inhibitory circuitry can be potentiated or depressed, a tetanic stimulation could indeed strengthen one or more synapses in an inhibitory circuit thereby resulting in a reduction in spontaneous activity. Conversely, repetitive stimulation could result in an increase in spontaneous activity.

Four major mechanisms may change spontaneous activity patterns: (1) Physical reconstruction of connections (i.e., changes in the number and/or location of synapses); (2) modification of intrinsic properties of the neurons themselves (a) presynaptic (alteration in inherent neuronal firing characteristics) or (b) postsynaptic (alteration of thresholds); (3) synaptic modification; (4) alteration in neuronal morphology (Getting, 1989; Kandel, 1991). Over long periods in time, all might be induced. Temporal limitations probably restrict changes in neuronal morphology and physical reconstruction during the time of our trials. Eve Marder and associates showed that intrinsic properties of the units may be altered in neuronal networks. However, that alteration was over a period of 1 hour (Turrigiano et al., 1994). Thus, synaptic modification seem the most likely mechanism underlying changing spontaneous activity patterns.

The primary hypothesis was that the same type of stimulation used to induce LTP and LTD *in vivo* and in slice prepartions can be used to induce significant changes in spontaneous activity in dissociated cultured networks over roughly the same time period. Although induction of storage mechanisms like LTP or LTD probably reveal themselves as changes in spontaneous network activity, the goals of this project did not include proof of induction of LTD/LTP mechanisms. The objective was only to determined if stimulation protocols that were known to induce LTD/LTP in other systems can alter spontaneous network activity in the system used.

The secondary hypothesis was that these changes have much of the same attributes as LTP and LTD (i.e. reversibility, saturability, lability) and are biased by pharmacological manipulation. The only attribute that was systematically tested was the reversibility of the effect. Because early trials with saturability and pharmacological manipulation yielded inconsistent results, experiments involving a thorough investigation of these properties were not pursued. However, the Appendix contains examples of results from some of these experiments.

Depotentiation is a phenomenon that, along with saturation of LTP and LTD, has been given a great deal of attention in recent years. Depotentiation is described by Stäubli and Chun (1996) as selective depression of potentiated inputs (i.e. reversal of LTP). It is generally accepted that the reversal of LTP and the induction of LTD occur via two distinct mechanisms (Stäubli et al, 1995). However, repetitive stimulation has been shown to induce both LTD and depotentiation. As noted by Bear and Malenka (1994), "...synapses that are depressed can be potentiated and vice versa, indicating that LTD is not a result of lasting damage to the stimulated synapses." As with LTP and LTD, the induction of depotentiation per se was not tested in this study. However, the reversal of both LTSAP via low frequency (repetitive) stimulation and LTSAD via high frequency (tetanic) stimulation was attempted.

The primary and secondary hypotheses are summarized in the specific questions listed below:

Specific Questions addressed:

1. Does HFS increase spontaneous activity?
2. Does LFS decrease spontaneous activity?
3. Can activity enhanced by HFS be depressed by LFS and vice versa?
4. Does stimulation on single channels give different results than stimulation on multiple channels?

The idea that application of the same stimulation pulse delivered at different frequencies can produce different physiological effects is not new. Many investigators have shown that excitation of the same cerebral point may produce different results depending on the frequency employed. For example, in dogs, stimulation of the same point in the orbital cortex produced slowing down of respiration with 6 Hz, respiratory arrest with 30 Hz, increase in respiratory amplitude and rhythm with 60 Hz, and no visible effect with 180 Hz (Delgado & Livingston, 1948).

The ongoing investigations of synaptic storage mechanisms by other researchers must eventually be linked to circuits where synergistic and antagonist interactions can produce results that cannot be predicted only from synaptic mechanisms. The effects of these synergistic and antagonistic mechanisms may be demonstrated only when observing the activity patterns of several individual units simultaneously before and after stimulation. It is hoped that this study will be a critical first step toward the elucidation of such network storage phenomena.

Because I observed network responses and not single synapses, the simultaneous induction of opposite storage mechanisms was expected (see Figure A-13 in Appendix). For example, it was possible that both LTP and LTD (or similar mechanisms) were induced at the same time but at different elements of the network. Even only LTP induction can be

problematic because it may be simultaneously induced in excitatory and inhibitory circuitry. The induction of both LTP (Kano, et al.,1992; Komatsu and Iwakiri, 1993) and LTD (Stelzer et al., 1987; Liu et al., 1993) of GABAergic inhibitory transmission has been reported. Hence an empirical approach was justified, because the systematic search for specific mechanisms seemed premature at this juncture. Thus the goal was to first determine basic network responses in order to ascertain whether this type of stimulation could be effective in the induction of storage mechanisms in this type of system.

The development of the preamplifier system, fabricated by the Electrical Engineering Department of Southern Methodist University in collaboration with the Center for Network Neuroscience at the University of North Texas, made it possible to computer-select any combination of electrodes for stimulation. This feature made a comparison of network responses to single and multiple electrode stimulation feasible. In all animals, input to networks is primarily multiunit. It already has been shown that stimulation in a slice culture from more than one electrode results in a greater expression of LTP than does stimulation with a single electrode (Nayak and Browning, 1994). This study also addressed the relative effectiveness of single versus multielectrode stimulation.

Using a system similar to ours, Jimbo et al. (1994) showed that tetanic stimulation of specific recording electrodes arranged in an array similar to the electrode array on our MMEPs increased the probability of evoking a synaptic current (P_{ev}) from <1/3 to almost 1. They concluded that "induction of synchronized bursting by localized tetanic stimulation is linked to a potentiation of excitatory synaptic currents". The modification of synaptic currents lasted for more than five minutes.

There are a multitude of researchers that are studying LTP/LTD and the factors that regulate these processes. When this study began in 1993, there was no one observing the effects that these changes may have on spontaneous activity (*in vitro* or *in vivo*). While it is

obvious that the complete understanding of how these mechanisms work is important, the effects of these mechanisms must also be considered.

Many investigators have reported both LTP and LTD in several brain structures (e.g. several distinct areas within the hippocampus, the amygdala, the cerebellum; and at least two neocortical areas). In addition, both LTP and LTD have been demonstrated in undissociated spinal tissue (Pockett and Figurov, 1993; Randic, et al.,1993;). The above examples provide support to the emerging view that there are "multiple memory systems" in the central nervous system (Macdonald and White, 1993; Squire, 1992). This idea that several different mechanisms in different locales of the nervous system may be involved in memory and learning promotes the prospect that storage mechanisms are functional regardless of architecture.

CHAPTER II

MATERIALS AND METHODS

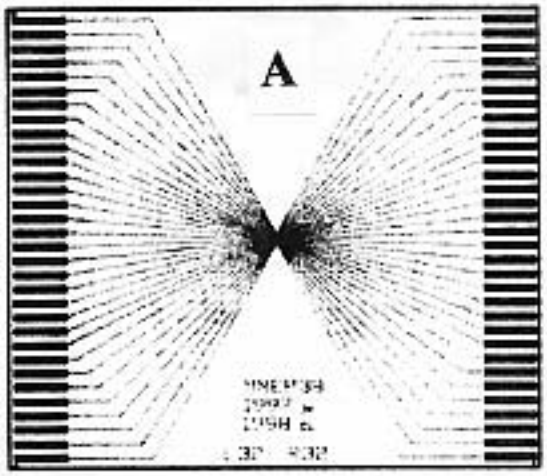
The techniques used for multimicroelectrode plate (MMEP) fabrication and preparation, as well as for cell dissociation, seeding, and culture maintenance have been described in previous publications (Gross, 1979; Gross and Lucas, 1982; Gross et al., 1982, 1985; Droge et al., 1986; Gross and Kowalski, 1991). Briefly, multielectrode plates (5 x 5 cm) were prepared from 1.2-mm-thick indium-tin oxide (ITO)-sputtered barrier glass (soda lime glass with a 100 nm quartz layer, Donnelly, Holland, MI). The electrode conductor pattern (Fig. 1), radiating from a central 0.8 mm^2 recording matrix of 64 microelectrodes (4 rows, 16 columns with 200 μm and 40 μm spacing, respectively) was photoetched with standard procedures at the University of North Texas (CNNS). The plates were spin-insulated with a 2-4 μm polysiloxane resin (DC 648, Dow Corning). An additional 40-60 μm-thick resin layer was hand-painted onto the entire plate (except for a 3 mm^2 culture area in the center, and the two 5-mm-wide contact strips at each edge). This procedure raised the conductor shunt impedance (at 1 kHz under saline) to - 40 MΩ, while preserving a thin resin layer required for deinsulation of electrode tips. After heat-curing the resin, the matrix microelectrode sites were deinsulated with single pulses from a nitrogen laser firing through a microscope (Gross, 1979). This resulted in a 20-30 μm diameter crater at the end of each ITO conductor (Fig. 2A & B). The conductors ranged in width from 8 μm to 12 μm depending on the photolithography mask used and fluctuations in the chemical etching.

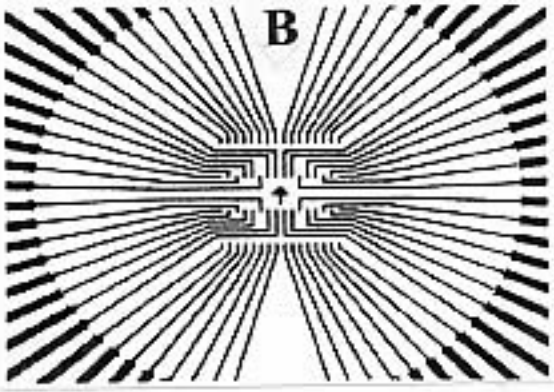
Figure 1. Electrode conductor pattern on multimicroelectrode plates (MMEPs).

A. Representation of electrode array plates used. The array consists of a 5 x 5 cm glass plate with 64 photoetched electrodes. The electrode leads on both sides interface with zebra strips (see Fig. 5) to transmit electrical signals to the preamplifiers (they also serve to deliver the stimulus pulses to the array). Electrode leads from channels 1-32 terminate on the left side, while leads from channels 33-64 terminate on the right.

B. Enlargement of center area of A showing pattern of 64 ITO conductors.

C. Electrode array recording area. All 64 recording channels (four rows of sixteen) are shown. Amplifier channels (CH) of the Plexon, Inc. data acquisition system were assigned in a horseshoe pattern starting with the lower left contact strip (CH-1 or electrode 4-8) to the uppermost contact strip (CH-32, electrode 1-8) and continuing on the right side with CHs-33 to 64.


A


B

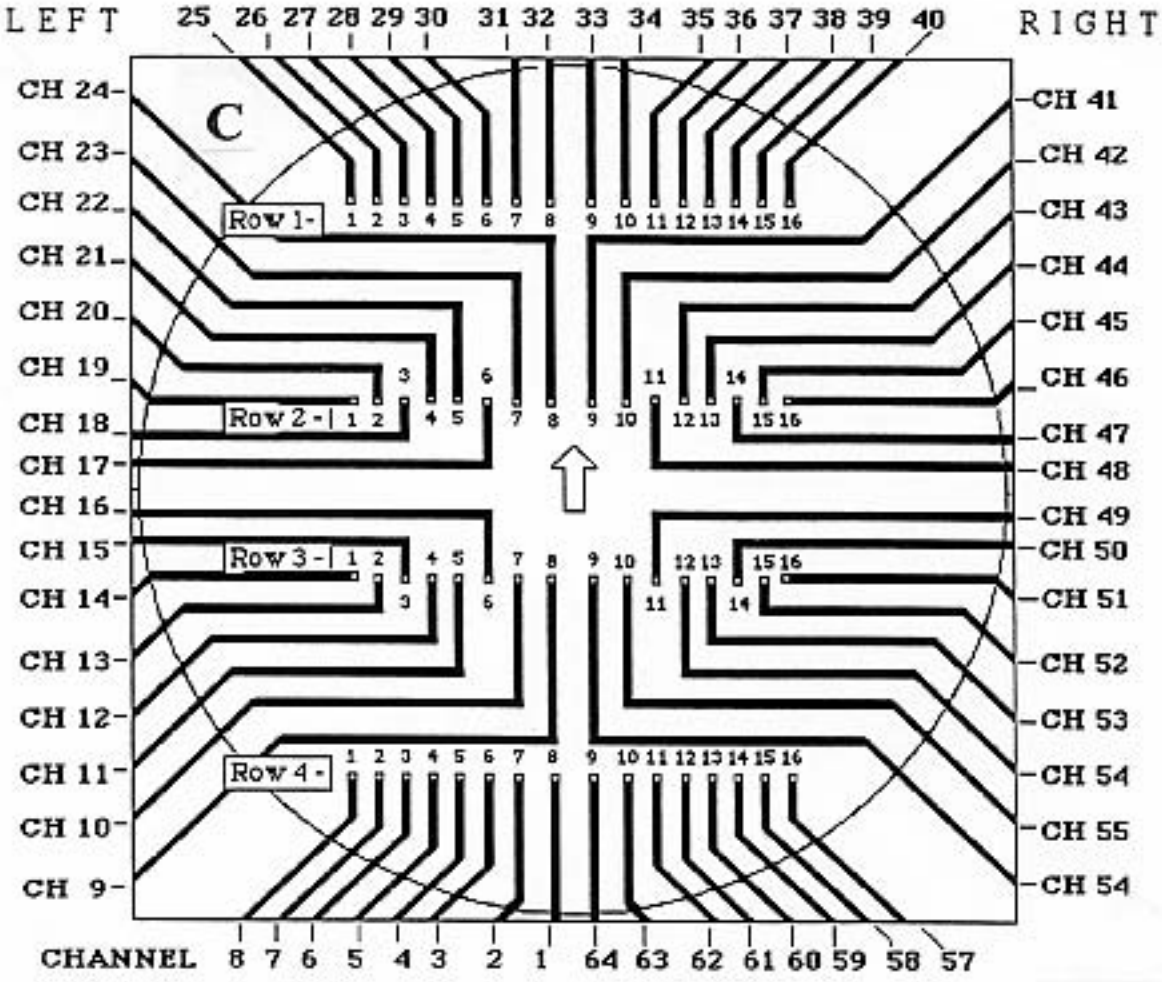

LEFT
RIGHT
C
Row 1-
Row 2-
Row 3-
Row 4-
CH 24
CH 23
CH 22
CH 21
CH 20
CH 19
CH 18
CH 17
CH 16
CH 15
CH 14
CH 13
CH 12
CH 11
CH 10
CH 9
CH 41
CH 42
CH 43
CH 44
CH 45
CH 46
CH 47
CH 48
CH 49
CH 50
CH 51
CH 52
CH 53
CH 54
CH 55
CH 54
CHANNEL 8 7 6 5 4 3 2 1 64 63 62 61 60 59 58 57

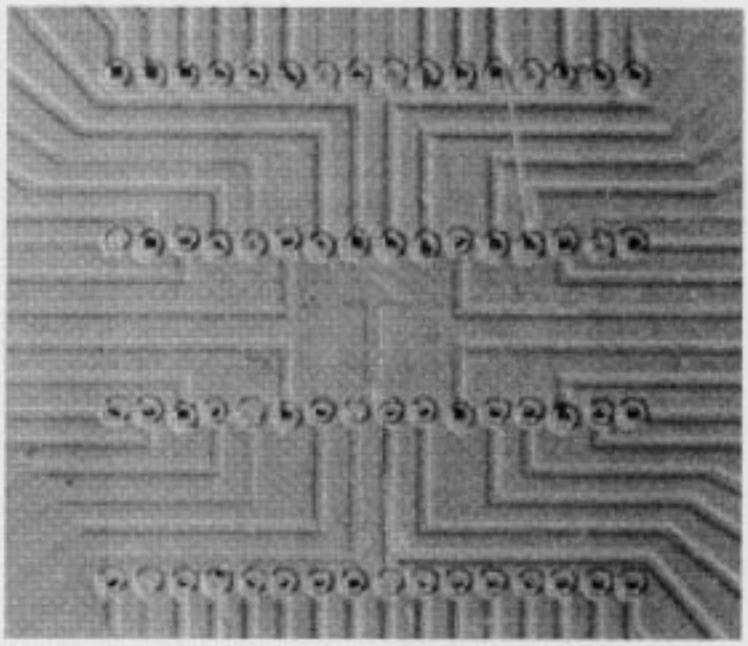
Figure 2. Matrix electrode sites.

A. Photograph of MMEP after deinsulation and gold-plating.

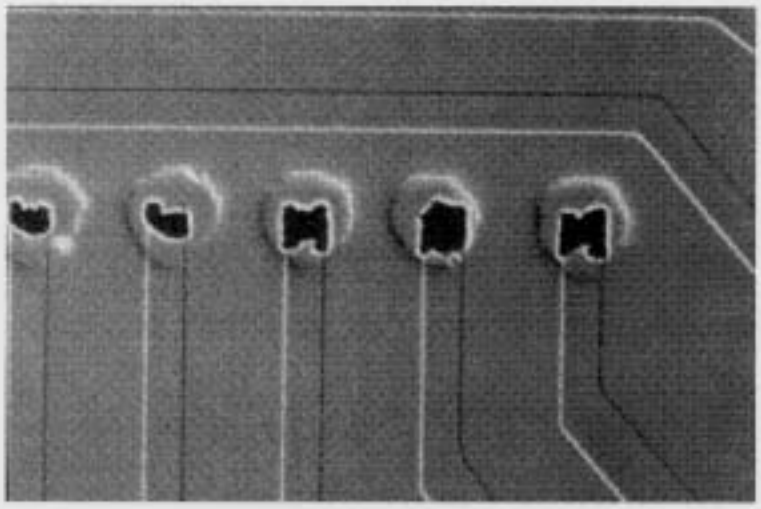
B. Higher magnification showing electrode craters. Recording craters were 15-20 μm in diameter and recessed by 2 μm. The indium-tin oxide metal exposed to the saline was electrolytically covered with gold. The ITO had an area of approximately 100 μm^2 and an impedance of 1-3 megohms at 1 KHz.

C. Diagram showing cross-section of MMEP after fabrication procedures. Plate material: soda-lime glass with a 10 nm (barrier film of quartz. Conductor material: indium tin-oxide (ITO), fully reduced, 10-20 ohms/square. Insulation material: polysiloxane resin 2-3 μm thick. Deinsulation: single laser shots that create 15-20 μm diameter craters and expose the terminal segment of the ITO conductor. Impedance adjustment: electrolytic gold plating of exposed ITO.

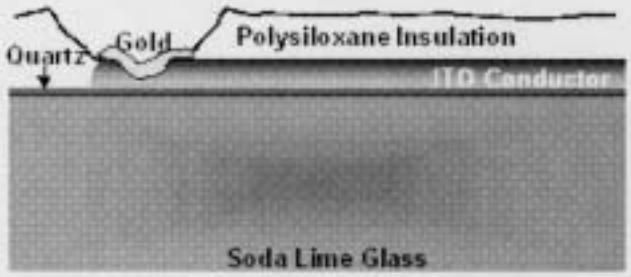
A.



B.



C.


Quartz
Gold
Polysiloxane Insulation
ITO Conductor
Soda Lime Glass

Electroplating of ITO

Recording crater impedances of 3 MΩ have been obtained from electroplating a thin layer of gold on each exposed ITO tip with a geometric area of 100-120 μm^2 (Gross et al., 1985). MMEPs used for this stimulation study featured 8-μm-wide conductors which reduced the gold-plated area to about 60-80 μm^2 and provided electrode tip impedances of about 4 MΩ. The stability of the ITO/gold interface was improved by the CNNS via acid striking the deinsulated MMEP (10 s exposure to 1 N HCI) and electroplating within 30 s with a potassium cyanide-gold chloride solution (SG-10, Transene, Rowley, MA) at a voltage of approximately 500 mV. This procedure produced a relatively stable ITO-gold interface that could be autoclaved (120˚ C, 30 psi, 10 min), flamed (see below), and maintained under culture medium for over 6 months without breakdown.

Culture Procedure and Maintenance

The MMEP insulation material (Dow Corning DC648 polysiloxane resin) presented a special problem because it was hydrophobic and had to be exposed to a brief (~1 s) pulse from a propane flame to make it hydrophilic (Lucas et al, 1986). Following this procedure, poly-D-lysine (25 μg/ml; 30-70 kD, Sigma) plus laminin (16 μg/ml) were added for substrate preparation. Flaming through an appropriate mask (Gross and Kowalski, 1991) generated two separate culture regions in which cells could adhere: a 1-3-mm-diameter island centered on the 1 mm^2 recording matrix and a 1 x 2 cm domain for a larger number of cells for the purpose of conditioning the medium for optimal growth of the smaller culture in the center.

ICR-Balb-C outbred white mice were obtained from Sprague-Dawley. The animals were maintained in the animal facility of the Department of Biological Sciences at the University of North Texas. Spinal cord neurons were obtained from fetal mice at E14-15

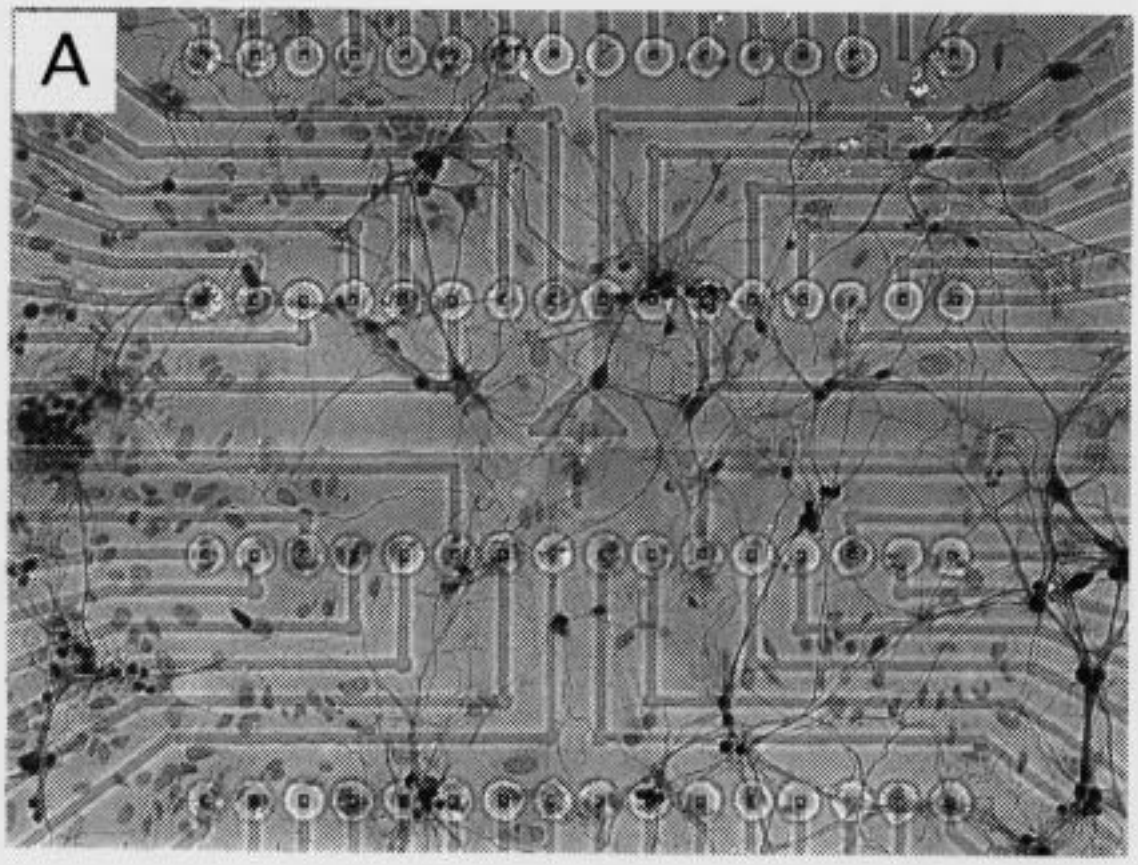
and cultured under sterile conditions (without antibiotics or fungicides) according to the methods of Ransom et al. (1977) with the addition of an enzymatic dissociation step [15 min in 20 units/ml papain (Huettner and Baughman, 1986) and 0.05% DNAse] and a 10% CO_2 atmosphere. Approximately 4 x 10^5 cells (glia and neurons) in a 1 ml aliquot were added to each MMEP with the medium confined to a 4 cm^2 area by a silicone gasket. This produced a monolayer neuronal network overlying a flat, fairly contiguous carpet of non-neuronal cells within each of the flamed adhesion areas. Some of the cultures received cytosine arabinofuranoside-cytosine (from Sigma) treatment on the fourth day *in vitro* to attenuate glial proliferation. Cultures were maintained with about 50% medium change biweekly using fresh medium every 2-3 days. Recording media was identical to culture medium. A low-density culture situated over the recording matrix is shown in Figure 3. Figure 4 shows higher magnifications from the same preparation.

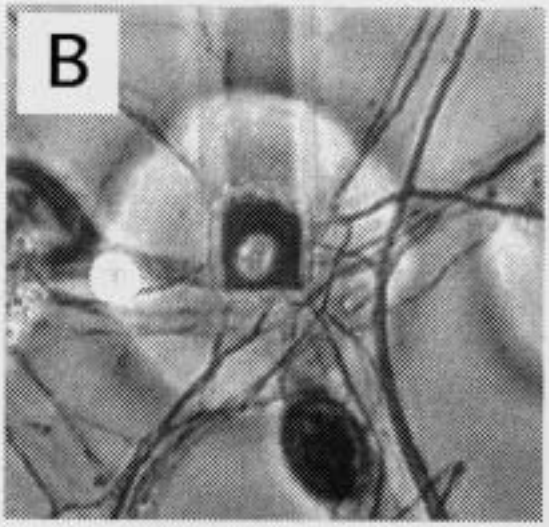
Figure 3. Cultured spinal cord neurons on MMEP.

A. Collage of photographs taken from a low density preparation. In addition to the neurons and neurites visible on the electrode array, many glial cell nuclei can be seen as faint blotches on the MMEP. Loots modified Bodian stain.

B. Greater magnification shows cell-electrode coupling, with focus on the processes crossing the electrode.

C. Focal point on the gold-plated electrode reveals that processes at this recording crater are out of focus and cross that crater at a higher level. This is usually the case when glial cells are situated between the exposed metal and the neurites.


A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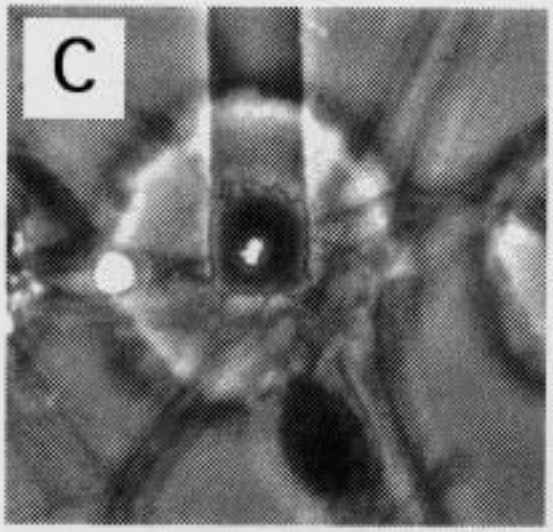
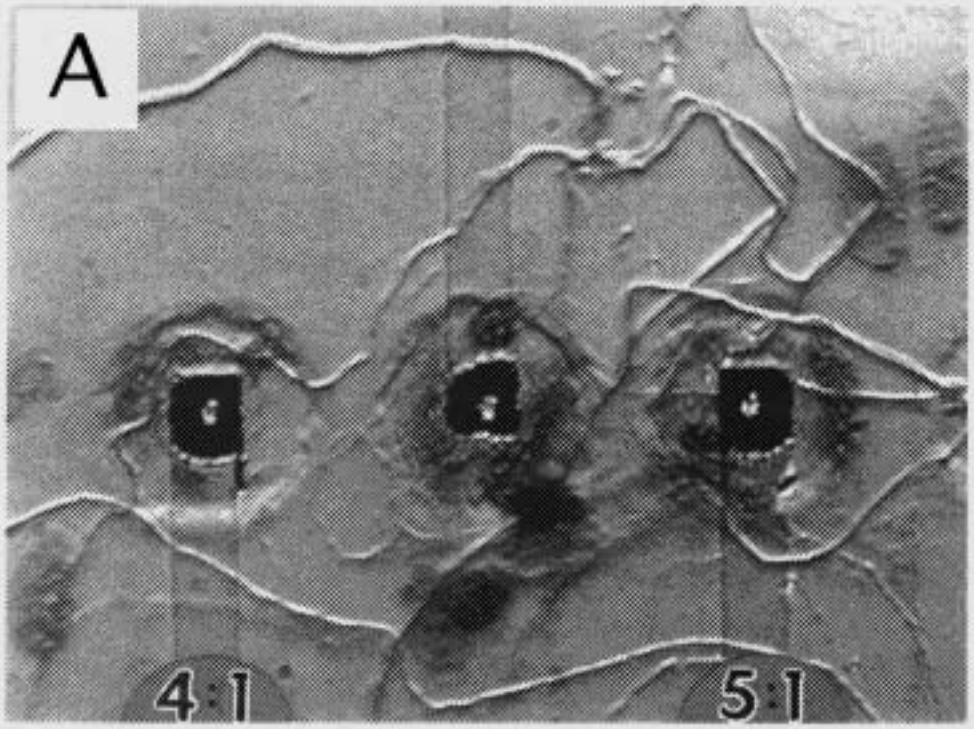
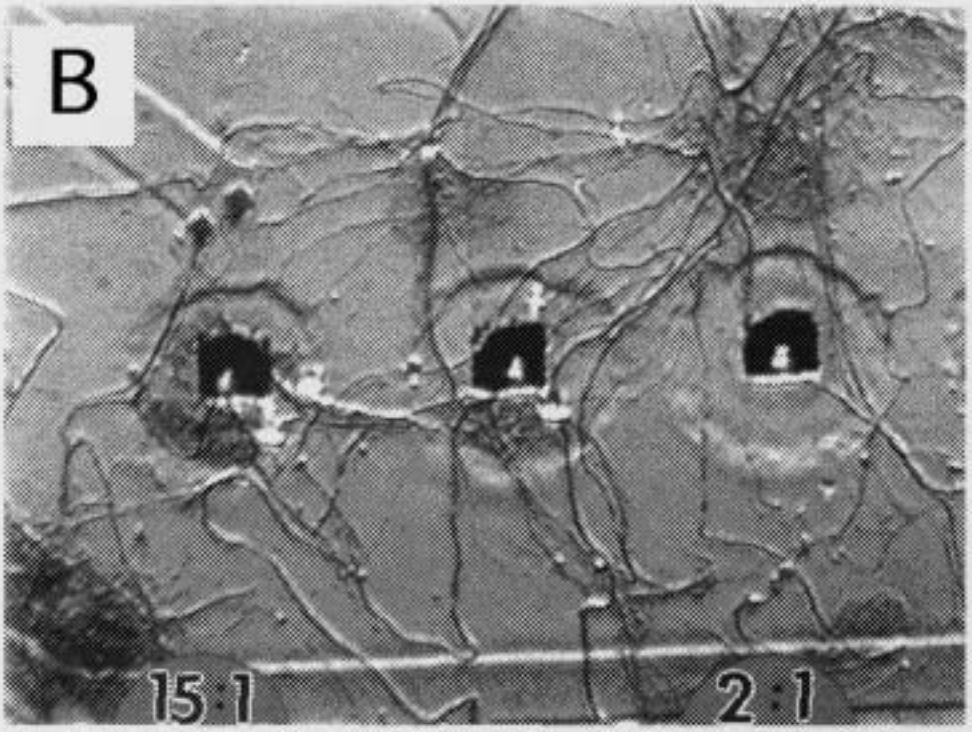

C

Figure 4. Hoffman modulation optics allows visualization of the topography on the MMEP surface. In each panel three gold-plated electrodes are shown along with several neuronal processes. The labels directly below four of the six electrodes shown represent the respective signal-to-noise ratios (SNRs) recorded for those recording channels. Most recordings were obtained from axons and not from cell bodies.


A
4:1
5:1


B
15:1
2:1

Recording Chambers

Both open and closed recording chambers have been developed (Gross and Schwalm, 1994) that allow, respectively, network maintenance in a constant bath of 1-2 ml medium or in a much smaller volume of 0.3 ml under a constant medium flow at 40 μl/min.

The former design was used more often because it allowed for a faster 'turn-around' time needed when a culture was determined to be a poor specimen for stimulation experiments. Most of the experiments were relatively short-term (less than 36 h total time on microscope). The MMEP assembly consisted of an aluminum base holding the MMEP, a stainless steel chamber, and a removable plastic chamber cover that contained a heated indium-tin oxide window. The chamber cover allowed medium changes and visualization of the culture medium color (for monitoring the pH) as well as the network through the microscope. The cover also contained most of the 15 ml/min CO_2 in air mixture for the maintenance of physiological pH. There was a small hole on the side of the cap for venting the air mixture. Two zebra strips (carbon-filled silicone elastomer, Fujipoly., Cranford, N.J) were pressed between the amplifier circuit board and the MMEP parallel ITO output strips to provide electrical contact with the recording matrix (see Figs 5 and 6).

Array Recording

Multielectrode recording was performed with a computer-controlled 64-channel amplifier system (Plexon, Dallas). VLSI preamplifiers (SMU) were positioned on the microscope stage to either side of the recording chamber. The amplifier bandwidth was usually set at 500 Hz to 6 kHz. Activity was displayed on oscilloscopes and recorded on a 14-channel Racal direct tape recorder. Spike data from active channels also were integrated (rectification followed by RC integration with a resulting time constant of 300 ms) and displayed on a 12-channel Graphtek strip chart recorder (Fig 7).

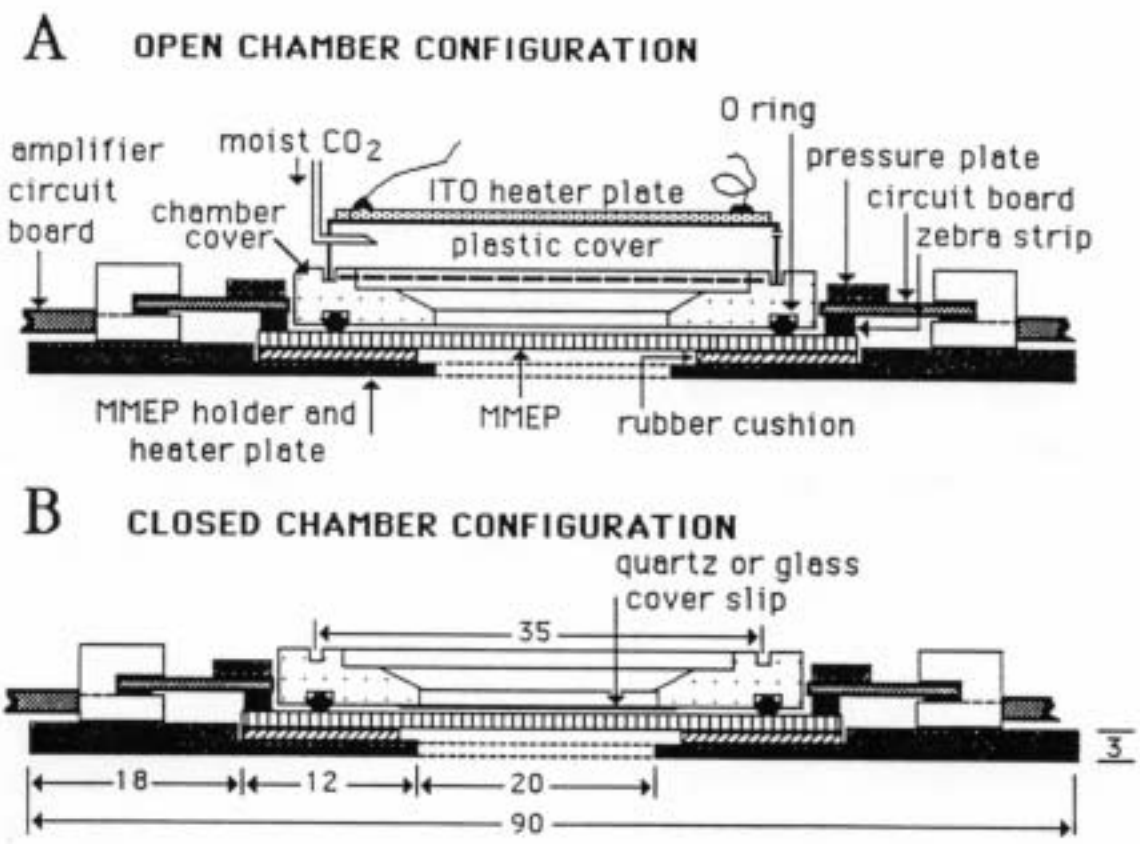


Figure 5. Recording chamber configurations.

A. Open chamber. Primarily used for relatively short-term experiments, or experiments requiring medium changes, or the addition of different pharmacological agents. Medium manipulation or exchange was accomplished by removing the cap and using syringes. The pH was kept stabilized within the physiological range by a gentle flow of 10% CO_2 in humidified air directed into the cap. Condensation within the cap was prevented by a heated ITO plate, making continual observation with inverted microscope possible.

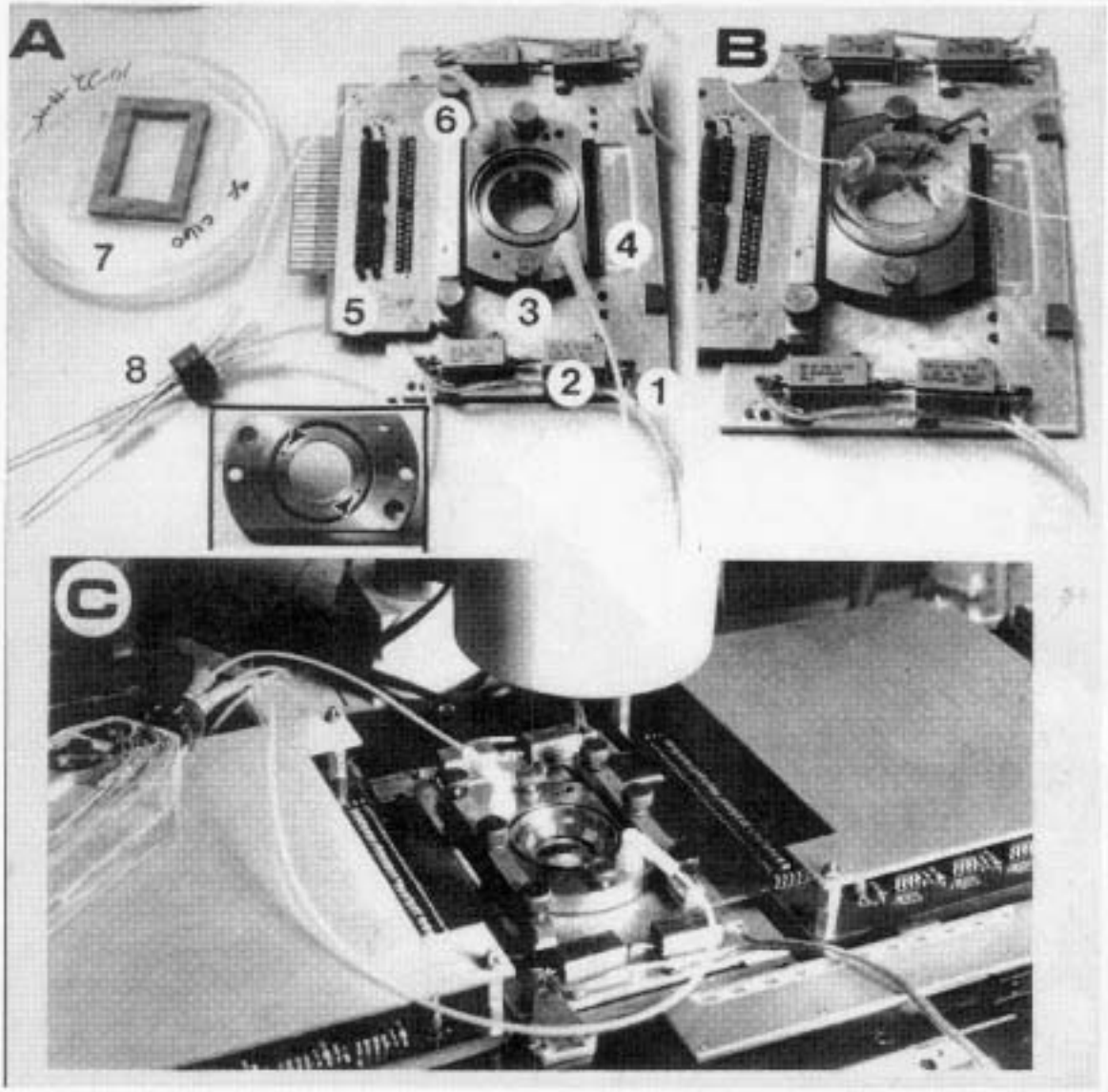
B. Closed chamber. The stainless steel chamber plate had 1 mm diameter channels exiting inside the O-ring and connecting to tubing at the edge the plate. The microscope port was closed with a 22 mm, 750 μm glass cover slip, producing a closed chamber when pressed against the electrode array plate and sealed with the O-ring. The chamber volume was approximately 0.3 ml. A supply flask for conditioned medium with connectors, appropriate tubing and a peristaltic pump completed a medium flow that provided life support in the closed chamber system for well over one week.

Figure 6. Chamber components and assembly (from Gross and Schwalm, 1994).

A. Photograph of a closed chamber during assembly. The chamber consists of a base plate (1) containing four power resistors (2), a stainless steel cover (3) with the microscope port, medium line connections, and set screws; two zebra strips (only the right side visible) placed on the contact strips of the MMEP (4), and two circuit boards (only the left one is shown) that serve to couple the chamber to different multiamplifier systems (5). Pressure bars (6) press the zebra strips between the circuit board and the MMEP. An inverted chamber cover is shown below the closed chamber, revealing the O-ring, the two medium ports (arrows), and the microscope window. Two 1 mm diameter internal conduits lead to the medium ports inside the O-ring domain. The chamber volume formed between the glass electrode plate and the chamber cover is approximately 0.3 ml. This panel also shows a 90 mm petri dish with a MMEP and gasket used for culture seeding and maintenance (7). A rubber cork housing 4 syringe needles (8) fits into a feeder flask of cultured spinal cord neurons and conditioned medium. Two conduits (syringe needles) provide intake and outflow of media while a third conduit connects to a 10% CO_2 humidified air hose. The fourth needle (not visible) provided an exhaust hole for air to escape.

B. Open chamber configuration with plastic cover and heater plate to prevent condensation.

C. Assembled chamber on an inverted microscope with 32 first-stage amplifiers attached to each side directly without a coupling board. A medium supply flask and tubing for medium circulation are also shown.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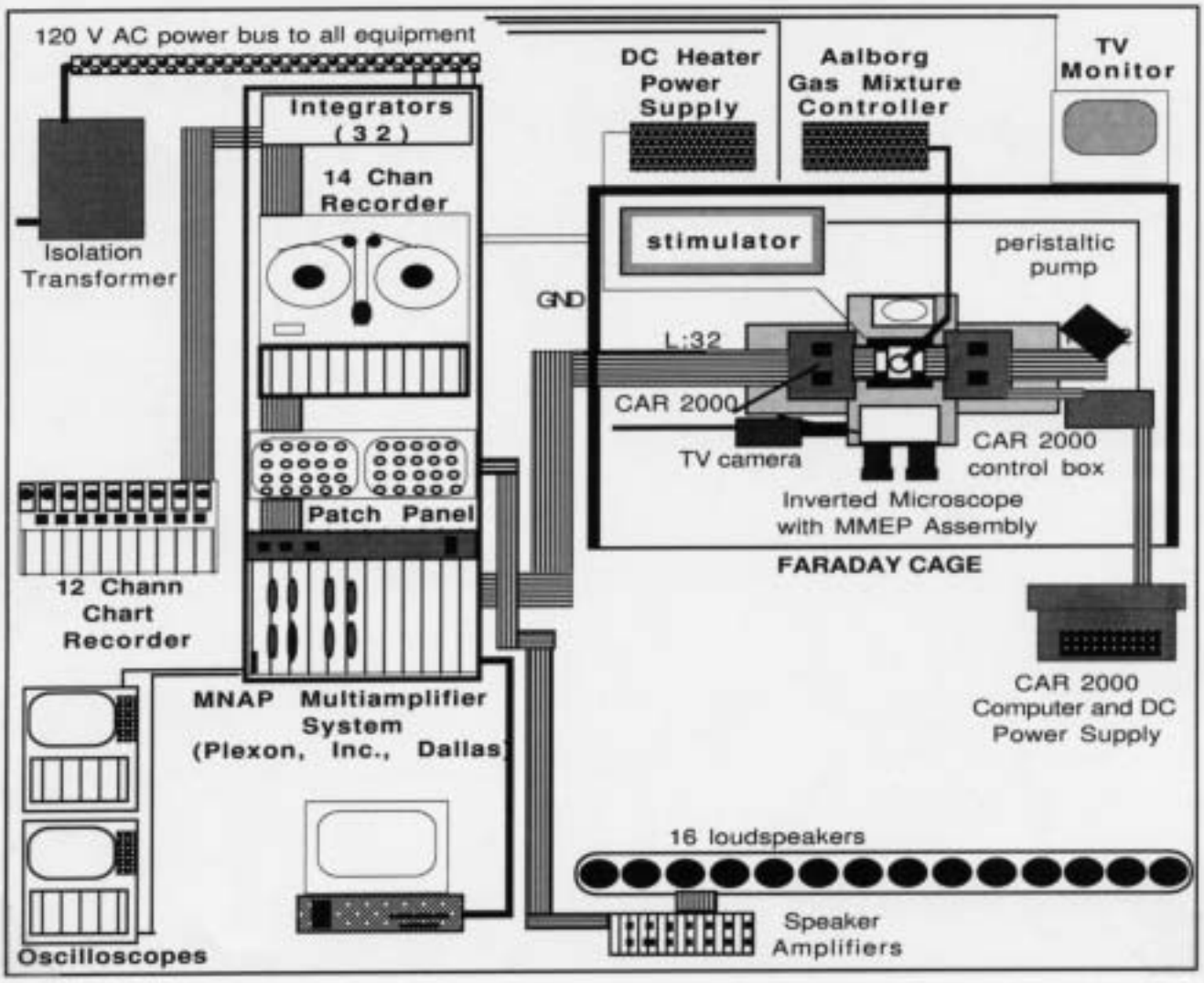


Figure 7. Schematic of multichannel recording station. The network inside the chamber was mounted on an inverted microscope and connected to 2 sets of 32 VLSI preamplifiers (SMU, Dallas) in a Faraday cage. Spike data were fed to 64 second stage amps (MNAP system, Plexon Inc., Dallas). All channels were scanned with computer control. Channels were selected and independently assigned to 4 oscilloscope traces, 32 patch panel locations, 14 tape recorder channels, and 14 computer channels (to a Masscomp 5700). The 32 patch panel channels were integrated (analog RC circuits) and assigned to another 32 patch panel location (left and right sides respectively, of a 64 connector panel). Selected integrated channels were displayed on a strip chart recorder. Raw data channels 1-16 were assigned to the analog tape recorder and to 16 speaker amplifiers. The MNAP system also allowed 16 channels to be digitized realtime for spike separation and spike statistics.

Electrical Stimulation

(A) Spectrum Scientific Preamplifiers. Pulses for electrical stimulation were fed from an isolated pulse stimulator (A-M Systems Model 2100) into the recording circuitry in front of the amplifier coupling capacitors. A series 10 MΩ resistor was used to prevent attenuation of spike signals on the electrodes connected to the stimulator. The reference electrode was attached to the amplifier ground, which was coupled to the stainless steel chamber holding the culture medium. Most stimulation was monopolar, biphasic with the cathodic pulse leading.

(B) SMU Preamplifier System. Stimulus pulses were fed from the A-M Model 2100 into a control box that could accept four different signal patterns simultaneously. Only one signal input line was used and assigned to specific electrodes (one or several) via a computer-controlled switching system. This controller connected the stimulator output directly to one or more electrodes without any serial resistances. The stimulus pulse was fed to the high impedance side of the circuit between the FET amplifier stage and the coupling capacitor that connects to the conductors of the MMEP. This created some distortion of the pulse wave but allowed effective stimulation. A disadvantage of the SMU system was the physical disconnection of input amplifiers from the electrode as soon as the stimulation mode was selected by the controller. This eliminated all responses from that channel until the channel was de-selected and switched to the recording mode (see Fig. 8).

The zebra strip adds a series resistance of 80 Ω as well as a shunt impedance to ground of approximately 30-60 MΩ, depending on the degree of zebra strip compression and proximity to the stainless steel chamber cover. Both the zebra and insulation shunt impedances are sufficiently large and do not cause major signal attenuation.

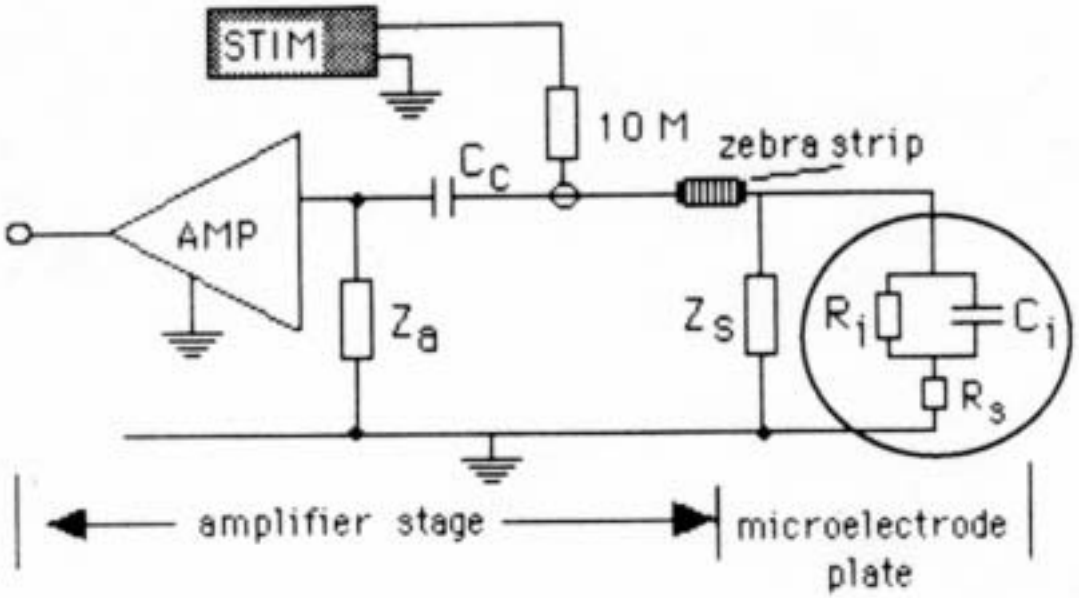


Figure 8. Circuit diagram showing connection of stimulator to the electrode plate and Plexon preamplifier. Unipolar stimulation of single electrodes was accomplished by feeding monophasic or biphasic pulses across a 10 MΩ resistor into the high-impedance side of the circuitry between the coupling capacitor Cc of the input stage and the zebra strip that makes contact with the MMEP. Za: amplifier input impedance; Zs: MMEP shunt impedance. The components of the metal-electrolyte interface at the electrode crater (R*i*, C*i*, and R*s*) represent, respectively, the interface resistance and capacitance, as well as the spreading resistance as described in Robinson (1968). For the ITO-gold-electrolyte interface, these values have not yet been determined (from Gross et al., 1993).

Recording and Stimulation

When the Plexon preamplifier system was used, voltages at the electrode were calculated from the voltage divider circuit shown in Figure 8 by using the following average values: (a) electrode crater impedance: 5 MΩ; (b) MMEP shunt impedance: 20 MΩ; and (c) range of amplifier input impedances: 12-18 MΩ. Studies requiring greater accuracy must take into consideration the variable amplifier input impedances, the differences in electrode impedances, and possible changes in impedances on electrodes used for extensive stimulation. In addition, input voltages beyond 4 V (at the 10 MΩ resistor) saturate the amplifiers for time periods up to 0.8 s. This prevents analyses of short responses from the stimulating channels. Nevertheless, the activity described in this study was derived primarily from network responses in which many units on other electrodes participated.

Experimental Stimulation Protocols:

1. Recorded spontaneously active channels, their maximum signal-to-noise ratios, and the approximate number of units that were detected per channel. If there were very few active channels (e.g., < 10) and/or very low signal-to-noise ratios (≤ 3:1) for the channels that showed activity, then the culture was determined to be a poor specimen and the experiment was terminated. However, sometimes bicuculline or fresh medium was added to the culture to see if the level of spontaneous activity would increase. If there was a sufficient enough elevation of the spontaneous activity, then the experiment would proceed. Bicuculline was thoroughly washed out before continuation of the experiment.

2. Single test pulses [biphasic square waves, 300 μsec each phase, 0.4 - 0.6 V] were given sequentially to each recording electrode and network responses to each stimulus were noted. Network responses indicative of a successful stimulation consisted of audible responses from several recording channels (within less than a second following stimulus pulse) that were patched to the speaker system, and of visual evidence of changed bursting

activity from several channels on the chart recorder. Stimulation channels meeting these criteria were logged along with a rough estimation of the response from the recording channels. Cultures producing very little or no responses to the test pulses were determined to be unsuitable for stimulation studies and the experiment was terminated (see Fig. 9).

3. Selection of specific stimulation protocol for experiment based on the culture's native activity and type of responses to test pulses. Completion of step two provided a list of electrodes that could be used for stimulation and which of this set were the most effective. The channels generating the greatest network response to stimulation were then further stimulated using either or both of the protocols shown in Fig. 10 (alternately) in order to induce changes in network activity. Usually, after a stimulation episode, a period of 15-20 min was allowed to elapse before another stimulation was commenced.

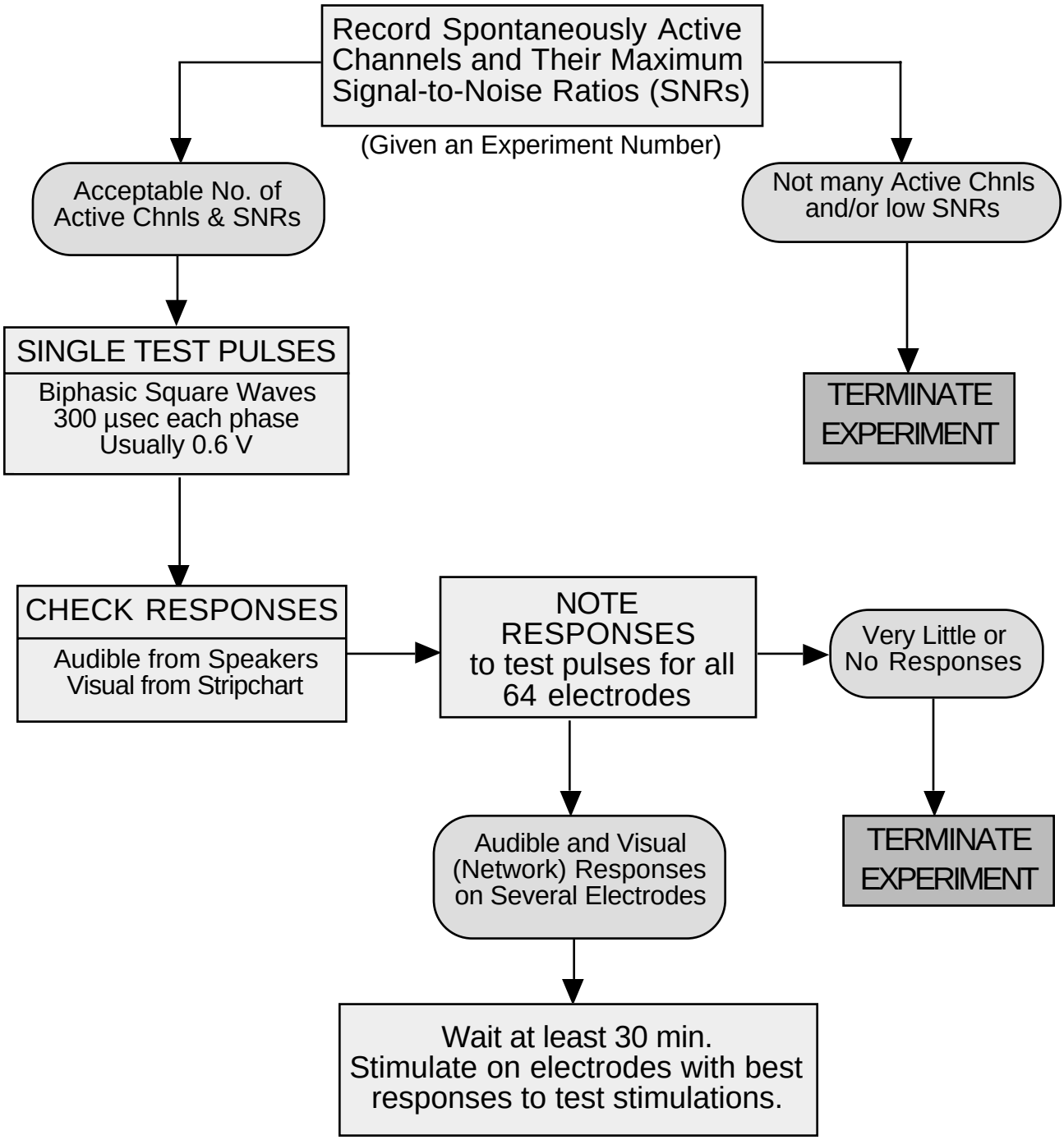
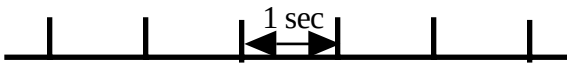


Figure 9. Flow diagram for stimulation experiments. Chart displays steps that were taken at the beginning of every experiment to determine whether a culture would be accepted or rejected. Because every culture for which SNRs were recorded was given an experiment number (for record-keeping purposes), the overall number of completed experiments is less than the total number of cultures used.

Conditioning stimulation protocols

The following stimulation protocols were used:

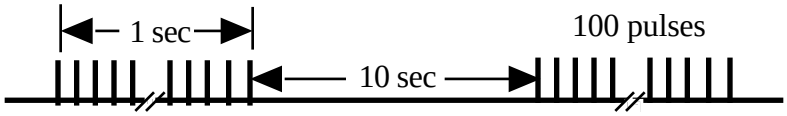
A. REPETITIVE STIMULATION



Single pulses @ 0.5 to 2 Hz.

Episodes are usually 15 -20 min. in duration.

B. TETANIC STIMULATION



Three 1 sec Pulses Trains @ 100 Hz delivered in 10 sec intervals

Figure 10. Schematic representation of stimulation patterns.

A. Repetitive Stimulation or Low Frequency Stimulation (LFS) @ 1 Hz:

B. Tetanic Stimulation or High Frequency Stimulation (HFS) @ 100 Hz:

Data Analysis

Time bins of 1 min were analyzed via a Masscomp 5700 computer. General variables that were examined include the spike rate, burst rate, mean burst amplitude, mean burst duration and mean burst area. However, changes in burst rate were much easier to detect by observation of the stripchart in real-time. Changes in the other variables were more difficult to assess via stripchart observation, particularly when trying to detect changes across 14 recording channels simultaneously. In addition, it was the simplest burst variable to evaluate. Therefore, detecting changes in burst rate was a primary goal in the analysis of network activity.

Real time integration was made possible by simple RC circuits (with integration constants of 0.5 sec,). This spike integration was used as a method of major feature extraction (Figure 11). Integration generated slow voltage changes that were proportional to the spike frequencies recorded and were easily graphed by chart recorders. Integration allowed extraction of bursts, which are generally more easily recognized and measured than spikes. In addition, burst patterns represent a simplified level of activity that often reveal major states or modes of the network activity without massive statistical calculations on very large spike data sets (Gross et al., 1994). If a channel recorded a single unit, then the integrated amplitude indicated the instantaneous spike frequency, and the integrated area under the curve represented the total spike production during the burst. However, most channels recorded more than one unit. Therefore, the integrated burst amplitude was influenced by the number of units, each instantaneous spike frequency, and the action potential size of the various units. Nevertheless, integration provided a useful and informative method of data extraction.

Figure 11. Examples of raw spike data, integration profiles, multichannel digitized data, and computer identification of bursts (from Morefield et al., 2000).

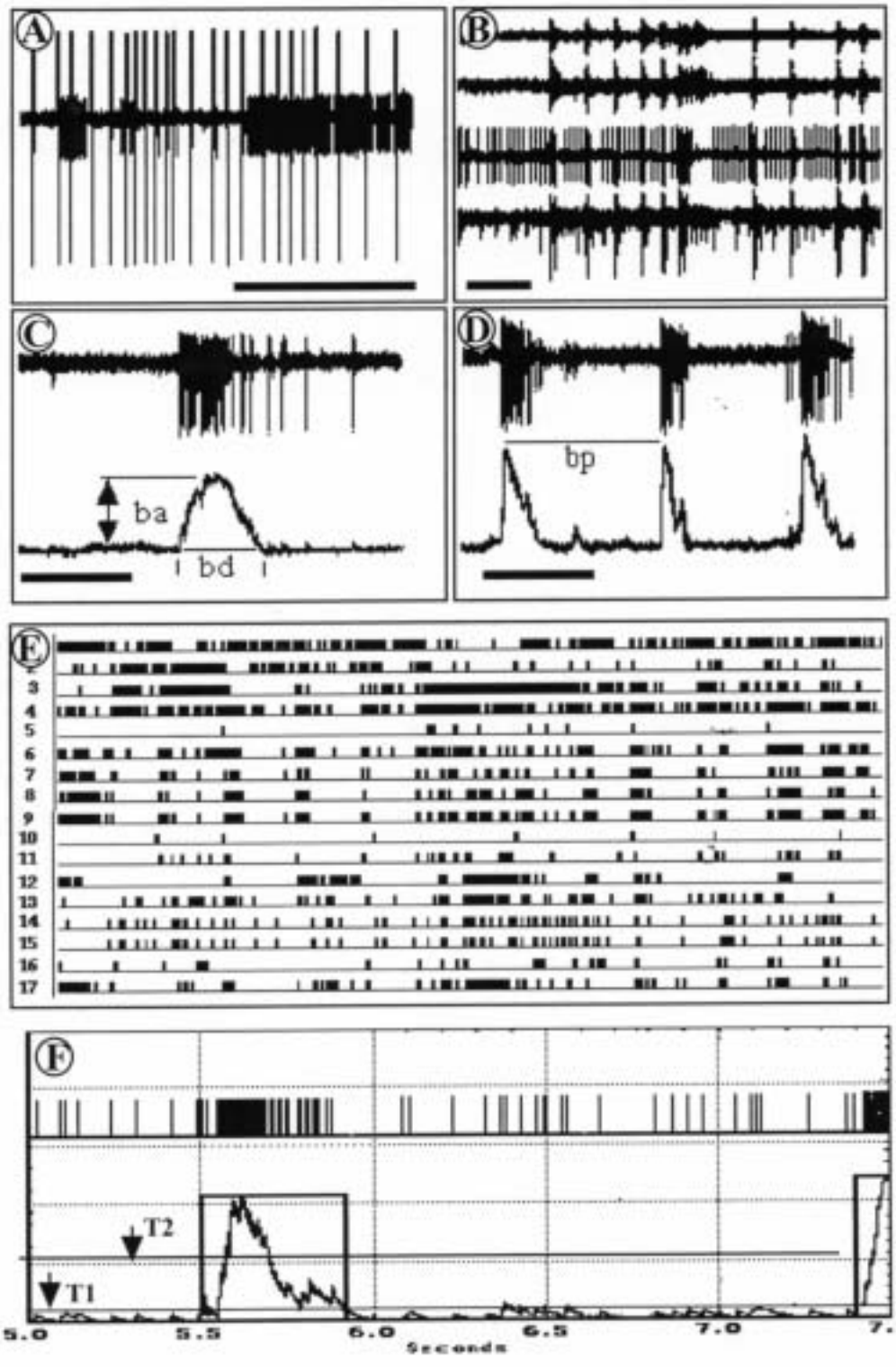
A. Two-unit recording on one channel with spike amplitudes of 700 and 200 μV.

B. Simultaneous, four-channel oscilloscope traces showing coordination of high-frequency spiking (bursts). Each channel is reporting several units.

C and D. Examples of spike data and corresponding integrated profiles (integration constant: 400 ms). Burst amplitude (ba), burst duration (bd), and burst period (bp) can be easily determined and quantified.

E. A 17-channel digital display with low coordination among channels.

F. Two-threshold method of burst identification and quantification. Digitized spikes are integrated and the resulting profile is subjected to two adjustable thresholds: a rapid integration threshold (T1) that determines the beginning and end of a burst (box) and a slow integration threshold (T2) that determines whether a T1-crossing event will be accepted as a burst.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A convenient parameter used to determine channel activity is the total integrated burst area per minute (burst rate * mean burst area), which approximates the total spike production of all units on a specific electrode. Tracing bursts by hand and using a program that then computes the total burst area is another method of analyzing burst data. Counting bursts by hand to determine burst rate was an alternative to computer-aided data analysis and was done periodically to check the data that the computer generated.

Time segments of larger intervals (e.g. 10-20 min) also were evaluated in order to minimize fluctuations in burst parameters that may occur on a minute-to-minute basis. These fluctuations were generally more pronounced in cultures that had an inherently slow burst rate.

Care was taken to allow sufficient time between stimulations in order to maximize differences between changes in spontaneous activity due to common fluctuations and network state drifts and changes due to stimulation. Whenever expedient, single channel activity was summed (or averaged) across all recording channels to obtain a comprehensive "view" of how the overall network was responding.

Spiking activity during stimulation was determined by generating a temporal record of all action potentials detected on each recording electrode within bins of one second duration via the Masscomp computer. The spike rate per second data was useful when analyzing short-term responses to stimulation. For longer term responses, spike rates were organized into one minute bins. These spike rate data were only available for six experiments.

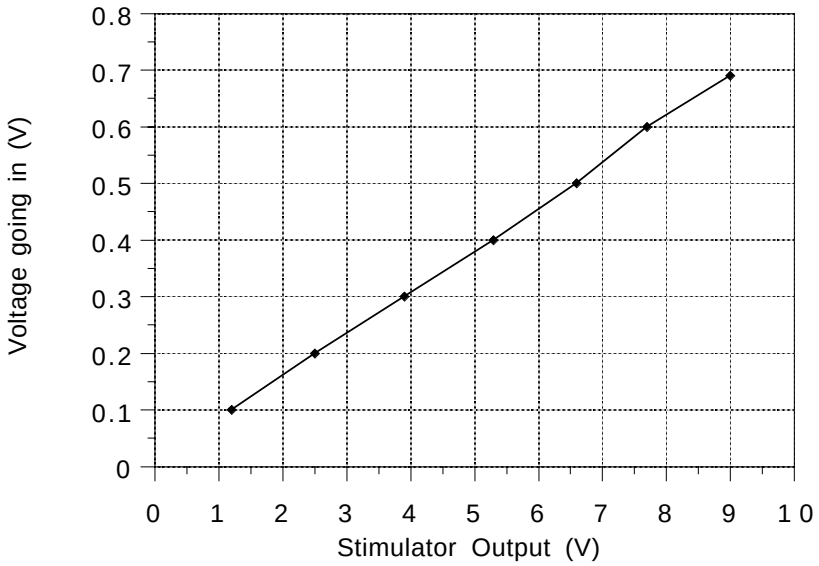
Statistical Analysis

The probability of a random increase in the burst rate has to be very close, if not the same, as the probability of a decrease in the burst rate as long as the magnitude of spontaneous activity is in the midrange levels. Under these circumstances of bidirectional random fluctuations, it is justifiable to assume that there is a gaussian distribution of data points. However, because of the added complexity of averaging across several channels often with non-uniform responses, I chose to use a non-parametric statistical test (i.e. Mann-Whitney analysis) that makes no assumptions about the data. Nevertheless, whenever prudent, statistical tests that assume normal distributions were employed, because analyses that assume normal distributions of responses are highly robust to the effects of nonnormal responses: "Two justifications have been given for applying these procedures when responses are not normally distributed: the central limit property and randomization. In general, these two justifications can be used to support the use of normal and t sampling distributions for the sample mean even with small samples for responses from many nonnormal probability distributions" (Mason, et al., 1989).

Stimulation Environment

To investigate the generation of action potentials at single stimulation electrodes, the Plexon amplifiers were employed. These amplifiers allowed the visualization of action potentials on the stimulating electrode while the electrode was selected and activated for stimulation. This could not be done with the SMU VLSI preamplifier system that was also available for these studies because of the loss of signals on the channel selected for stimulation. The SMU VLSI preamplifier system, however, was technically more efficient, primarily because of computer selection of single or multiple stimulation channels. Unless otherwise stated, all other types of experiments were performed with the SMU system. With the Plexon system the stimulus pulse was delivered through a 10 MΩ resistor in front of the coupling capacitors to prevent loss of biological signal due to the voltage dividing properties created by the low input impedance stimulator (see Fig. 8). A series of biphasic pulses were measured with a digital oscilloscope to ascertain the voltage actually delivered to the Plexon system. This voltage was measured to be 8% of the output voltage from the stimulator (see Fig. 12A). The magnitudes of the pulses were fairly precise (ranging from 7.6-8.3% of the stimulator output--resulting in a variance of 0.24 V) as long as the stimulator output was at or below nine volts (see Fig. 12B). Above 9 V the amplifiers saturated. For most of the experiments performed the stimulus voltage was 8 V (0.64 V at the electrode). Resistance of zebra strip (only 80Ω) was ignored.

A.



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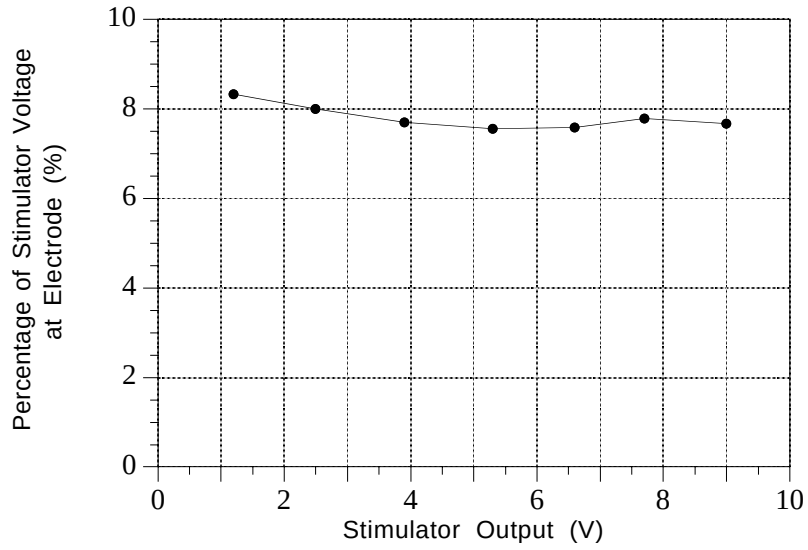

Figure 12.

A. Stimulator output vs actual voltage delivered to the electrode through the 10 MΩ resistor as measured on the oscilloscope (refer to Fig. 8).

B. Percentage of voltage at electrode as a function of stimulus output voltage. The percentage of voltage at the electrode changed little from ~1-9 volts.

Saturation of Amplifiers

For these trials, the stimulus pulse was monitored at the patch panel (see Fig. 7). The maximum amplification factor used was 10,000 with the Plexon system (10X at stage 1) and 10,000 with the SMU system (50X at stage 1). The amplification factor at stage 2 was 100-1000X. The Plexon amplifiers saturated at ~2 mV. There are two levels of saturation: (1) amplitude saturation (resulting in signal "clipping") and (2) amplifier saturation resulting in "blocking" of all signals.

Although I was able to monitor the spike activity on the stimulus channel while using the Plexon preamplifiers, I was not able to monitor responses immediately following the stimulus. The saturation of amplifiers obscure other signals during and immediately after pulse delivery. The duration of saturation is related to the magnitude of the stimulus voltage (see Figs. 13 and 14). This was problematic because relatively large stimulation voltages were often required to elicit network responses.

Short-term responses

These trials were carried out to determine optimal stimulation parameters for single channels before proceeding to network responses. It was considered unlikely that the network would respond to stimuli which failed to elicit responses from single units. Trials with different stimulation parameters showed that evoked responses varied depending on the type of stimulating pulse including pulse duration and voltage. It was found that the optimal pulse duration was ~300 μs. Pulse durations longer than 300 μs did not generate greater responses and pulse durations much longer actually produced a smaller number of evoked action potentials (see Fig. 15). The stimulus pulse intensities at or around 8 V (640 mV at the electrode) reliably produced the maximal response.

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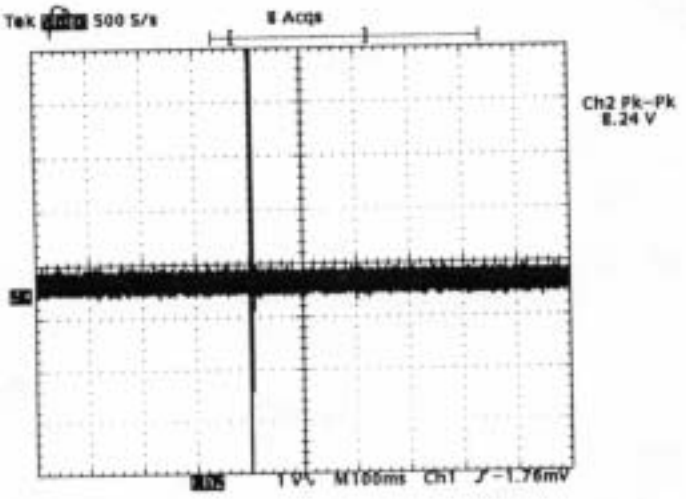

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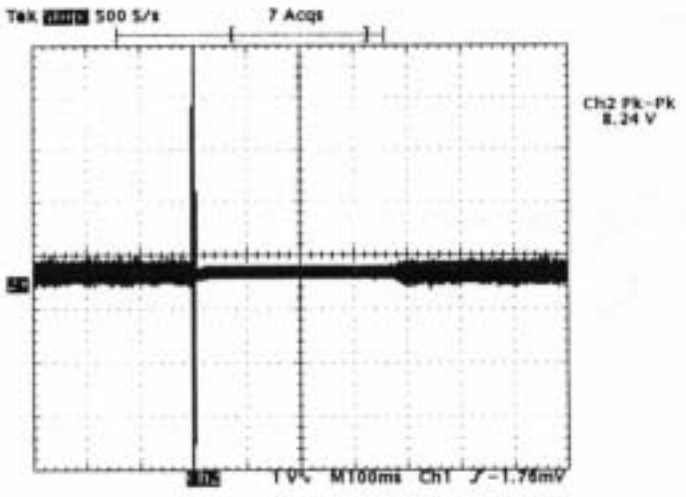


Figure 13. Demonstration of saturation on stimulus channel.

A. At an output of 1.2 V from the stimulator (96 mV at the electrode), there was no saturation of the amplifiers.

B. At 2.5 V (200 mV at the electrode) the stimulus channel was saturated for 380 ms.

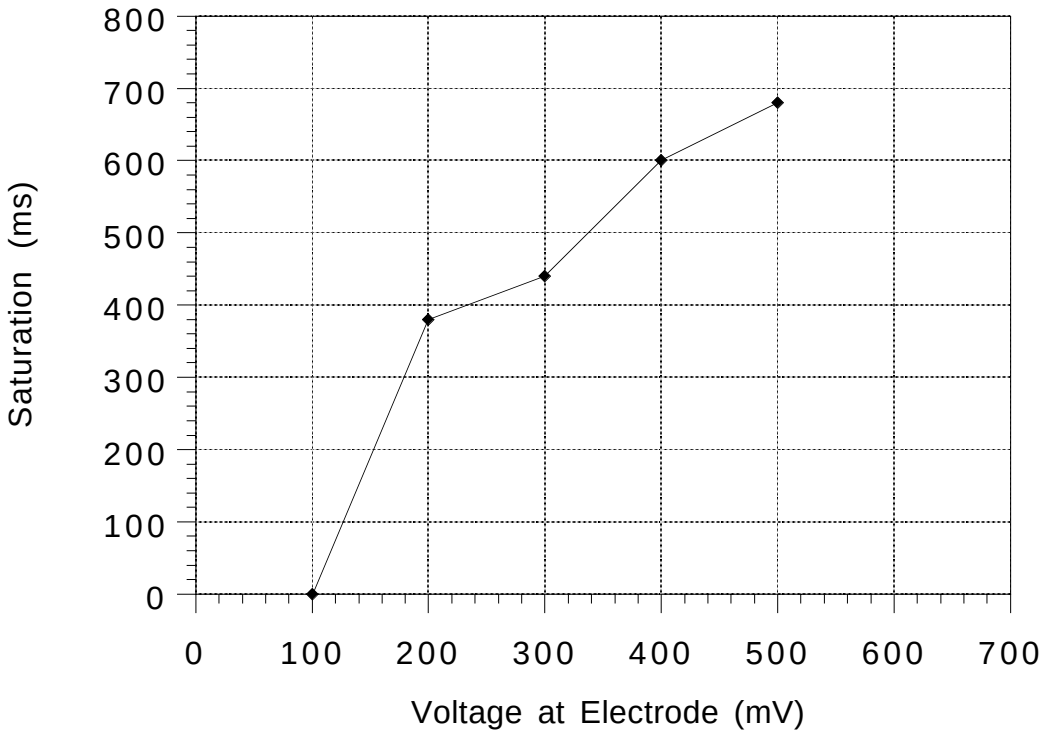


Figure 14. Duration of saturation as a function of stimulus voltage at the electrode. Single, biphasic, 300 μsec (both phases) pulses were delivered at different intensities.

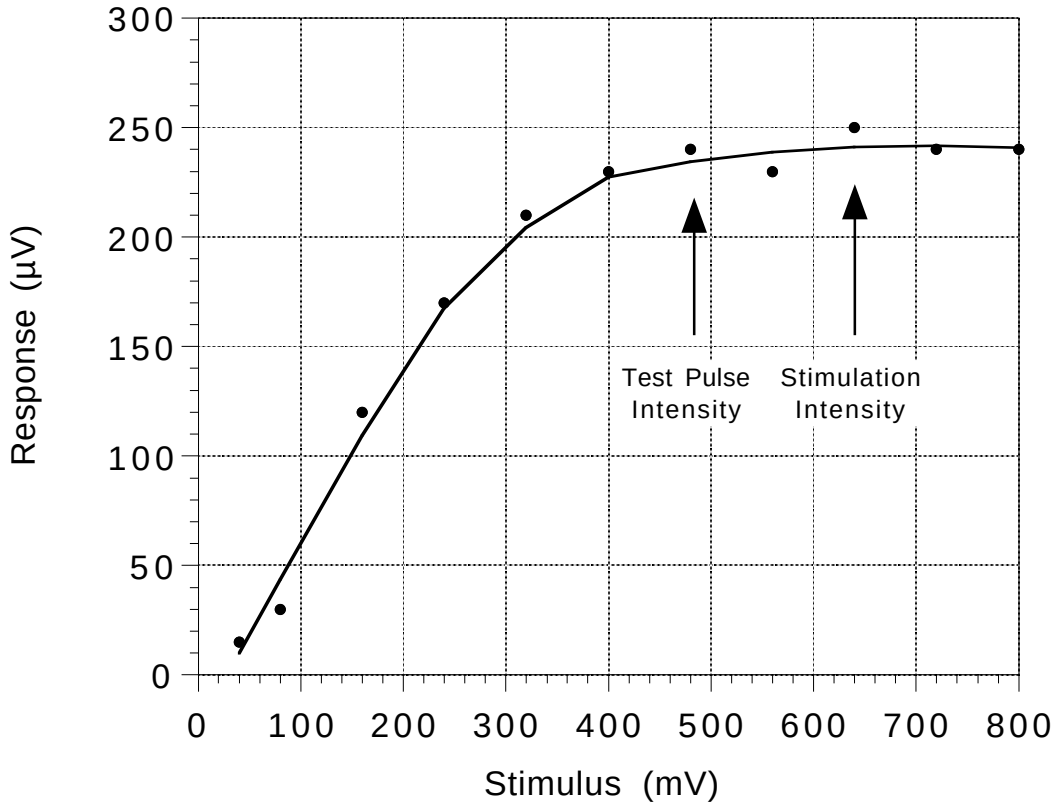


Figure 15. Stimulus-response curve for short-term responses to stimulus intensity. Amplitude of dendritic spike (or CAP) immediately following stimulus pulse increases proportionately with voltage until the maximum amplitude is reached. Arrows show stimulus intensity for test pulses, and normal stimulation.

Curve was fitted to points (weighted 50%).

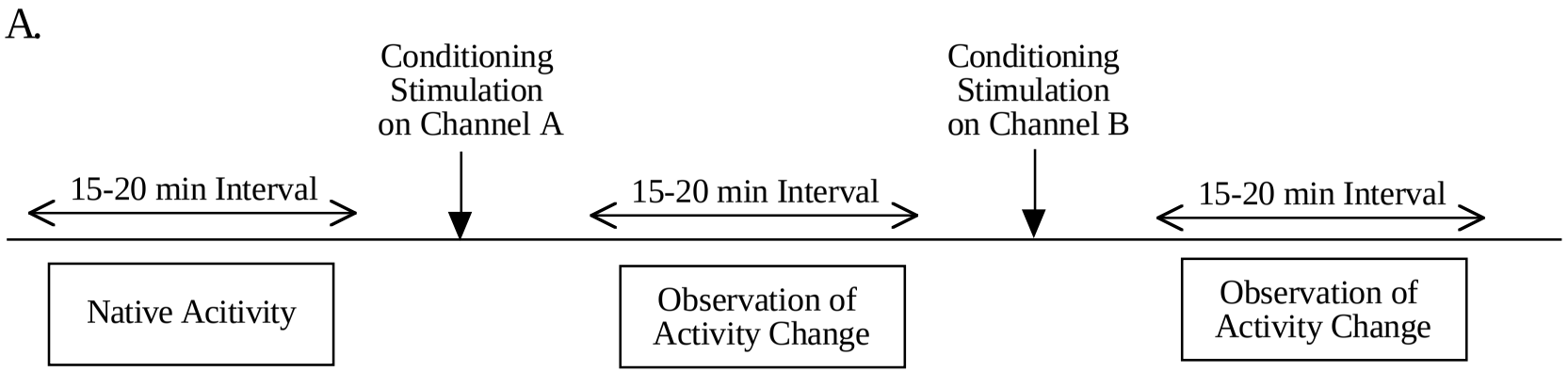
Using the Plexon amplifiers, one can deliver a stimulus of up to 1.2 V without incurring amplifier blocking. However, 1.2 V (which was only ~0.1 V at the electrode) was not an effective stimulus intensity. Nevertheless, stimulus-response experiments with monitoring periods of 100 ms were performed. The time window of 100 ms was considered to be prudent because evoked responses [to which slow PSPs and feedforward and feedback activity from recurrent connections contribute (Buonomano and Merzenich, 1995)] can range in the hundreds of milliseconds (also see Reich et al., 1997). An example of one of the experiments using the longer time window to count the spikes is shown in the Appendix (Figure A-15).

Figure 16 is a generalized time line for the conditioning stimulation experiments. In some trials, the conditioning stimulation on "Channel A" (see Fig 16A) was used as a control stimulation.

Because the primary focus of this project was aimed at changes that occurred after tetanic or repetitive stimulation, other test-pulse/dose-response experiments were performed before and after HFS or LFS. The results from those experiments will be presented later in this manuscript.

Figure 17 is a graphical representation of the general method for the test-pulse experiments. Values from pre-conditioning stimulation test-pulse episodes were compared to post-conditioning stimulation test-pulse episodes to determine if there was a significant change in the number of mean evoked action potentials (MEAPs). These differences in evoked responses were used to supplement the results from changes in spontaneous activity in the same experiment.

Figure 16. General Protocol for Conditioning Stimulation Experiments



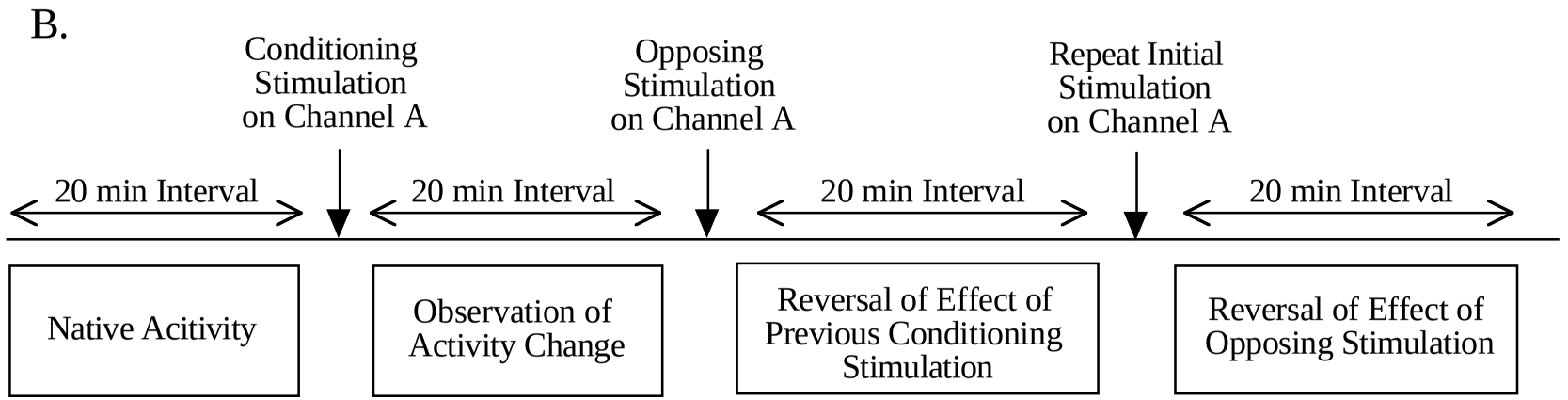
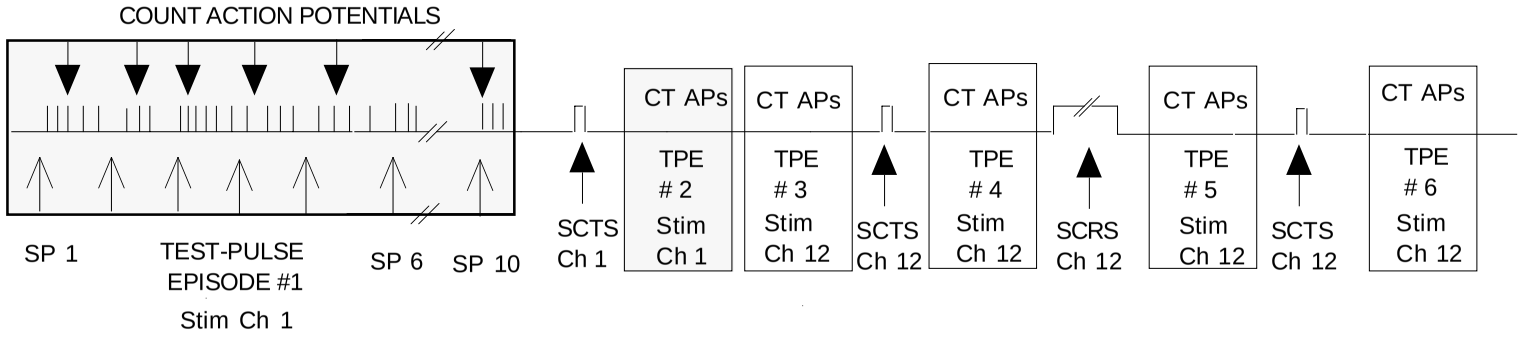


Figure 17. Protocol for test-pulse experiments. Ten to twenty-two test-pulses were delivered before and after low- or high-frequency stimulation. When 22 test-pulses were delivered, the highest and lowest counts were thrown out, and the mean of 20 pulses were used in calculating MEAPs. The stimulation channel(s) for the single test-pulses was the same stimulation channel(s) designated for the conditioning (LFS or HFS) stimulation. Following each test-pulse, all action potentials above the noise-line were counted and logged. Following conditioning stimulation on the same channel (s), the procedure of delivering test-pulses and counting action potentials was repeated.

Shaded area represents stimulation on channel 1--unshaded area, channel 12.

Figure 17. Sequence of Events for Test-Pulse Experiments



Legend: SP = Stimulus Pulse
Stim Ch = Stimulus Channel
SCTS = Single Channel Tetanic Stimulation
SCRS = Single Channel Repetitive Stimulation
TPE = Test-Pulse Episode

CHAPTER III

RESULTS

Chapter 3 Section 1

EFFECTS OF TETANIC STIMULATION ON CULTURED NETWORKS

3.1.1 Background and Specific Methods

Tetanic stimulation has been shown by a number of electrophysiologists to induce changes in synaptic efficacy (for review, see Shors and Matzel, 1997). Most of the time, these modifications result in the enhancement of synaptic transmission. This enhancement, often referred to as long-term potentiation (LTP), is thought by many to be a mechanism for learning and memory. While the vast majority of research involving tetanic stimulation is focused on showing changes in the evoked postsynaptic potentials (EPSPs), little attention is devoted to how these changes may affect the spontaneous activity of small networks. Presented in this section are the results of tetanic stimulation trials delivered to spinal cord networks in culture for the purpose of altering spontaneous activity.

In this study, part of the quantification process consisted of determining the duration of each response to tetanic stimulation from integrated spike data displayed on stripcharts. The beginning of a response was identified as a clear change of activity from the pre-stimulus activity. The cessation of the response was characterized as the return of spontaneous activity to pre-stimulus levels. Responses were classified as short or long-term based on the duration of responses. Long-term responses to HFS were defined as a 10% change in mean spontaneous activity over a 15 min interval relative to pre-stimulus activity, with the expected response being an increase in activity.

Single exploratory pulses of 480 mV were given sequentially on all electrodes at the beginning of every experiment to determine which electrodes could trigger a network response. These pulses should not be confused with test stimulation pulses delivered before and after a tetanic stimulation episode. The purpose of test pulses was to determine whether or not there was a change in the number of evoked action potentials following tetanic stimulation in comparison to pre-tetanic stimulus activity.

For single vs. multichannel tetanic stimulation, usually two to four single electrodes were selected from the pool of effective stimulation electrodes. Tetanic stimulation [three 1-second trains delivered at 100 Hz at 10-second intervals] was then delivered to different channels to determine if specific stimulation sites were critical to the nature of the response. Following stimulation on single channels, two or more of the selected channels were stimulated simultaneously. As in almost all stimulation experiments, at least 15 min were allowed to elapse before the next stimulation was begun to allow time for a response and to establish a new baseline of pre-stimulus spontaneous activity for the next stimulation.

Test-pulses were usually delivered 10-15 min before the stimulation to determine pre-stimulus mean evoked action potential (MEAP) values and 10-15 min following tetanic stimulation to allow some modification of the circuit before the onset of the test-pulse trials to collect post-stimulus MEAP values. While this methodology may miss the short-lived responses, it was deemed more important not to interfere with the natural progression of events leading to changes in spontaneous activity by further stimulating the tissue. It is well documented that single pulses delivered in a repetitive manner often result in the depotentiation of potentiated synapses (Turner and Miller, 1982; Bramham and Srebro, 1987; Xiao, et al., 1994; Barr, et al,. 1995). The means of the test pulse responses from all four recording channels were averaged to determine a grand mean in order to facilitate quantification of the changes. Because there was usually more than one unit counted on the different recording channels, a grand mean probably is more representative of network

evoked responses.

3.1.2 Overview of Responses to Tetanic Stimulation

Below are two common examples of responses to tetanic stimulation. Figure 18 shows that tetanic stimulation accelerated the bursting activity across several channels. Figure 19 exhibits a myriad of responses that can occur on different recording channels following tetanic stimulation.

The most common change following tetanic stimulation was an increase in the overall spontaneous activity. The burst variable most easily detected was the burst rate (see Fig. 18). Although the figure represents the most common nature of the change, most of the changes in burst rates were not as pronounced as this example. Indeed, in many trials the change in burst rate was relatively subtle. On some occasions, there was an obvious increase in spike production but not a comparable increase in the burst rate (see Fig. 19).

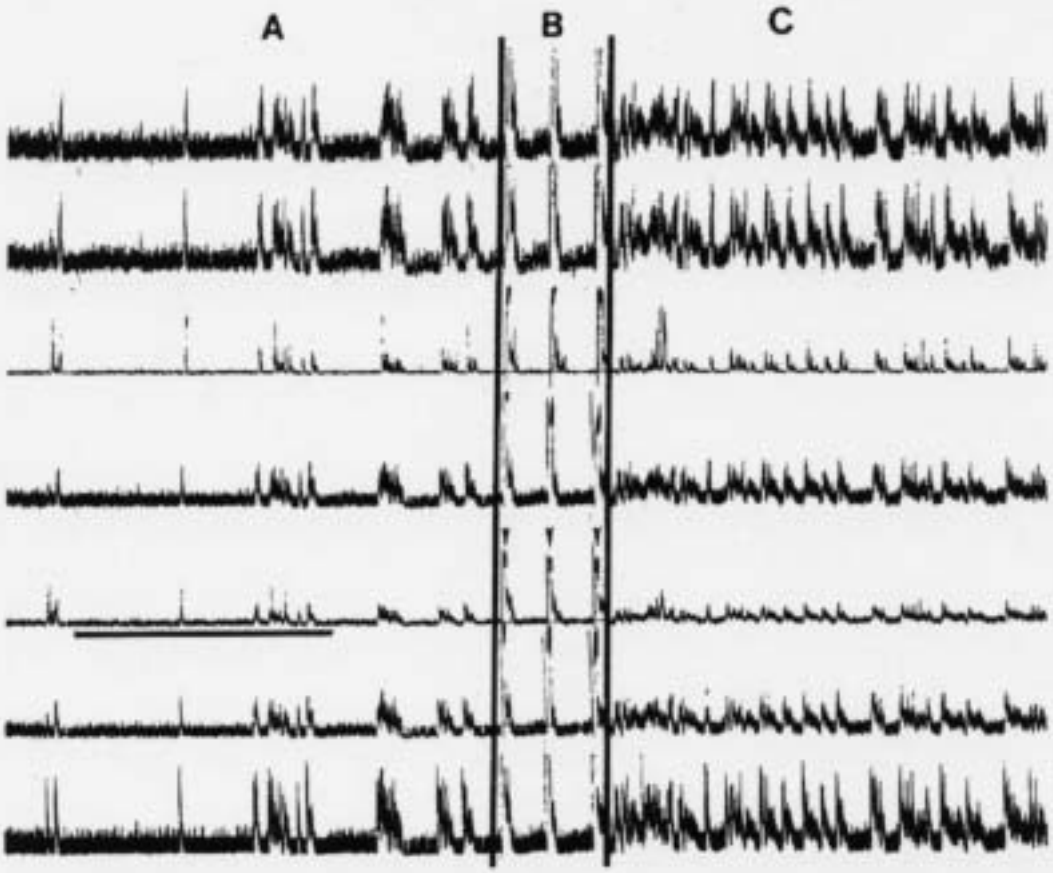
Figure 18. Example of response to single channel tetanic stimulation. Spontaneous bursting activity was integrated and recorded on a stripchart. Approximate integration constant: 700 ms.

A. Pre-stimulus activity recorded on seven channels simultaneously.

B. Characteristic stimulus artifacts (arrowheads) of the tetanic stimulation pattern used in these experiments.

C. Network response showing an immediate increase in burst rate on all channels.

Time bar = 1 min.


A
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C

Figure 19. Six-channel integrated spike data on stripchart showing short-term changes in activity following multi-channel tetanic stimulation.

A. Native activity. The bottom trace represents activity on one of the stimulus channels, and shows amplifier blocking (between arrows) during the time the channel was activated for stimulation.

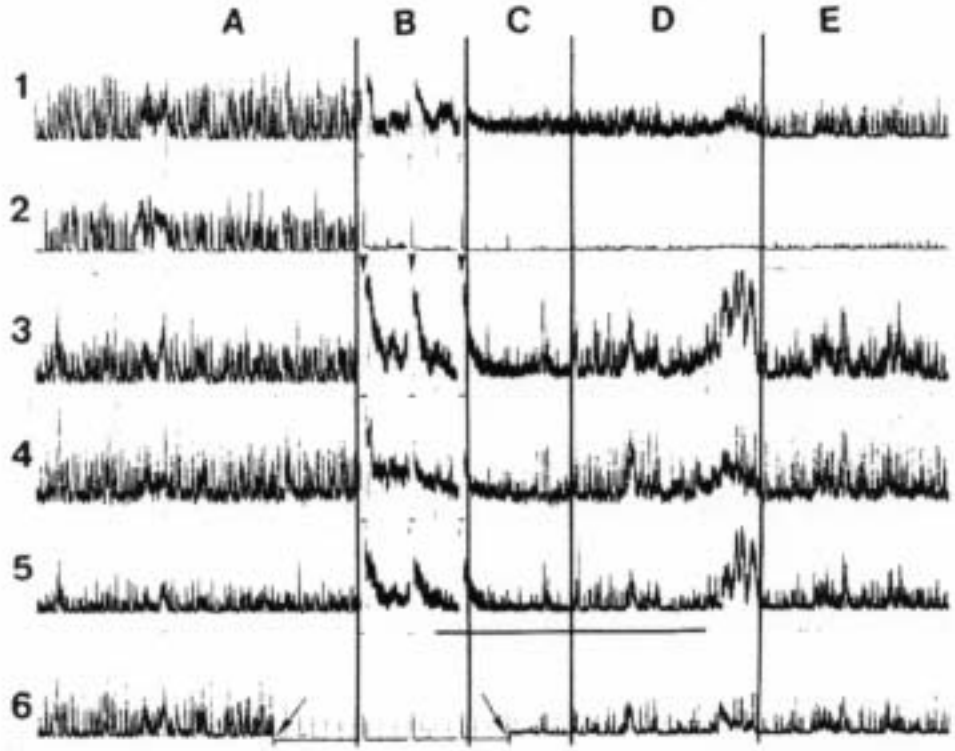
B. Activity during tetanic stimulation (at arrows). Notice inhibition during and following MCTS on lane 2.

C. Short period (20 sec) of inhibition displayed across all channels, and a delay to onset of post-tetanic paroxysmal bursting on some channels.

D. Increase in bursting activity that erupted into paroxysmal bursting in lanes 3-5.

E. As activity begins to return to pre-stimulus patterns, activity in lane 2 begins to slowly reappear. The activity in lane 2 returned to pre-stimulus levels after 3 min (data not shown).

Time bar = 1 min.


A
B
C
D
E
1
2
3
4
5
6

In Figure 19, lane 1 shows an obvious reduction in organized bursting following MCTS; however, the activity seen in segments C and D reveals intense, tonic spiking. Thus, although this response shows little bursting activity, it is not clear whether it reflects an actual reduction in overall neuronal output. The only channel that showed a distinct decrease in overall activity following MCTS for the entire data segment is shown in lane 2. This trace exhibits an example of almost total depression of activity on a recording channel following stimulation. Lanes 3-5 display a pronounced depression of bursting activity after MCTS followed by a pattern of activity similar to pre-stimulus activity, and paroxysmal bursting activity (D). This type of activity, also referred to as epileptiform activity, introduces a problem when only burst rates are measured as the computer detected only a single burst on lane 3 because of the failure of integrated activity to reach baseline. Activity patterns on lanes 4 and 5 return to pre-stimulus profiles in period E. Lane 6 depicts an example of how activity is blocked when a stimulus channel is selected for stimulation (SMU amplifiers). Thus, although changes in activity are evident on all channels, quantifying these changes is problematic. For the channels that responded with paroxysmal bursting to TS, there is a clear overall increase in spike activity. However, the number of bursts per minute actually decreased during this period due to the increased duration of the paroxysmal bursts.

Short-term responses to tetanic stimulation occurred much more frequently (~80% of trials) than long-term responses. This short-term enhancement of spontaneous activity was often in the form of paroxysmal bursting (see Fig. 19), which resulted in increased burst durations. Because of this, the mean burst duration (mbd/min) was also taken into consideration when assessing changes in activity. These data, shown in Figure 20, are an example of how drastically the burst duration can be affected. To counterbalance the occurrences of long burst durations, the total burst area per minute (TBAPM) was calculated. Since this parameter takes into account the mean burst area per minute as well as the burst rate, it is representative of the total spike production or overall "output" per minute of the units being recorded (see methods section-Chapter 2). Because the spike rate could not be assessed directly in every experiment, the TBAPM was calculated as an indicator of spontaneous activity.

Another interesting feature encountered when evaluating network responses to stimulation was the different effects seen on different channels (Fig. 19). One can clearly observe the almost total inhibition of activity immediately following the MCTS in lane 2, while simultaneously lane 3 displayed paroxysmal bursting. In these instances, calculating a "network" response to tetanic stimulation meant a reduction of the maximum responses in the averaging process. Nevertheless, to maintain a consistent data analysis approach, the averaging of responses across channels was performed in every experiment that was analyzed quantitatively.

Figure 20. Mean burst duration (per minute) as a function of time for each recording channel. Notice that only one channel showed a large increase in burst duration after the first MCTS. While the second MCTS failed to produce such an effect on the same channel, there were more channels that showed an increase in burst duration.

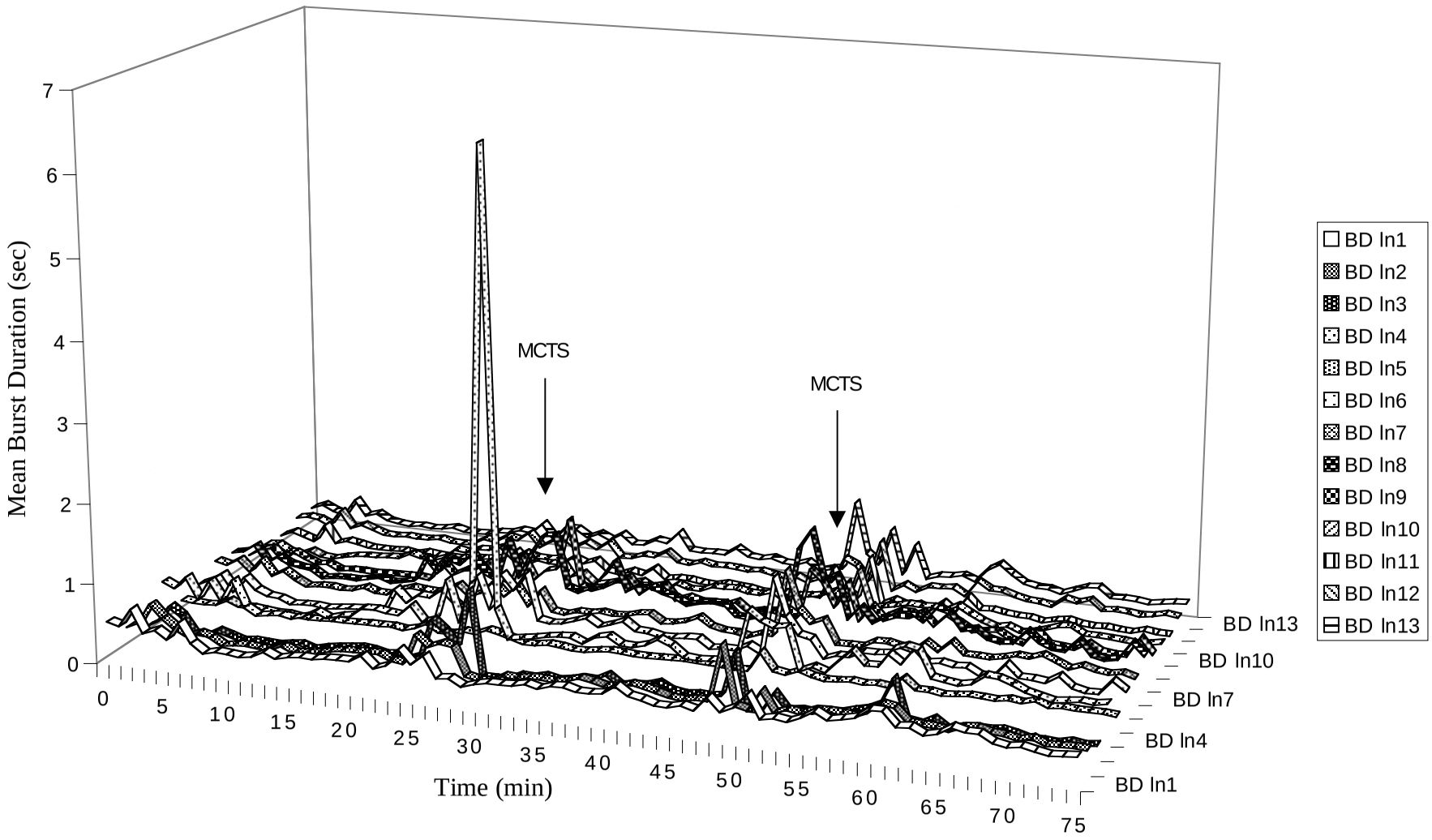

Mean Burst Duration (sec)
0
1
2
3
4
5
6
7
MCTS
MCTS
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
Time (min)
BD In13
BD In10
BD In7
BD In4
BD In1
BD In1
BD In2
BD In3
BD In4
BD In5
BD In6
BD In7
BD In8
BD In9
BD In10
BD In11
BD In12
BD In13

Table 2 shows the data set of experiments from which tetanic stimulation results were compiled. Network responses were collected directly from the stripchart for a total of 34 cultures. The total number of trials was 96 (59 single channel and 37 multichannel).

Table 2. Tetanic Stimulation Data Set

Culture #	Expt. Date	Age (days)	SNR	Active Elec (%)	NRE %	SCTS #	MCTS #
30	8/10/94	72	2.8	55	39		1
31	8/16/94	86	2.3	70	52		1
50	3/15/95	76	3.1	25	33		2
51*	3/22/95	111	3.0	78	52	1	5
62*	8/3/95	133	3.8	76	48		1
66*	9/14/95	132	6.0	69	64	3	1
70	10/4/95	89	4.0	41	41	3	3
76	10/22/95	41	3.3	50	57	5	3
79*	10/28/95	56	3.4	43	52	5	1
80*	10/30/95	39	2.7	66	48	5	2
91	3/31/96	103	1.9	66	51	1	
99*	4/14/96	81	3.0	72	72	4	2
123*	8/20/96	60	3.9	66	40	1	
124	9/4/96	102	2.6	51	49		1
126*	9/11/96	81	2.1	55	53		
130	10/16/96	75	3.8	40	39	1	
131*	10/23/96	68	3.2	40	30	1	4
133	11/6/96	134	4.4	92	65	4	1
135*	11/13/96	43	4.3	88	39	4	
136	12/11/96	68	4.2	41	39	4	
140	1/8/97	72	3.0	30	2	2	
147	1/29/97	68	1.8	59	20		4
148	1/30/97	56	1.8	26	12	1	4
149*	2/5/97	60	4.5	78	70	6	
150*	2/12/97	40	2.6	44	49		

152*	2/26/97	40	4.7	49	33		
153*	3/9/97	53	4.2	56	42		
154*	3/26/97	50	3.0	45	47	1	
159*	4/17/97	84	3.5	58	19	1	
165	4/21/97	75	3.2	49	28	1	
166*	4/23/97	84	5.7	93	63		
167*	5/7/97	54	3.3	40	33	3	1
173*	7/21/97	114	2.8	30	26	1	
176*	9/10/97	60	2.6	61	59	1	
34		Mean: 75 ± 27.0	Mean: 3.4 ± 1.01	Mean: 56 ± 18.7	Mean: 43 ± 16.6	*59*	*37*

Legend: SNR = Mean signal-to-noise ratios (includes values of 1.5:1.0); Active Elec (%) = percentage of electrodes recording active unit(s); NRE = Network response electrodes (percentage of the recording electrodes that, when stimulated with a test pulse, caused a network response--includes electrodes on which no spontaneous activity was detected); SCTS # = Number of single channel tetanic stimulations delivered; MCTS = Number of multi-channel tetanic stimulations delivered. Starred items represent experiments in which data were collected on computer. Italicized values represent a summation of the column. Values following means represent the standard deviation of the means.

Visual inspection of integrated bursting activity following tetanic stimulation led me to the conclusion that changes in burst rate was the simplest activity parameter to detect from stripchart data, so that parameter was selected for evaluation. The total number of trials for the stripchart analysis was 89 (57 single channel and 32 multichannel). Table 3 summarizes these responses from qualitative, visual inspection of this stripchart data (experiments C-30-149). The totals differ from Table 2 because the Table 3 only contains trials for which there was a response to tetanic stimulation. Table 2 includes all tetanic stimulation trials.

Table 3A. Tetanic Stimulation Summary

Stim Type	Number of Trials	Increase in Activity‡ (>50% of Chs / <50%)	Decrease in Activity	No Effect	Mean Network Delay Time
SCTS	57	47% (39% / 9%)	11%	42%	1.1 ± 3.2 min
MCTS	32	59% (47% / 12%)	0	41%	0.3 ± 0.6 min
Both	89	52% (42%/ 10%)	8%	42%	

‡Burst rate

SCTS=Single channel tetanic stimulation; MCTS=multi-channel tetanic stimulation

Legend: The values do not include changes in other burst parameters (i.e. burst area, burst duration, etc) or other pattern changes. Values in parentheses provide a crude interpolation of how widespread the effect was throughout the network. For example, 47% of the 57 SCTS trials resulted in an increase in the burst rate. In 39% of the 57 trials, over 50% of the recording channels showed an increase, whereas 9% of the trials showed an increase on less than half of the recording channels.

Table 3B. Durations of Effects of HFS

Stim Type	Mean Duration of Increase (min)	Mean Duration of Spontaneous Decay (min)
SCTS	9.0 ± 20.10* (n= 22)	2.2 ± 4.17 (n=15)
MCTS	5.9 ± 15.2* (n=15)	1.2 ± 1.18 (n=12)

Legend: Starred values include durations (measured from the beginning of the effect to the decay back to baseline) that were interrupted (i.e. cut short) by another (repetitive) stimulation, and not allowed to decay spontaneously. The duration of spontaneous decay is the time from the beginning of the effect to the time that the burst rate decays back to the pre-stimulus level.

3.1.3 Short-term Responses to Tetanic Stimulation

Increases in activity following tetanic stimulation ranged from a few seconds to over an hour. Based on empirical data in Figure 21A, the durations were divided into two categories: those less than 15 min were termed short-term spontaneous activity potentiation (STSAP), while durations of 15 min and longer were designated as long-term spontaneous activity potentiation (LTSAP). Data for response durations were collected from trials in Tables 2A and 2B. That is, any recorded instance in which there was a noticeable increase in activity following tetanic stimulation was included in Figure 21A. The cessation of the response was defined as a return to pre-stimulus activity (or baseline) levels assessed by visual inspection. There were 48 instances in which an activity increase was noted in 96 trials (50%). Of this total, 39 responses were classified as STSAP (81%). Figure 20B shows a histogram of the STSAP durations. As one can determine from the graph, durations of less than one minute occurred with the greatest frequency (59% of all STSAP responses).

The onset of the response was considered to be an obvious elevation (or depression) of spontaneous activity relative to baseline levels. Most of the time, there was no delay between the cessation of the stimulus and the onset of the response (see Fig. 22). However, on some occasions, several seconds to a few minutes elapsed before commencement of the increase of bursting activity (see Fig. 19C & D). As suggested by Table 3A, the likelihood for a delay following tetanic stimulation was increased if the stimulus was on a single channel rather than on several channels.

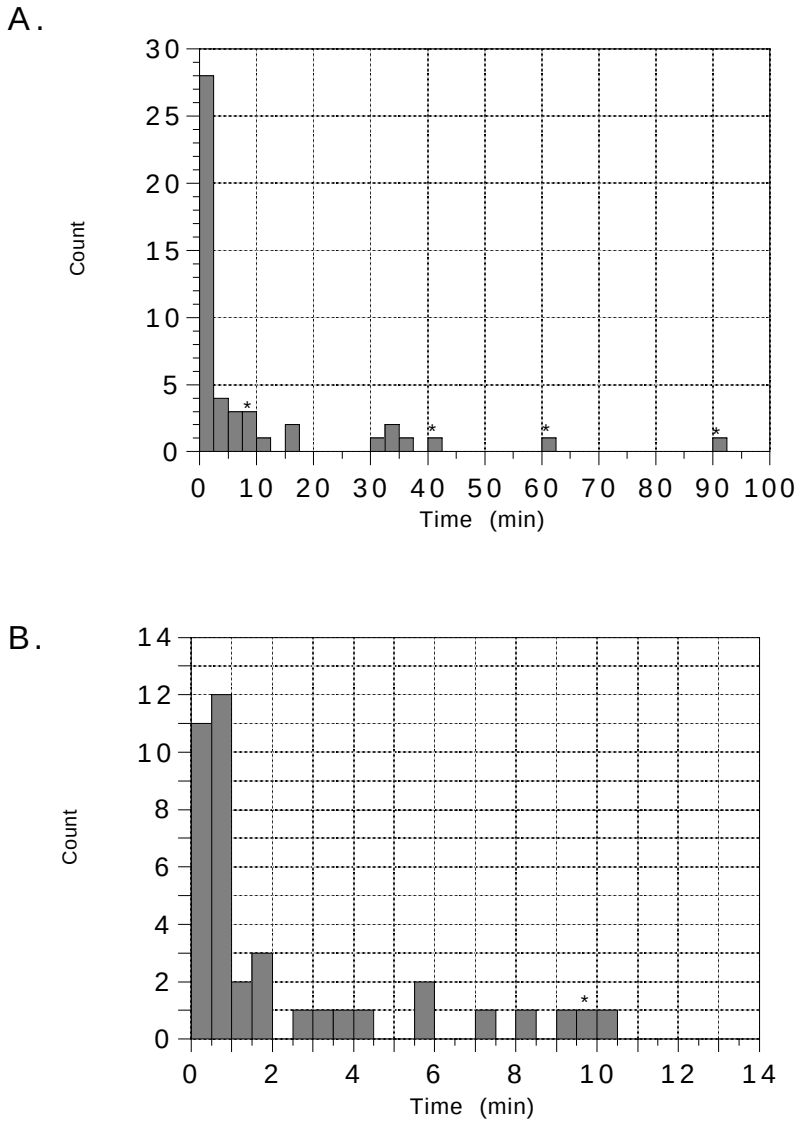


Figure 21. Frequency histograms of response durations (increases only) to tetanic stimulation. A. Graph includes durations of responses to both SCTS and MCTS. The activity parameter measured was burst rate (48 trials total). Starred items represent occasions when the response was cut off by repetitive stimulation.

B. Short-term responses only (subset of Graph A).

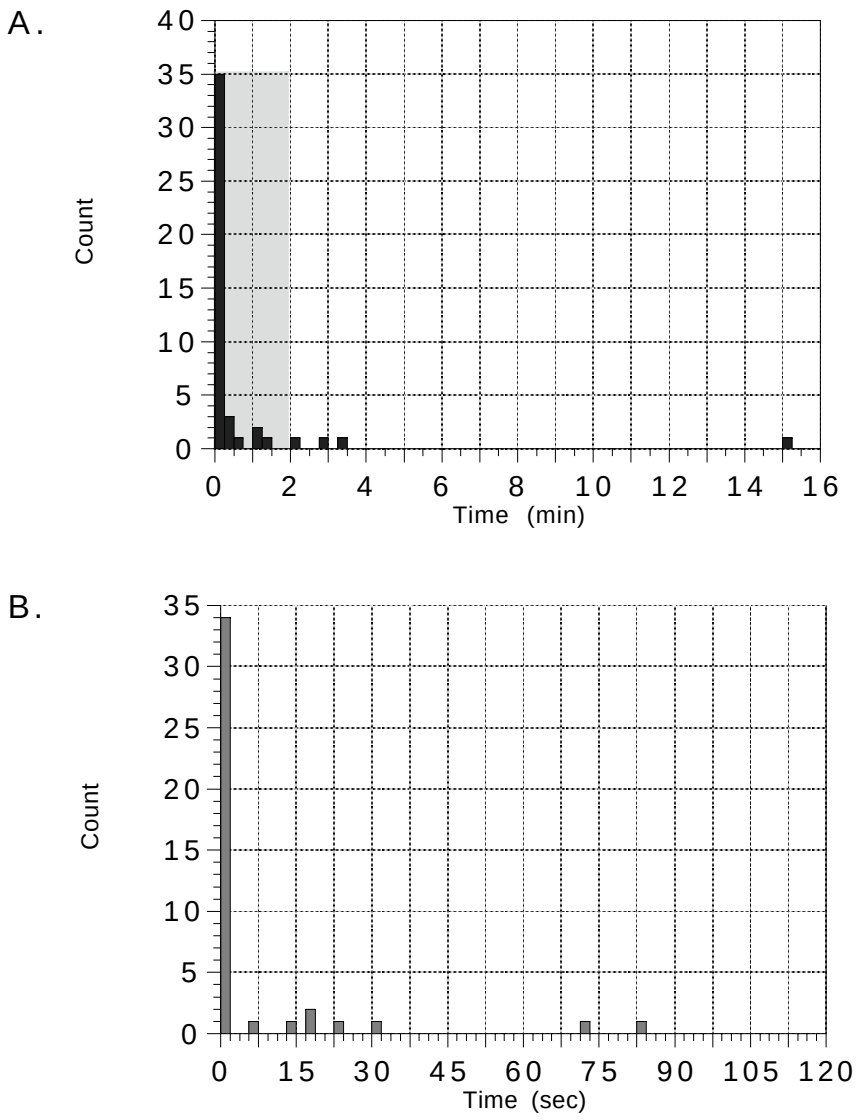


Figure 22. Response delay histograms. Frequencies of time intervals between cessation of stimulus and onset of response (increase in burst rate) to SCTS and MCTS. A. All delay intervals recorded (46) that were linked to a response. The data point at 15 min was included because spiking responded immediately to SCTS, increased over a period of 15 min, and triggered bursting over a 15 min interval (see Fig. 32). B. Subset of Graph A (shaded area) to show that in 72% of recorded intervals, the response delay was less than two seconds.

There were some occasions when the level of spontaneous activity did not increase following tetanic stimulation. Two different examples of short-term responses to tetanic stimulation are shown in Figure 23. The top panel, depicting the averaged burst rate for the entire network, shows a noticeable change in spontaneous bursting activity following SCTS on two different channels. In the bottom panel showing spiking activity, the results are not as clear for channel 54 because of test pulse stimulations before and after each SCTS. However, Figures 24 and 25 indicate that the evoked activity from test pulses on channels 54 and 3 before and after each SCTS reflected the respective changes in spontaneous activity. The duration of the effect for both the first and second tetanic stimulation was 9 min. In Figures 26 and 27, the early phase of the responses are shown across all 13 recording channels. There was no delay between the cessation of the stimuli and the two responses. Figure 28 shows the effect of SCTS on four different activity parameters.

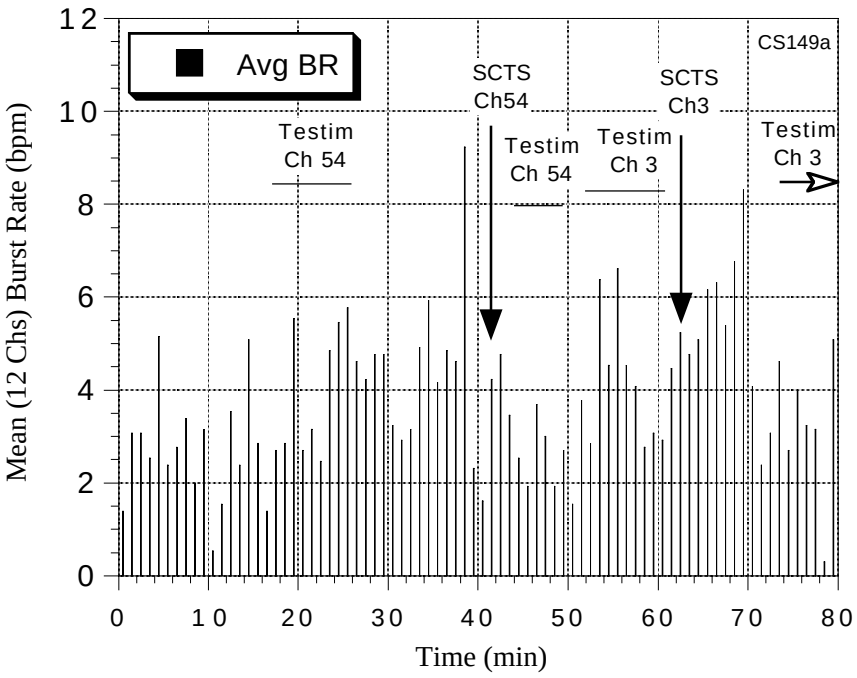
Figure 23. Short-term responses to SCTS.

A. Mean (12 channels) burst rates before, during, and after test-stimulation (single pulses delivered for the purpose of counting evoked responses, see Fig. 16) and SCTS episodes on channels 54 and 3.

B. Mean spontaneous spike rates of the same time period.

Following SCTS on channel 54 there was a decrease in spike rate (although the spontaneous activity --partially corrupted by counting action potentials after single pulses before and after SCTS-- had already begun to decline prior to the stimulation). Stimulation on channel 3 resulted in an increase in burst and spike rates. The changes in spontaneous activity lasted ~9 min in both instances.

A.


Avg BR
SCTS
Ch54
SCTS
Ch3
CS149a
Testim
Ch 54
Testim
Ch 54
Testim
Ch 3
Testim
Ch 3
Mean (12 Chs) Burst Rate (bpm)
0
2
4
6
8
10
12
0
10
20
30
40
50
60
70
80
Time (min)

B.

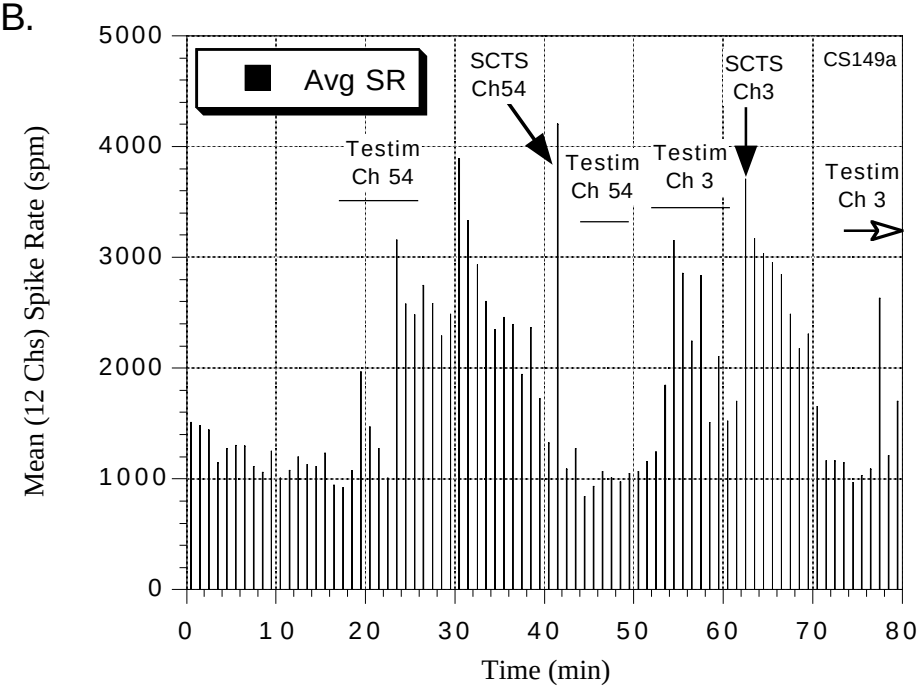
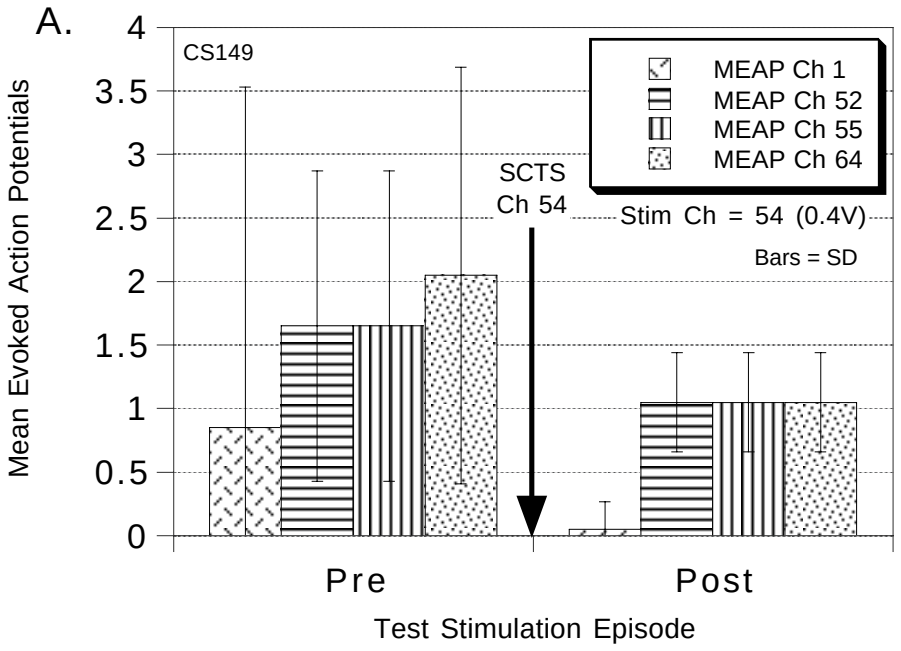
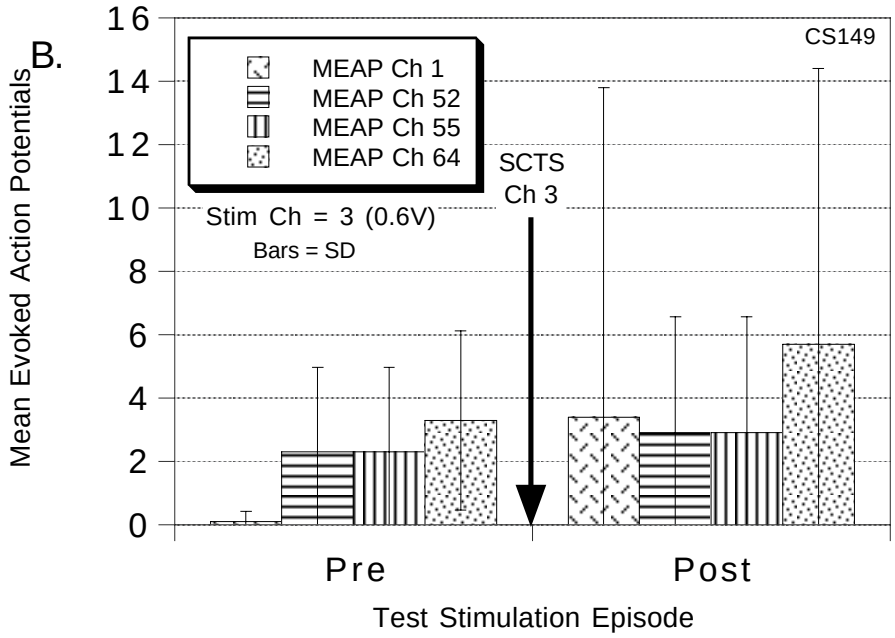

Avg SR
SCTS
Ch54
SCTS
Ch3
CS149a
Testim
Ch 54
Testim
Ch 54
Testim
Ch 3
Testim
Ch 3
Mean (12 Chs) Spike Rate (spm)
0
1000
2000
3000
4000
5000
0
10
20
30
40
50
60
70
80
Time (min)

Figure 24. Responses to test pulses following SCTS.

(A) Test pulses (0.4 V) were delivered to channel 54 before and after SCTS on the same channel. Evoked action potentials from 20 pulses were counted and averaged for each of the recording channels. Following SCTS on channel 54, the mean evoked action potentials (MEAPs) decreased across all four recording channels. Although unpaired student t tests showed the MEAPs before versus after SCTS were significantly different (all P values ≤ 0.001, df = 38) for each recording channel, this probably resulted from the overall variance after SCTS. Consequently, when data were evalulated with Mann-Whitney U procedures, MEAPs before and after were significantly different only for channel 64 (U =110.50, P ≤ 0.014)

(B) MEAPs from ten test pulses (0.6 V) before and after SCTS on recording channel 3. The Mann-Whitney U test revealed no signficant differences between the before and after groups (P > 0.05). There appeared to be an increase in the variability after stimulation on channel 3.


A.
CS149
MEAP Ch 1
MEAP Ch 52
MEAP Ch 55
MEAP Ch 64
SCTS
Ch 54
Stim Ch = 54 (0.4V)
Bars = SD
Mean Evoked Action Potentials
4
3.5
3
2.5
2
1.5
1
0.5
0
Pre
Post
Test Stimulation Episode


B.
CS149
MEAP Ch 1
MEAP Ch 52
MEAP Ch 55
MEAP Ch 64
SCTS
Ch 3
Stim Ch = 3 (0.6V)
Bars = SD
Mean Evoked Action Potentials
16
14
12
10
8
6
4
2
0
Pre
Post
Test Stimulation Episode

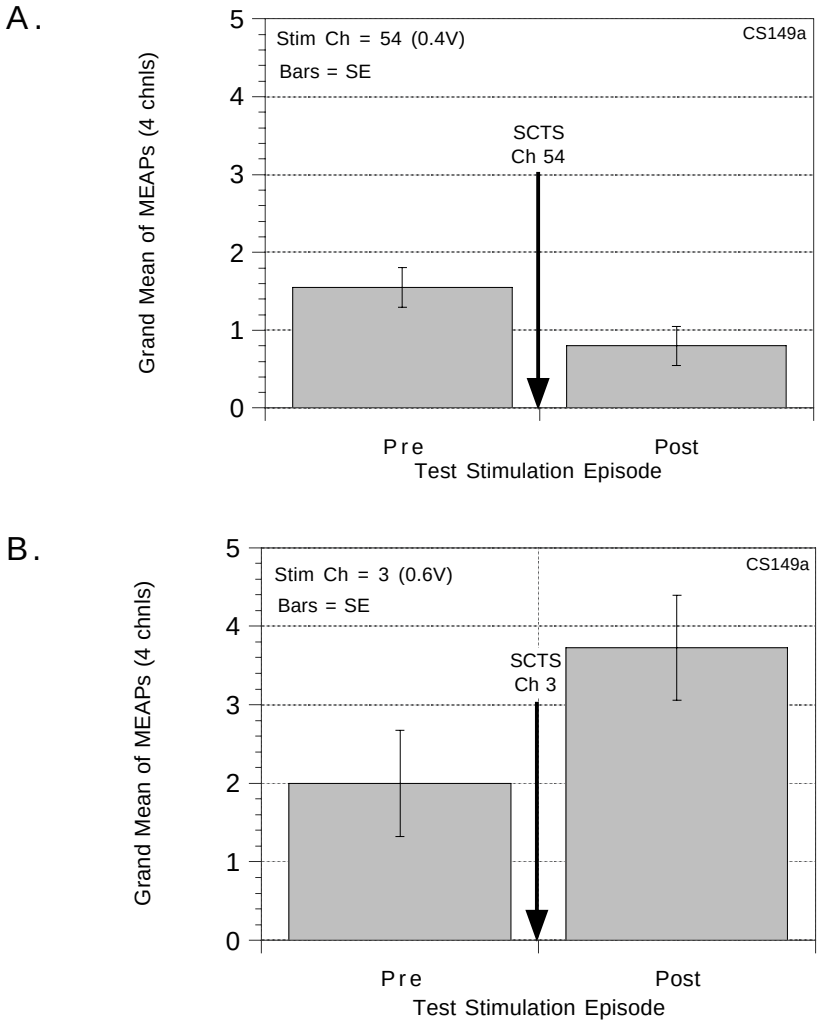


Figure 25. Effect of stimulation site on response.

A grand mean of the MEAPs was determined for all four of the recording channels.

A. Although there was a 48% reduction in the grand mean following tetanic stimulation on channel 54, the difference was not significant (student's t, 6 df, $P > 0.05$).

B. Following SCTS, the grand mean of the MEAPs increased by 86%. The difference between grand means before and after SCTS was not significant ($P > 0.05$) according to student's t (6 degrees of freedom).

Figure 26. Immediate responses to SCTS on channel 54 monitored on 14 channels. Prior to stimulation, spiking activity erupted quasi-periodically across most of the channels (~23 sec intervals). These episodes represent bursts. Following tetanic stimulation, the spontaneous periodic spiking was depressed for over 2 min.

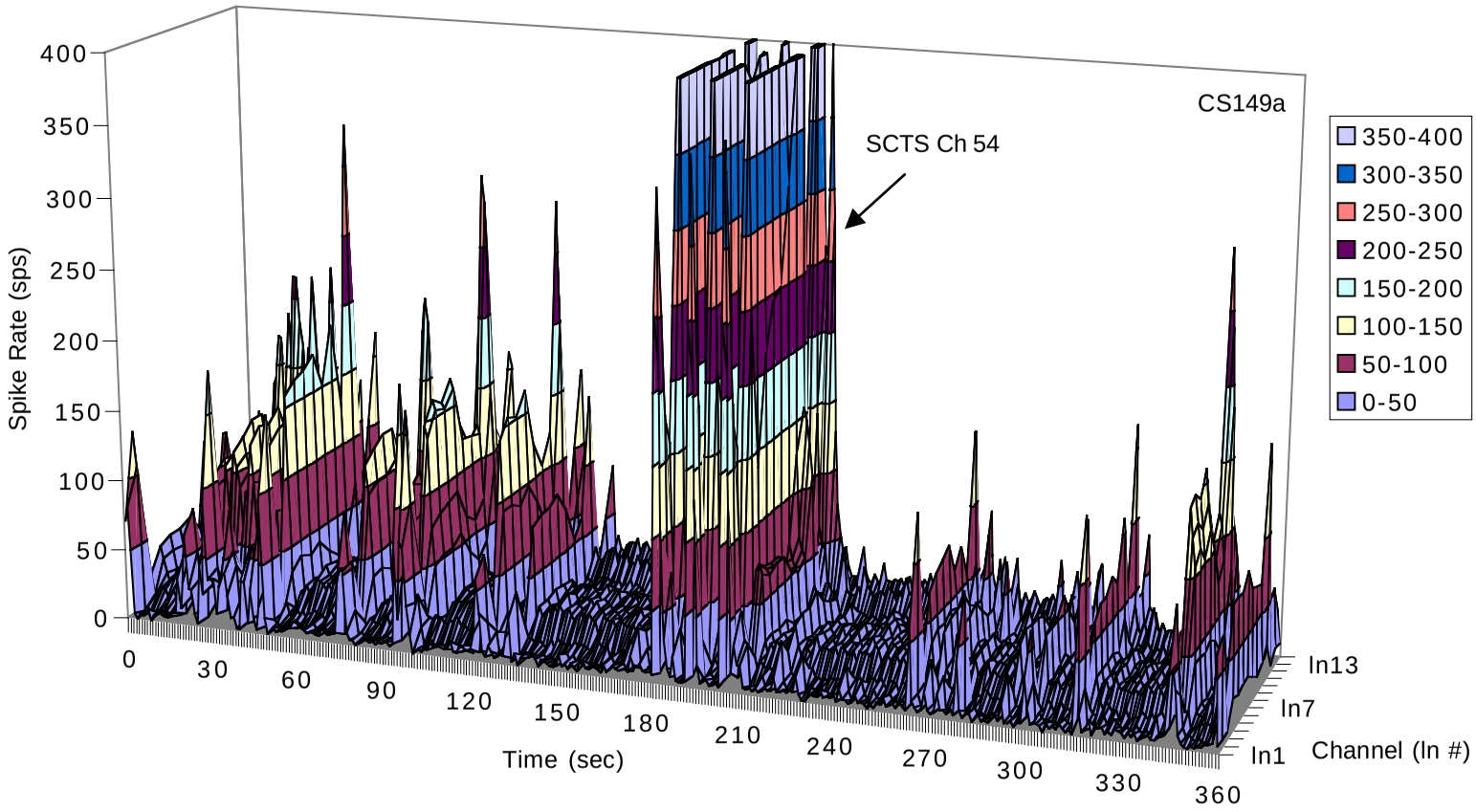
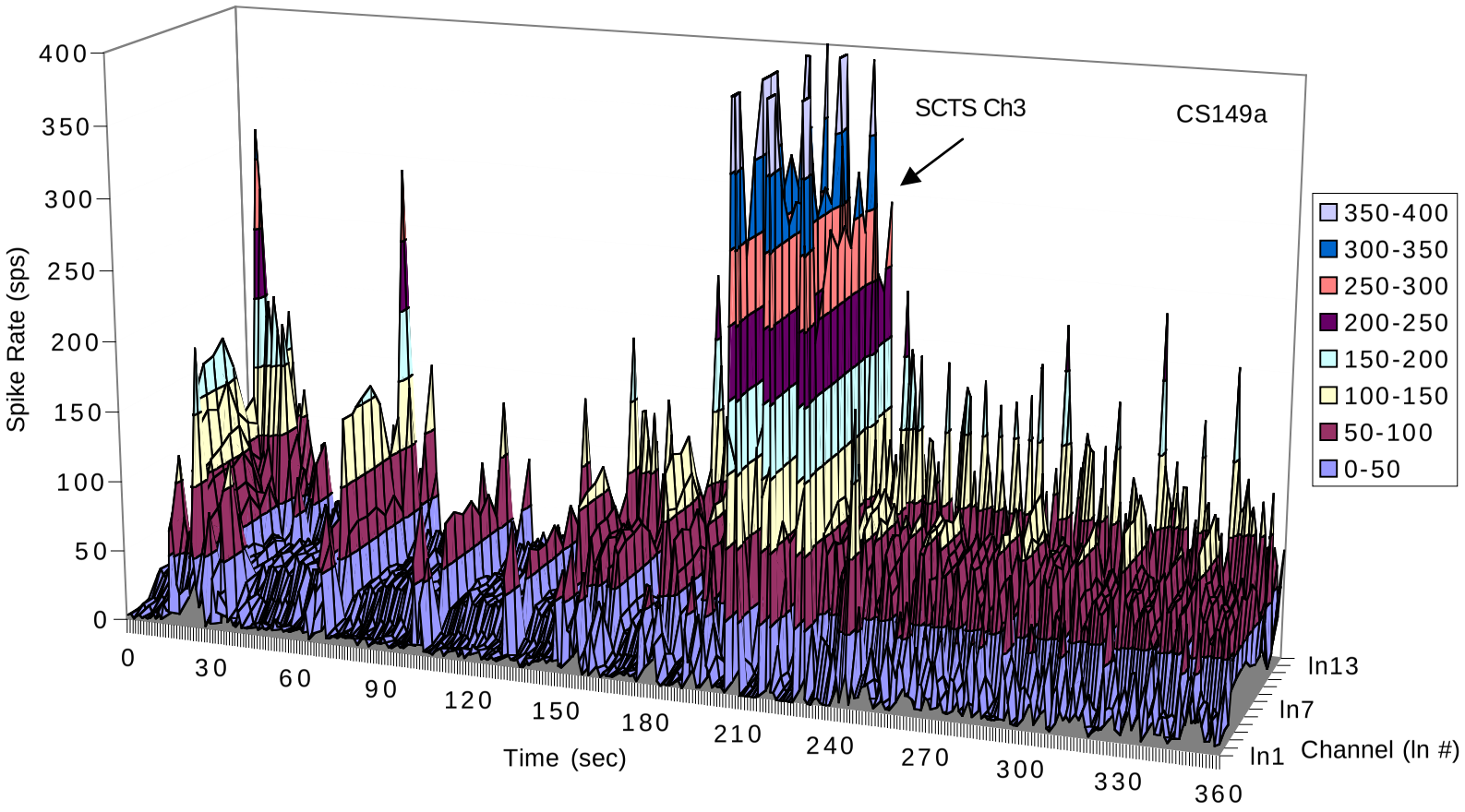

CS149a
SCTS Ch 54
Spike Rate (sps)
400
350
300
250
200
150
100
50
0
0
30
60
90
120
150
180
210
240
270
300
330
360
Time (sec)
In13
In7
In1
Channel (In #)
350-400
300-350
250-300
200-250
150-200
100-150
50-100
0-50

Figure 27. Immediate responses to SCTS on channel 3. About 15 min after the initial SCTS on channel 54 (see Fig. 24), tetanic stimulation on channel 3 caused an immediate increase in the spontaneous spike rate.


SCTS Ch3
CS149a
Spike Rate (sps)
400
350
300
250
200
150
100
50
0
0
30
60
90
120
150
180
210
240
270
300
330
360
Time (sec)
350-400
300-350
250-300
200-250
150-200
100-150
50-100
0-50
In13
In7
In1
Channel (In #)

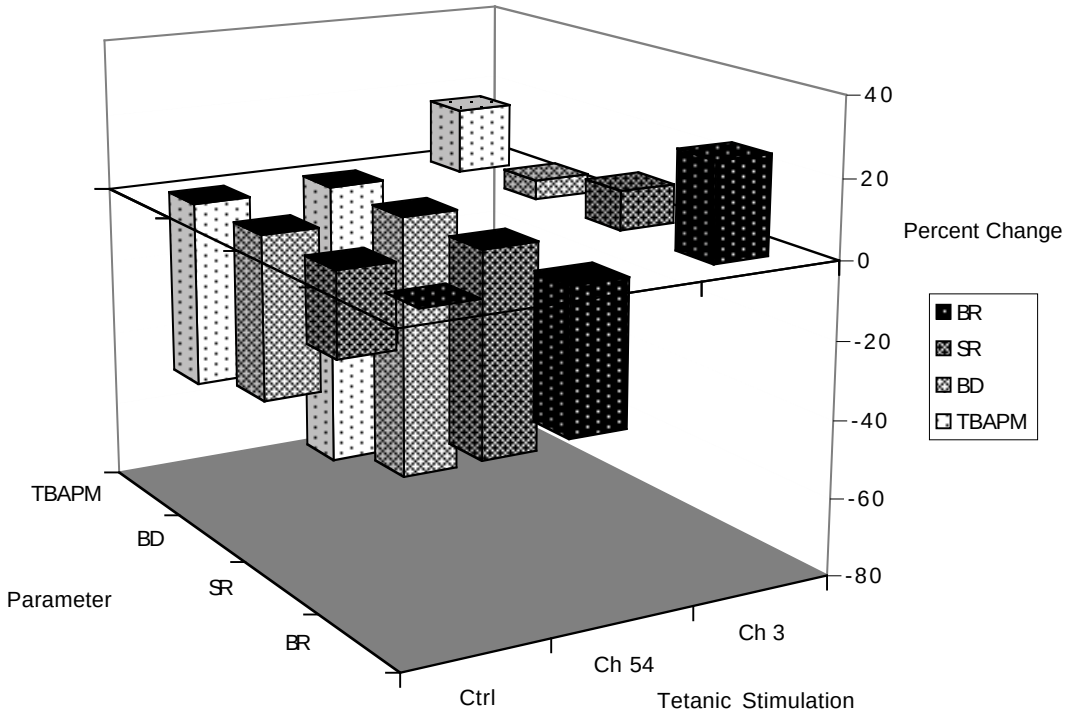


Figure 28. Tetanic stimulation-dependent changes in four different activity variables: burst rate (BR), spike rate (SR), burst duration (BD), and total burst area per minute (TBAPM). Data are expressed as percent change in mean activity intervals after SCTS using the pre-SCTS activity as baseline. Three levels of mean values were used: (1) minute means for BD and TBAPM, (2) network means by averaging across 12 recording channels per minute, (3) interval means for all variables over 9 min time periods (before and after SCTS). The detailed method for calculating the means was presented in Chapter 2.

For the control (ctrl), a 9 min activity interval was compared to a subsequent 9 min activity interval following a mock stimulation (no stimulation was applied). Note that a slight negative trend in the control period was greatly enhanced by SCTS on channel 54 and reversed to the positive direction by stimulation on channel 3.

3.1.4 Long-term Responses to Tetanic Stimulation

Of the 48 tetanic stimulation episodes that showed an increase in spontaneous activity (see Fig 21A), 19% (9) were classified as long-term responses (>15 min). Because of the relatively low frequency of long-term responses to tetanic stimulation, and because the reversal of its effect was an important element of this study, the maximum duration of LTSAP is still unknown (see Fig. 29). The longest observed duration of enhanced activity before the initiation of repetitive stimulation was 90 min (experiment 79, Fig. 30). Although the burst rate and spike rate increased substantially in this trial, the parameter that was most affected following both stimulations was the burst duration (Fig. 31). The burst rate did not change following a control (Ctrl) episode (no stimulation between the 15 min pre and 15 min post intervals during the native activity segment). There was a decrease in the spike rate (SR), burst duration (BD) and total burst area per minute (TBAPM) by 26% during this same period. Following SCTS on channel 12, the burst rate decreased by 6%, but the spike rate increased by 7%, burst duration by 23%, and TBAPM increased by 14%. Although the responses to SCTS on channel 12 were mostly positive compared to the control, the magnitude of the change was not above the "random" fluctuations during the pre-stimulus native activity of the preparation. The SCTS on channel 24 however, produced significant changes in all 4 activity parameters (burst rate increased by 66%, spike rate by 50%, burst duration by 233% and the TBAPM by 198%). Once again, the stimulation site appears to be a critical factor in the magnitude of the change in spontaneous activity via tetanic stimulation. The difference in percent increase between the spike rate and the TBAPM was largely due to the way in which TBAPM was calculated. Because of increases in both the burst rate and the mean burst area, the product of these two variables often was more than the increase in the single variable of spike rate. Several double variable plots (like Fig. 30) showed that the change in TBAPM paralleled the change in spike rate on a minute-to-minute basis (see Fig. A-10 in Appendix).

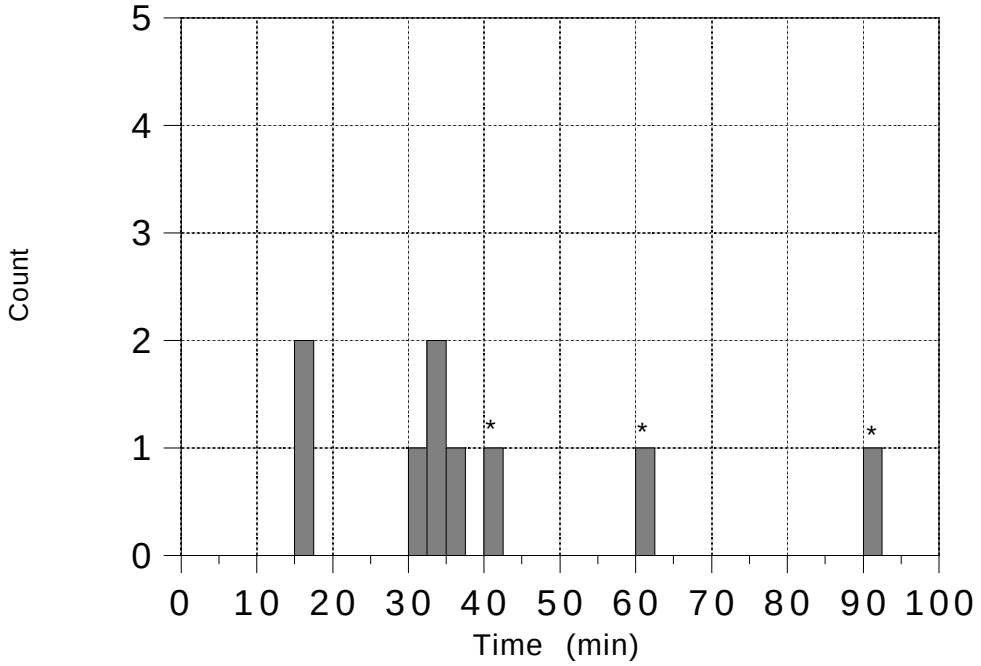


Figure 29. Frequency histogram of long-term durations of responses to tetanic stimulation (subset of Fig. 20A). Graph includes durations of responses to both SCTS and MCTS. The activity parameter measured was burst rate. Starred items represent occasions when the response was cut off by repetitive stimulation.

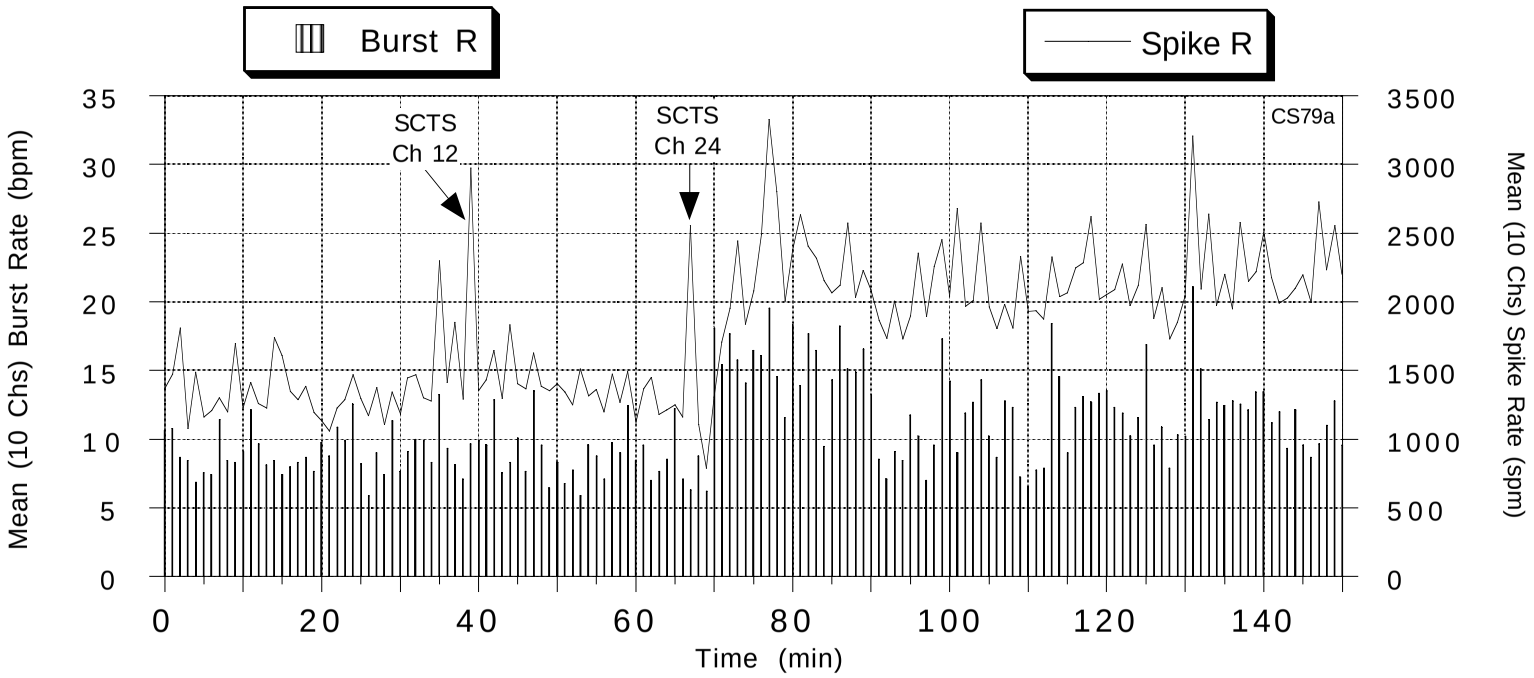
Table 4 shows quantitative data for LTSAP. Because one of the goals of this study was to determine if tetanic stimulation could alter spontaneous activity on a long-term basis, only durations of at least 15 min were included in the table. Calculations for C-126 and C-135 are not shown because the experiments were not recorded on the computer (stripchart data only). Experiment C-126 was a test-pulse experiment only, and the stimulus pulse for C-135 was constant current (instead of constant voltage).

Table 4. Summary of Results for Long-Term Increases in Resonse Duration Following Tetanic Stimulation

Expt.	Stim	Response	% Δ BR	% Δ SR	% Δ TBAPM
C-66b	SCTS	16 min	11,150	80	61,958
C-66b	MCTS	60 min*	83,500	481	220,467
C-79a	SCTS	90 min*	41	54	156
C-99	SCTS	33 min	38	61	63
C-123a	SCTS 1	42 min*	*-76*	*-85*	*-90*
C-123a	SCTS 2	37 min	*-82*	*-80*	*-84*
C-126	SCTS	17 min	NCDA	NCDA	NCDA
C-136	SCTS	35 min	NCDA	NCDA	NCDA
C-149a	SCTS 3	31 min*	106	99	392
Means and SE		40.1 ± 7.61	18,967± 16,276%	155 ± 81.88%	56,607 ± 42,675%

Legend: % Δ = Percent Change; BR = Burst Rate; SR = Spike Rate; TBAPM = Total Burst Area Per Minute; NCDA = No Computer Data Available. Starred values represent durations that were cut off by another stimulation. Italicized values were not included in calculation of means.

Figure 30. Long-term response to SCTS (mean of 10 channels). Single-channel stimulation on channel 12 had little effect on burst rate (Burst R) and spike rate (Spike R) and could be considered as a control stimulation. However, SCTS on channel 24 had a significant effect on both the burst rate and spike rate. The spike rate was increased by over 50% and maintained at that level for 90 min before it was cut off by a repetitive stimulation trial (not shown--see Chapter 3.3). The burst rate was almost doubled for 20 min before it diminished suddenly to a level that was ~30% above the pre-stimulus activity.


Burst R
Spike R
SCTS
Ch 12
SCTS
Ch 24
CS79a
Mean (10 Chs) Burst Rate (bpm)
Mean (10 Chs) Spike Rate (spm)
Time (min)
0
5
10
15
20
25
30
35
0
500
1000
1500
2000
2500
3000
3500
0
20
40
60
80
100
120
140

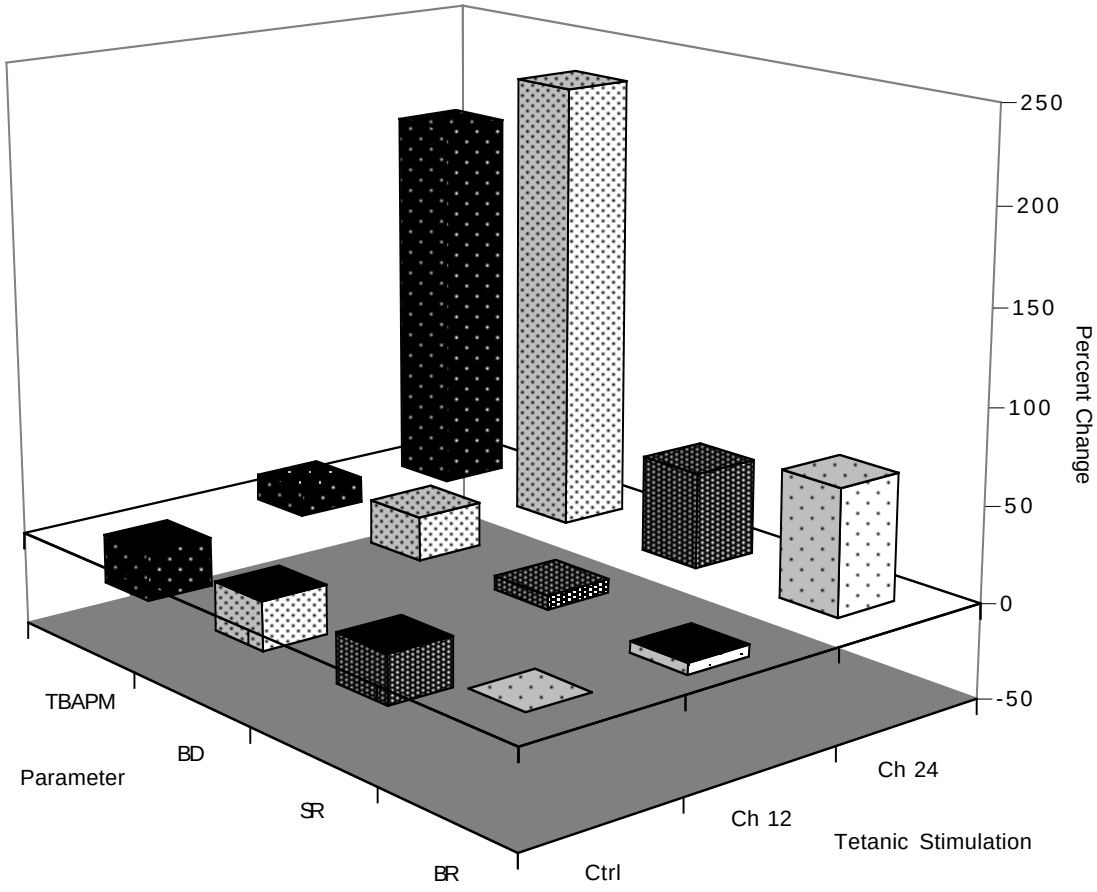


Figure 31. Percent change of four different activity parameters. Grand means of 10 channels (20 min intervals) before and after SCTS on channel 12 and 24 respectively. Control values also represent the percent change following a mock tetanic stimulation between the two 20 min time bins.

Figure 32 displays some of the data used to create the graph in Figure 30. The former figure represents the overall network response, whereas this figure shows activity for each recording channel. This figure provides another example of the range of responses across several recording channels to the same stimulation and the loss of information associated with averaging across all channels.

Not only was there a difference in long-term responses to the two different stimulation sites, but the short-term responses were dissimilar as well. Stimulation on channel 12 produced a subtle but immediate effect on spike frequency (data not shown). However, Figure 33 shows that following stimulation on channel 24, many seconds passed before the enhancement of spontaneous activity became evident. This difference indicates that stimulation site may not only determine whether or not there is a response (and the direction in which the response goes), but also the timing of the response (i.e. whether or not there is a delay following stimulation).

Figure 32. Three-dimensional graph of same experiment shows range of responses. Dark traces represent large increases following SCTS. Gray traces depict recording channels with little change in spontaneous activity. Light traces signify channels on which there was a decrease in activity following SCTS.

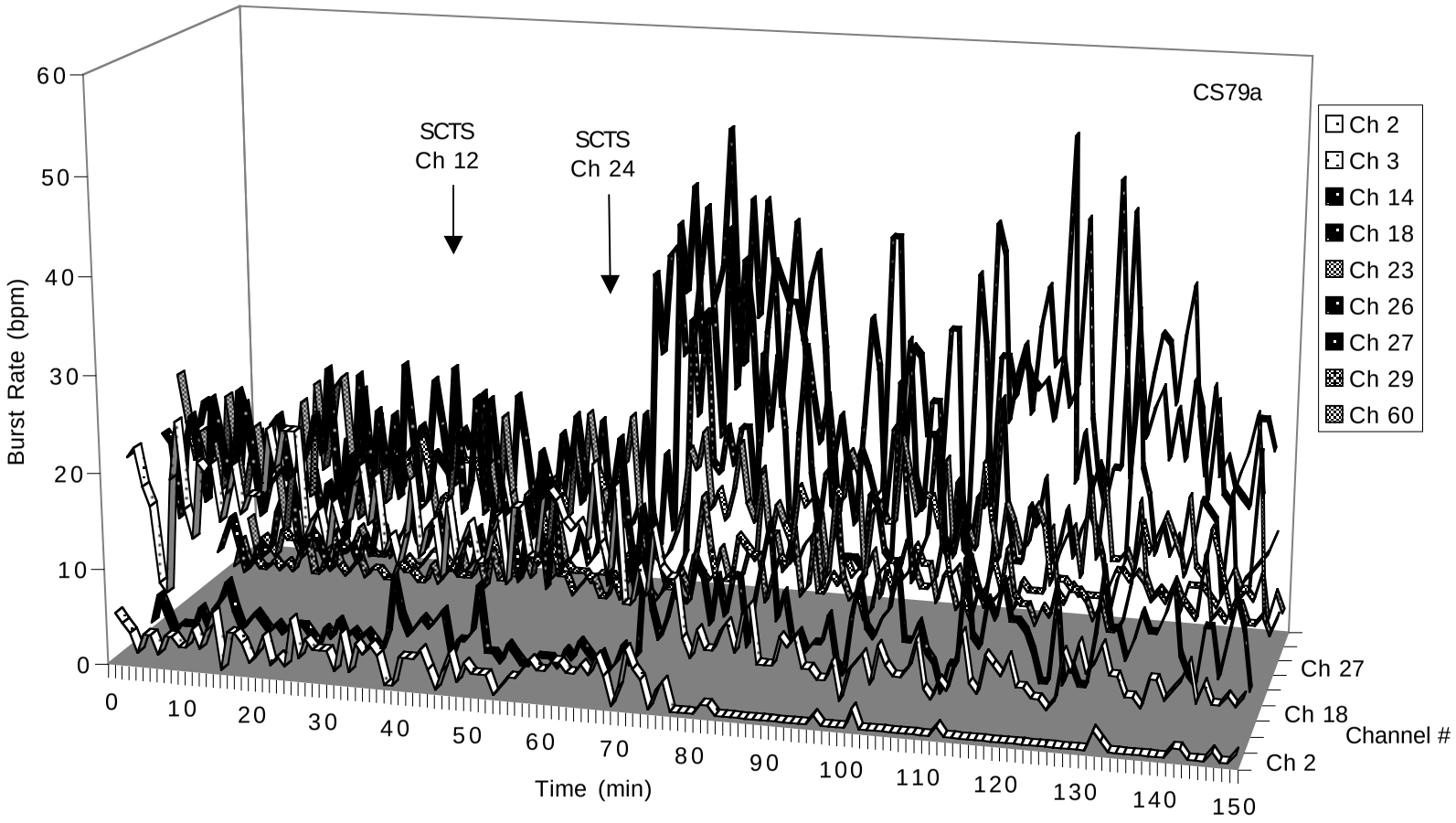
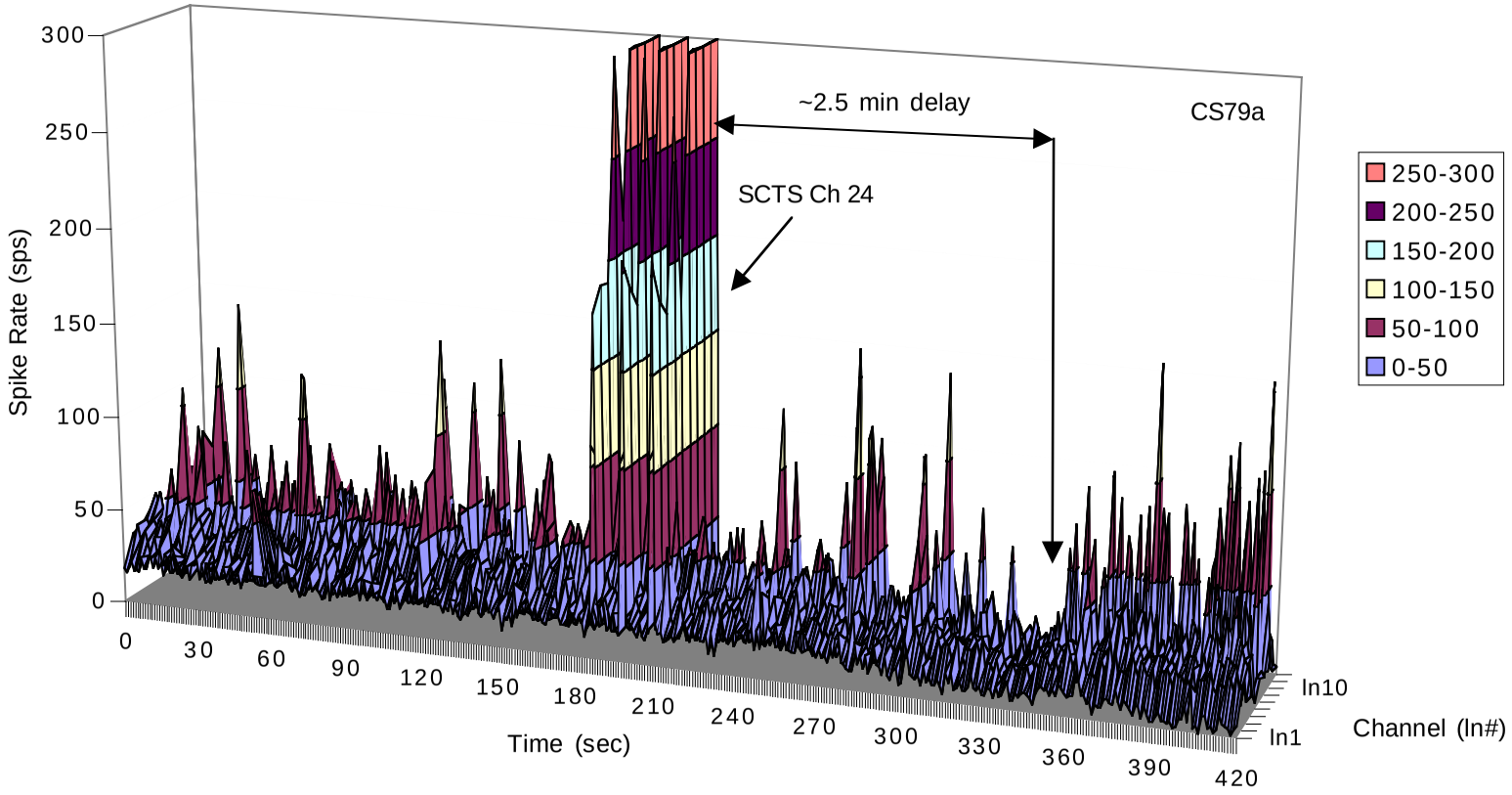

CS79a
SCTS
Ch 12
SCTS
Ch 24
Ch 2
Ch 3
Ch 14
Ch 18
Ch 23
Ch 26
Ch 27
Ch 29
Ch 60
Burst Rate (bpm)
0
10
20
30
40
50
60
Time (min)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
Channel #
Ch 27
Ch 18
Ch 2

Figure 33. Early phase of response to second stimulation. This short-term profile shows seven minutes of spiking activity across several channels in order to increase the resolution of the early phase of the long-term response. Thirty-eight minutes after the first SCTS on channel 12, a second stimulus was delivered to channel 24. After about a 2.5 min delay, the spike rate began to rapidly increase.


300
250
200
150
100
50
0
Spike Rate (sps)
~2.5 min delay
CS79a
SCTS Ch 24
250-300
200-250
150-200
100-150
50-100
0-50
0
30
60
90
120
150
180
210
240
270
300
330
360
390
420
Time (sec)
In10
In1
Channel (In#)

3.1.5 Single vs. Multiple Channel Stimulation

Some of the differences between the responses to SCTS and MCTS (i.e. differences in delay before onset of response and differences in duration of effect) can be found in Table 3. The best singular example of the difference in the effectiveness of the two stimulation types is shown in Figure 34. Not only is the magnitude and duration of the response to MCTS greater, but the delay between the stimulus and the response (particularly in the top panel) is much shorter. This delayed increase in bursting following SCTS on channel 36, however, is probably not directly related to the stimulation, but likely a result of facilitation of bursting activity by increased spiking following SCTS. One must note, however, that the difference in durations is contrary to the trend in Table 3 where responses to SCTS tended to result in longer durations. The examination of much shorter segments of the same experiment reveals that there was even a difference between SCTS and MCTS during the stimulation event (Fig. 35). However, the results from other trials were less obvious.

Figure 34. Single vs. multiple channel tetanic stimulation.

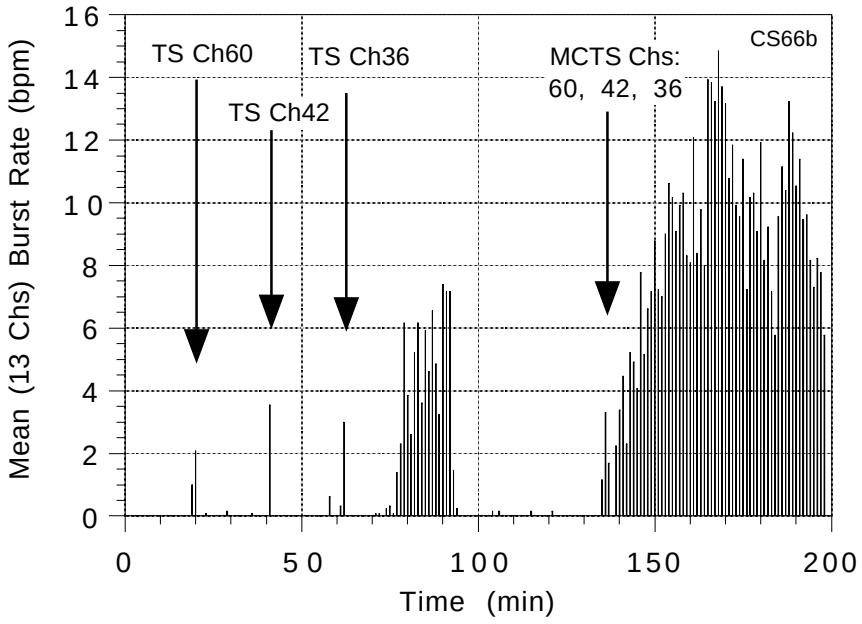
A. Mean burst rates of 13 channels.

B. Mean spike rates of the same 13 channels.

Stimulation on channels 60 and 42 had little effect on spontaneous activity. However, SCTS on channel 36 caused an increase in the spike rate ~4 min after the stimulation. At ~8 min after the SCTS, the spike rate had increased by 50%. At 15 min after SCTS, the burst rate began to increase. The enhanced bursting activity lasted for 16 min before it decayed. The duration of the increase in spiking was ~25 min. Stripchart activity also showed that there was a complete decay of bursting activity such that the activity preceding MCTS was very similar to activity prior to all three SCTS episodes. Stimulation on all three channels resulted in an immediate enhancement of spontaneous activity that lasted for over 60 min before repetitive stimulation (not shown) was initiated.

Gaps in data line (at 42, 63, and 137 min in B) are the result of data dropout of large spikes due to the stimulation artifacts.

A.


Mean (13 Chs) Burst Rate (bpm)
16
14
12
10
8
6
4
2
0
TS Ch60
TS Ch42
TS Ch36
MCTS Chs:
60, 42, 36
CS66b
0
50
100
150
200
Time (min)

B.

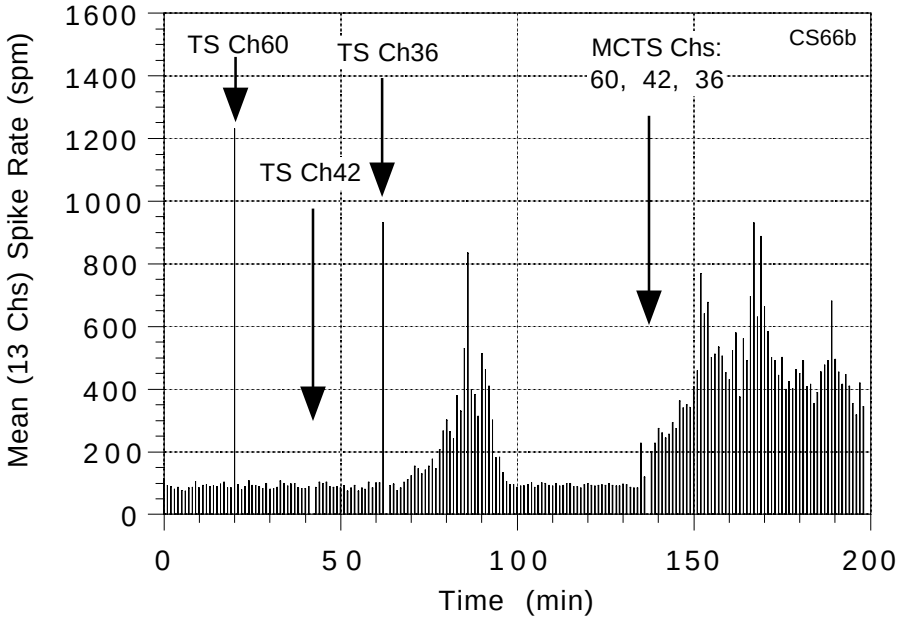

Mean (13 Chs) Spike Rate (spm)
1600
1400
1200
1000
800
600
400
200
0
TS Ch60
TS Ch42
TS Ch36
MCTS Chs:
60, 42, 36
CS66b
0
50
100
150
200
Time (min)

Figure 35. Comparison of single and multichannel tetanic stimulation on spike rates on an expanded time scale. Mean spike rates (13 channels) are shown before, during and after stimulation.

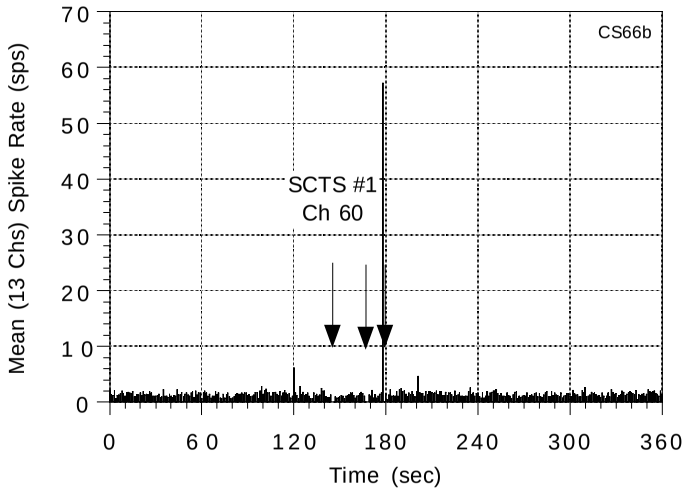
A. SCTS on channel 60.

B. SCTS on channel 42.

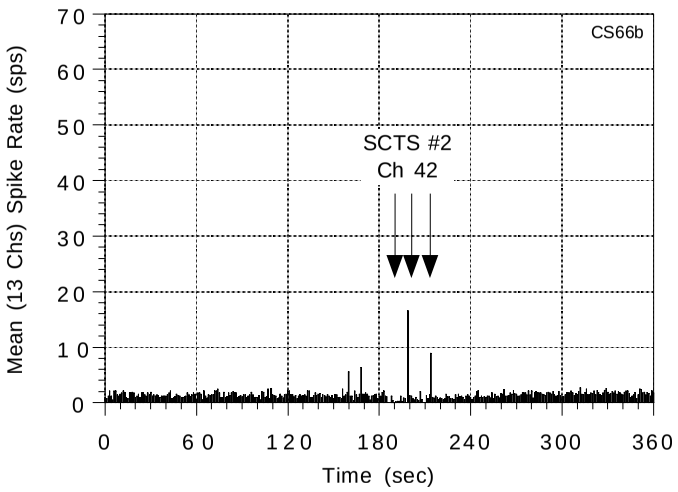
C.: SCTS on channels 36.

D. Simultaneous stimulation on all three channels. Stimulus artifacts are not shown. Notice the evoked spiking activity after each stimulus train during MCTS, the "exponential" decrease of the response with each train, and the increased tonic spontaneous spiking after such stimulation.

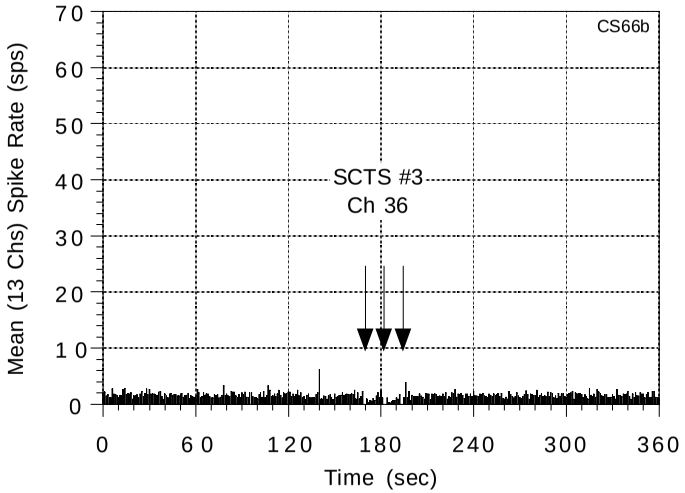
A.


CS66b
SCTS #1
Ch 60
Mean (13 Chs) Spike Rate (sps)
Time (sec)
0
10
20
30
40
50
60
70
0
60
120
180
240
300
360

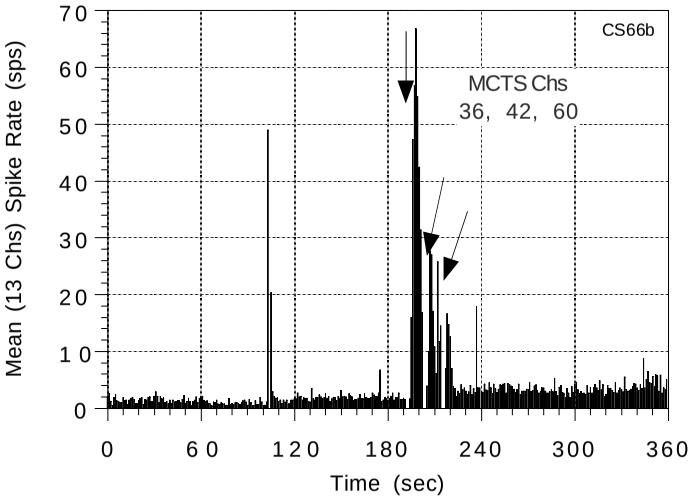
B.


CS66b
SCTS #2
Ch 42
Mean (13 Chs) Spike Rate (sps)
Time (sec)
0
10
20
30
40
50
60
70
0
60
120
180
240
300
360

C.


CS66b
SCTS #3
Ch 36
Mean (13 Chs) Spike Rate (sps)
Time (sec)
0
10
20
30
40
50
60
70
0
60
120
180
240
300
360

D.


CS66b
MCTS Chs
36, 42, 60
Mean (13 Chs) Spike Rate (sps)
Time (sec)
0
10
20
30
40
50
60
70
0
60
120
180
240
300
360

3.1.6 Summary of Responses to Test Pulses

Perhaps the most convincing evidence of the difference between SCTS and MCTS was the data from test-stimulation experiments, which involved the counting of action potentials before and after the stimulus episode. Figure 36 is a summary graph of the data set for test-stimulation experiments. This graph indicates that SCTS was just as likely to increase the MEAP value as it is to decrease the MEAP value. However, the magnitude of the changes was greater for increases. The graph also indicates that both SCTS and MCTS increased the number of action potentials recorded after test pulses. However, MCTS trials had a statistically significant higher percentage of experiments that showed increases (5 out of 7) in action potential production to test pulses compared to SCTS positive responses. These differences in magnitudes of effects and ratio of increases suggest that MCTS is more effective than SCTS in changing the response to test pulse stimulation.

There was a statistically significant difference between the SCTS and MCTS MEAP responses. That is, a significantly greater percentage of MCTS test-pulse stimulation trials resulted in an increase in evoked responses following HFS than SCTS trials. In addition, statistical tests were run on HFS trials that showed an increase in grand MEAP values in order to determine if there was a significant difference in the magnitude of responses between SCTS and MCTS stimulation. There was no significant difference found between these two groups.

The site of stimulation was not only critical to the magnitude of the change in spontaneous activity, but it was also decisive in the nature of the change in spontaneous activity (e.g. Fig. 25). Because some parameters were affected more than others following stimulation on one site, while other parameters were more sensitive to the same stimulation at a different site, one can assume that the site of stimulation has an influence on how the activity will change as defined by the parameters being measured, rather than just an increase or decrease in the spontaneous activity.

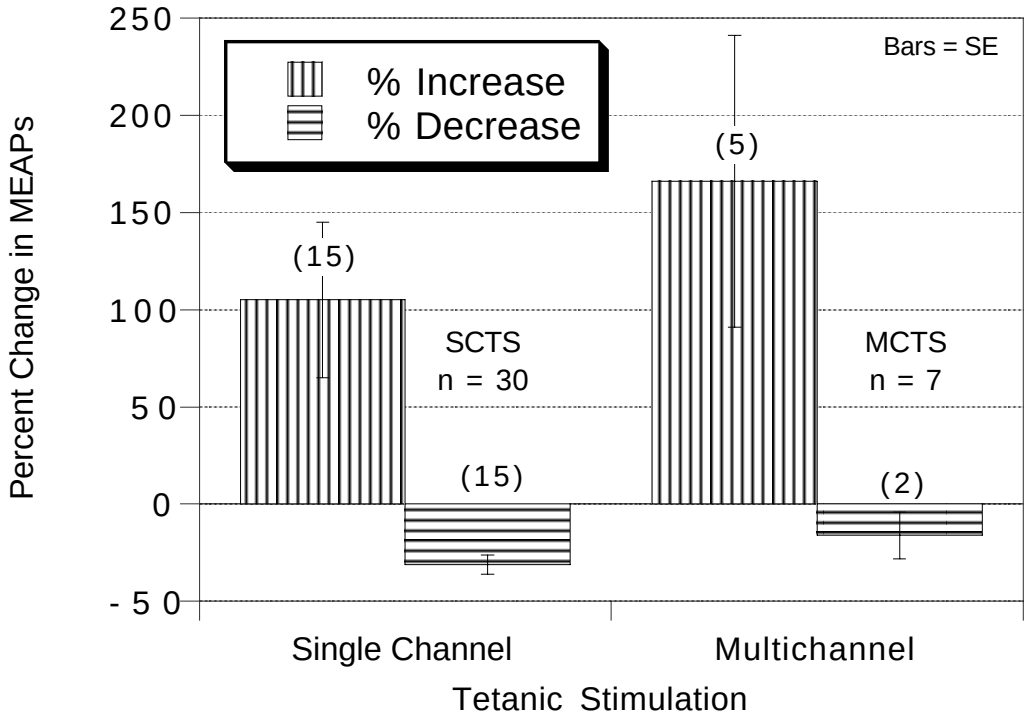


Figure 36. Changes in responses to test pulses after HFS.

The data in this graph represent 15 experiments (37 trials). Mean evoked action potentials (MEAPs) were summed across all four recording channels for each test-stimulation episode before and after a tetanic stimulation event. All of the occurrences in which there was a net percent increase in MEAPs following SCTS (15 of 30) were averaged to obtain a grand mean. The same was done for the other half of the SCTS trials for which there was a net percent decrease in the MEAPs. The same procedure was carried out for the MCTS trials. Shown are means ± SEM.

Student t tests showed no significant difference between the percent increase values for SCTS and MCTS ($P > 0.5$). There were too few decreases following MCTS for a statistical comparison.

Figure 37 shows that SCTS on four different channels either produced low MEAP (mean evoked action potentials) values or a reduction in the MEAPs following the stimulation event. Stimulation on all four channels simultaneously not only reversed the negative trend, but produced a significant increase in the MEAP value (Fig. 38). Even though the MEAP value increased by 170% following MCTS, the spontaneous activity showed no noticeable change during the same time period (Fig. 39).

There were other instances where changes in the MEAP value were not reflected in the spontaneous activity. Direct comparison of percent change of MEAP values versus percent change in burst rates revealed that in 18 out of 20 trials, the MEAP values were more sensitive to HFS. There were 5 trials where there was a decrease in the burst rate, yet the MEAP value increased. Even when there was reduction in both the burst rate and MEAP value (3 trials), the percent change in MEAP value was greater. Figure 40A and B shows that successive stimulation on the same channel produced increases in MEAPs by an average of almost 20% following each stimulation event. Panels C and D of the same figure illustrate that the spontaneous activity actually decreased during the corresponding time intervals. This decrement in spontaneous activity following SCTS indicates that synapses within inhibitory circuitry were likely potentiated. These two examples of direct comparison of MEAPs and spontaneous activity indicate that tetanic stimulation can have a meaningful effect on the former while having little or even the opposite effect on the latter.

Figure 37. Grand means of MEAPs (mean evoked action potentials) for four different stimulus channels.

A. Ten test pulses (0.64 V) were delivered to channel 14 before and after SCTS on the same channel.

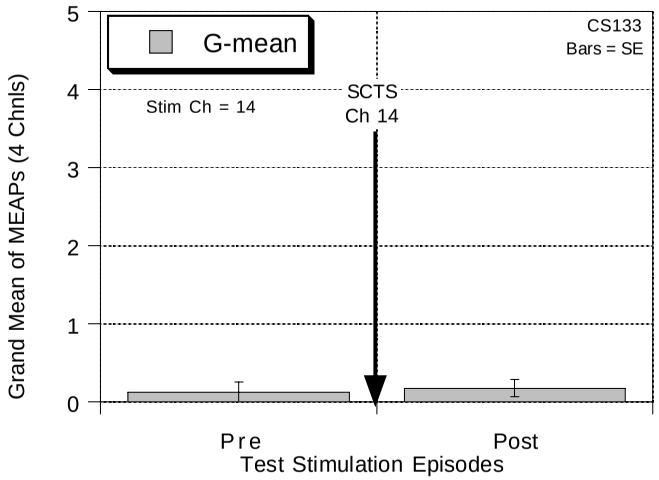
B. Twenty test pulses were delivered to channel 37. Following SCTS on the same channel, there was a 63% reduction in the MEAPs.

C. The number of test pulses delivered to channel 43 before and after SCTS was 10; and the percent change in MEAPs was -48%.

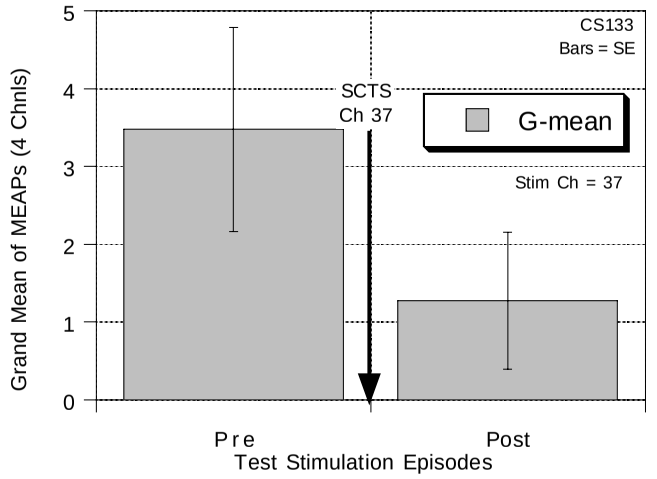
D. Ten test pulses were also delivered to channel 54. The percent change was -42%.

There were no significant differences between the pre- versus post MEAP values for all four graphs shown (Welch's approximate t).

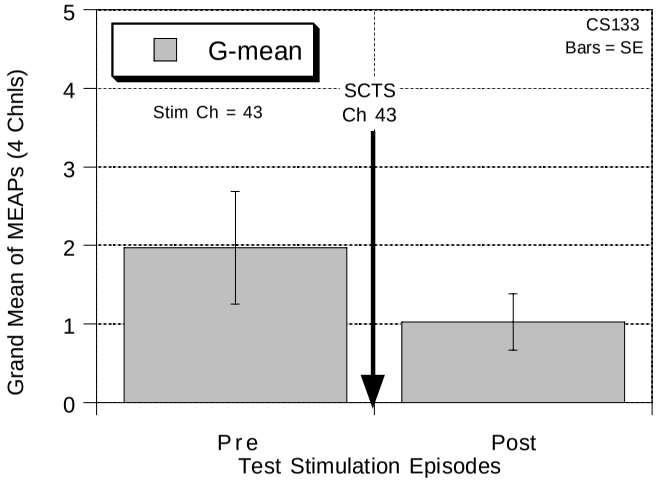
A.


G-mean
CS133
Bars = SE
Stim Ch = 14
SCTS
Ch 14
Grand Mean of MEAPs (4 Chnls)
0
1
2
3
4
5
Pre
Post
Test Stimulation Episodes

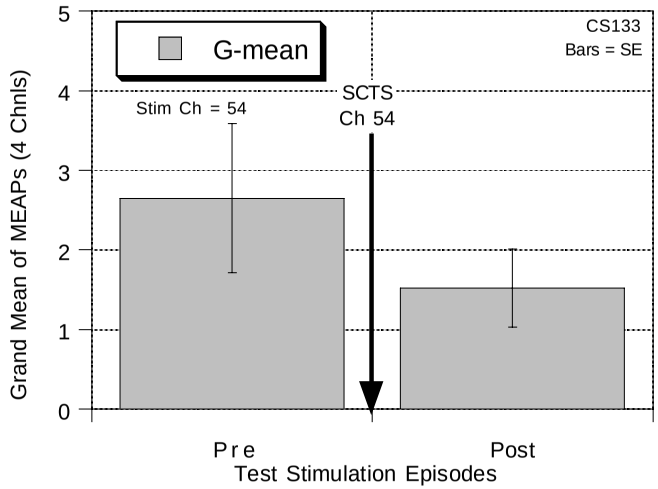
B.


CS133
Bars = SE
SCTS
Ch 37
G-mean
Stim Ch = 37
Grand Mean of MEAPs (4 Chnls)
0
1
2
3
4
5
Pre
Post
Test Stimulation Episodes

C.


G-mean
CS133
Bars = SE
Stim Ch = 43
SCTS
Ch 43
Grand Mean of MEAPs (4 Chnls)
0
1
2
3
4
5
Pre
Post
Test Stimulation Episodes

D.


G-mean
CS133
Bars = SE
Stim Ch = 54
SCTS
Ch 54
Grand Mean of MEAPs (4 Chnls)
0
1
2
3
4
5
Pre
Post
Test Stimulation Episodes

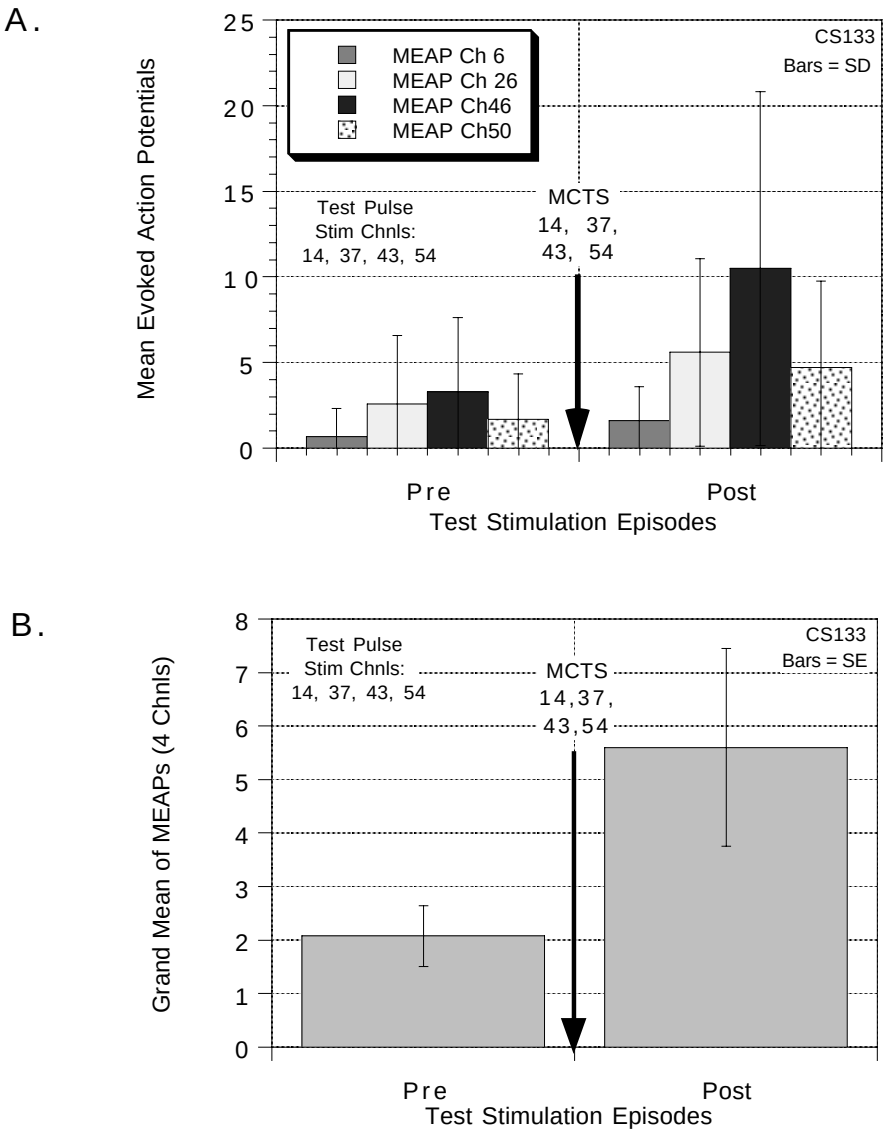


Figure 38. Multi-channel test-pulse and tetanic stimulation.

A. Ten test pulses were delivered to all four of the channels that were previously stimulated singularly before and after MCTS on the same channels. There was an increase in the MEAPs following MCTS on all four recording channels.

B. The grand means of the four channels showed a 170% increase following MCTS. However, this increase was not significant (student's t, df = 6, $P > 0.05$).

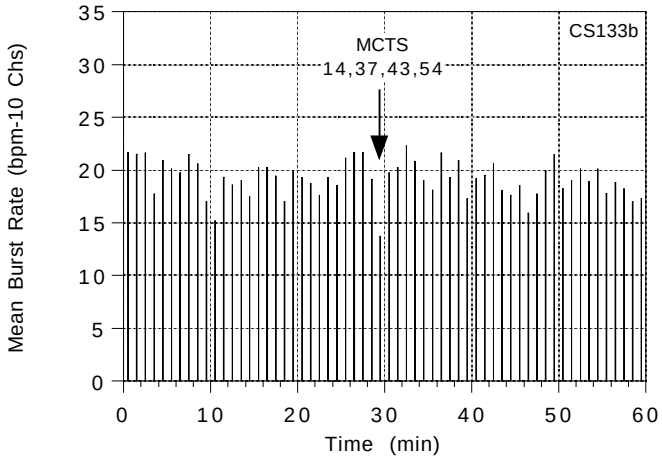
Figure 39. Mean spontaneous activity changes following MCTS.

A. Mean (10 channels) burst rate before and after MCTS on channels 14, 37, 43, and 54.

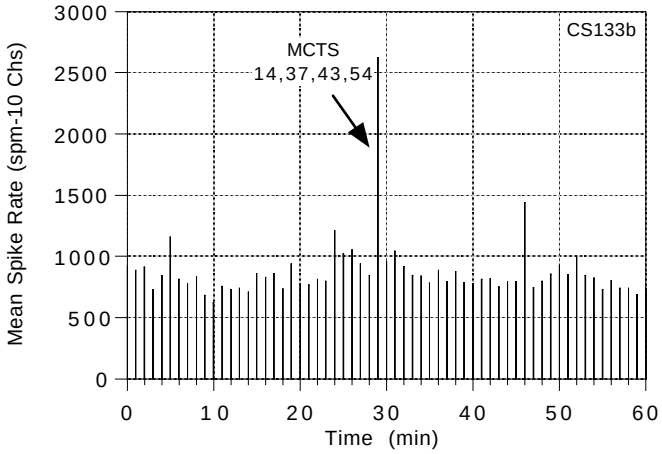
B. Mean spike rate of same channels during same episode. Following MCTS there was no noticeable change in any of the parameters that are usually measured (burst duration and TBAPM not shown).

C. Grand means of spontaneous activity parameters (10 recording channels averaged over 20 min bins before and after MCTS) reveal that no single parameter increased over 10 percent following MCTS.

A.


CS133b
MCTS
14,37,43,54
Mean Burst Rate (bpm-10 Chs)
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
Time (min)

B.


CS133b
MCTS
14,37,43,54
Mean Spike Rate (spm-10 Chs)
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
Time (min)

C.

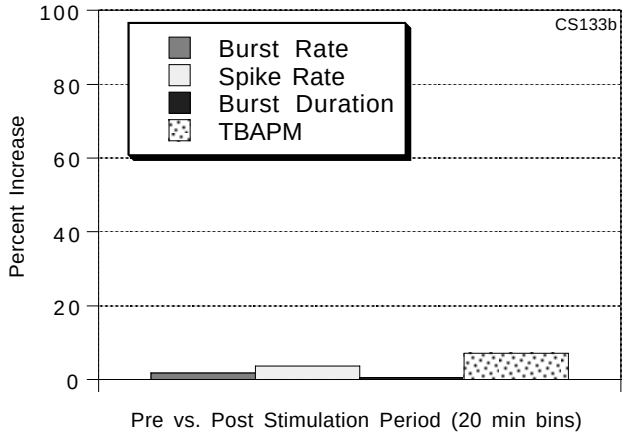

CS133b
Burst Rate
Spike Rate
Burst Duration
TBAPM
Percent Increase
100
80
60
40
20
0
Pre vs. Post Stimulation Period (20 min bins)

Figure 40. Changes in MEAPs vs changes in spontaneous activity following SCTS.

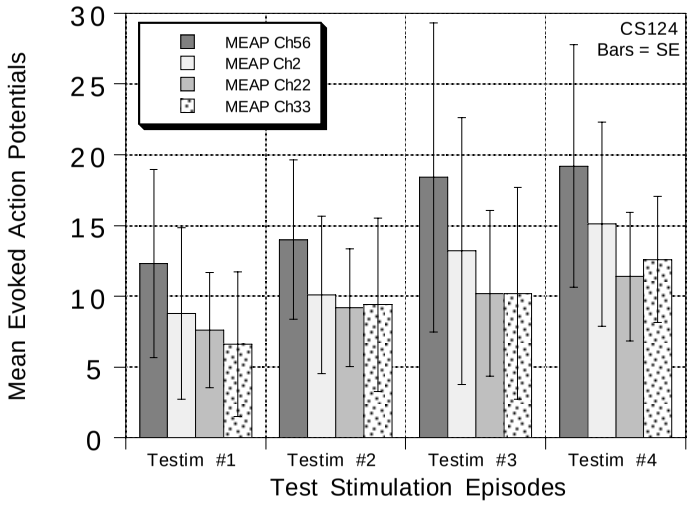
A. Twenty test-pulses were delivered to channel 28 and evoked action potentials were recorded on channels 2, 22, 33, and 56 before and after SCTS on channel 28. Test stimulation 1 serves as a control. Following each SCTS episode, there was an increase in the MEAPs on all four channels.

B. Grand means of the four channels show a 21, 22, and 12% increase following each episode of SCTS respectively. There was no significant difference between the MEAP values of the first and second, second and third, and third and fourth test stimulation episodes. However, there was a significant difference between the MEAP values of the first and fourth stimulation episodes (student's t, 6 degrees of freedom, $P \leq 0.05$).

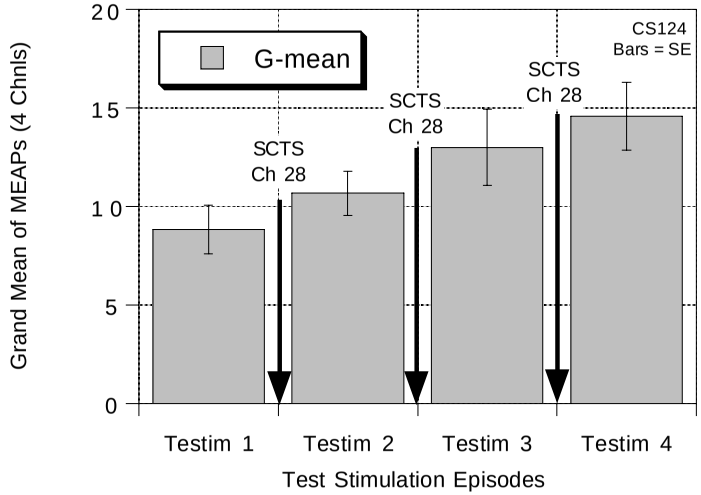
C. The mean spike rate (12 channels) depicts an overall decrease following each SCTS episode.

D. The mean spontaneous bursting activity, prior to the first stimulation, was relatively stable during the native activity episode. Following the first SCTS on channel 28 there was an increase in the mean burst rate for ~15 min then a downward trend began which continued following (or was facilitated by) each subsequent stimulation on the same channel.

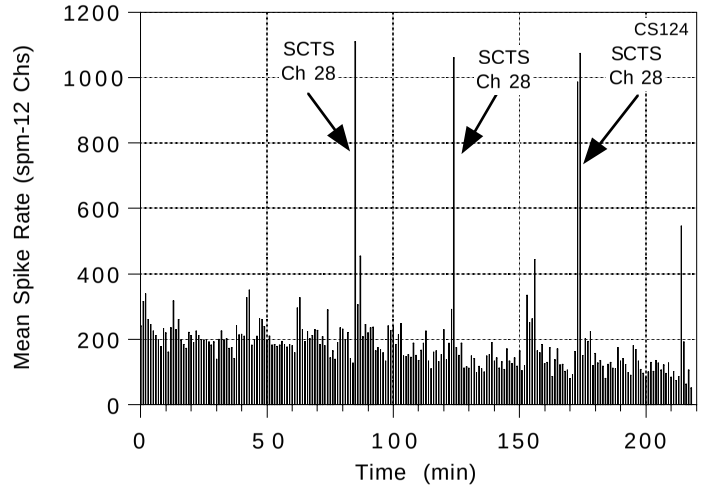
A.


MEAP Ch56
MEAP Ch2
MEAP Ch22
MEAP Ch33
CS124
Bars = SE
Mean Evoked Action Potentials
0
5
10
15
20
25
30
Testim #1
Testim #2
Testim #3
Testim #4
Test Stimulation Episodes

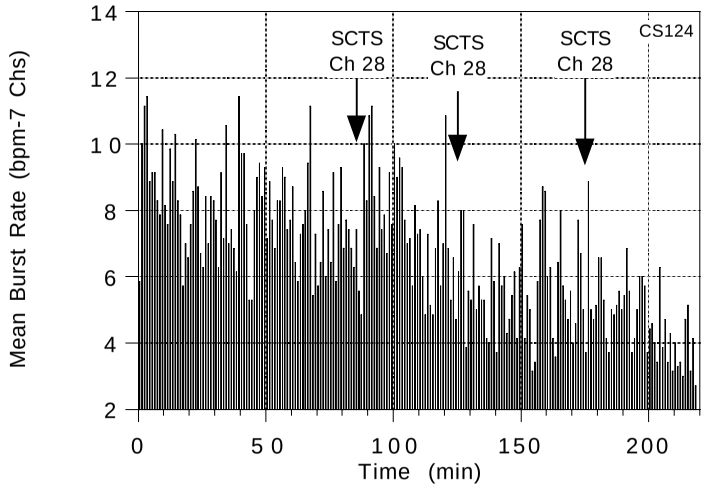
B.


G-mean
CS124
Bars = SE
SCTS
Ch 28
SCTS
Ch 28
SCTS
Ch 28
Grand Mean of MEAPs (4 Chnls)
0
5
10
15
20
Testim 1
Testim 2
Testim 3
Testim 4
Test Stimulation Episodes

C.


CS124
SCTS
Ch 28
SCTS
Ch 28
SCTS
Ch 28
Mean Spike Rate (spm-12 Chs)
0
200
400
600
800
1000
1200
0
50
100
150
200
Time (min)

D.


CS124
SCTS
Ch 28
SCTS
Ch 28
SCTS
Ch 28
Mean Burst Rate (bpm-7 Chs)
2
4
6
8
10
12
14
0
50
100
150
200
Time (min)

Chapter 3 Section 2

EFFECTS OF REPETITIVE STIMULATION ON CULTURED NETWORKS

3.2.1 Background and Specific Methods

Single pulses delivered in a repetitive manner, usually at frequency of 0.5-2 Hz have been shown to induce synaptic depression in several preparations (Artola and Singer, 1993). Like LTP, LTD of GABA-ergic inhibitory transmission has been reported (Stelzer et al., 1987; Liu et al., 1993). This synaptic alteration is similar to LTP because its induction has been shown to be dependent upon postsynaptic depolarization, activation of NMDA receptors, and the elevation of internal calcium levels (Linden, 1994). The cascade of biochemical events that bring about synaptic depression involves the activation of calcium-dependent phosphatases, triggered primarily by the influx of extracellular calcium (Cohen, et al., 1998).

Repetitive stimulation, or low frequency stimulation (LFS) was used in this study to determine if the spontaneous activity in spinal cord networks could be effectively decreased by such a stimulation method. Different frequencies ranging from 0.5-2 Hz were attempted in early experiments. It was decided later that 1 Hz stimulation would be the frequency for the rest of the LFS experiments because it gave results that were comparable to or better than the other frequencies. One Hertz stimulation was also the most common low frequency stimulation found in the literature (for review, see Wagner and Alger, 1996).

The rationale was that, if repetitive stimulation is successful in the induction of synaptic depression in this preparation, then that synaptic depression should lead to a decrease in spontaneous transynaptic signalling. Depending on the number of synapses modified, the "importance" of the synapses in the network, and the magnitude of the depression, this form of stimulation could lead to a noticeable change in spontaneously occurring action potentials or changes in action potential firing patterns.

Data presented below indicate that LFS did lead to a clear decrease in spontaneous activity; and in some cases caused a total cessation of spontaneous bursting activity following termination of the LFS. The beginning of a response was identified as a distinct change of activity from the pre-stimulus activity. The cessation of the response was characterized as the return of spontaneous activity to pre-stimulus levels. Responses were classified as short or long-term based on the duration of responses. Long-term responses to LFS were defined as a ten percent change in mean spontaneous activity over a 15 min interval relative to pre-stimulus activity, with the expected response being a decrease activity. Total cessation was determined to be a period of 5 min or more with no evidence of bursting activity. Spiking activity, however, was never nullified totally.

Single, biphasic pulses (300 μsec per phase) were delivered at a rate of 1 Hz usually for 15 min periods. In experiments involving test pulses, the same general methodology used in the previous section was employed--single test pulses were delivered to the designated stimulus channel before and after the actual stimulation episode in order to count the number of evoked action potentials immediately following each test pulse. Pre-stimulus mean evoked action potentials (MEAPs) were compared to post-stimulus MEAPs to determine if units recorded on selected channels were more or less responsive to the test pulses following the LFS episode. In four out of six trials, the percent change in MEAP values were found to be more sensitive to LFS than percent changes in burst rate.

Stimulus histograms were obtained post-hoc from some of the taped experiments because the capability to employ this type of analysis became available only recently in this laboratory. The figures containing stimulus histograms were presented below primarily for two reasons: (1) to demonstrate that specific units within the network were, indeed, being stimulated and did respond to the stimulation, and (2) to show how the response of discriminated units within the preparation changed over time *during* the stimulation episode. It is logical to assume that changes in responses to the same stimulation over time is an

indicator that (1) the intrinsic properties of these units, or (2) the synapses themselves, or (3) both the units and the synapses, are in the process of being modified throughout the course of the stimulation episode (see Turrigiano, et al., 1994). Therefore, stimulus histograms of different segments of the stimulation episode are presented below to observe the changes in evoked responses to LFS over time. It should be noted that the products of these data analyses were not technically stimulus histograms, but peri-event histograms. That is, the stimulus pulse itself was identified and discriminated apart from the neuronal electrical activity. This waveform was then used as the reference unit for the peri-event histogram calculations.

3.2.2 Overview of Responses to Repetitive Stimulation

Qualitative data were collected directly from the stripchart for a total of 23 cultures. A subset of these (19 cultures) had additional quantitative information (data collected on the Masscomp and analyzed via KaleidaGraph™). The total number of trials for the RS data set was 75 (58 single channel and 17 multichannel). As summarized in Table 4, 34% of the SCRS and 41% of the MCRS trials showed decreases in one or more channels.

Repetitive stimulation on single channels was observed to stop spontaneous activity on all channels in four different experiments. Selected portions of one such experiment is shown in Figure 41. The spontaneous bursting activity was suppressed for over 10 min with a gradual recovery ranging from 7-18 min across recorded channels (Fig. 41C). In some cases, complete depression lasted for more than 40 min. There was no apparent damage to the cells since the return of activity was similar to pre-stimulus activity following the tetanic stimulation that interrupted the depression period (data not shown). Repetitive stimulation on multiple channels has never resulted in complete network depression.

Figure 41. Integrated Spontaneous Activity.

A. Native spontaneous activity from seven different recording channels depicted as integrated bursts on a stripchart.

B. Spontaneous (and evoked) activity during a repetitive stimulation episode (single pulses delivered to a single channel @ 0.2 Hz.

C. Absence of spontaneous bursting activity on all recording channels (including 7 more channels not shown) following cessation of 0.2 Hz repetitive stimulation.

Time bar (beneath the uppermost trace in C) = 1 min

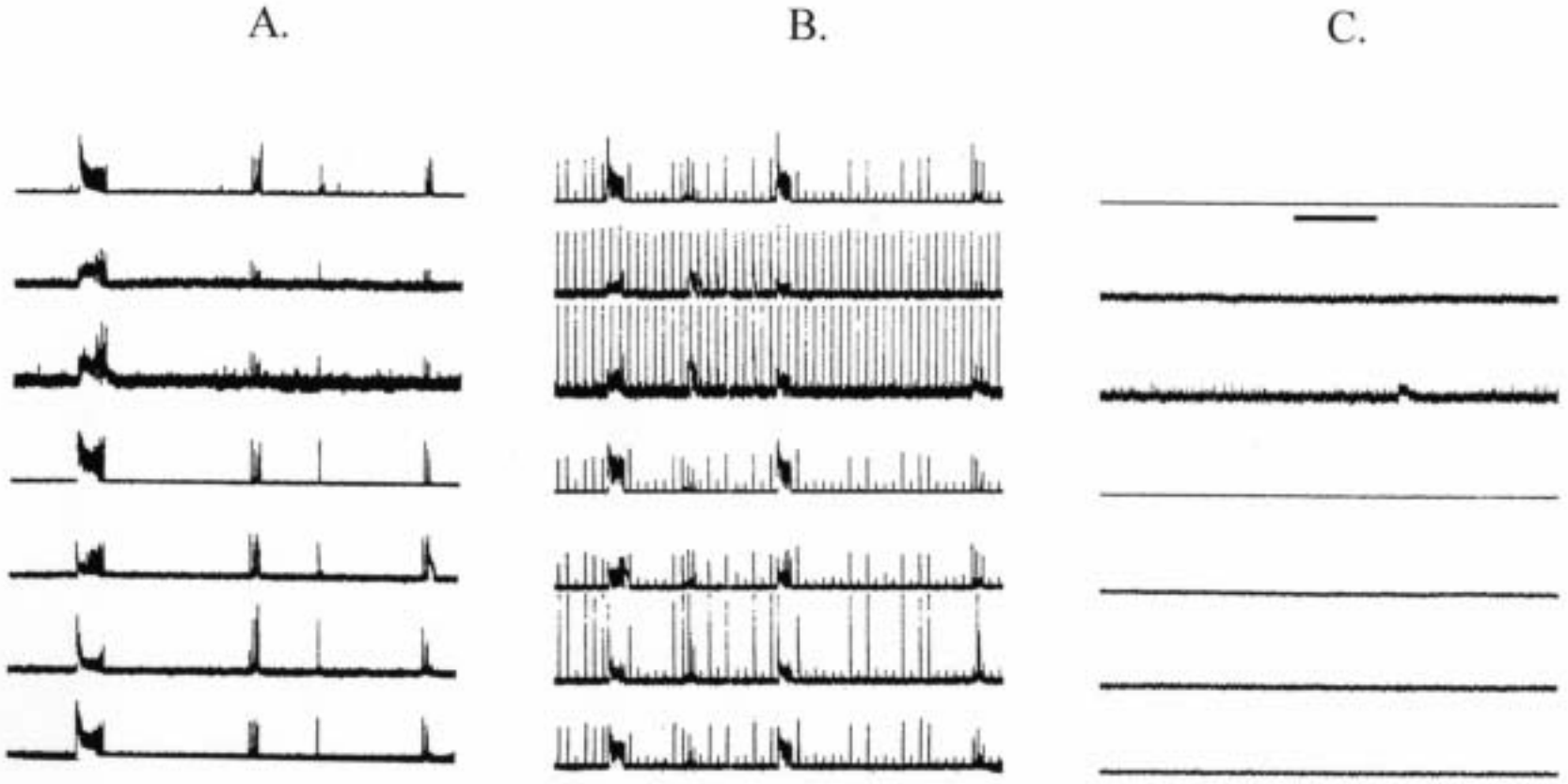

A.
B.
C.

Table 5 lists specifications of the cultures used in the repetitive stimulation data set. The bottom row shows the total number of cultures and the means for each column.

Table 5. Repetitive Stimulation Data Set

Culture #	**Expt. Date**	**Age (days)**	**Avg SNR**	**Active Elec (%)**	**NRE %**	**SCRS #**	**MCRS #**
33	10/19/94	104	4.8	18	15	3	1
34*	11/30/94	103	2.4	33	23	1	
37*	12/21/94	123	3.4	33	35	1	2
51	3/22/95	111	3.0	78	52	3	8
56	5/3/95	62	6.0	54	28	5	1
58	7/10/95	72	2.9	56	70	7	
66	9/14/95	132	6.0	69	64	1	1
67*	9/21/95	63	3.0	63	27	4	
70*	10/4/95	89	4.0	41	41	3	
76*	10/22/95	41	3.3	50	57	3	
77	10/25/95	41	3.5	83	53	6	
79	10/28/95	56	3.4	43	52	1	
80	10/30/95	39	2.7	66	48	7	1
90*	3/27/96	158	2.2	77	19	1	
91*	3/31/96	103	1.9	66	51	1	
99	4/14/96	81	3.0	72	72	3	2
123	8/20/96	60	3.9	66	40	1	
124*	9/4/96	102	2.6	51	45		1
131	10/23/96	68	3.2	40	30	1	
132*	10/30/96	41	4.7	19	38	2	
135	11/13/96	43	4.3	88	39	1	
140*	1/8/97	72	3.0	30	2	1	
149	2/5/97	60	4.5	78	70	3	
23		Mean: 81 ± 32.8	Mean: 3.6 ± 1.09	Mean: 55 ± 20.4	Mean: 42 ± 18.4	*58*	*17*

Legend: Avg SNR = Mean Signal-to-noise ratios (includes values of 1.5:1); Active Elec (%) = percentage of electrodes with active unit(s); NRE = Network Response Electrodes (percentage of electrodes that, when stimulated with a single test pulse, caused a network response); SCRS # = Number of SCRS stimulations delivered; MCRS = Number of MCRS stimulations delivered. Starred items represent experiments in which data was collected on computer. Italicized values represent a summation of the corresponding column. Values following means represent the standard deviation of the means.

Table 6 is a summary table of qualitative results from repetitive stimulation trials (experiments: 30-149) derived from stripchart data only.

Table 6A. Repetitive Stimulation Summary

Stim Type	Number of Trials	Decrease in Activity† (>50% of Chs / <50%)	Increase in Activity	No Effect	Mean Delay Network Time
SCRS	58	34% (28% / 11%)	17%	49%	3.4 ± 6.95 min
MCRS	17	41% (41% / 0)	6%	53%	0.2 ± 0.37 min
Both	75	35% (26% / 9%)	15%	50%	

†Burst rate SCRS=Single channel repetitive stimulation; MCTS=multi-channel repetitive stimulation

Table 6B. Durations of Effects of LFS

Stim Type	Mean Duration of Decrease (min)	Mean Duration of Spontaneous Decay (min)
SCRS	12.8 ± 14.31* (n = 16)	5.0 ± 3.87 (n = 8)
MCRS	5.2 ± 7.70* (n = 7)	2.4 ± 2.53 (n = 6)

The values in Table 6 do not include changes in other burst parameters (i.e. burst area, burst duration, etc) or other pattern changes. The values in the category of mean duration were calculated from the trials where the effect was allowed to decay on its own. Starred values include durations that were cut short by another stimulation. The maximum duration of decreased spontaneous activity following SCRS (which was cut short) was 44 minutes. The maximum recorded length of depressed activity following MCRS (also cut short) was 20 minutes.

The histogram in Figure 42 portrays the durations of recorded responses to repetitive stimulation in which there was a decrease in spontaneous activity. Twenty-seven percent of responses (14 trials) were classified as long-term (>15 min).

In only 30% of repetitive stimulation trials was there a delayed response following the stimulation episode (Fig. 43). This percentage is less than half of the 72% value for delayed responses seen following tetanic stimulation. Figure 44 portrays the longest of such delays for all recorded repetitive stimulation trials. The response is considered to be caused by the stimulus because of a similar delayed response to tetanic stimulation on the same stimulus channel in the same culture (see Fig. 34).

The large difference in the number of delayed responses for repetitive stimulation trials and the number of delayed responses for tetanic stimulation trials is likely due to the large disparity between the durations of the stimulus episode (~23 sec for HFS vs 15 min for LFS). That is, the network has ample time to respond to the stimulus during the repetitive stimulation episode (see Fig. 10).

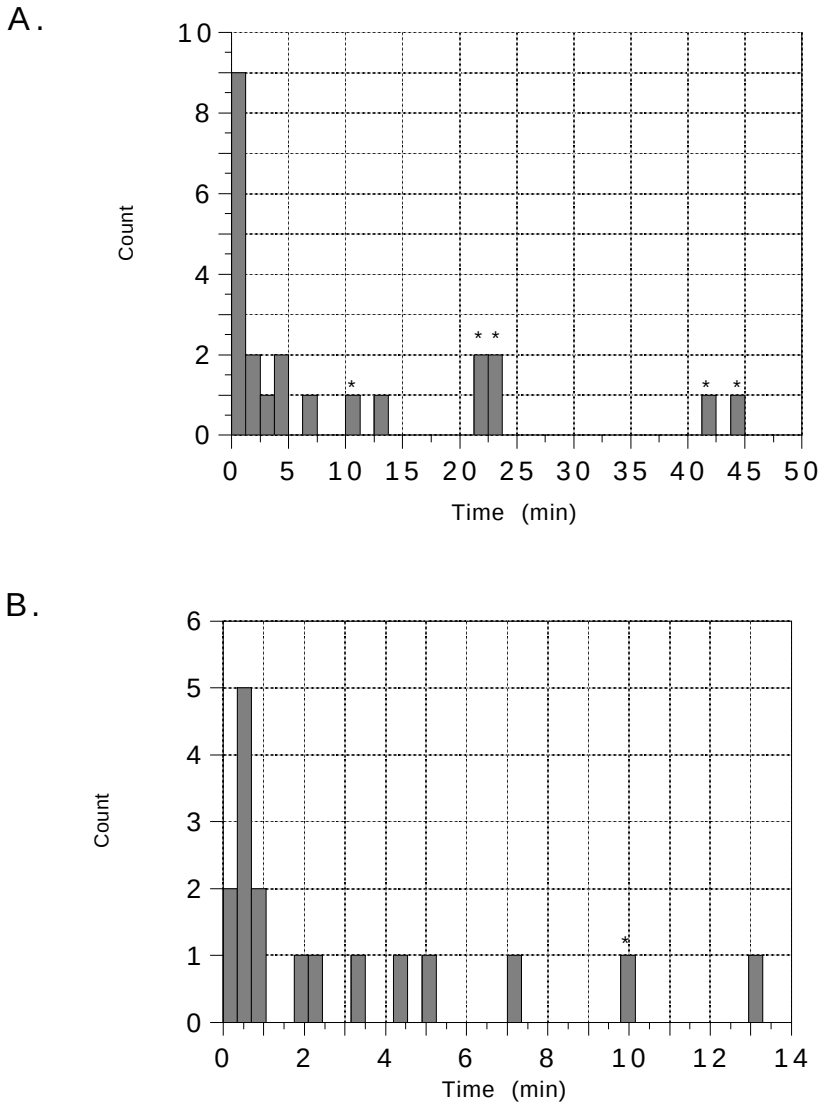


Figure 42. Frequency histograms of response durations to repetitive stimulation.

A. Graph includes durations of responses to both SCRS and MCRS. The activity parameter measured (via visual inspection of stripchart and computer data) was burst rate (75) trials total). Starred items represent occasions when the response was cut off by tetanic stimulation. Bins = 1.25 min

B. Short-term responses (subset of Graph A). Bins = 0.33 min

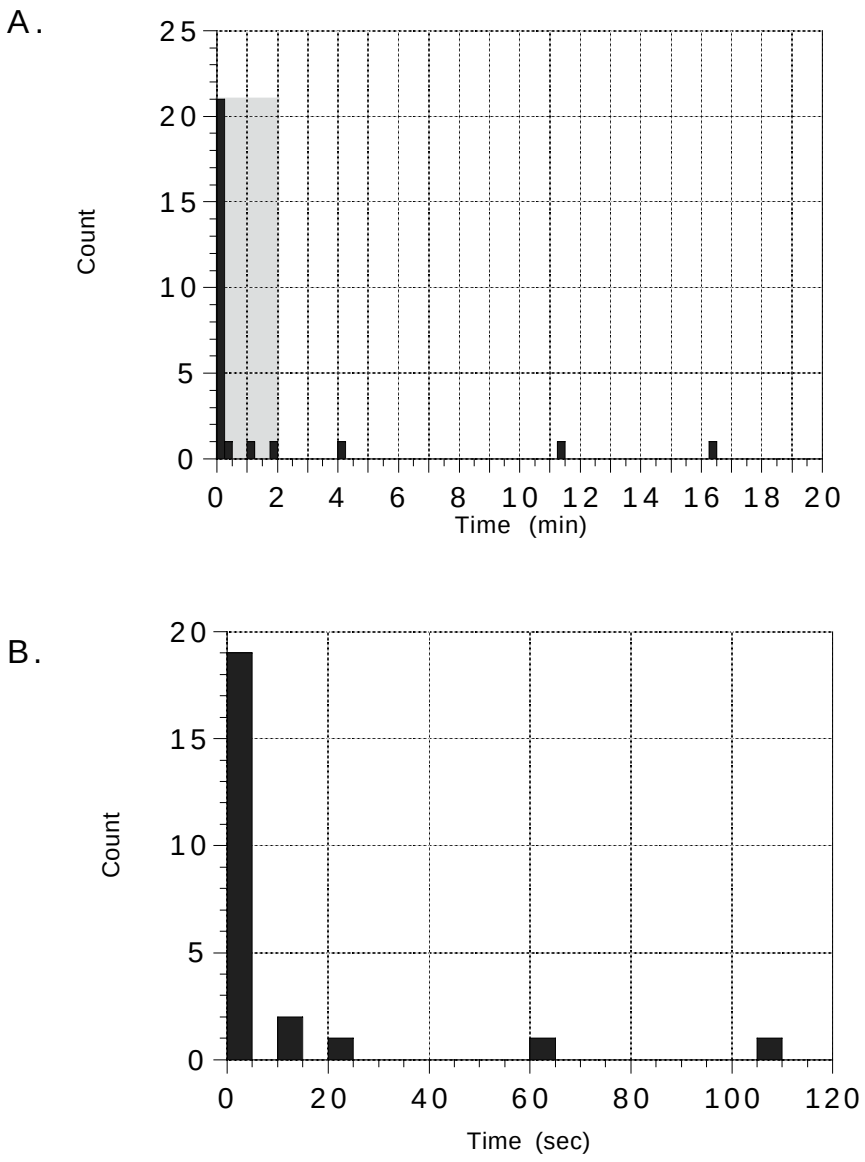


Figure 43. Response delay histograms. Frequencies of time intervals between cessation of stimulus and onset of response (decrease in burst rate) to SCRS & MCRS. A. All delay intervals recorded linked to a response. The delay of 16 min was included because of stripchart data and an earlier delay following SCTS on the same electrode. B. Subset of Graph A (shaded area) to show that in 70% of recorded intervals, the response delay was less than 2 seconds.

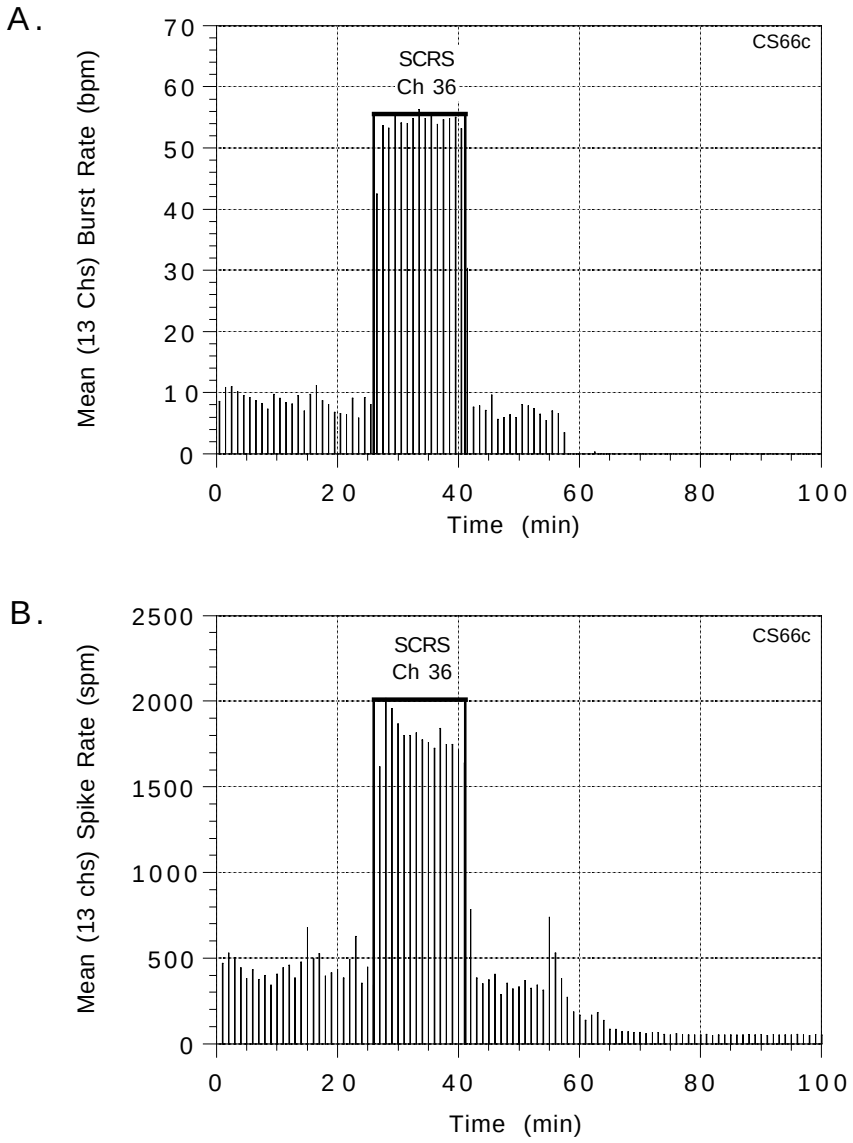


Figure 44. Delayed Response to Single Channel Repetitive Stimulation.

A. Mean burst rates for 13 recording channels. Sixteen minutes after SCRS, the network ceased all bursting activity. The duration of depressed activity lasted for over 40 min with no recovery, until another stimulation episode was initiated.

B. Mean spike rates for 13 channels. After the delay, the spike rate decreased to ~60 spikes per minute. [See Fig. 33 for a similar delayed response to stimulation on the same electrode in the same culture.]

3.2.3 Network Activity During Repetitive Stimulation

Because the repetitive stimulation episode is so long in comparison to the tetanic stimulation episode, efforts were made to determine network activity (spontaneous and evoked) during the stimulation episode that might be used to predict whether or not there would be a change in the spontaneous activity following the cessation of the repetitive pulses. Mean spike rates for several stimulation trials were obtained and examined in order to determine if there was a noticeable change in the spiking activity during LFS. Examples of three such trials from C-149 are depicted in Figure 45. The changes in evoked (and spontaneous) activity over the course of the different stimulus episodes seemed to warrant further investigation of how the network was responding during this 15 minute time period.

A classic and more specific approach to characterize this evoked activity is the stimulus histogram. However, during the course of these experiments, computer generated stimulus histograms were not available. Therefore, six experiments that were recorded on analog tape were replayed back into the updated Plexon ™ data collection and analysis system in order to generate stimulus histograms. Stimulus histograms from nine different LFS trials (5 SCRS trials, and 4 MCRS trials) were generated post hoc. The mean number of recorded units per LFS trial was 15 ± 1.2.

Individual units were qualitatively evaluated as to their change in spontaneous activity following LFS, by inspection of spike rate plots (see Fig. 46), as well as their responsiveness to the stimulus pulse during LFS via stimulus histograms. Classification of responses depicted by rate histograms included: (1) decrease in spontaneous activity (the expected response); (2) no response; and (3) increase in spontaneous activity.

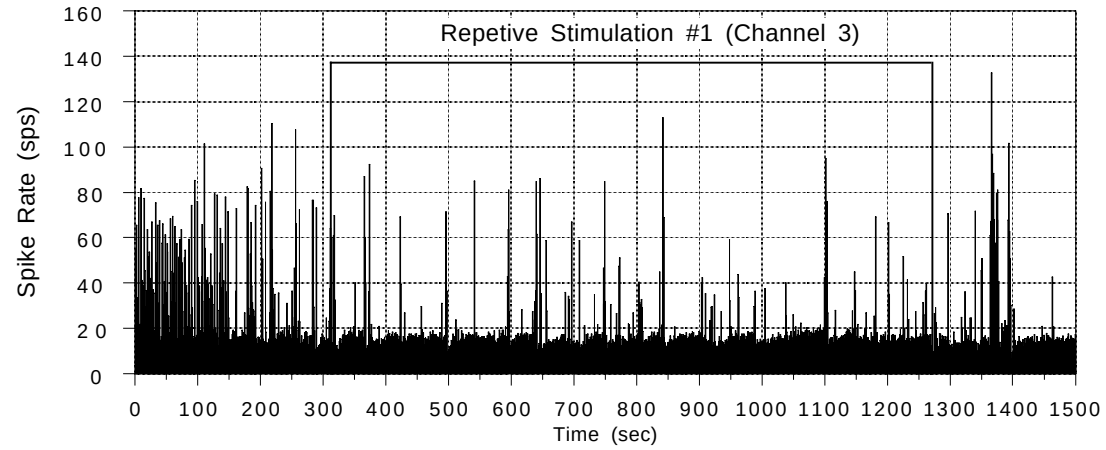
Figure 45. Changes in spiking activity during repetitive stimulation.
Mean (14 recording channels) spike rates of before, during and after 3 different single channel repetitive stimulation episodes are depicted. Total length of activity shown for each panel equals 25 min.

A. Spiking activity that had already begun to decrease prior to the onset of SCRS, diminished more so during the LFS episode, and remained at that level for ~10 min (later activity not shown) following the cessation of the stimulus episode. Notice the quasi-periodic baseline (<20 sps) activity associated with bursts.

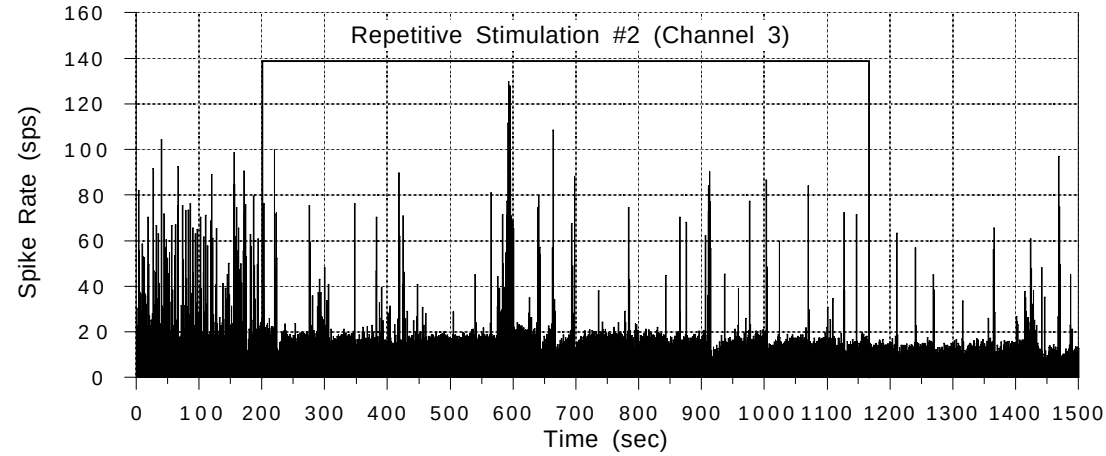
B. Higher frequency spiking activity was almost immediately attenuated at the onset of the second LFS episode on the same stimulus channel (~25 min after the first LFS episode). The depressed spiking activity remained depressed after SCRS for over 20 min until interrupted by a tetanic stimulation on the same stimulus channel which doubled the spike rate for 20 min (not shown). Notice the slow, but relatively constant rate of decline of baseline activity 10 min after the onset of LFS (starting from about the 800 s mark).

C. The third SCRS episode attenuated spiking activity at the beginning of the episode and to a greater extent ~3.7 min into the LFS. This time, the nature of the activity changed during and after the LFS. After the high burst of action potentials at ~390 sec, the baseline activity dropped to ~12 sps, then gradually rose to above 20 sps and remained at that level for ~600 sec (10 min), during and after which the action potentials were clustered in to high frequency bursts. The depressed activity remained at or near those levels for ~40 min after SCRS despite three subsequent attempts to reverse the depressed activity (via SCTS).

A.


Repetive Stimulation #1 (Channel 3)
Spike Rate (sps)
0
20
40
60
80
100
120
140
160
0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500
Time (sec)

B.


Repetitive Stimulation #2 (Channel 3)
Spike Rate (sps)
0
20
40
60
80
100
120
140
160
0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500
Time (sec)

C.

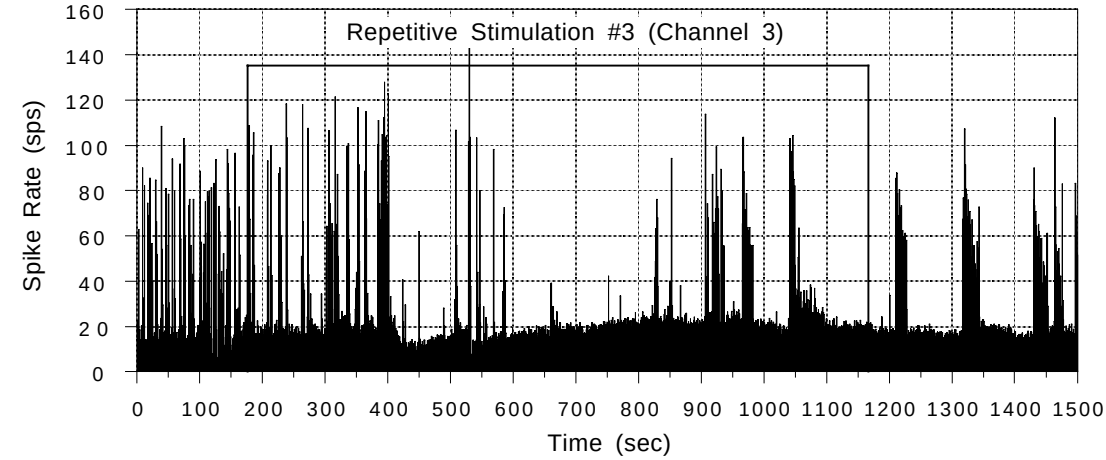
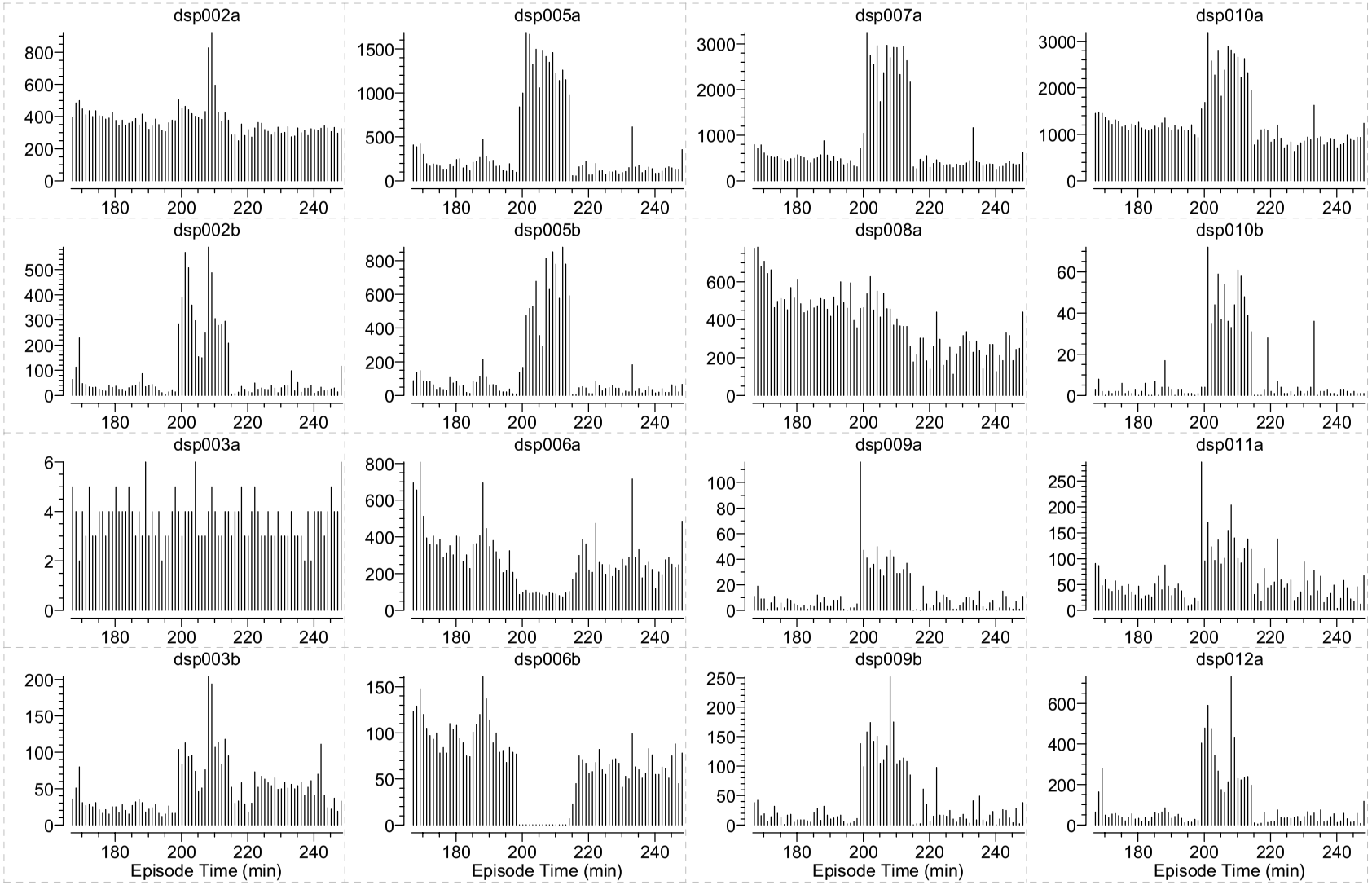

Repetitive Stimulation #3 (Channel 3)
Spike Rate (sps)
0
20
40
60
80
100
120
140
160
0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500
Time (sec)

Figure 46. Spike rate plots for 16 units before, during and after MCRS.

Each graph represents the activity of a discriminated unit (single neuron). The action potentials for each single unit were recorded on separate digital signal processors (dsp) and counted for the duration of the episode (10,000 to 14,900 sec). The time bins were 1 min in duration (i.e. spikes per minute on y-axis). Three channels were repetitively stimulated at 1 Hz from 199 to 214 min.

Responses during the MCRS episode were strongly excitatory. Only units 006a and 006b, derived from the same physical electrode channel, showed a clear inhibition. Unit 3a (zeros dropped for brevity) must be ignored because of a lack of activity.

Responses after the MCRS were mostly inhibitory when compared to pre-MCRS activity levels, with the greatest inhibition seen in units 6b and 8a (36% and 50% decrease in spike rate, respectively). Most decreases ranged from 15% to 30%. Only two units showed increases in activity (3b: 134% and 9b 53%).


dsp002a
dsp005a
dsp007a
dsp010a
dsp002b
dsp005b
dsp008a
dsp010b
dsp003a
dsp006a
dsp009a
dsp011a
dsp003b
dsp006b
dsp009b
dsp012a
Episode Time (min)
Episode Time (min)
Episode Time (min)
Episode Time (min)

Classification of responses depicted by stimulus histograms were as follows: (1) association: histogram shows a clear pattern of evoked responses to the stimulus pulse, with a majority of action potentials following the pulse rather than preceding the pulse, and one or more peaks in the histogram immediately following the stimulus pulse; (2) weak or no association: a slight change (or no change) in the pattern of spikes before and/or after the stimulus pulse; (3) negative association: when the stimulus pulse actually seems to inhibit action potentials immediately following it in time (in these cases, there is often a greater number of action potentials preceding the pulse than following it). Examples of these descriptions (except for negative correlations) are shown in Figure 47.

Figure 48 (A, B, & C) displays an example of how responses to LFS can change over time. To reiterate, these graphs delineate how (or whether) spiking activity was influenced by the stimuli. That is, stimulus histograms are a graphical representation of the timing of evoked responses for each unit to the stimulus pulse.

The classification of responses characterized by the stimulus histograms were used to formulate the graph shown in Figure 49. Figure 49 represents a normalization of responses during and after LFS for the experiments that were replayed through the Plexon data analysis system. The evaluation of responses during LFS were combined with the evaluation of responses after LFS, in order to determine whether a high degree of association (of evoked activity to the stimulus pulse) tended to influence the change in spontaneous activity following the cessation of repetitive stimulation. Based on the data from the nine LFS trials, there was no clear evidence that responses during LFS influenced the change in spontaneous activity following the stimulation episode. That is, unit-by-unit analysis revealed no relationship between responsiveness to stimuli during LFS and the alteration of spontaneous activity following LFS.

Figure 47. Stimulus histograms for the multichannel repetitive stimulation episode. Stimulus histograms were generated for the same 16 units (as in Fig. 46) during the entire 15 min MCRS episode (i.e. data derived from 900 single pulses). Action potentials from the 16 channels were counted for each 1 ms time bin for 200 ms immediately prior to the single pulse and for 200 ms following the pulse. Thus, the x-axis represents time in seconds, with the zero point designating the time of the stimulus pulse. The y-axis represents the number of action potentials counted for each bin.

The most apparent feature of this figure is the differential responses to the same stimuli. Units 2a, 3a, 3b, 5a, and 9a showed weak or no association with the stimulus pulse. Units that were associated were 2b, 5b, 7a, 8a, 10a, 10b, 11a, and 12a. As was indicated on the previous set of graphs, units 6a and 6b showed an inhibition of activity during the stimulus episode.

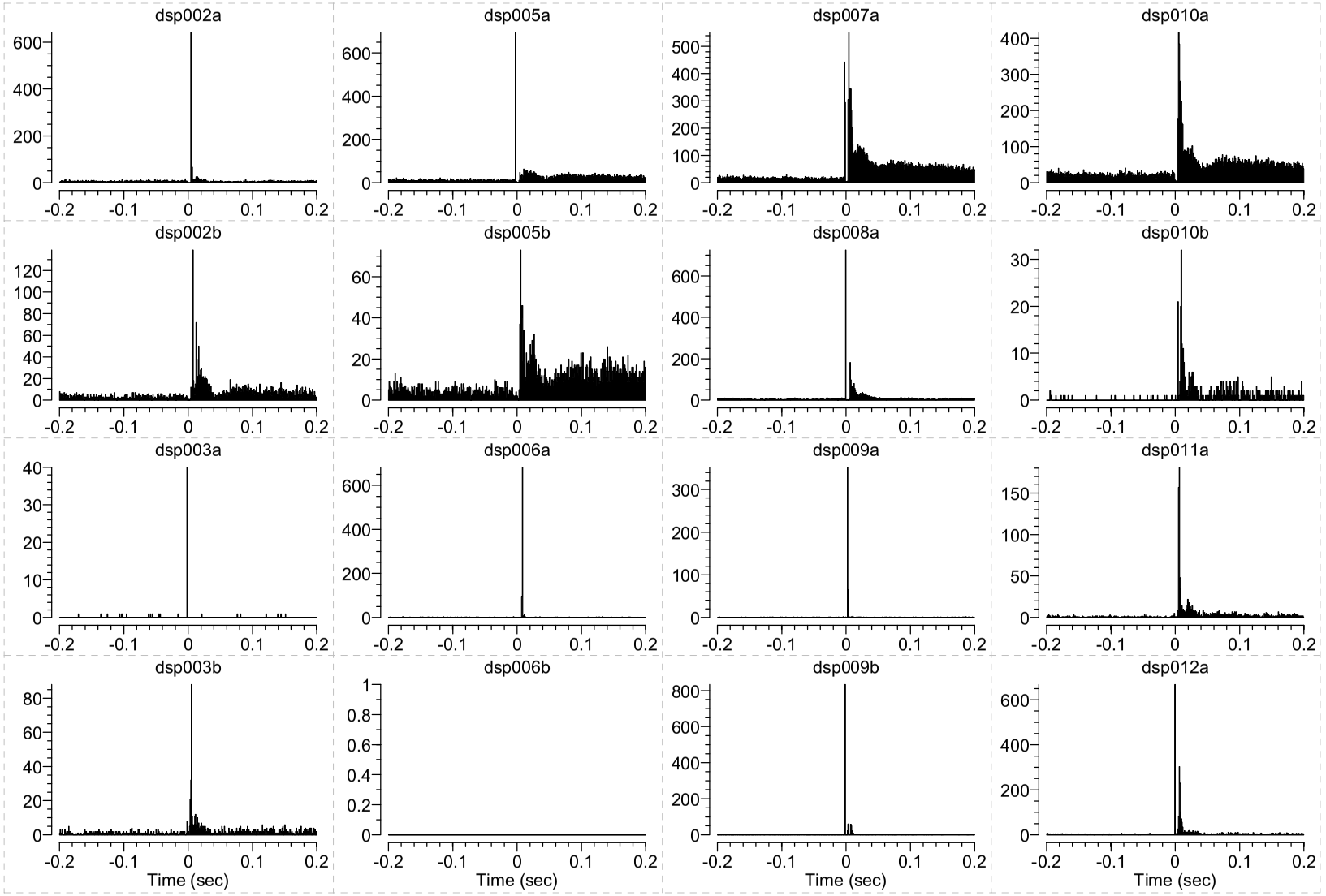

dsp002a
dsp005a
dsp007a
dsp010a
dsp002b
dsp005b
dsp008a
dsp010b
dsp003a
dsp006a
dsp009a
dsp011a
dsp003b
dsp006b
dsp009b
dsp012a
Time (sec)
Time (sec)
Time (sec)
Time (sec)

Figure 48. Change in Stimulus Histogram profiles over time. The next three panels depict stimulus histograms for eight of the sixteen discriminated units for experiment 66b, in which three channels were repetitively stimulated simultaneously with single pulses at 1 Hz for 15 min. The entire repetitive stimulation episode was divided into five 3 min intervals (180 pulses). Each figure represents a 3 min interval. Action potentials were collected in 1 ms bins (200 ms before and 200 ms after the stimulus pulse). Scale for the y-axis was dependent on the total spikes per bin. [dsp (digital signal processor) = discriminated unit or single neuron.]

A. First 3 min interval (0-3 min). All units showed a greater number of spikes in the 200 ms following the stimulus than the 200 ms prior to the pulse. Unit 7a showed a high number of spikes within the first 10 ms following the pulse as well as a peak of spikes less than 5 ms immediately before the pulse (which may be an artifact), but very few around the time of the pulse. The histogram for unit 10a was similar to 7a except there was no peak preceding the stimulus. Unit 12a also displayed a peak of action potentials within 10 ms of the stimulus. The peak at the zero point may be the unit being stimulated by the pulse, or it may be the artifact of the pulse itself that was counted along with the biological responses (the latter is less likely because of single unit discrimination, and because the maximum count per bin is less than 180, which is the total number of pulses in the 3 min interval). None of the other units had clearly defined peaks following the stimulus pulse.

A.

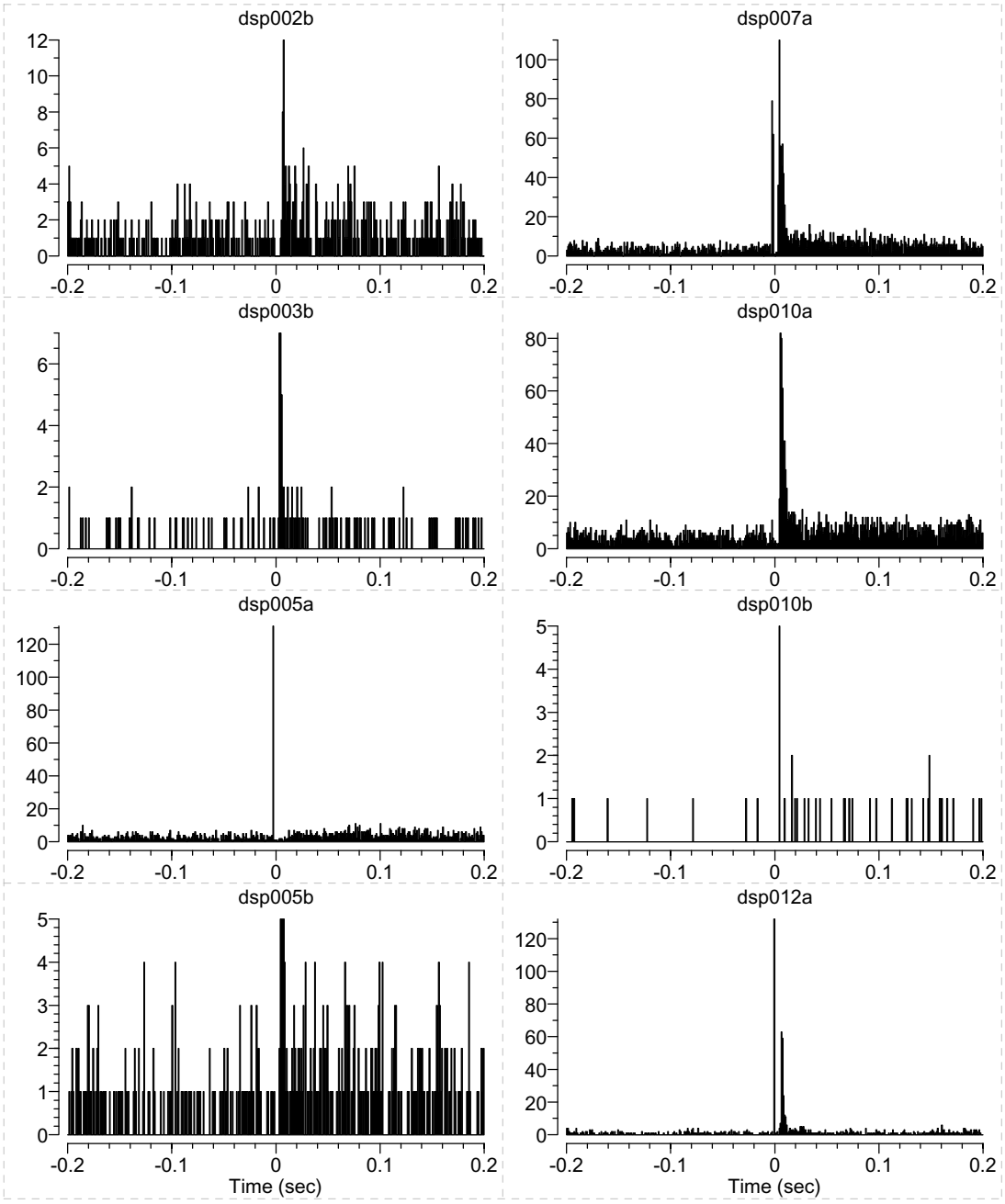

dsp002b
dsp007a
dsp003b
dsp010a
dsp005a
dsp010b
dsp005b
dsp012a
Time (sec)
Time (sec)

Figure 48

B. Third 3 min interval (6-9 min). Notice the secondary peaks within 40 ms after the stimulus for units 7a and 10a. All units showed a relative decrease in the number of spikes preceding the pulse compared to the number of action potentials following the stimulus, indicating a marked increase in evoked responses associated with the stimulus during this 3 min interval. Notice the doubling of the scale on the y-axis for units 2b and 3b. The scale for unit 5b increased almost 3-fold.

B.

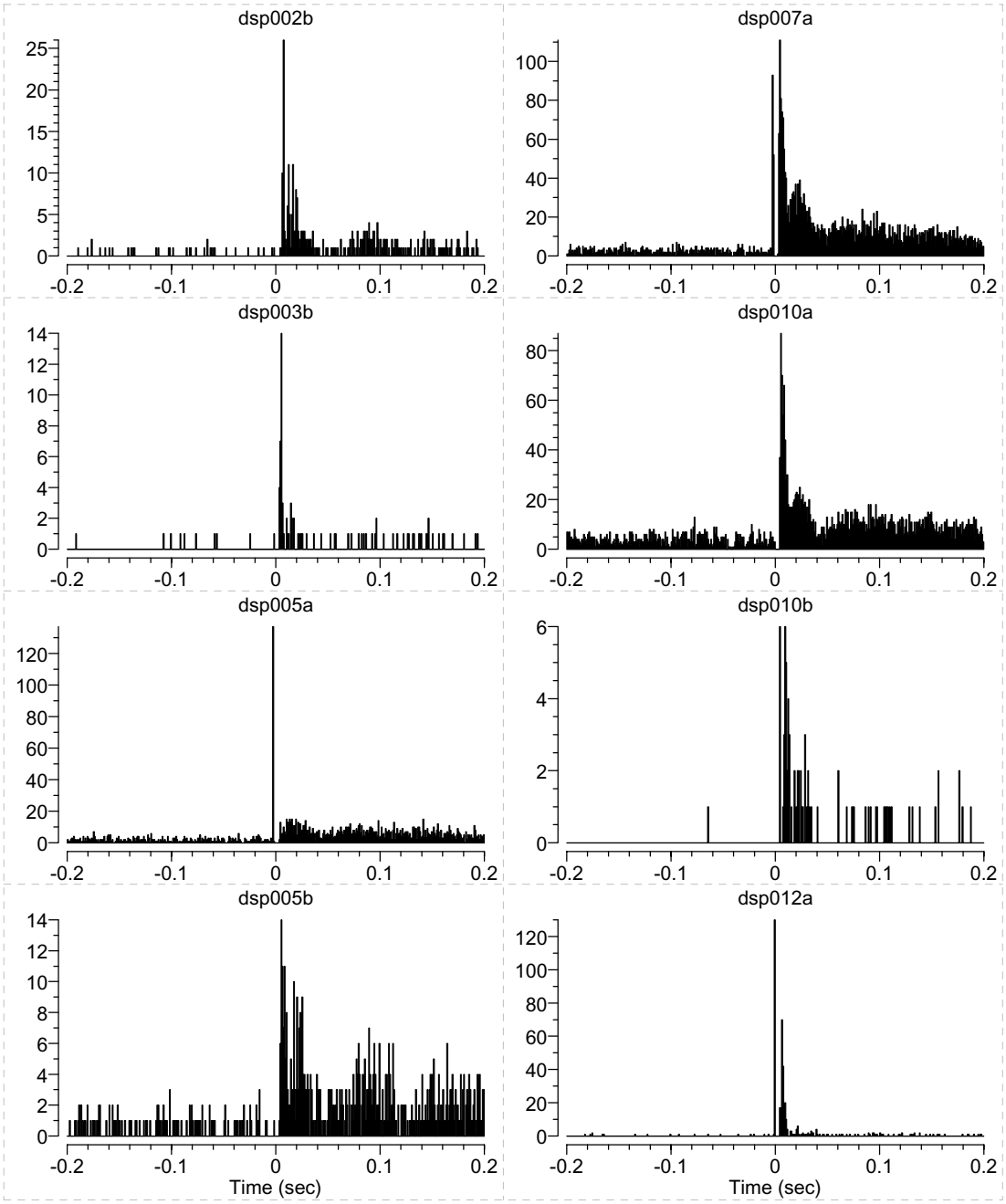

dsp002b
dsp007a
dsp003b
dsp010a
dsp005a
dsp010b
dsp005b
dsp012a
Time (sec)
Time (sec)

Figure 48

C. Fifth 3 min interval (12-15 min). The overall change in the features of the histograms between the third and final interval was not as noticeable as the difference between the first and third intervals. Nevertheless, there were notable differences. For example, the secondary peaks found in units 7a and 10a were less distinctive, and began to merge with the primary peak. A secondary elevation became apparent in the histogram for unit 12a, and a primary peak was slightly more defined for units 3b and 5a. Perhaps the most noticeable change was the increase in magnitude of the maximum peaks on the y-axis. For unit 2b, the increase was 56%; for 3b, the increase was 107%; and for 10b, the increase was 50% (note scale change).

C.

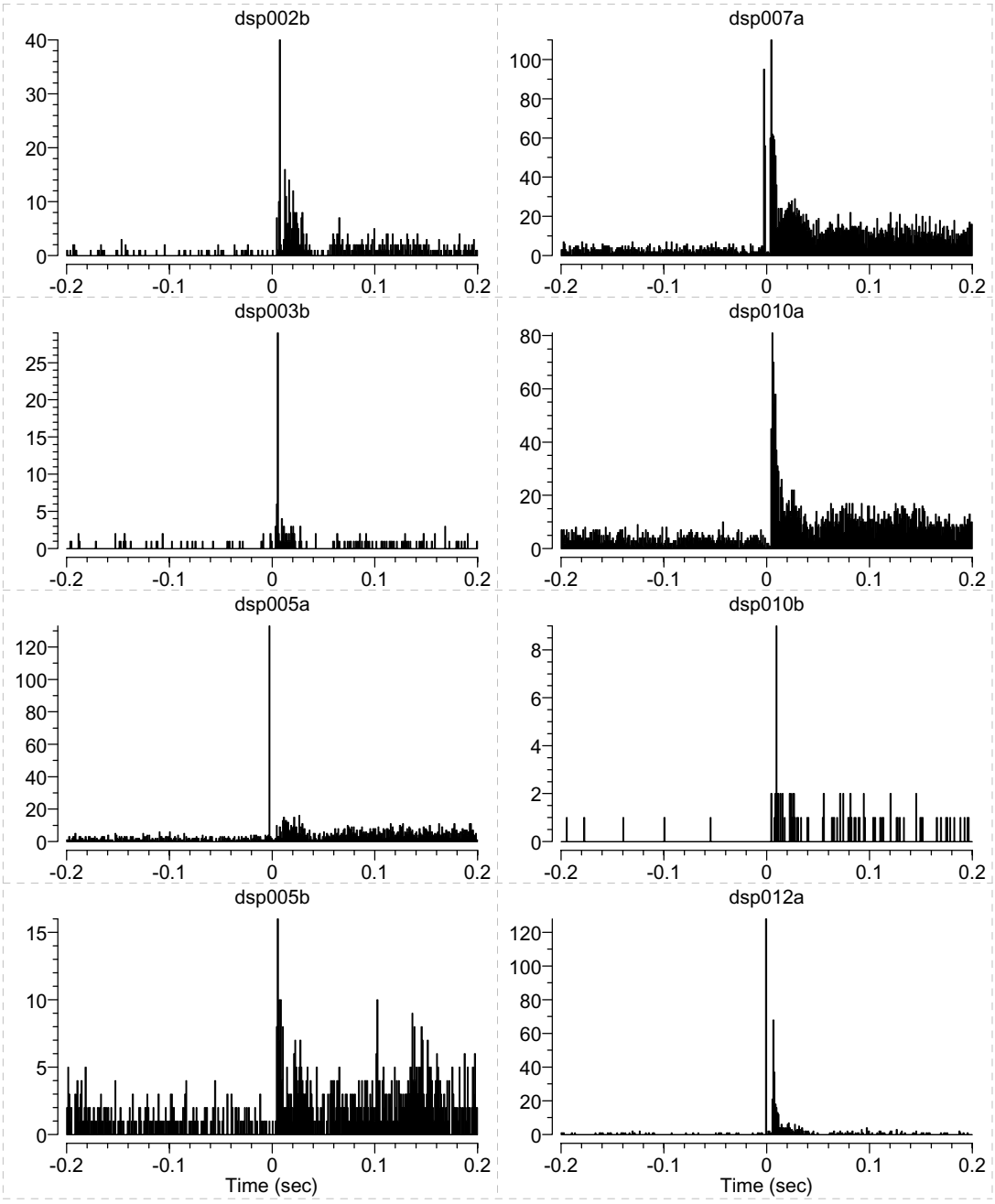

dsp002b
dsp007a
dsp003b
dsp010a
dsp005a
dsp010b
dsp005b
dsp012a
Time (sec)
Time (sec)

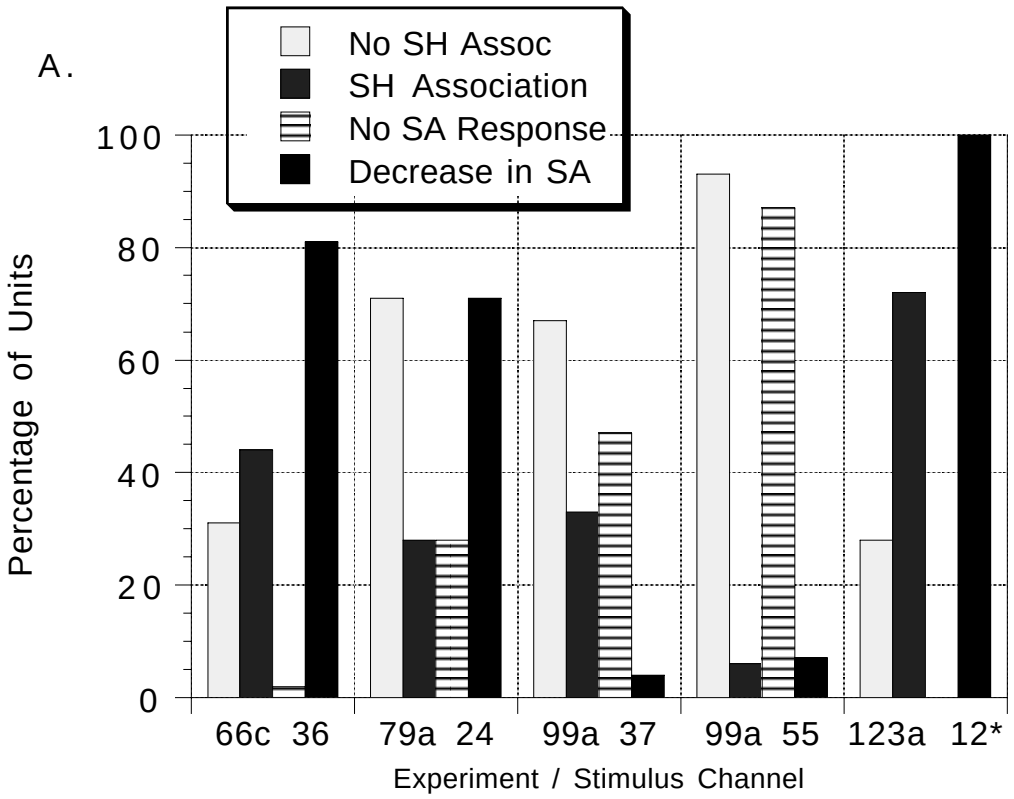
Figure 49. Summary graph of responses of single units during and after repetitive stimulation. Qualitative evaluations of changes in spontaneous activity following LFS were plotted on the same graph with evaluations of stimulus histogram profiles for 9 different stimulation trials.

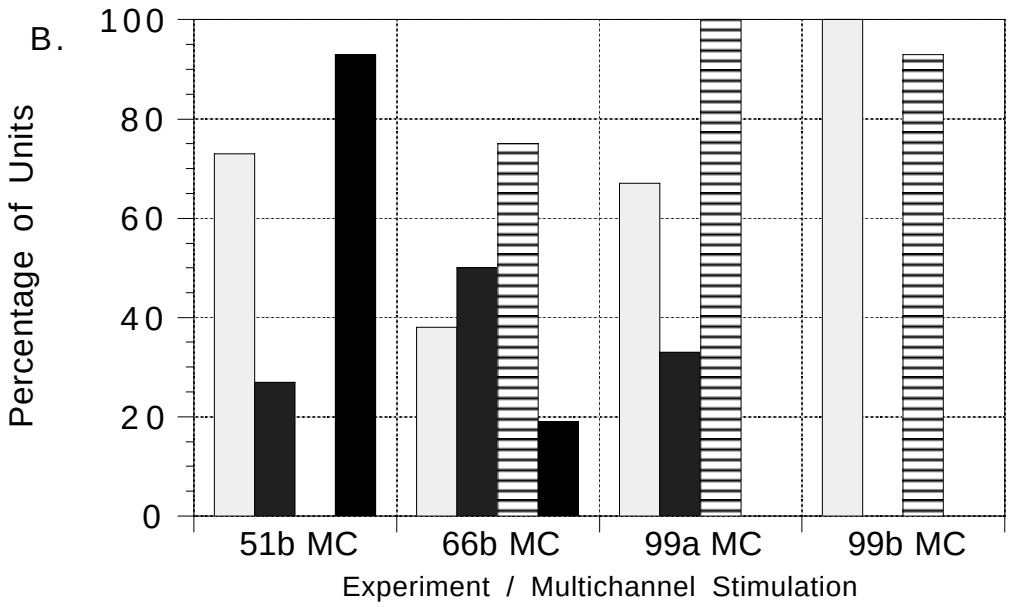
A. Responses to single channel repetitive stimulation. Following SCRS, the percentage of units showing a decrease in spontaneous activity (labeled Decrease in SA) were calculated and plotted. The same procedure was carried out for units showing no, or very little change in spontaneous activity (No SA Response). In addition, evaluations of stimulus histogram profiles for each single unit were grouped and plotted as a percentile of all recording units. No association profiles were labeled No SH Assoc. Profiles showing an association with the stimulus pulse were labeled SH Association. Negative associations were omitted (as were increases in spontaneous activity).

B. Responses to multichannel repetitive stimulation. The same procedures noted above were carried out for these stimulations.

SA: spontaneous activity responses

SH: stimulus histogram profiles


A.
No SH Assoc
SH Association
No SA Response
Decrease in SA
Percentage of Units
100
80
60
40
20
0
66c 36
79a 24
99a 37
99a 55
123a 12*
Experiment / Stimulus Channel


B.
Percentage of Units
100
80
60
40
20
0
51b MC
66b MC
99a MC
99b MC
Experiment / Multichannel Stimulation

3.2.4 Single versus Multiple Channel Stimulation

Statistical analyses of the values represented in the graphs above revealed no significant difference between LFS on single channels when compared to multiple channels. However, direct comparison of responses in the same preparation revealed that a much higher percentage of units showed a clear decrease in spontaneous activity following SCRS in experiment C-66 than did MCRS in the same experiment. Moreover, SCRS on channels 37 and 55 in experiment C-99a, showed a small percentage of units with a clear decrease in spontaneous activity. Stimulation on both channels simultaneously (99a MC) showed no decrease in spontaneous activity for any units. As was the case with C-66, the percentage of units associated with the stimulus pulse was not as clear when comparing single channel to multi-channel LFS.

Repetitive stimulation on multiple channels has never resulted in a complete or total depression of bursting activity. However, SCRS has resulted in total depression of bursting activity in 2 separate cultures. An example of the contrast between responses to MCRS and SCRS is shown in Figure 50. Not only was the magnitude of depression greater, but the mean duration of responses for SCRS was more than twice the mean duration for MCRS responses (see Table 6).

3.2.5 Effect of Repetitive Stimulation on Evoked Responses

To assess the effect of LFS on evoked responses, test pulses (usually 0.5 V) were delivered to the same stimulation channel(s) before and after LFS (methods reported earlier in this section as well as in chapter 2). Figure 51 shows how MEAP (mean evoked action potential) values decreased following two successive SCRS trials.

Figure 50. Repetitive stimulation on multiple and single channels.
Spontaneous bursting and spiking activity recorded on a single channel (channel 40) shows that simultaneous tetanic stimulation on 3 channels resulted in a very large increase in burst and spike rates that lasted for ~60 min until the initiation of LFS on the same channels. The MCRS, applied to the same 3 channels after the stabilization of elevated activity, effectively decreased the burst and spike rates by ~50%. The network response (mean of 13 channels--not shown) also showed a reduction in activity by the same percentage. The reversal of the effect of MCTS by MCRS (also known as depotentiation) lasted for ~31 min. Following MCRS, SCRS on channel 36 resulted in a total depression of bursting activity (on every recording channel--data not shown) following a 16 min delay. This depression lasted for over 40 min until the initiation of another stimulation episode.

[The 6 min gap between graphs encompasses the time it took to rewind the analog tape and set up another reel for recording.]

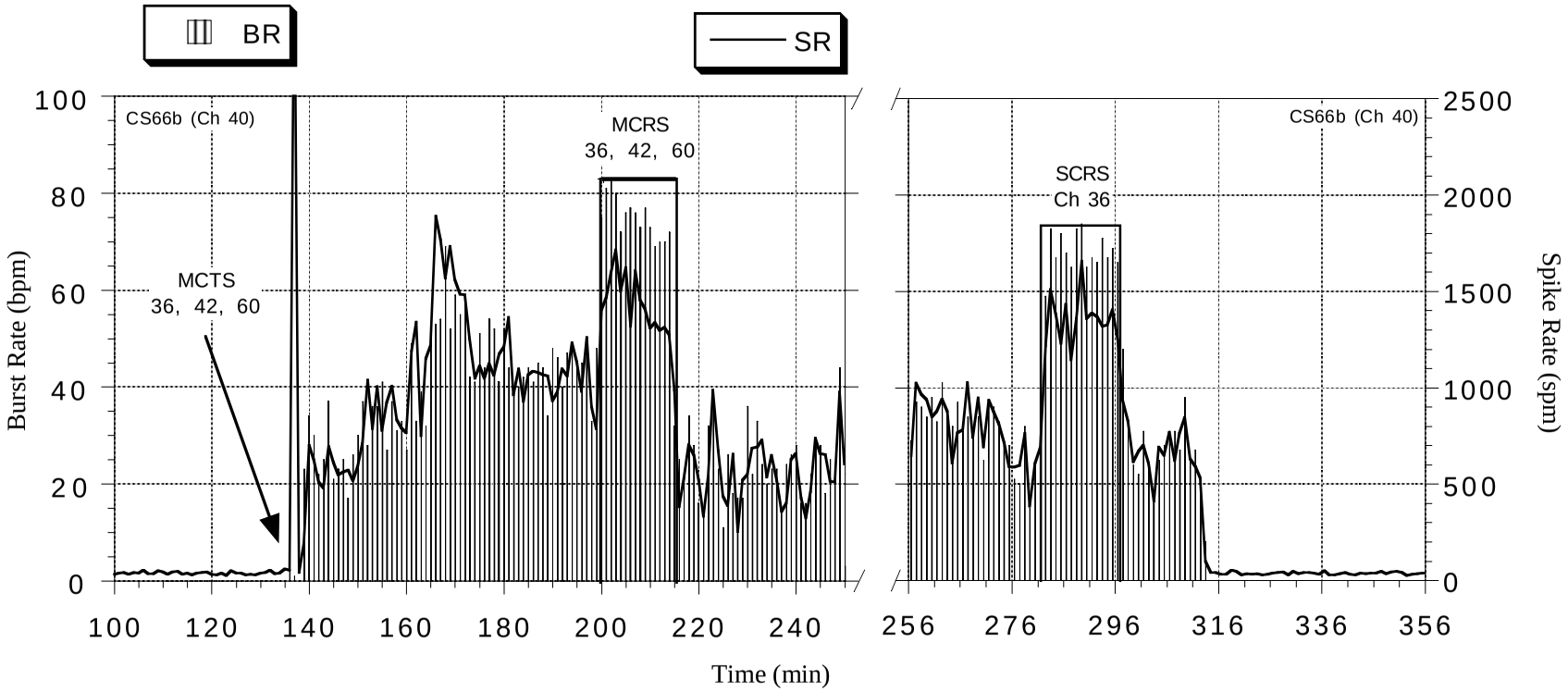
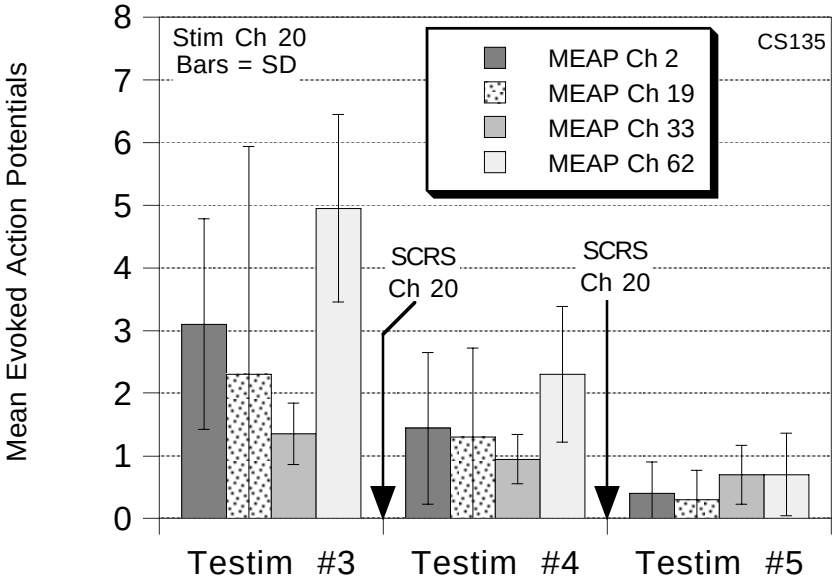

BR
SR
CS66b (Ch 40)
MCRS
36, 42, 60
MCTS
36, 42, 60
SCRS
Ch 36
Burst Rate (bpm)
Spike Rate (spm)
Time (min)
0
20
40
60
80
100
500
1000
1500
2000
2500
100
120
140
160
180
200
220
240
256
276
296
316
336
356

Figure 51. Changes in mean evoked action potentials following repetitive stimulation. Single test pulses (0.5 V) were delivered to stimulus channel 20. Evoked responses for each pulse were recorded on four separate recording channels via cathode ray oscilloscopes.

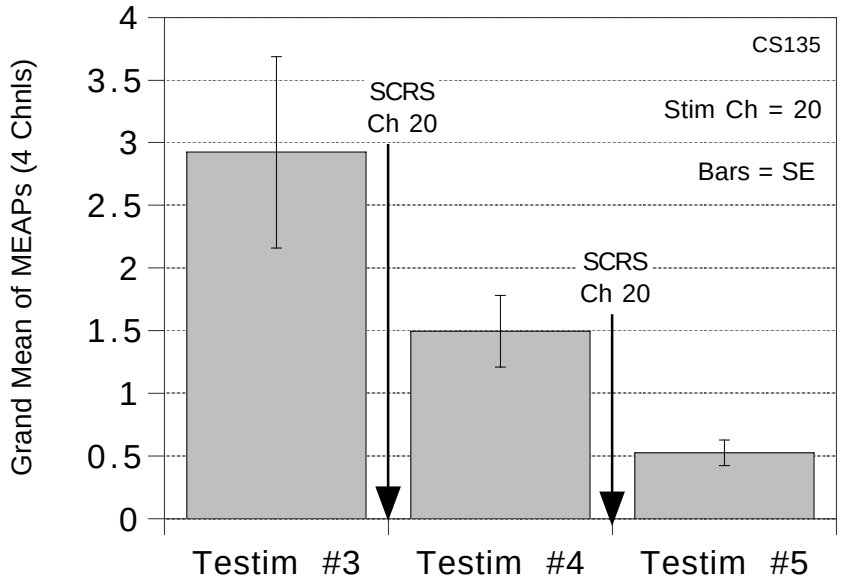
A. Means of responses to 20 test pulses are shown. Between each series of test pulses, LFS was delivered to the same stimulus channel (channel 20). The mean evoked action potentials (MEAPs) decreased for each recording channel following each LFS. [Bars = standard deviation]

B. Grand Mean of MEAPs. Grand mean of MEAPs for all four recording channels were calculated and plotted. Statistical analysis (student's t test) showed no significant difference between the grand means of MEAP values following the first SCRS (P value ≤ 0.10, 6 degrees of freedom). The difference between the grand means of MEAPs following the second SCRS was significant (P value ≤ 0.02, 6 degrees of freedom). [Bars = standard error]

A.


Mean Evoked Action Potentials
8
7
6
5
4
3
2
1
0
Stim Ch 20
Bars = SD
CS135
MEAP Ch 2
MEAP Ch 19
MEAP Ch 33
MEAP Ch 62
SCRS
Ch 20
SCRS
Ch 20
Testim #3
Testim #4
Testim #5

B.


Grand Mean of MEAPs (4 Chnls)
4
3.5
3
2.5
2
1.5
1
0.5
0
CS135
Stim Ch = 20
Bars = SE
SCRS
Ch 20
SCRS
Ch 20
Testim #3
Testim #4
Testim #5

The percent decrease of grand means of MEAPs for all 5 SCRS trials ranged from 10 to 65% [mean = 38% ±10.7 (SE)]. The percent decreases for the two MCRS test pulse trials were 4 and 11%. There was never an increase in the grand mean of MEAPs following LFS on single or multiple channels. The low number of MCRS trials prevented a statistical analysis for the comparison of SCRS versus MCRS trials. However, the above values from the data collected showed that decreases in the grand mean of MEAPs following SCRS were generally larger than decreases in MCRS grand mean values.

3.2.6 Quantitative results of long-term spontaneous activity depression (LTSAD)

Quantitative data for LTSAD is summarized in Table 7. The values for C-123 were not included in the means, because the likely depression of inhibitory circuitry resulted in an increase in activity. Inclusion of these large, positive numbers would have confounded the values of the means. Moreover, it is logical to exclude increases in activity when evaluating the extent of depression. The duration of effect for C-79a was italicized because this value was not included in the duration histogram (it was also excluded from the mean duration value in the Table 7). As noted earlier, the durations were determined by visual inspection of stripchart data (see Table 6). Because, in C-79a, the decline in activity over time was relatively subtle following SCRS, it was not readily detected; thus the duration of this LTSAD was not recorded. But as one can see in Figure 52, there was a substantial depression of spontaneous activity over time following SCRS that was likely due to the stimulus. As seen in Table 7, the smallest decrease in an activity parameter was a 29% decrease in the spike rate. The largest decrease was a total depression of bursting activity resulting in a 100% decrease in activity. The time intervals for percent change calculations (pre-stimulus versus post-stimulus) were 20 min (except for C-66b and C-66c, which were 15 min). In all cases, the post-stimulus time intervals were taken from a part of activity segment following LFS, at which the decrease in activity had leveled off (see Fig. 53).

Table 7. Summary of Results for Long-Term Decreases Following Repetitive Stimulation

Expt.	Stim	Response	% Δ BR	% Δ SR	% Δ TBAPM
C-51b	MCRS	22 min*	-46	-73	-76
C-66b	MCRS	31 min*	-38	-29	-38
C-66c	SCRS	44 min*	-100	-88	-100
C-79a	SCRS	*77 min*	-48	-45	-73
C-123a	SCRS	42 min*	*283*	*490*	*1137*
C-149a	SCRS 1	23.2 min*	-47	-52	-80
C-149a	SCRS 2	23.6 min*	-99	-36	-71
Means and SD		29 ± 9.2	-62 ± 28.6%	-73 ± 20.1%	-53 ± 22.6%

Legend: % Δ = Percent Change; BR = Burst Rate; SR = Spike Rate;
TBAPM = Total Burst Area Per Minute. Starred values represent durations that were cut off by another stimulation. Italicized values were not included in calculation of means.

Another reason that the decline in overall network activity was not detected in C-79 was the fact that not all channels responded to the LFS to the extent that channel 18 did. The spontaneous activity on some channels changed very little and the activity on three of the recording channels actually increased (data not shown).

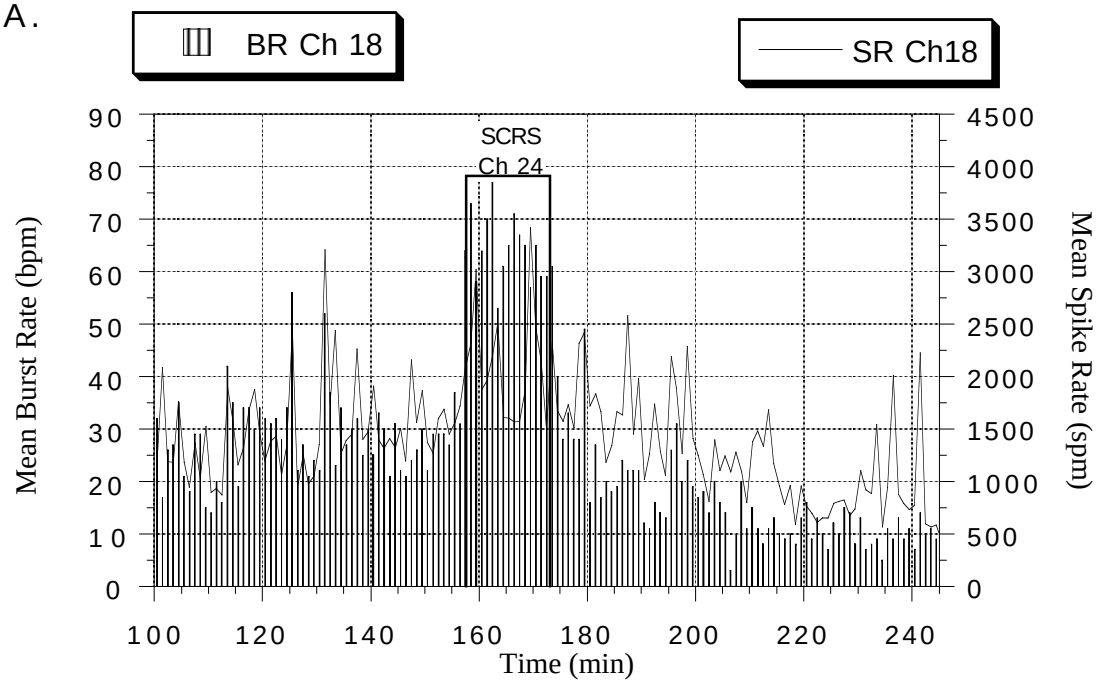
In contrast to the multiplicity of responses to LFS in C-79, there was a relatively uniform depression of activity across all channels in C-51b (Fig. 54). As illustrated in Figure 55, 20 minute time intervals of spontaneous activity across all recording channels were averaged and calculated in order to create Table 7. As was the case in the determination of network responses to tetanic stimulation in the previous section, averaging across all channels may diminish or even negate the magnitude of the effect manifested in single recording channels (see Fig. 52). However, the benefits of averaging across channels, in order to simplify the assessment of network responses to stimulation patterns, were deemed to outweigh the costs.

Figure 52. Long-term depression of network spontaneous activity over time. Single channel repetitive stimulation on channel 24 resulted in a gradual decrease in spontaneous activity across five of nine recording channels.

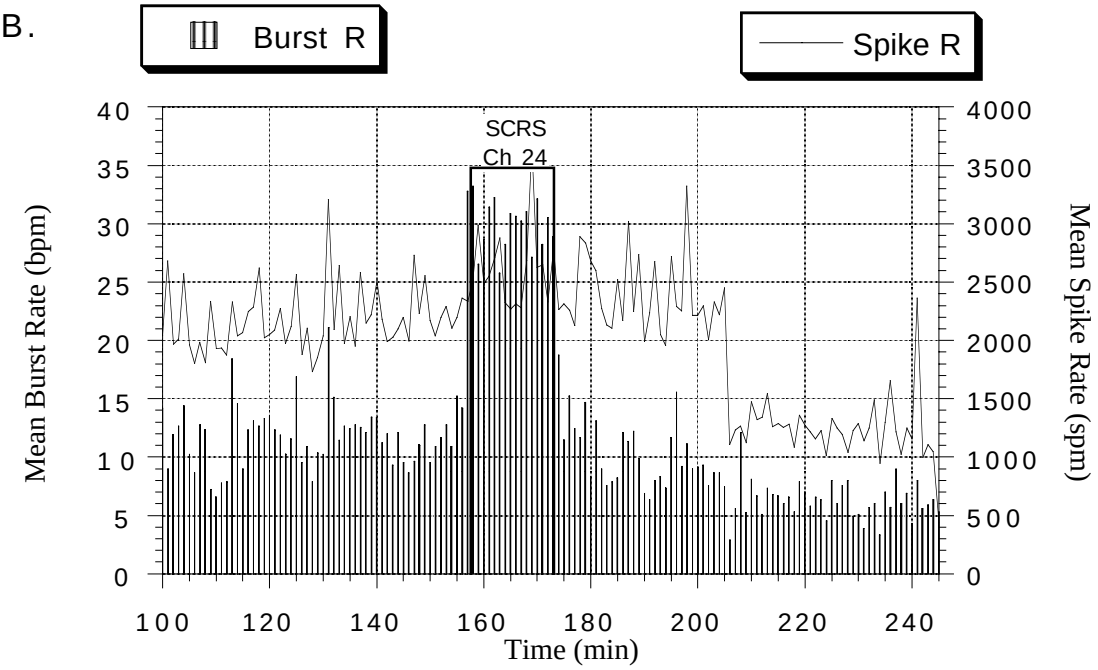
A. Single recording channel data. Channel 18 showed the maximal amount of depression following LFS. The burst rate declined to about one third of its pre-stimulus level (62% decrease), while the spike rate decreased by 46%. Notice how the burst rate tended to decrease in steps until it leveled off.

B. Average responses of spontaneous bursting and spiking activity. The magnitude of depression in burst rate was more modest when calculating the mean of nine recording channels (45% reduction). However, the ultimate reduction in the mean spike rate was comparable to channel 18.

A.


BR Ch 18
SR Ch18
SCRS
Ch 24
Mean Burst Rate (bpm)
Mean Spike Rate (spm)
Time (min)
0
10
20
30
40
50
60
70
80
90
500
1000
1500
2000
2500
3000
3500
4000
4500
100
120
140
160
180
200
220
240

B.


Burst R
Spike R
SCRS
Ch 24
Mean Burst Rate (bpm)
Mean Spike Rate (spm)
Time (min)
0
5
10
15
20
25
30
35
40
500
1000
1500
2000
2500
3000
3500
4000
100
120
140
160
180
200
220
240

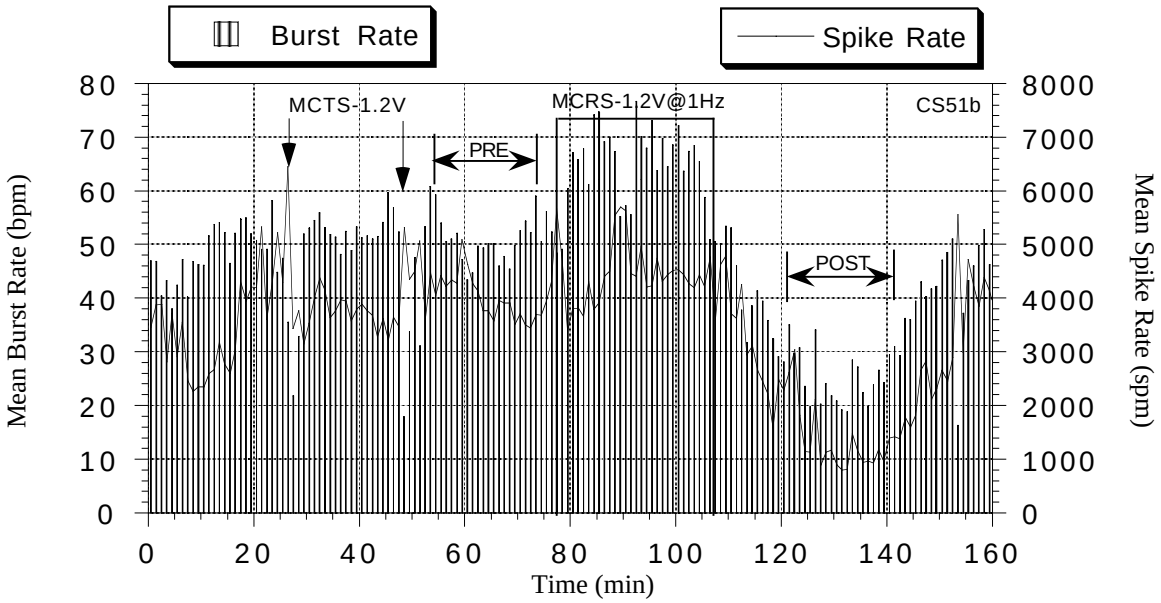


Figure 53. Effect of multichannel repetitive stimulation on network activity.

Two successive tetanic stimulations on six different recording channels had no long-term effect on network (mean of 13 recording channels) burst or spike rates. Following ~25 min of LFS, on the same channels, the burst rate declined steadily for ~26 min until it reached a low of ~20 bpm.

Response characteristics in terms of time:

Delay: 4 min

Decay: 15 min

Stable Depression: 15 min

Recovery: 10-15 min

Figure 54. Changes in four different activity parameters following LFS in one experiment. Recording channels are shown on the x-axis. Each bar represents 20 min of activity means, average obtained from 1 min bins (see "PRE" and "POST" segments in Fig. 53). The speckled bars represent pre-stimulus activity and the gray bars represent post-stimulus activity. In each panel, there is a significant decrease for each recording channel [(Mann-Whitney unpaired analysis ($p<0.0001$)].

A. Mean spike rates

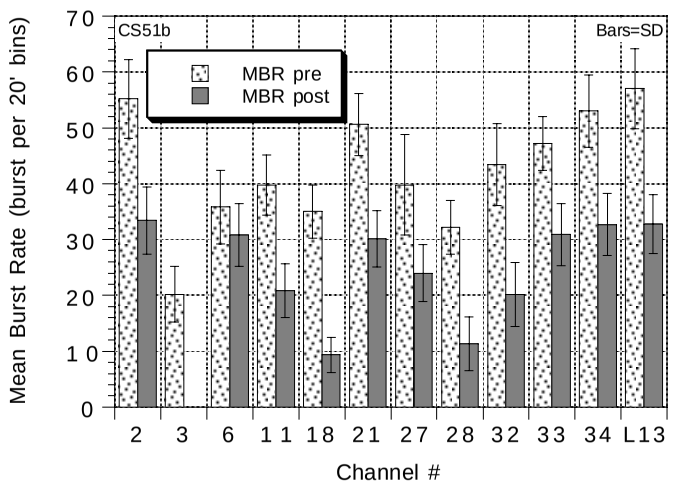
B. Mean burst rates

C. Means of mean burst duration per minute

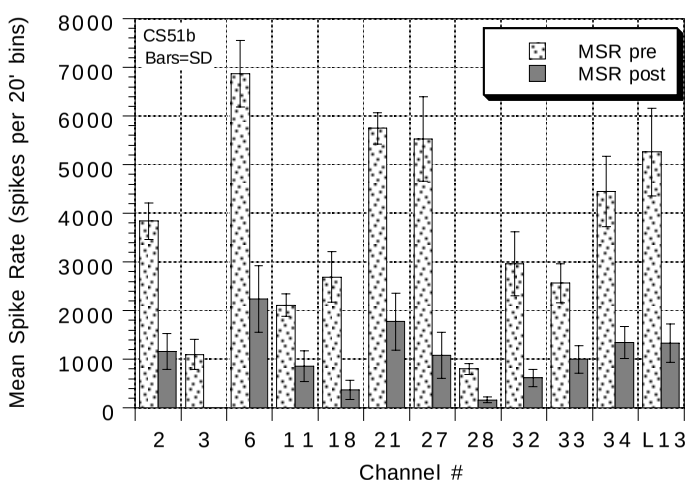
D. Means of total burst area per minute

Standard deviations were used for A and B because the averaging of burst rate and spike rate for interval means was a single calculation. Standard error bars were used in C and D because the interval means were calculated from mean burst variables per minute.

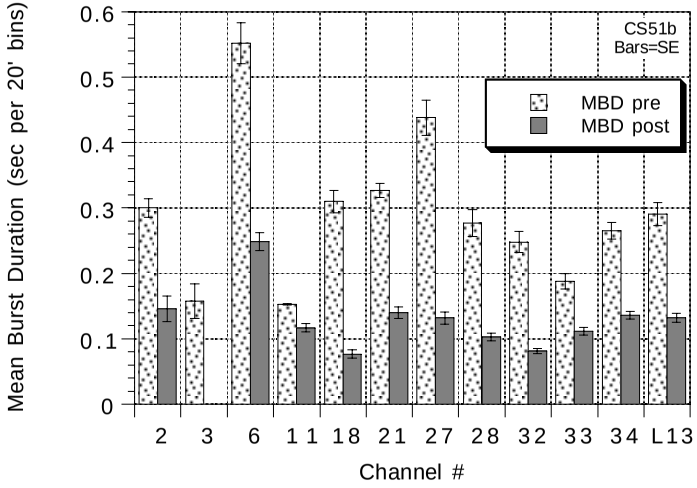
A.


CS51b
Bars=SD
MBR pre
MBR post
Mean Burst Rate (burst per 20' bins)
0
10
20
30
40
50
60
70
2 3 6 11 18 21 27 28 32 33 34 L13
Channel #

B.


CS51b
Bars=SD
MSR pre
MSR post
Mean Spike Rate (spikes per 20' bins)
0
1000
2000
3000
4000
5000
6000
7000
8000
2 3 6 11 18 21 27 28 32 33 34 L13
Channel #

C.


CS51b
Bars=SE
MBD pre
MBD post
Mean Burst Duration (sec per 20' bins)
0
0.1
0.2
0.3
0.4
0.5
0.6
2 3 6 11 18 21 27 28 32 33 34 L13
Channel #

D.

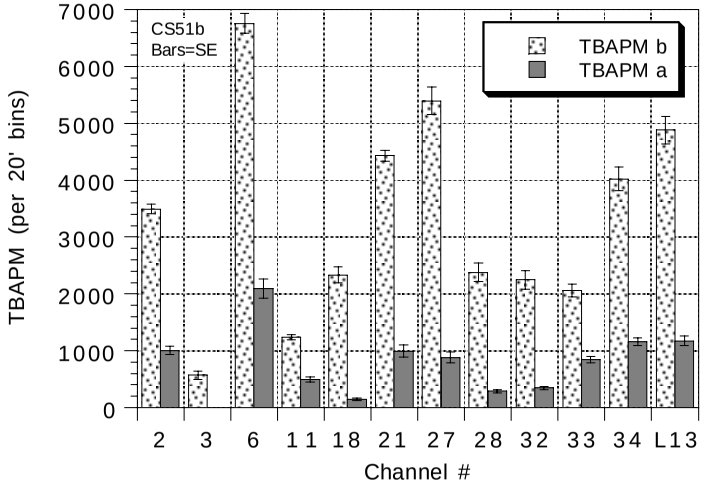

CS51b
Bars=SE
TBAPM b
TBAPM a
TBAPM (per 20' bins)
0
1000
2000
3000
4000
5000
6000
7000
2 3 6 11 18 21 27 28 32 33 34 L13
Channel #

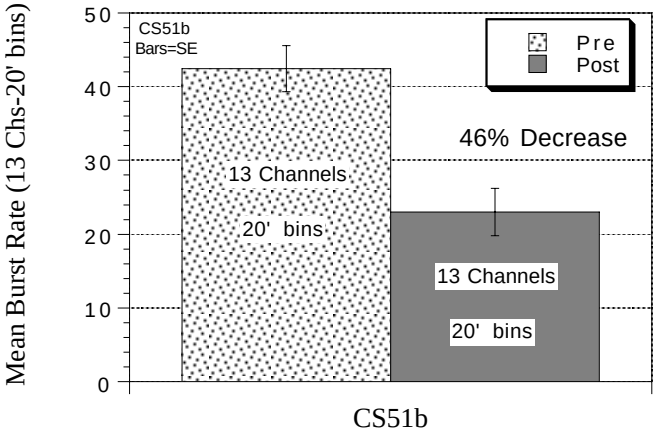
Figure 55. Quantitative results of changes in spontaneous activity following LFS. For the same experiment as in Fig. 54 (51b), a channel mean was calculated in order to determine an overall (or network) percent change in activity.

A. Mean values were determined by averaging across 13 recording channels for 20 minutes before and after repetitive stimulation episode. Mean burst rate decreased by 46% following MCRS (960 mV @ 1 Hz) on six channels simultaneously. Note: Immediately following the repetitive stimulation episode, the activity declined steadily for ~10 min. Therefore, the 20 min period following MCRS was started after the activity had leveled off.

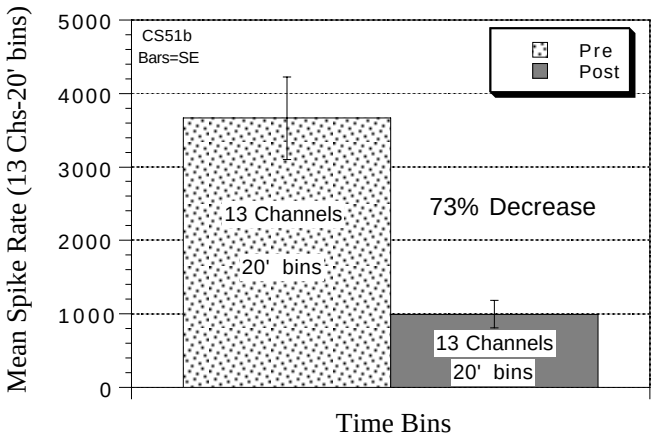
B. Mean spike rates before and after MCRS show a much larger percent decrease (73%). Statistical analysis of pre- vs. post time bins determined that both the burst rate and spike rate were significantly different ($p < 0.0001$ Mann-Whitney unpaired analysis).

C. Percent change of four activity parameters. Reduction in spontaneous activity ranged from 46% to 76% following multichannel repetitive stimulation. The percent change in TBAPM was similar to the percent change in the spike rate.

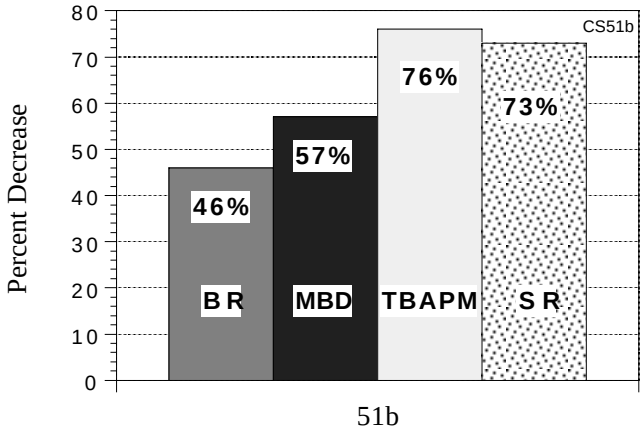
A.


CS51b
Bars=SE
Pre
Post
46% Decrease
13 Channels
20' bins
13 Channels
20' bins
Mean Burst Rate (13 Chs-20' bins)
0
10
20
30
40
50
CS51b

B.


CS51b
Bars=SE
Pre
Post
73% Decrease
13 Channels
20' bins
13 Channels
20' bins
Mean Spike Rate (13 Chs-20' bins)
0
1000
2000
3000
4000
5000
Time Bins

C.


CS51b
46%
57%
76%
73%
BR
MBD
TBAPM
SR
Percent Decrease
0
10
20
30
40
50
60
70
80
51b

Chapter 3 Section 3

REVERSIBILITY OF EFFECTS

3.3.1 Background and Specific Methods

As indicated by the data presented in the previous sections, tetanic stimulation (or HFS) tends to increase spontaneous activity, and repetitive stimulation (or LFS) tends to decrease spontaneous activity. Therefore, it can be inferred that HFS and LFS are opposing stimulation influences. It then follows that, as long as the stimulus intensity and duration, and the designated stimulation channel(s) remain the same, the effects of these two stimulation modes can be reversed by each other. Such data would provide strong support that the observed stimulus-induced changes in spontaneous activity result from manipulations of plasticity mechanisms. This last section involves experiments in which this question was explored.

Selected channels were stimulated with either HFS or LFS. If there was a clear response to the initial stimulus, at least 15-20 min were allowed to elapse before the opposing stimulation pattern was applied to the same stimulation channel(s). Responses to HFS and LFS were defined in section 2.1 and 3.1 respectively. A response that lasted at least 15 min was categorized as long-term spontaneous activity potentiation (LTSAP) or its counterpart long-term spontaneous activity depression (LTSAD). If there was an effect following the opposing stimulation, then approximately the same amount of time was allowed to elapse before repeating the initial stimulus. Again, if there was an effect following the second initial stimulation pattern, then a second opposing stimulation was delivered (see Fig. 15B). This cycling back and forth was carried out until the response following the stimulus diminished to the extent that there was no clear alteration of spontaneous activity. There were several occasions where the opposing stimulation was delivered in the absence of a clear, distinct response to the initial conditioning stimulation. Both stimulation types were delivered in order to determine if one type of stimulation was

more effective at eliciting a response than the opposing stimulation. For seven of these trials, MEAP data were collected before and after each stimulation.

State-space graphs (or scatter plots) were also included in this section in order to show how burst duration was affected depending on the type of stimulation pattern. Mean burst durations (1 min bins) were computed by Masscomp programs for each recording channel. The value (in seconds) are given along with the standard deviation (SD). The points on the scatter plots are calculated by using the mean burst duration (MBD) as the x-component. The coefficient of variation (CV, the quotient of the SD divided by the MBD), makes up the y-component. For network results, the MBDs per minute for each recording channel were averaged across channels to obtain a network grand-mean per minute. The SD used for each channel-grand-mean was the SD calculated by averaging the MBDs across 12-14 recording channels.

3.3.2 Results of Reversibility Trials

In the vast majority of these stimulation trials, it was found that if the network was unresponsive to one type of stimulus pattern, it was largely unresponsive to the opposing stimulation. However, when there was a response to one pattern, but not the other, the more effective stimulation pattern was tetanic stimulation (see Table 8). The response was usually very brief. The mean duration of the four responses when only one stimulation mode was effective was less than 2 min.

Table 8. Data Set and Results for Reversibility Trials

Culture #	Stim Channel #	SCTS as Initial Stimulus			SCRS as Opposing Stimulus		
		Duration (min)	% Δ (BR)	% Δ (SR)	Duration (min)	% Δ (BR)	% Δ (SR)
79	24	(80)	41	54	(77)	-48	-45
91*	59	7.2	NCDA	NCDA	9	NCDA	NCDA
99	55	5.7	20	6	11	13	-15
99	37	1.6	-10	14	NR	7	-15
123	12	(47.6†)	-85	-90	(42.3†)	344	453
131*	41	NR	----	----	NR	----	----
132*	22	NR	----	----	NR	----	----
132*	48	NR	----	----	NR	----	----
135*	20	NR	----	----	NR	----	----
149	3	(11)	66	92	10	-32	-37
149	3	(*30.9*‡)	106	92	(*23.6*‡)	-99	-36
		SCRS as Initial Stimulus			**SCTS as Opposing Stimulus**		
76*	38	NR	----	----	0.5	NCDA	NCDA
80*	14	NR	----	----	0.6	NCDA	NCDA
123	12	(42.3†)	344	453	60†	82	76
135*	20	NR	----	----	NR	----	----
149	3	(*23.2*)	-47	-52	(*30.9*‡)	106	92
149	3	(*23.6*‡)	-99	-36	0.8	----	----
		MCTS as Initial Stimulus			**MCRS as Opposing Stimulus**		
51		1	-7	14	22	-46	-73
66	36, 42, 60	(60)	11,150	80	(44)	-38	-29
124*	45-48	NR	----	----	NR	----	----
99	37 & 55	NR	5	5	NR	6	-4
99	46 & 64	NR	7	-10	NR	-5	-12
145*	33-64	4.2	NCDA	NCDA	NR	----	----

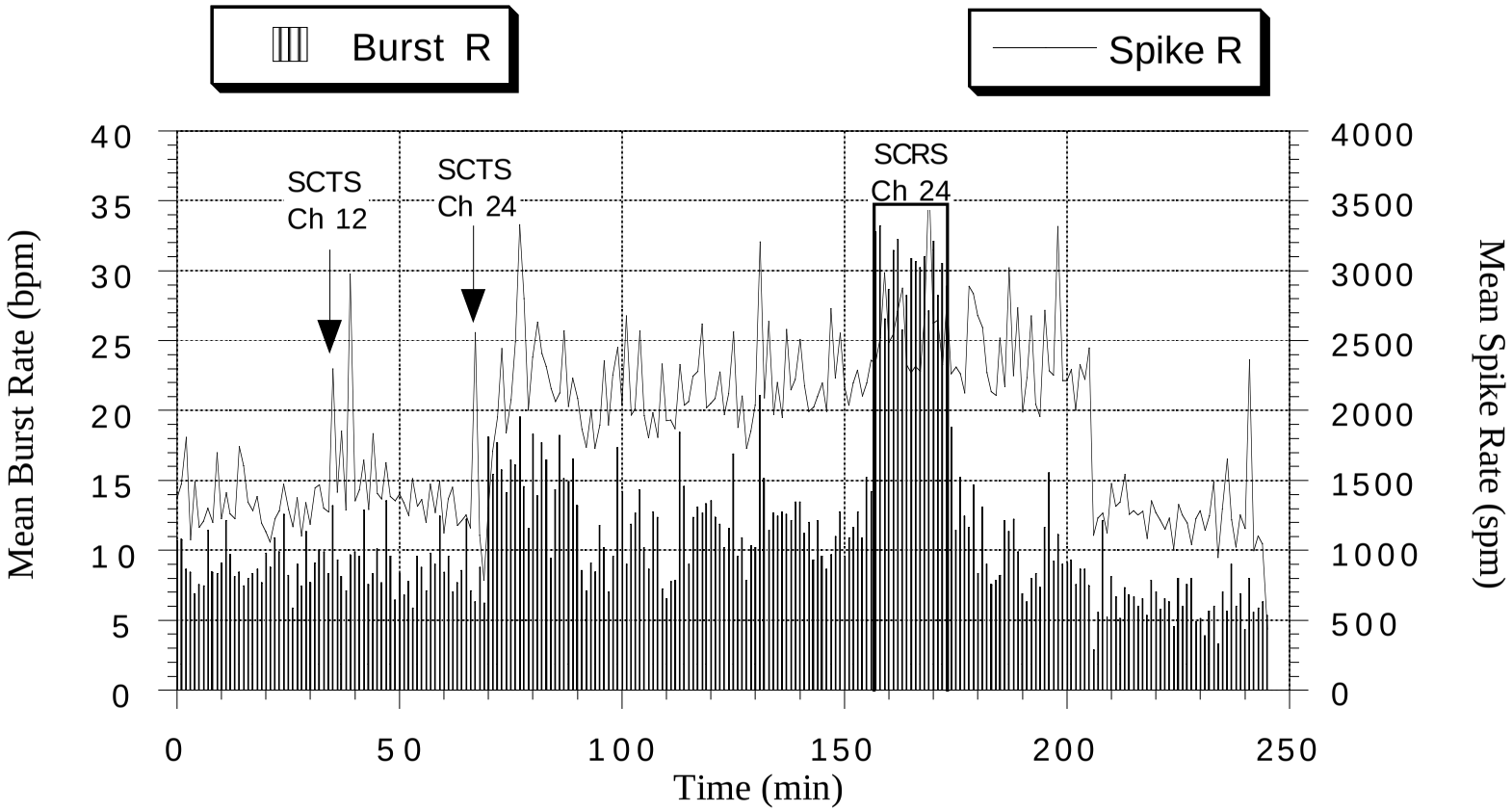
* No Computer data available (NCDA); ‡ Duplicate duration because part of a stimulation cycle; † Direction of change in spontaneous activity opposite of expected results;
Values in the columns under the stimulation type represent the duration of the expected response; (NR = no response was detected); Durations in parentheses were interrupted.

Table 8 shows the data set for reversibility trials. Results were grouped according to the type of stimulation delivered initially and the opposing stimulation. The durations of the response from each stimulation mode, measured from stripchart data, were listed along with the percent change in spontaneous activity, calculated from activity intervals (usually 15 min) of computer data. When computer data were unavailable, no percent change in activity was calculated. Two duration values were listed twice because they were part of a 4-part stimulation cycle. That is, the same response to an opposing stimulation was also listed as a response to the initial stimulation in another group because another opposing stimulation followed it.

When there was a response to both stimulation patterns, analysis of data suggested that the duration of the effect of the opposing stimulation tended to mirror the duration of the prior stimulation. When the duration of the response to a particular stimulation was relatively long, the duration of the opposing stimulation was comparable to the length of the previous stimulation (Fig 56). In most cases, the responses to the stimuli diminished rapidly after the first opposing stimulation. There were only two occasions when there was a response to the repeated initial stimulus (following the opposing stimulus), and there were no recorded responses to the second opposing stimulus (following the repeated initial stimulus).

Despite these limitations, eight long-term (duration ≥ 15 min) responses to the initial stimulus were recorded. Of these trials, only one (C-149) of the responses to the opposing stimulus failed to last longer than 20 min (and the opposing stimulation that failed to last longer than 20 min is one that was a second opposing stimulus--i.e. 4th consecutive stimulation) That is, 88% of the responses to the opposing stimulation lasted for longer than 20 min. These data suggest that when a stimulation electrode was capable of altering spontaneous network activity for 15 min or more, the opposing stimulation on the same electrode was just as effective at long-term modification of network activity.

Figure 56. Reversal of elevation of spontaneous activity following opposing stimulation (C-79). Tetanic stimulation on channel 12 did not affect the mean (13 channels) burst rate, and only slightly elevated the mean (13 channels) spike rate that lasted ~15 min. About 30 min later, SCTS on channel 24 resulted in a two-fold increase in the burst and spike rate. The doubling of the burst rate lasted for 30 min before falling to a level that was 40% above the pre-stimulus level. The spike rate remained elevated for 90 min. Following SCRS on the same stimulus channel, the mean burst rate gradually declined until it reached a level that was ~30% below that of the native activity. After about a 30 min delay, the spike rate abruptly decreased to a level that was very near to the native mean spike rate. These reductions in spontaneous activity lasted for at least 40 min (the total duration was not recorded because the analog tape on which the data were being recorded came to an end).


Burst R
Spike R
SCTS
Ch 12
SCTS
Ch 24
SCRS
Ch 24
Mean Burst Rate (bpm)
Mean Spike Rate (spm)
Time (min)
0
5
10
15
20
25
30
35
40
0
500
1000
1500
2000
2500
3000
3500
4000
0
50
100
150
200
250

Data evaluation also suggested that when the magnitude (percent change) of the initial effect was large, the likelihood of reversing the effect was increased. In addition, if the overall change in spontaneous activity following the initial stimulation episode was relatively small; the probability of reversing the effect was diminished. Figure 57 shows the magnitude of change in spontaneous activity across four different activity variables for the same trials (in expt. C-79) shown in Figure 56.

These results not only suggest that the effect of a particular type of stimulation pattern can be reversed by the opposing stimulation pattern, but that the initial change in spontaneous activity was actually caused by the initial stimulation. A common method employed in scientific investigation is to show that if a certain response can be blocked or reversed, then the response itself was real and not just happenstance. That is, in an effort to prove cause and effect, the effect must be susceptible to nullification, or reversal by a method or action that is considered to be the opposite of, or blocks the effect of the initial action.

3.3.3 Single versus Multichannel Stimulation

The results highlighted in this section compare the differences in response to LFS and HFS with respect to single versus multichannel stimulation in general (i.e. in all trials--not just trials in which the opposing stimulation was delivered following the initial stimulation). Tables 3A (summary of HFS responses) and 6A (summary of LFS responses) show that stimulation on multiple channels resulted in a higher percentage of predicted responses (MCTS was 12 percentage points higher than SCTS, and MCRS was 7 points higher than SCRS) compared to single channel stimulation. In addition, multichannel stimulation resulted in a lower percentage of unpredicted responses. That is, in SCRS and MCRS trials, the percentage of increases in spontaneous activity was 17% and 6% respectively. In the same fashion, the percentage of decreases in activity following

SCTS was 11%, and there were no observed decreases in activity following MCTS. While this would seem to suggest that stimulation on multiple channels was more effective, one would first have to consider the mean durations of the shifts in activity. The mean durations of effect were longer following single channel stimulation, than the mean durations following multichannel stimulation (see Tables 3B and 6B). This was true for SCTS versus MCTS (mean enhancement was 44% longer for SCTS), as well as for SCRS versus MCRS (mean depression was over twice as long following SCRS). Although this was a tendency, ranked sum tests showed that the difference between median values of single channel stimulation and multichannel stimulation were not great enough to exclude the possibility that the difference was due to random sampling variability. There was also no significant difference found between the duration of effect following tetanic stimulation and repetitive stimulation.

The mean delay times for the single and multichannel stimulation trials also seemed to indicate that the delay following stimulation was influenced by the number of channels being stimulated. The mean delay time for a response to HFS on single channels was almost three times as long as the mean delay for a response on multiple channels, whereas mean delay time for LFS on single channels was 20 times longer than LFS on multiple channels. However, statistical tests showed no significant difference between any groups of delay times following different stimulation patterns.

Figure 57. Quantification of changes in spontaneous network activity parameters. Channel means (spontaneous activity values averaged across 13 recording channels) were averaged over 20 min time intervals of relatively stable spontaneous activity to produce grand means (GM) of "network" activity. LFS episode was 17 min.

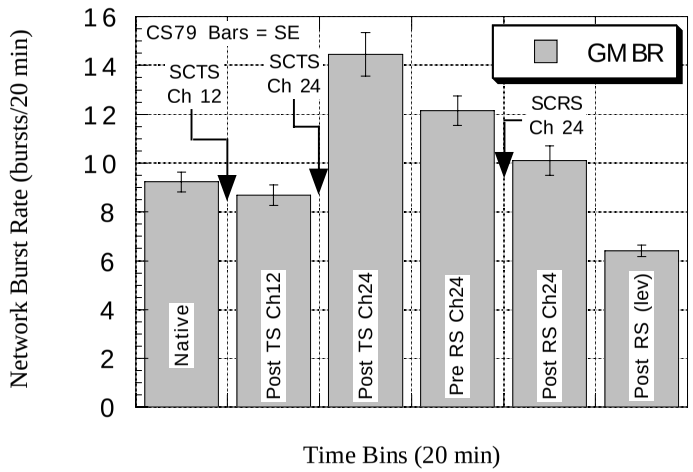
A. A negligible reduction in network burst rate followed SCTS on channel 12. SCTS on channel 24 resulted in a 66% increase in the burst rate that decayed to 40% above pre-stimulus levels. The 20 min segment following SCRS on channel 24 exhibited a 17% reduction that continued to decrease until it bottomed out with a 47% reduction from the pre-stimulus level and a 31% reduction from the native state.

B. The network spike rate showed an insignificant increase following SCTS on channel 12. There was a 50% increase in spike rate following SCTS on channel 24 that continued to increase during the time interval that showed decay of the effect in the panel A. This increase even persisted during the next 20 min segment immediately following SCRS on channel 24. However, the final 20 segment (after activity had leveled off) showed a reduction in spike rate by 49% (3% below native activity).

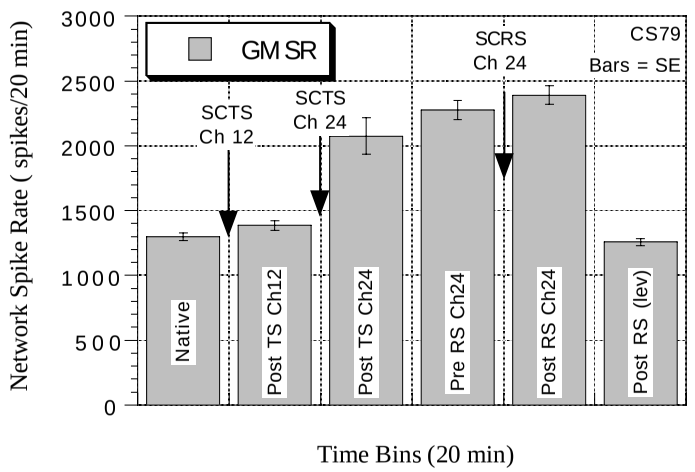
C. Tetanic stimulation on channel 12 did not affect network burst duration. Tetanic stimulation on channel 24 resulted in a three-fold increase in network burst duration. The next 20 min time interval showed an increase in the burst duration (four-fold above pre-stimulus levels). Repetitive stimulation on the same channel shortened bursts by 39%. The final interval depicts a network burst duration that is 92% below that of pre-SCRS levels and 66% shorter than the native state.

D. The trend for changes in the TBAPM intervals are similar to the trend seen in panel A. There was little or no change following the first tetanic stimulation. The second SCTS resulted in an increase of 198% that decayed to 173%. Repetitive stimulation facilitated a further reduction (by 14%) that ultimately decreased to a level 72% less than that of the pre-stimulus level and 27% below the native level.

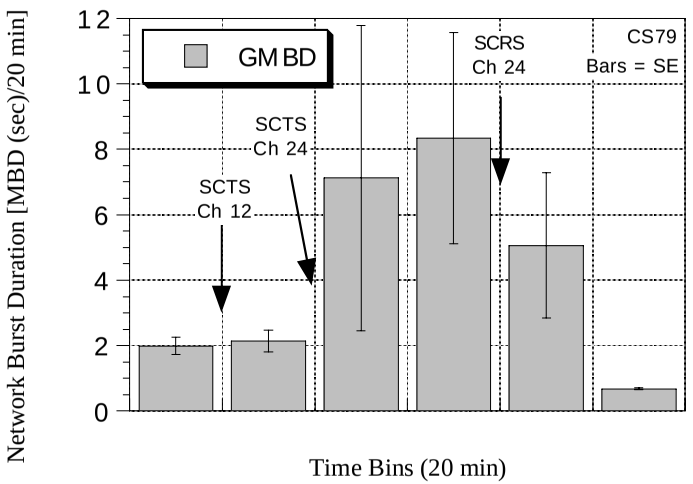
A.


CS79 Bars = SE
GM BR
SCTS Ch 12
SCTS Ch 24
SCRS Ch 24
Native
Post TS Ch12
Post TS Ch24
Pre RS Ch24
Post RS Ch24
Post RS (lev)
Network Burst Rate (bursts/20 min)
Time Bins (20 min)

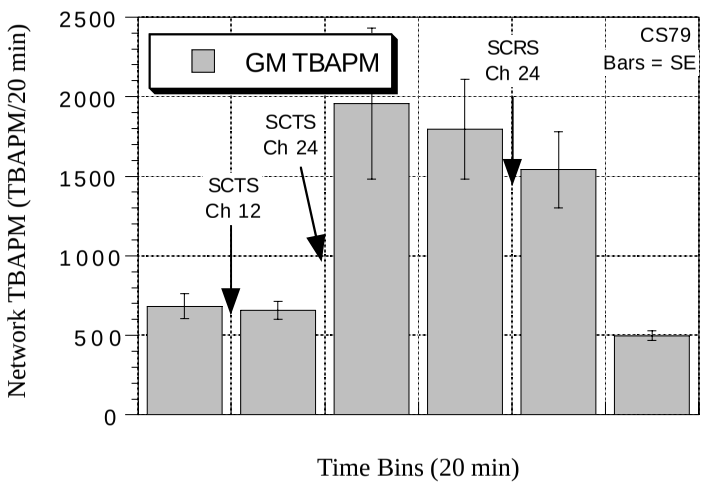
B.


GM SR
CS79
Bars = SE
SCTS Ch 12
SCTS Ch 24
SCRS Ch 24
Native
Post TS Ch12
Post TS Ch24
Pre RS Ch24
Post RS Ch24
Post RS (lev)
Network Spike Rate (spikes/20 min)
Time Bins (20 min)

C.


GM BD
CS79
Bars = SE
SCTS Ch 12
SCTS Ch 24
SCRS Ch 24
Network Burst Duration [MBD (sec)/20 min]
Time Bins (20 min)

D.


GM TBAPM
CS79
Bars = SE
SCTS Ch 12
SCTS Ch 24
SCRS Ch 24
Network TBAPM (TBAPM/20 min)
Time Bins (20 min)

3.3.4 Effect of Conditioning Stimulation on Burst Duration

Reversal of the effects of conditioning stimulation was also attempted with stimulation patterns delivered to several channels simultaneously. Of the six MCRS trials listed in Table 8, there was only one instance when the effect was reversed. However, the lack of successful reversals probably had more to do with the lack of clear, sustained responses to the initial stimulation, than the inability of the opposing stimulation to negate the effect. The trial in which the effect was reversed was also the only trial in which the conditioning stimulation caused a response (see appendix Fig. A-12).

Scatter plots of spontaneous activity intervals were made in order to depict the reversal of burst durations following the application of the opposing stimulation. These graphs clearly show (1) that the nature of the spontaneous activity (enhanced or depressed) depended upon the type of stimulation pattern delivered to the network, and (2) that this effect was nullified (or reversed) by the opposing stimulation. There were four cultures in which clear, sustained responses followed the conditioning stimulation and this same response was, in turn, reversed by the opposing stimulation. A graph from a typical experiment is shown in Figure 58. Tetanic stimulation increased the burst duration on single channels as well as network-wide, whereas repetitive stimulation shortened the duration of the bursts, also on both levels.

Results of statistical analysis (Mann-Whitney Unpaired) of these trials are depicted in Table 9. As in the experiment shown in the Figure 58, ~15 points (representing approximately 15 min time intervals of activity) were used in the statistical tests. The burst durations for C-66 were included because the durations during the LFS were significantly different than pre-stimulus levels even though those shortened durations did not "hold" following the cessation of stimulation--that is, the change in the burst duration imposed on the network during MCRS was not retained after the stimulation episode. The inability of the MCRS episode to cause a lasting effect on the burst duration following the cessation of the stimulation may be in part due to the difference between MCRS and SCRS.

Extra intervals ["Post (2) TS-Ch24" and "Post (2) RS-Ch24"] were added for C-79 because of the extended period between stimulation episodes. The second 20 min intervals allowed time for the establishment of delayed effects, as well as the decay of the effect following the stimulation episode.

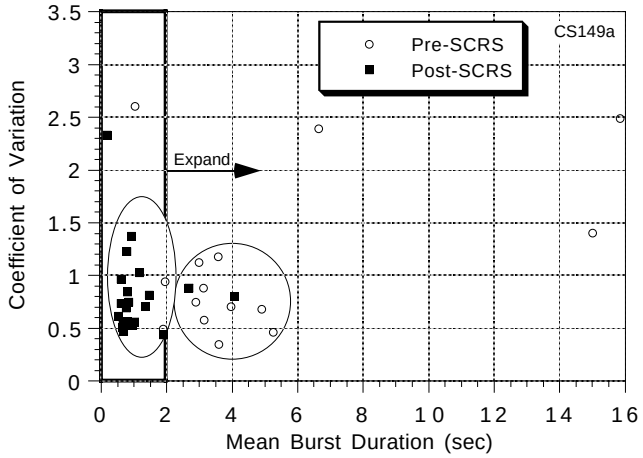
Figure 58. Network-wide burst duration changes. State-space plots depict changes in burst duration on a network-wide level following conditioning and opposing stimulation protocols. Mean burst duration (per minute) values for each recording channel (12 channels total) were averaged to obtain a "network" grand mean burst duration per minute. Each time interval represents ~15 minutes of activity--corresponding to 15 points for each one minute time bin plotted on the graph. Four time intervals in all (each 15-16 minutes in length) are plotted on the following three graphs. [Pre SCRS; during SCRS; post SCRS/pre SCTS; and post SCTS]

A. Open circles represent the 15 min time interval prior to SCRS (on channel 3). Closed squares represent the "network" burst durations after the cessation of the SCRS stimulus episode. Note: Prior to SCRS, over 50% of the time, the burst duration of the network was between 2 and 4 sec long (on two occasions, the network burst duration was over 14 sec). Following SCRS, all but two of the averaged burst durations were less than two seconds in duration.

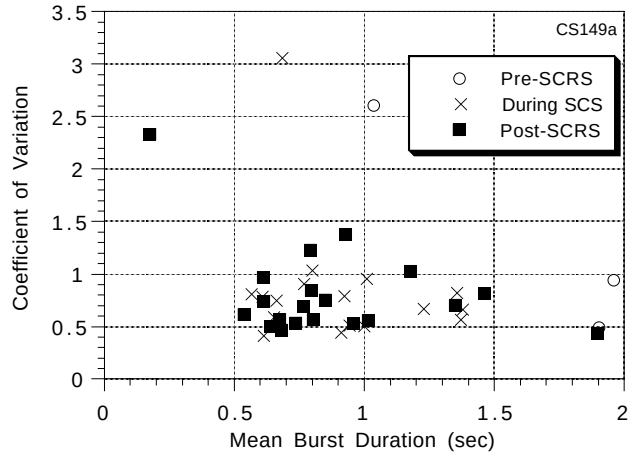
B. Mean burst durations during and after SCRS. An expanded view of the first two seconds shown in panel A allows the inclusion of data points representing the mean "network" burst duration during the 15 min repetitive stimulation episode. Note: all 16 of the 1 min time bins representing MBD during SCRS had a duration between 0.5-1.5 sec.

C. Mean burst durations (12 channels) before and after tetanic stimulation. The mean durations of the bursts increased significantly ($P < 0.0001$, Mann-Whitney U) following the tetanic stimulation episode. The effect of the stimulation largely reversed the effect of the previous repetitive stimulation episode on the same stimulus channel. Note: the post-RS markers and the pre-TS markers represent the same activity (same time interval).

A.


CS149a
Pre-SCRS
Post-SCRS
Expand
Coefficient of Variation
Mean Burst Duration (sec)
0
0.5
1
1.5
2
2.5
3
3.5
0
2
4
6
8
10
12
14
16

B.


CS149a
Pre-SCRS
During SCS
Post-SCRS
Coefficient of Variation
Mean Burst Duration (sec)
0
0.5
1
1.5
2
2.5
3
3.5

C.

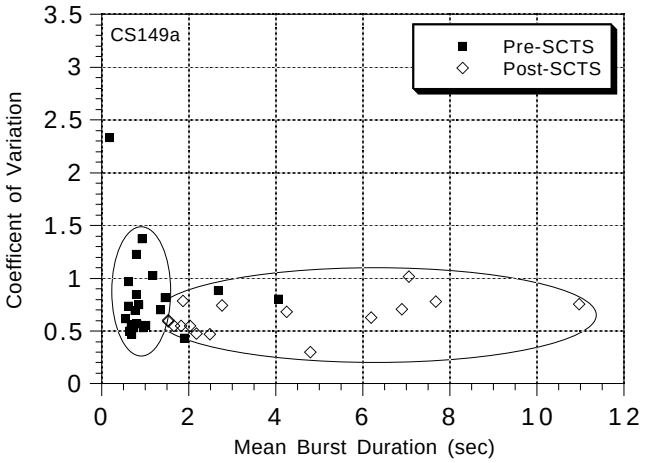

CS149a
Pre-SCTS
Post-SCTS
Coefficent of Variation
Mean Burst Duration (sec)
0
0.5
1
1.5
2
2.5
3
3.5
0
2
4
6
8
10
12

Table 9. Changes in Network Burst Duration Based on Stimulation Pattern

Expt.	Comparison of Intervals	Network BD	P value	Results
C-66	Pre MCTS vs Post MCTS	171% increase	< 0.0001	Significant
	Post MCTS vs During MCRS	43% increase	0.0005	Significant
	During MCRS vs Post MCRS	38% decrease	< 0.0001	Significant
	Post MCTS vs Post MCRS	10% decrease	0.1362	Not Sig
C-79	Pre TS-Ch24 vs Post TS-Ch24	134% increase	0.012	Significant
	Post TS-Ch24 vs Post (2) TS-Ch24 *	74% increase	0.0003	Significant
	Post (2) TS-Ch24 vs Post RS-Ch24	6% decrease	0.2914	Not Sig
	Post RS-Ch24 vs Post (2) RS-Ch24 ‡	70% decrease	< 0.0001	Significant
C-123	Pre TS-Ch12 vs Post TS-Ch12	38% decrease †	< 0.0001	Significant
	Post TS-Ch12 vs Post RS-Ch12	285% increase †	< 0.0001	Significant
	Post RS-Ch12 vs Post TS-Ch12	57% decrease †	0.0001	Significant
C-149	Pre RS-Ch3 vs Post RS-Ch3	89% decrease	< 0.0001	Significant
	Post RS-Ch3 vs Post TS-Ch3	734% increase	< 0.0001	Significant

* No stimulation took place, yet enough time had elapsed so that the effect [change in burst duration (and burst rate)--but not change in spike rate] had begun to decay.

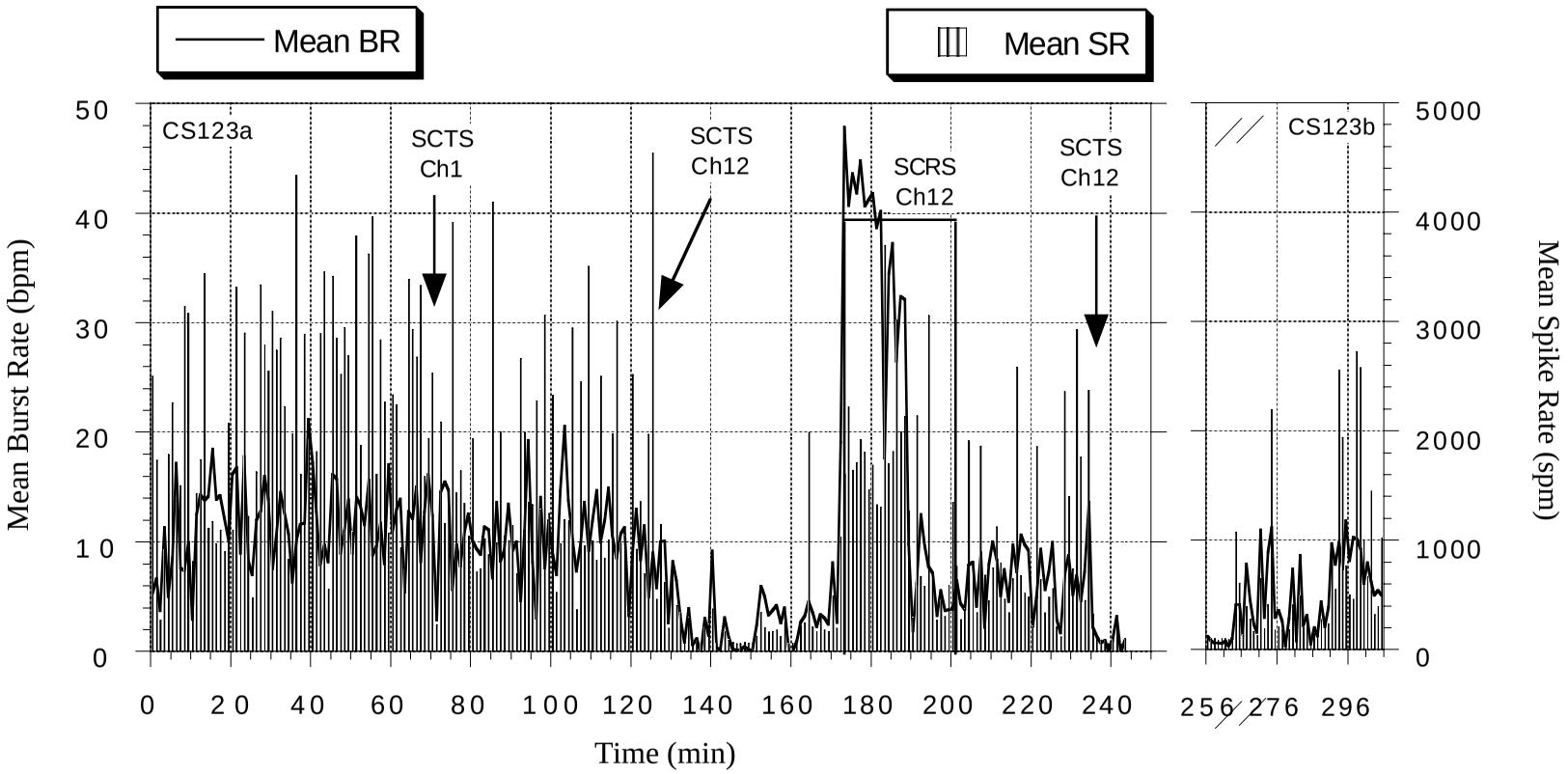
‡ [Same as (*) but this time BR and SR had a delayed effect.]

† Opposite effect of expected results.

The change in burst duration following conditioning and opposing stimulations in experiment C-123 turned out to be opposite from the expected results (see Table 9). That is, tetanic stimulation tended to increase burst duration, whereas repetitive stimulation tended to decrease burst duration. However, as shown in Figure 59, the conditioning and opposing stimulation apparently activated inhibitory circuitry (also see Fig. A-17 in Appendix). This circumstance happens to be a revealing one, because it indicates that "network" burst duration is dependent more upon the direction of change in spontaneous activity (i.e. burst and spike rate) than the type of stimulation pattern. In other words, if there was an increase in burst rate, there tended to be an increase in burst duration. Likewise, the burst duration tended to shorten with decreases in burst rate.

Figure 59. Stimulation of inhibitory circuitry. Mean (13 channels) burst and spike rates show that SCTS on channel 1 resulted in a slight dampening of spontaneous activity (by 15%-BR, 36%-SR). Tetanic stimulation on channel 12 caused a depression of bursting (85% decrease) and spiking (90% decrease) activity. While still in the "depressed" state, LFS on the same channel reversed the effect of the prior stimulation (BR: 344%, SR: 453% increase). Finally a second HFS on the same channel "repotentiated" the inhibitory circuitry (BR: 82%, SR: 76% decrease). This enhancement of inhibitory circuitry lasted for 28 min. However, full recovery did not occur until another 25 min had elapsed. The decay of the effect is seen in the adjacent graph. [All percent change values based on 20 min continuous intervals (except for the last calculations, which used 9 min from the end of C-123a and 11 min from the beginning of C-123b).]

[The 6 min gap between graphs encompasses the time it took to rewind the analog tape and set up another reel for recording.]


Mean BR
Mean SR
CS123a
SCTS
Ch1
SCTS
Ch12
SCRS
Ch12
SCTS
Ch12
CS123b
Mean Burst Rate (bpm)
Mean Spike Rate (spm)
Time (min)
0
10
20
30
40
50
1000
2000
3000
4000
5000
0
20
40
60
80
100
120
140
160
180
200
220
240
256
276
296

As in the previous sections, test pulses were delivered to the same stimulation channel as the conditioning stimulation channel before and after conditioning stimulation, in order to determine if there were changes in the number of evoked responses. These changes in evoked responses would indicate some type of alteration of the synapses associated with the units being stimulated.

To review, two of the hypotheses of this study were (1) that HFS causes an increase in spontaneous activity (i.e., an increase in the rate of bursting and spiking), and (2) LFS causes a decrease. If this increase in spontaneous activity is due to an increase in synaptic efficacy, then there might be an increase in responsiveness to test pulses following a tetanic stimulation event. Likewise, a decrease in evoked responses following a repetitive stimulation event could be an indication of a decrease in synaptic efficacy. There were only 5 reversibility trials that had MEAP values recorded [four single channel trials C-123 (2-in succession) C-135 (2-not in succession), and one multichannel trial (C-124)]. Below, Table 10 represents percent changes in MEAP values for each conditioning stimulation. Following the line of reasoning presented above, the expected results following HFS is an increase in evoked responses. In the same manner, the expected results following LFS is a decrease in evoked responses. Thus, HFS followed by LFS should lead to an increase and decrease in evoked responses, respectively. Likewise, LFS followed by HFS should lead to a decrease and subsequent increase in evoked responses. This reversal (or turnaround) of evoked responses is shown in Table 10.

The values shown under the heading of "Calculations" are the respective percent change in MEAP values for the conditioning stimulation in the column to the left ("Transition"). If the opposing stimulation was LFS, then the percent change was subtracted from the HFS. If the opposing stimulation was HFS, then the percent change was added to the LFS to determine the percent turnaround. The actual value of the percent turnaround may not be as important as the fact that in every reversal trial (except C-124) the

change in MEAP value was negative if LFS was delivered and positive if HFS was delivered. One should note that the one unexpected result was an MCRS stimulation. This type of stimulation does not seem to be as effective as SCRS, probably because it affects excitatory and inhibitory circuitry directly and simultaneously.

Table 10: Changes in MEAP Values Based on Stimulation Pattern

Experiment	Transition	Calculations	Percent "Turnaround"
C-123	SCTS to SCRS	195% - (-15%)	-210%
C-123	SCRS to SCTS	-15% + (+15%)	30%
C-135	SCTS to SCRS	2% - (-49%)	-51%
C-135	SCRS to SCTS	-255% + (+65%)	320%
C-124	MCTS to MCRS	28% - (+4%)	-24%

Experimental results highlighted in this section involve the enhancement (LTSAP) and depression (LTSAD) of inhibitory circuitry (Fig. 59). Although data from other trials indicate that inhibitory circuitry had been enhanced (and depressed), this experiment was the best example of alteration of spontaneous activity via activation of inhibitory units within the network. In addition to the substantial changes in spontaneous activity, changes in responses to test pulses (see Fig. A-16) support the inference that potentiating mechanisms were activated, followed by the activation of depotentiating mechanisms. That is, even though the SCTS on channel 12 resulted in a decrease in spontaneous activity, the same stimulation caused an increase in the MEAP value on all four recording channels.

The apparent change in evoked bursting (and spiking) activity during the SCRS episode in Figure 59 seemed to indicate that the network response to the single pulses were diminishing with time. Stimulus histograms of that LFS episode were plotted to determine if this was indeed the case. Figure 60 tends to confirm the argument that desensitization for the 12 recorded units did occur during SCRS on channel 12.

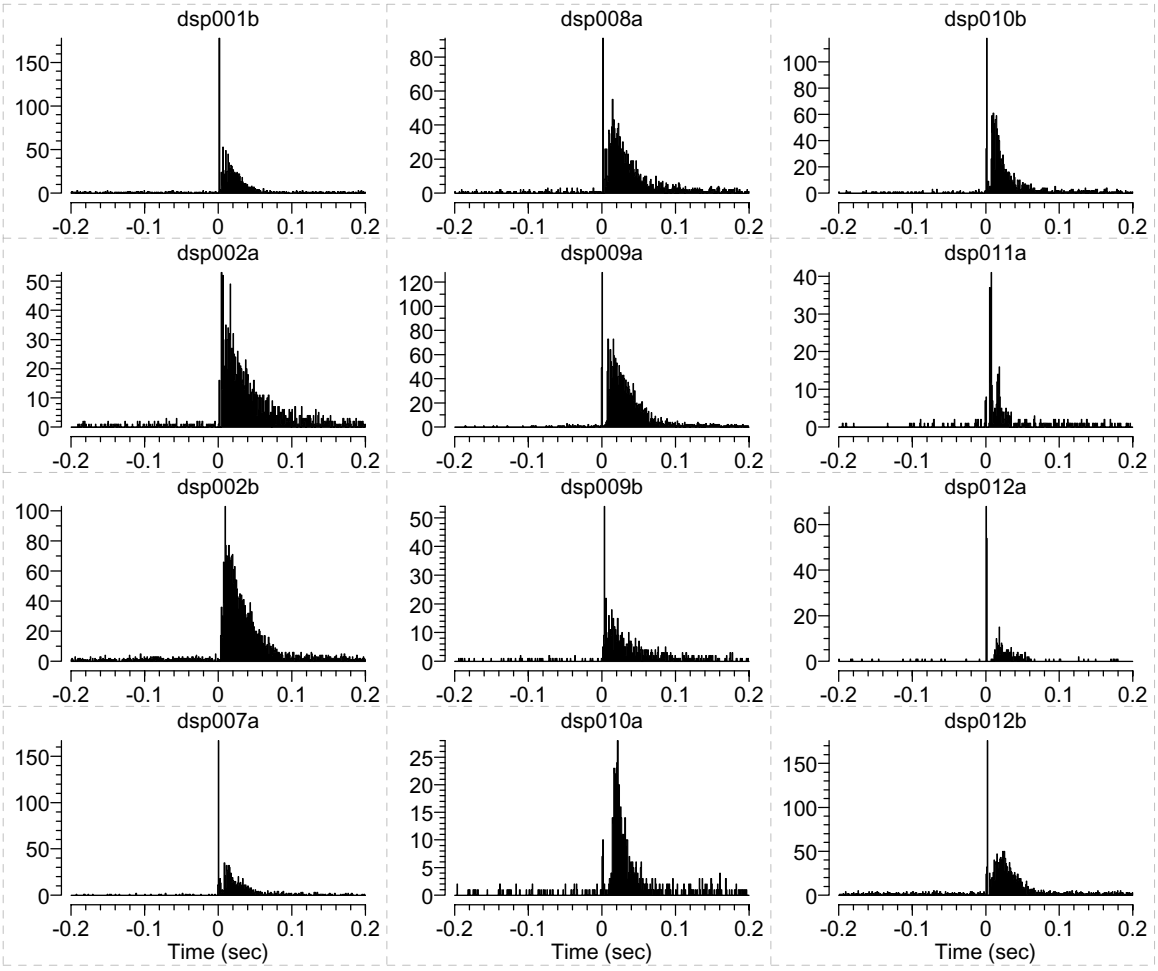


Figure 60. Changes in Stimulus Histogram Profile Over Time. As in the previous section, the 15 min repetitive stimulation period was divided into five 3-min segments in order to determine whether or not responses to single pulses changed over time. Six units were omitted because they showed no, or weak association to the stimulus pulse throughout the SCRS episode. Reference electrode (5b) is not shown.

A. First 3 min segment of the SCRS episode. Following SCTS on the same channel, the responses at the beginning of the repetitive stimulation period were well associated in time with the stimulus pulse.

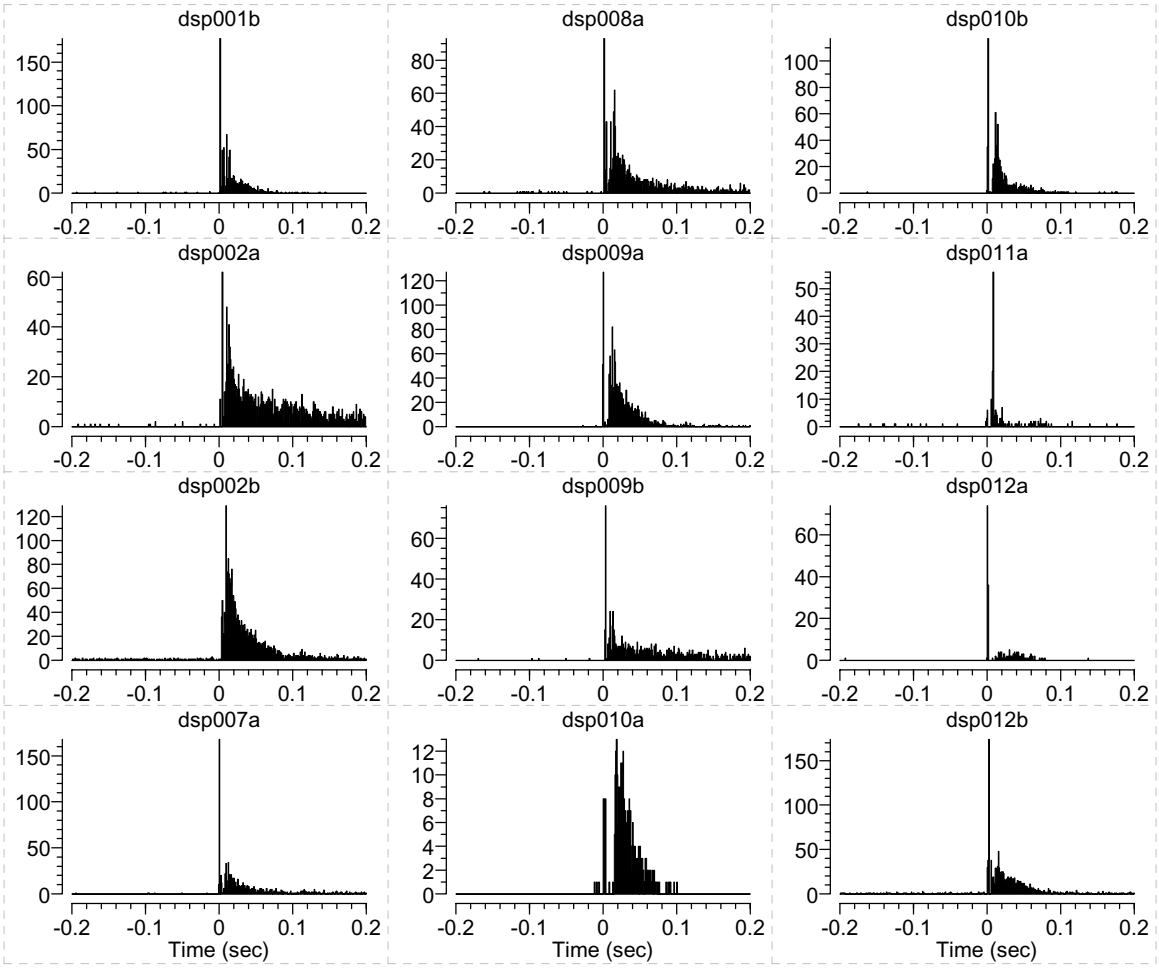


Figure 60B. Third 3 min segment of the SCRS episode. During this middle segment of the LFS episode, every unit depicted (except 2b and 10b) showed a decrease in the amplitude of the peak following the stimulus (e.g. unit 12b) and/or a decrease in the slope of the curve (e.g. unit 2a). For every unit shown, there was a noticeable reduction in the number of action potentials preceding the stimulus. While this could indicate an increase in association to the pulse, it may also indicate an overall reduction in spontaneous spiking activity.

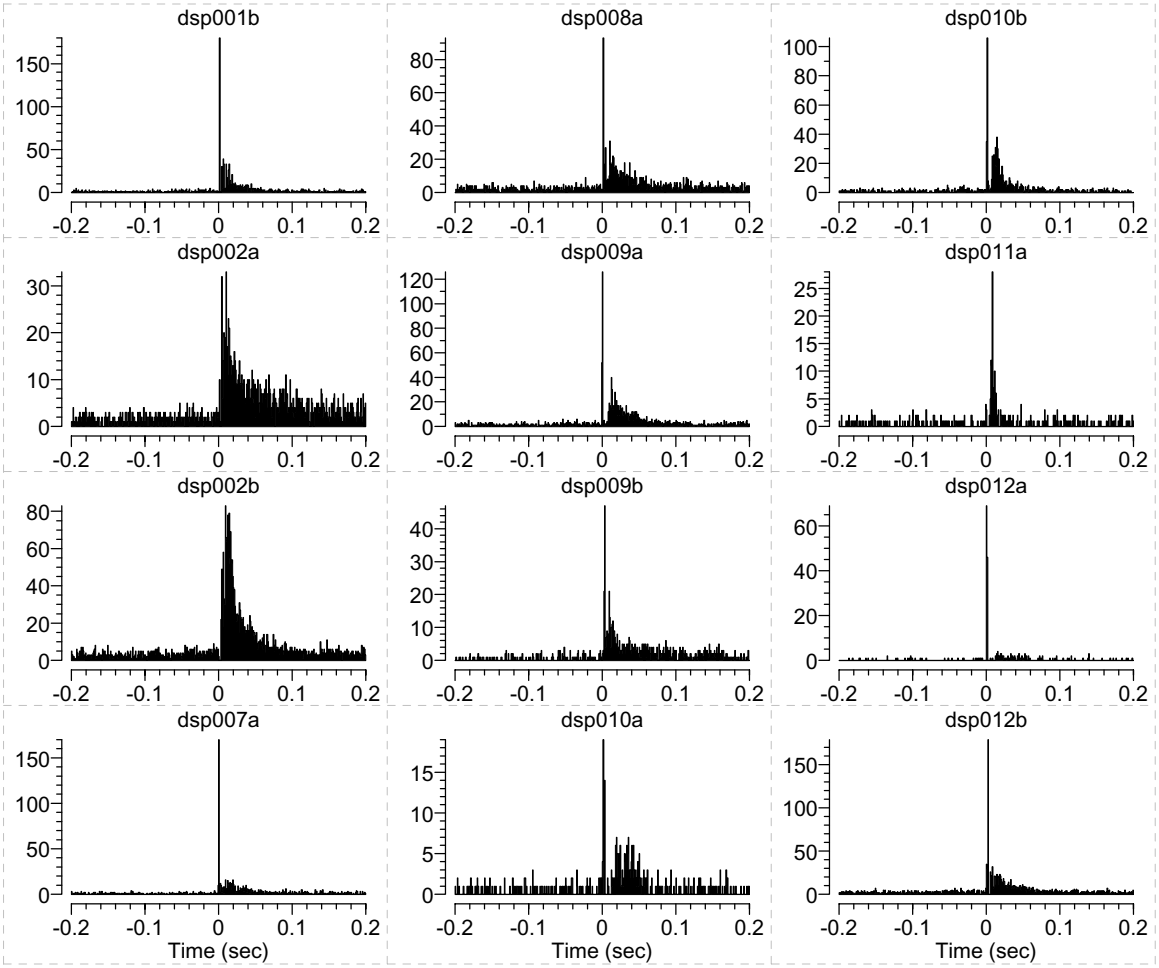


Figure 60C. Fifth 3 min segment of the SCRS episode. The final segment of the repetitive stimulation episode shows the deterioration of the association of evoked spikes with the stimulus pulse. The trend of decreases in the peak continued for all units shown.

The scale for many of the histograms decreased, including the scale for units 2a and 11a which showed a reduction of ~50%. The trend of reduced slopes also persisted. However, the trend of decreased action potentials preceding the stimulus was reversed, indicating an increase in random spiking.

CHAPTER IV

DISCUSSION

Introduction

The key to understanding how the nervous system works depends on the experimental investigation of the dynamics of neuronal networks. Neuronal information processing is best studied on a network level, primarily because of the dynamics and the relationship to biocommunication to specific targets that neuronal networks provide. Although activity patterns and their regulation are determined in part by the final architecture of the associated units in each distinct network, there has been a shift in thinking from hard-wired circuits to multifunctional networks (Katz, 1996; Naisberg et al., 1996; Canepari et al., 1997; Gross and Kowalski, 1999).

One of the primary functions of neuronal networks is considered to be the storage of the engram or memory trace. There are many different types of memory described in the literature. In Vanderwolf and Cain's 1994 review article, 18 different varieties of memory were named and these authors noted that there were other types not included in the list. Electrophysiological and neuroimaging techniques as well as neurobehavioral studies indicate that long-term memory as well as working memory all share the same substrate: a large network of partly overlapping and interconnected cells. In the neocortex, working memory can be described as the sustained activation of one widely distributed network of long-term memory (Fuster, 1998). According to Lisman and Idiart (1995), around 7 (± 2) short-term memories (stored by patterns of neuronal activity) can be stored in a single human neural network. Because memory is encoded in the activity of many neurons across a population of cells, it has been suggested that a neural population code is used to store and retrieve this information (Lewis and Kristan, 1998).

In the cerebral cortex (and likely other areas) memory networks are formed by associative processes that follow Hebbian rules of synaptic modulation (Fuster, 2000). More than 50 years ago, Donald Hebb (1949) revived and consolidated older hypotheses and proposed that learning was associative. In short, the modern-day hypothesis is that afferent stimulation combined with simultaneous postsynaptic depolarization leads to an enhancement of synaptic efficacy. In Fuster's 1995 article, he describes memory as essentially associative in its generation, structure and retrieval, and that depending on the level of their biological relevance, stimuli, both external and internal, can at any time activate the neuronal network to which they are part of by previous association. He goes on to assert that this process is the basis of knowledge and remembering.

A primary factor in the modulation of neuronal networks is altered synaptic function or efficacy, often referred to as synaptic plasticity (or synaptic modification). The study of synaptic plasticity comprises a substantial fraction of the investigative work in neuroscience. As the primary candidates for the mechanism of learning and memory, synaptic modification, principally long-term potentiation (LTP) and long-term depression (LTD), has been the focus of study throughout the last quarter of this century. The current definition of LTP put forth by Bear and Malenka (1994) describes LTP as a synaptic enhancement that follows brief, high-frequency electrical stimulation in the hippocampus and neocortex. It seems that most definitions of LTP in the literature imply that the mechanism is confined to the hippocampus. While most researchers recognize that the phenomenon was first discovered there (Bliss and Lomo, 1973), this definition of LTP is rather limited in its scope. Nevertheless, the preparation of choice for the study of the cellular basis of learning and memory has historically been the hippocampal slice and the hippocampal area *in vivo*; this is evident because the vast majority of experiments involving synaptic plasticity have

been carried out using this tissue. This dominance of the hippocampus in LTP studies is primarily a result of the area's documented importance in memory acquisition, consolidation, and retrieval (Berman and Kesner, 1976; Fair, 1992; Fletcher, et al. 1997). Long-term depression, as defined by Bolshakov and Siegelbaum (1994), is an activity-dependent decrease in synaptic efficacy that together with its counterpart, LTP, is thought to be a critical cellular mechanism for learning and memory in the mammalian brain.

While the hippocampus (and hippocampal slice) is a convenient preparation for the study of plasticity mechanisms, it is becoming more and more obvious that the concept of strengthening and weakening of synaptic function is applicable throughout the CNS (and likely throughout the nervous system as a whole). Researchers have reported both LTP and LTD in several distinct areas within the CNS including (but not limited to) the hippocampus, the amygdala, the cerebellum; and at least two different neocortical areas). In addition, both LTP and LTD have been demonstrated in the undissociated spinal cord slice (Pockett and Figurov, 1993; Randic, et al., 1993;).

The above examples give credence to the view that there are "multiple memory systems" in the central nervous system (Macdonald and White, 1993; Squire, 1992). The idea that several different mechanisms in different locales of the nervous system may be involved in memory and learning promotes the prospect that storage mechanisms are similar and functional regardless of architecture.

What the vast majority of these preparations have in common is the level of organization or complexity—they are all, by and large, neuronal networks. Whether slices or dissociated cultures, the neuronal network remains as the standard preparation for the analysis of synaptic plasticity. Even with *in vivo* studies, the locus of the stimulating and recording electrodes were within a neuronal network of specific architecture inside a discrete

brain structure (e.g. hippocampus). Furthermore, when investigators have focused on modification of single synapses, most of those single synapses were studied within network preparations.

Experimental investigations of synaptic plasticity on a molecular and cellular level have yielded a wealth of information about how the efficacy of transmission can be enhanced or diminished, depending on the technique used to induce these plasticity mechanisms. However, the complex behavior of neuronal ensembles cannot be explained by the concepts that apply to cellular and molecular electrophysiology. Rose and Siebler (1995) studied the global network spike activity of dissociated hippocampal cultures as a function of synaptic interaction. They presented evidence that, at a critical level of synaptic connectivity, the firing behavior undergoes a phase transition that cannot be ascribed to the spike threshold of individual units. They reported that a tremendous increase in the firing level came about following an increase of synaptic efficacy by lowering the extracellular magnesium concentration. These findings led them to assert that the "on-off" aspect of their preparation demonstrated that even in small neuronal ensembles collective behavior can emerge which is not explained by the characteristics of single neurons.

There are some that contend with the mostly accepted relationship between synaptic plasticity (LTP, LTD, and depotentiation—a reversal of LTP not related to LTD) and learning and memory. An article by McEachern and Shaw (1996) points out that this relationship has not been vigorously challenged, particularly in light of the lack of an unambiguous correlation of LTP/LTD with either learning or memory. In their review, they examined experimental evidence supporting the linkage between LTP/LTD and memory, and deemed it wanting. Instead, they proposed that synaptic modification as a whole is a continuum of events in the realm of neuroplasticity/pathology. They go on to suggest that

the regulation of neurotransmitter receptors may be a pivotal element leading to synaptic modification.

Although Holscher (1997) may be counted among those who dispute the likelihood of synaptic modification as mechanisms used by the nervous system for learning and memory processes, he stated that *in vitro* experiments were essential in the investigation of cellular and subcellular mechanisms that underlay synaptic transmission. But Holscher shows he is clearly an advocate of confining learning and memory studies to *in vivo* experiments with his opinion that measurements of LTP, LTD, or DP (depotentiation) are not reliable models for learning processes and should not replace experiments with intact animals that learn spatial tasks. He cited several reports showing that the elimination of LTP via blocking agents and the use of mutant mice with gene deletions that prevent LTP, did not significantly impair learning *in vivo*. In addition, in their 1997 publication, Holscher, et al. chimed in with Bliss and Lynch (1988) and Barnes (1995) in voicing their concerns about the conditions in which LTP/LTD were obtained using *in vitro* preparations. Among their concerns were the temperature and buffer contents. While many (perhaps most) experiments using *in vitro* preparations use HEPES buffer and are conducted at room temperature (the temperature of the preparation is often omitted from the Methods section in publications), the only buffer in our medium was NaHCO2 / NaHCO3 , and the temperature was maintained at 37º C. Maintenance of the pH and temperature added a level of difficulty throughout the experiment, but we felt it important to record in conditions that were as close to physiological as possible.

The current studies differed from classical experiments in this area in two ways. First of all, the preparation was different. These experiments were carried out with networks derived from spinal cord tissue. While this part of the CNS is not immediately recognized

for its demonstration of plasticity mechanisms, there have been (and continue to be) experiments showing storage capabilities in the spinal cord (Svendsen, et al., 1999; Ikeda, et al., 2000; Chen and Sandkuhler, 2000). Indeed, as early as 1985, Durkovic, and in 1987, Wolpaw reported evidence of a memory trace in the spinal cord. [Also see Levita et al. (1986) regarding memory improvement in patients that had undergone spinal cord stimulation.] Reviews of memory traces in the spinal cord have been published by Wolpaw and Carp (1990) and Windhorst (1996).

In addition to the preparation being derived from the spinal cord, the integrity of the tissue was also different. As mentioned earlier, the bulk of *in vitro* experiments involving LTP and LTD have used hippocampal slices as the preparation. Neuronal cell cultures of dissociated tissue have not garnered as much attention. And of this group, dissociated tissue from the hippocampus and cortex have received the lion's share of experimental focus. Yet, even in this subgroup of network preparations, as stated by Jimbo et al. (1999), little is known about the effects of synaptic plasticity on network activity. Using electrode arrays very similar to the ones used in this study, they investigated how a single tetanic stimulus affected the firing of up to 72 cortical neurons through the 64 different stimulation electrodes on the array. They found that the same tetanus induced potentiated transmission in some stimulus pathways and depressed transmission in other pathways. They go on to report that the responses were homogeneous (i.e., depending on the electrode stimulated, the responses were either all enhanced or all depressed). My results were only partly consistent with these findings. While I found the stimulation site to be critical to the type of response, I rarely found the response to be homogeneous. This is probably due to the difference in preparations. Culturing techniques could also be a factor. However, more work like this needs to be done for other dissociated cultures derived from different areas of the CNS, in

order to compare and contrast the effects of different stimulus patterns on spontaneous activity. These types of studies would go a long way toward a better understanding of the information processing on the network level. Jimbo and colleages concluded with a generalized statement alleging that the "spatial responses following localized tetanic stimuli, although complex, can be accounted for by a simple rule for activity-dependent modification." While I am currently reluctant to support that statement for all dissociated neuronal networks, it is worth further testing in our particular environment.

The second way that my studies differed from most experiments on storage mechanisms was that the primary measurement of the manifestation of those mechanisms was the alteration of spontaneous activity (not synaptic efficacy). I recorded spontaneous activity (primarily population bursts) from non-invasive planar electrode arrays. While the conventional way to measure those changes has been to deliver test pulses through a stimulating electrode and record the change in response (e.g. changes in EPSP, population spike amplitude, and latency) with a recording electrode, very few experiments measured changes in synaptic efficacy via monitoring changes in spontaneous activity. The paucity of experiments using this approach is likely due to the difficulty of measuring changes in synaptic modification via changes in spontaneous activity as opposed to changes in evoked activity. It is also very likely that because many experiments focusing on synaptic plasticity were performed at room temperature, there was little or no ongoing spontaneous activity. In our preparations, the spontaneous activity at room temperature is practically nonexistent. The fact remains that there is spontaneous activity *in vivo*, and in spite of this "interference" problem, the percentage of "successful modifications" obtained were still similar to the published results from *in vitro* preparations with little or no spontaneous activity.

Studies of the effects of changes in synaptic efficacy on spontaneous activity must be conducted because these effects are important to the understanding of information processing, particularly on the network level. As discussed above, LTP and LTD are thought by many to be mechanisms involved in learning and memory processes, and electrical (or conditioning) stimulation is often used to induce LTP and LTD. Yet electrical conditioning stimulation has also been used to affect memory processes in behavioral studies. In these *in vivo* experiments, conditioning electrical stimulation has been shown to both enhance (Sos-Hinojosa, et al., 2000) and disrupt (Bierly and Kesner, 1980; Penna, et al., 1998) memory processes in the CNS. In addition, several types of sensory memory processes have been modified by electrical stimulation. For example, visual STM (Kovner and Stamm, 1972), both visual STM and verbal LTM (Sherder, et al., 2000), and olfactory memory (Gorkish, et al., 1985) have all been altered via electrical stimulation.

Working memory can be activated via electrical stimulation. The evidence of short-term or working memory activation is the elevation of ongoing spontaneous activity for a period of time after the cessation or removal of the stimulus (Fuster and Alexander, 1971; Fuster and Jervey, 1982, Curtis et al., 1992; Zisper, et al., 1993). Alan Baddely and Graham Hitch proposed that short-term memory is a part of a "working memory" system that briefly stores and processes information needed for planning and reasoning (Wickelgren, 1997). In these experiments the electrical stimulation actually changed the level (or character) of spontaneous activity. The results of the electrical stimulation studies are supported by experiments involving physiologically relevant stimuli. For example, Bodner, et al. (1996) used auditory stimuli to show sustained elevated firing levels (higher than spontaneous discharge) in the dorsolateral prefrontal cortex of monkeys; Miyashita and Chang (1988) used colors to show the same effect in an anterior ventral part of the temporal

lobe; and Zhou and Fuster (1996) used tactile stimulation to demonstrate the effect in the hand area of the somatosensory cortex.

The modification of spontaneous activity in cultured neuronal networks may be very important because this is ultimately the modification of a "basal" physiological state. In fact, according to Arhem and Johansson (1996), spontaneous activity is necessary for normal brain function. Therefore, an understanding of those mechanisms responsible for modifying spontaneous activity may provide clues for identifying and treating pathological conditions.

Recording changes in spontaneous activity in our networks following electrical conditioning stimulation may actually be more physiologically relevant than thought at the outset of this study. In Barnes' 1995 review, he states that most experiments performed in intact animals that focused on activity-dependent changes in hippocampal evoked responses found changes only in the population spike component of the response, with no reliable effects on the synaptic response. The events that occurred (alteration of spontaneous activity) during some learning experiments seem to have been replicated in this study. Just as spontaneous activity has been found to be important during development in the formation, elimination, and "fine-tuning" of synaptic contacts, ongoing spontaneous activity likely plays a role in changing and redefining synaptic contacts in mature networks as the organism learns to adapt to and process novel stimuli (Kandel, 1991). Taken together, these issues indicate that the increase (or decrease) in spontaneous activity seen following the stimulus may be the activity aspect of "activity dependent" plasticity, and not necessarily the stimulus (or the evoked response to the stimulus) itself—at least not in the intact animal. This notion is bolstered by Kavanau's (1997) opinion that oscillatory firing is a practical method used by higher organisms to dynamically stabilize synapses without having to

undergo repeated stimulations of relevant circuits for the purpose of enhancement of synaptic efficacy.

Primary Focus of Investigation

The primary goal of this study was to determine if stimulation patterns consistent with those used to induce modifications of synaptic efficacy in conventional preparations (i.e. slices and *in vivo*) could also change the level of spontaneous activity in dissociated spinal cord network preparations. Below are the specific questions raised in Chapter 1:

1. Does HFS increase spontaneous activity?
2. Does LFS decrease spontaneous activity?
3. Can activity enhanced by HFS be depressed by LFS and vice versa?
4. Does stimulation on single channels give different results than stimulation on multiple channels?

Thus, the effects of tetanic and repetitive stimulation on spontaneous activity or network plasticity, and not synaptic plasticity, per se, was the focus of this investigation. However, modifications of several synapses may indeed be involved in the alteration of spontaneous activity. For example, Bliss and Lomo (1973), Andersen, et al. (1980), and Abraham et al. (1985) all measuring field potentials, reported a potentiation (increased amplitude and reduction in latency) of both the population EPSP and population spike in the hippocampal area. Andersen et al. showed an increased EPSP via intracellular recordings. Abraham et al. showed that tetanization of the perforant path of the dentate gyrus led to long-term changes in the relation between the EPSP and the population spike. They concluded that the effects likely reflect a generalized post-synaptic change. Although none of the researchers above recorded spontaneous activity, they did record evoked suprathreshold

potentials. The test pulse experiments that were performed in this study, although not identical, were similar to the population spike measurements in the studies listed above. Just as a relationship was found between the EPSPs and evoked action potentials, the test-pulse experiments often supported apparent alteration of spontaneous network activity.

Many neuroscientists reason that LTP is a mechanism associated with memory formation, and that its counterpart, LTD, is somehow related to forgetting or perhaps to some type of a resetting mechanism. Wolf, et al. (1995) stated that memory traces permanently modify the behavior of neurons and networks in the form of activity patterns and gene expression. Following this statement, they go on to question the current knowledge of the stability of the synapse under normal circumstances. They review evidence that synapses may be remodeled and removed within the time frame of hours to weeks. Evidence is presented that suggests the elimination of 250 million synapses per hour in area 17 in the cerebral cortex of Marmoset monkeys.

Review of Basic Findings

The basic findings of this study were that spontaneous activity was increased in 52% of tetanic stimulation trials. Activity was increased in 47% of single channel stimulation trials and 59% of multichannel tetanic stimulation trials. The spontaneous activity decreased following 35% of repetitive stimulation trials (see Table 3A). As was the case in tetanic stimulation trials involving enhancement of spontaneous activity, repetitive stimulation on several channels simultaneously resulted in a higher percentage of depression of spontaneous activity. In 41% of multi-channel repetitive stimulation trials, the activity was depressed. Following 34% of single channel repetitive stimulation trials, there was a decrease in activity (see Table 6A). These percentages are consistent with or

higher than results obtained in conventional preparations for experiments involving long-term potentiation and long-term depression (references listed later). These findings seem to provide sufficient evidence that HFS and LFS do increase and decrease spontaneous activity, respectively.

In trials to reverse the effect of a particular stimulation pattern, a reversal occurred 80% of the time (only trials where an initial change of activity were recorded). Of the eight long-term responses, the response was reversed seven times. In the analysis of the reversal trials data set, responses with a recorded percent change in burst rate below 20% of prestimulus activity were not considered to be a true response. These data show that activity enhanced by HFS can be depressed by LFS and vice versa.

Examination of both tetanic and repetitive stimulation trials reveals that stimulation on several channels simultaneously resulted in a higher percentage of predicted results than single channel stimulation. In addition, the occurrence of unpredicted results (a decrease in activity following HFS, or an increase in activity after LFS) was lower with multichannel stimulation. However, the difference between stimulation on single and multiple channels was not great enough to assert conclusively that stimulation on single channels give different results than stimulation on multiple channels.

No significant difference was found between the duration of effect following tetanic stimulation and repetitive stimulation. The results from the latter test tend to support the observation made in Chapter 3.3 denoting how the duration of the opposing stimulation tended to mirror the duration of the initial stimulation. These particulars may add a component of parity to the opposing nature of the two stimulation patterns used in this study. In other words, the two different stimulations produce relatively equal as well as opposite changes in spontaneous activity. This notion of equality may reinforce the idea

that the same mechanism is (or similar mechanisms are) involved in the enhancement and depression of spontaneous activity. This mechanism gets activated in one direction or another depending on the type of stimulation delivered.

Durations shorter than 0.2 minutes (or 12 sec) were not included in the analysis. The percentage of short-term (<15 min) responses for tetanic stimulation was comparable to the percentage of short-term responses in repetitive stimulation trials. In 75 repetitive stimulation trials, a large majority (73%) were short-term; and of the tetanic stimulation trials, 81% of the responses were characterized as short-term. The longest duration (when the response was allowed to decay spontaneously) was not recorded in trials where the change in activity lasted beyond 60-90 min. The opposing stimulation was delivered while the change in activity was still elevated (or depressed) in order to assess the capacity of the opposing stimulus to reverse the effect of the original stimulus.

The range of the durations of the response was comparable to the range of depression (and enhancement) reported in the literature. For example, Lovinger, et al. (1993) noted that once depression of glutamatergic synapses in slices of the neostriatum was induced, the responses returned to baseline levels within 10 min in most slices, but lasted for up to 60 min in others. They also inferred that the form of synaptic depression expressed in their studies involved a decrease in the release of glutamate. Brown et al. (1991) assert that potentiation can be produced in other areas of the CNS but the potentiation lasts for only a few minutes or at most hours.

Delays in Response Following Stimulation

Most (72%) of the time following HFS there was no noticeable delay between the cessation of the stimulation episode and the response. Even when there was a delayed

response to tetanic stimulation, the delay interval was often very brief. Of all the delay intervals recorded (46), only four were over 2 min. In 70% of recorded intervals following LFS, the response delay was less than 2 seconds. The longest recorded delays were 15 (SCTS) and 16 (SCRS) min. These outlyers were not discarded because both delays were recorded following stimulation on the same electrode in the same preparation. The underlying spiking activity, which was not visible in the burst domain (stripchart data), began to increase shortly after the SCTS episode. As the spiking reached a certain level, the bursting activity became apparent (see Fig. 34). This particular response was very evident, primarily because the pre-stimulus bursting activity was nonexistent (i.e. no bursts were displayed on the stripchart). The results of this stimulation trial reveal one of the problems associated with these studies—if there had been "normal" levels of spontaneous activity prior to tetanic stimulation, it probably would have been more difficult to detect the response to that stimulation among the ongoing spontaneous activity, particularly when the long delay following the stimulus is taken into account. Later in the same preparation LFS on the same channel resulted in a similar delay after which there was a total depression of bursting activity (see Fig. 44). Other electrophysiologists have recorded delays between the stimulus and the response. Huerta and Lisman (1996), recording from hippocampal slices, reported that the interval between the initial potentiating tetanus and the induction of theta-LTD could be as long as 90 min. This form of LTD was also reversible via tetanic stimulation.

One key factor that tended to influence all aspects of the response was the site of the stimulation in the network. While one would expect that depending on which electrode was stimulated, there might or might not be a response, it was not anticipated that the duration and magnitude of the response, as well as the delay of the response were also biased by which electrode was selected for stimulation. In hindsight it seems logical that in a network

not homogeneous in cell type, glial coverage (particularly on and near specific electrodes), relative arrangement of neurons, and circuitry, the same stimulation delivered at any given point within the network may give very different responses.

In my spinal cord network cultures, the response to stimulation (either tetanic or repetitive) greatly depended on which perspective electrode(s) were stimulated. Although the perspective electrodes were chosen via test pulses at the beginning of every experiment, there were still numerous times when stimulation of a particular electrode produced no discernible response. There were other occasions when the same stimulation pattern produced a different response when stimulated at a different electrode (not to be confused with a single stimulation that causes a different response depending on which electrode is being monitored). Turner and Miller (1982) also showed the importance of stimulation site, when they stimulated two afferent systems in the hippocampal slice. They reported that LFS (1 Hz) delivered to the Schaffer collateral-commissural fibers produced a short-term potentiation, while the same stimulation pattern delivered to the perforant path evoked a short-term depression.

Assessing the effect of conditioning stimulation by calculating the percent change in spontaneous activity was often questionable. In many cases it was an exercise in oversimplification. The means of channel activity were often misleading. Most of the time there was a wide range of responses; and for several trials, values had to be omitted due to infinity values. These omissions led to a much smaller percent change in tetanic stimulation trials. Thus HFS seemed to be much less effective in regards to overall percent change (particularly burst and spike rates) than LFS (see Tables 4 and 7).

Responses to Test-Pulse Stimulation.

In 44 stimulation trials, test pulses were delivered before and after conditioning stimulation to the same stimulus electrode in order to determine if the response to the single pulses were potentiated following tetanic stimulation or, in the case of repetitive stimulation, depressed. Testing between the subgroups of single channel stimulation revealed that there was a statistically significant difference between the SCTS and SCRS mean MEAP values. Because there was a concern about potential long-term effects caused by the test pulse stimuli alone, the voltage was decreased to 480 mV (from 640 mV: regular stimulation voltage). This seemed to be a valid concern when one considers that, for each test pulse episode, no fewer than 10 (not exceeding 22) pulses were delivered through one or more stimulus electrodes.

As referred to above, test pulses were delivered before and after the conditioning stimulation for the purpose of assessing the effect of the conditioning stimulation on evoked responses. As mentioned previously, there was some concern that the administration test pulses might confound the response to the conditioning stimulation because the pulses themselves might have some a lingering effect on the spontaneous activity or at least may affect the way the network responded to the conditioning stimulation. Rick and Milgram (1996), showed that prior stimulation did influence the response to subsequent conditioning stimulation. This concept is not new, researchers for years have known about primed burst stimulation (see Diamond, et al., 1988) and other techniques that affect the response to conditioning stimulation. Nevertheless, these studies focus more on the long-term effect of prior stimulation and its ability to bias synaptic plasticity (i.e. metaplasticity). Rick and Milgram delivered the following tetanic stimuli: 3, 6, 12.5, 25, 50, 100, 200, and 400 Hz in ten minute intervals (3 trains each) to freely moving rats. The trains were delivered in

ascending and descending order. They found that potentiation was observed at high frequencies regardless of the order, but that depression occurred at low frequencies only in ascending order.

Data collected from these experiments, to compare the relative effectiveness of single versus multiple channel stimulation, produced equivocal results. The increase in MEAPs for MCTS was higher than the increase in MEAPs following SCTS, whereas the mean percent decrease in MEAPs following SCRS was greater than the decrease in MEAPs following MCRS. These data suggest that repetitive stimulation is more reliable at depressing evoked responses than tetanic stimulation is at potentiating evoked responses following the respective conditioning stimulation. This difference is a clear indication that the type of conditioning stimulation delivered influenced the way the network responded to test pulses. Thus, for future studies of network plasticity, MCTS should be used for maximum enhancement of evoked responses. Yet, SCRS should be utilized for optimum depression of evoked responses.

The calculation of MEAP values proved to be crucial in determining whether or not a modification (likely synaptic) had taken place following conditioning stimulation. The implication became particularly evident when the MEAPs were averaged across the four recording channels. In addition to the problem of the test pulses themselves affecting the spontaneous activity (mentioned above—also see Fig. 23), the test pulses could also be recorded as biological activity by the Masscomp 5700 (but not with the Plexon System). Thus test pulses might have presented a major problem in instances where there was total depression following a LFS episode. These potential problems were outweighed by the fact that results from some test pulse trials showed changes in MEAP values when there was no noticeable change in spontaneous activity. Thus, test pulse experiments provided evidence

of an effect following conditioning stimulation even when this effect was "masked" by ongoing spontaneous activity. This "unmasking" of the effect of conditioning stimulation was also valuable when trying to verify that inhibitory circuitry had been potentiated or depressed (see Figs. 40 and A-16 for examples).

Because the basic attributes of the response depended on the stimulation site, this site-specificity aspect of the response to stimulation, in addition to the example given above regarding the comparable delay times of the responses following conditioning stimulation on the same electrode, invites speculation on whether or not there may be a connection between the delay of response and the duration of the response. Because of the complexity and range of the responses, this possible connection was not investigated in this study. However, investigators pursuing these types of studies may wish to tailor their experiments to determine if there is some sort of association between the delay and the duration of the response.

Tetanic Stimulation

Alteration of Activity

Some common responses to tetanic stimulation include: paroxysmal bursting, elevated burst rate, changes in burst pattern (with no apparent change in burst rate), and decreases in burst rate. Because of this diversity of responses to a single tetanic stimulation event, quantitative evaluation of changes in spontaneous activity was somewhat of a challenge. Still, of the responses previously listed, an increase in overall spontaneous activity most often manifested itself in the form of an increase in the burst rate. However, it became obvious that in some stimulation trials, when the increase in overall spontaneous

activity was manifested in the form of paroxysmal bursting,that measuring the burst rate alone was inadequate.

Paroxysmal bursting (shown in Fig. 19) is clearly an increase in overall spontaneous activity. Yet, paroxysmal bursting rarely yields an increase in burst rate. To the contrary, the hallmark of this type of bursting is very long burst durations, because the high frequency spikes are organized into large, complex bursts. Paroxysmal bursting is a distinctive response to tetanic stimulation. It is a clear departure from the normal mode of spontaneous bursting activity. It has also been described as epileptiform activity. Indeed, tetanic stimulation is closely related to the stimulation protocols used to induce kindling leading to experimental epilepsy *in vitro*. Kindling represents the progressive development of generalized seizures and results from the repeated application of low-level electrical stimulation to limbic structures. It is generally accepted as a good model of epilepsy (Baudry 1986). Johnston and Brown (1986) produced experimental evidence showing that paroxysmal bursting activity was the result of a large, network driven EPSP.

Evaluation of Changes

There were many cases when changes in spontaneous activity were not quantifiable with present methods, but visual inspection revealed pattern changes. In other words, there was no net increase or decrease in the burst rate, yet the pattern of burst packages, or even the shape of the bursts themselves was altered. Those trials were omitted from the data set. One can assume that the stimulation had an effect on the spontaneous activity, but without quantitative appraisal of that effect, it is difficult to describe (or categorize).

Repeatability

Even though the experiments performed involved the stimulation and recording from a population of cells, the percentage of changes in spontaneous activity was comparable to percentage of change in synaptic efficacy (EPSP and IPSP) using single cell recordings or field potentials from a population of cells. For example, Pennartz, et al. (1993) recorded intracellularly as well as extracellularly in slices from the nucleus accumbens. They reported that in extracellular recordings, LTP was induced in the population spike in 20 out of 53 slices (38%). For intracellularly recorded EPSPs from 54 cells, LTP was expressed in 16 cells (30%), decremental potentiation in eight cells (15%), and LTD in six cells (11%). In a report on synaptic plasticity of the spinal cord, Pockett and Figurov (1993) recorded field potentials in slices from the ventral horn. Tetanic stimulation produced LTP in 25% of the slices, LTD in 33%, and no lasting change in the remaining slices. The long-term changes lasted at least 2.5 hours. Wang, et al. (1994), recording extracellularly from chick forebrain slices, reported population spike potentiation in 25% of the tested neurons following afferent tetanic stimulation. They also reported a long-term depression following the tetanic stimulus in some units in the same area. In these studies activity was increased in 52% of HFS trials and was decreased in 35% of LFS trials.

Single vs. Multichannel Stimulation.

The reason MCTS tended to be more potent (aside from the duration of the effect) than SCTS may have had to do with the associativity factor of MCTS. Tetanic stimulation at a single electrode may stimulate several units simultaneously, but MCTS stimulates many more units within the network simultaneously causing action potentials to flow in both directions (orthodromically and antidromically) at several sites on the array.

The nature of the high frequency stimulation is such that, for all units which have axons that are directly depolarized by the electrical current, the entire neuron (including all axon terminals, the soma and dendrites) is being stimulated by the strong tetanic stimulus. Thus many units within range of the depolarizing effect of the active electrodes will have coactive synapses, and even more units downstream from the stimulation electrode(s) will have active presynaptic terminals. Many of these presynaptic terminals may be "nontraditional" presynptic sites. That is, signals traveling antidromically through the axon will be carried back to the axon hillock, invade the soma and after some integration may be back-propagated into the dendrites and may (depending on decay constants) depolarize adjacent units. Thus, normally presynaptic sites become postsynaptic sites (Magee and Johnston, 1997). This associative stimulation is occurring within several circuits within the network, so there is simultaneous electrical and physiological stimulation not only within the neuronal network, but also throughout the glial cell substrate. Glial cell stimulation could (often does) induce a higher level of calcium in the overall network (milieu)—the contribution of glial cells will be discussed later in this chapter.

Multichannel tetanic stimulation ultimately results in a net increase in the area of the network affected by the high frequency pulses. High frequency stimulation (HFS) tends to override the magnesium ion blockade at the NMDA channel thus allowing more calcium influx. In addition, HFS depresses GABAergic synaptic inhibition, by mechanisms initiated by $GABA_B$ autoreceptors (Davies, et al, 1991). Thus not only is there a greater amount of Ca2+ influx into the network as a whole, but the attenuation of inhibition is expanded as well. That is, stimulation of more excitatory circuits could provide enough disinhibition of inhibitory circuitry to facilitate the induction of plasticity mechanisms leading to the potentiation of synapses that would ultimately lead to an increase in spontaneous activity.

There is corroborating evidence that stimulation from several inputs is more effective than stimulation from a single input. For example, White, et al. (1990) found that conditioning stimulation at four electrodes produced a stronger LTP than stimulation at each electrode alone. They then determined that the extent of associative LTP or LTD depended on the extent of overlap between the terminal fields of pathways. That is, coactivation of two pathways that overlapped by 51-100% led to LTP, and coactivation of pathways that overlapped by 0-50% did not. The results were essentially the same for LTD induction.

If one assumes that MCTS is much more likely to simultaneously stimulate afferent circuitry as well as depolarize certain units within the network than SCTS, then this "Hebbian rule" of coactive synapses goes a long way towards accounting for the trends seen in HFS trials. In addition, Konig, et al. (1996) assert that neurons are naturally more sensitive to coincident inputs, because PSPs from synchronous inputs are more apt to combine, thus driving a neuron to reach firing threshold. Postsynaptic potentials generated from asynchronous inputs decay more rapidly and their amplitude is smaller and is somewhat less effective.

In Chapter 3.1 experiment 66b was highlighted as an example of differing results from SCTS versus MCTS. While the use of three electrodes to achieve a more global stimulation is the likely explanation for the greatly increased response and prolonged effect, one must not overlook the possibilty that other mechanisms may have been involved. For instance, priming the pathway or saturation, two different but related phenomena that involve a repeat of an initial stimulus to produce a greater effect following the application of the second stimulus, could have been factors. In the experiment mentioned above, each channel was stimulated singularly and later, all three channels were stimulated simultaneously.

Thus, each channel was being stimulated for a second time. In addition, it is possible that some fibers were stimulated in all four episodes.

Researchers studying memory mechanisms have largely focused on LTP and LTD as markers of learning-induced synaptic plasticity. The results from many of these LTP/LTD experiments show a large magnitude of change in synaptic strength. The convincing results from these experiments, however, may not necessarily translate into large changes on the widespread distributed network level, where it is generally considered that the representation of memory traces reside. Thus, it is more likely that one would only observe small distributed changes within a network, which would prove to be formidable task to measure (Davis and Laroche, 1998).

Consequently, it is possible that LTP/LTD was induced in at least 75% of all trials in one or more synapses within the network. But in some of those cases, other, perhaps stronger processes in the network masked that change in synaptic efficacy. This masking effect is likely due to the activation of antagonistic processes within the network. However, ongoing spontaneous activity may in some cases be sufficient to "drown out" the effect of the change in synaptic strength in these "weak" or relatively unimportant, perhaps even previously "silent" (or inactive) synapses. The presence of inactive synapses in spinal cord cultures was reported by Pun, et al., (1986). During preliminary stimulation trials in these studies, there were many cultures that initially had a high level of spontaneous activity and these preparations were resistant to changes in spontaneous activity via conditioning electrical stimulation. If these modest synaptic modifications (or large changes in "insignificant" synapses) did occur, it is likely that the effects of those changes were "drowned out" by ongoing activity.

Another possibility is that the effects of the conditioning stimulation were negated (in the case of tetanic stimulation, depotentiation), before any changes in synaptic strength were "consolidated." In the absence of pharmacological manipulation, changes in synaptic efficacy are mostly, if not totally, activity-dependent. Thus there were no long-lasting changes in synaptic strength because of a "washing out" effect. That is, the alteration of synaptic weights was not sufficient to overcome the inertia of the network's spontaneous activity to a degree that would alter the existing bursting/spiking. This explanation may account for the fact that more than half of all potentiated responses to HFS was less than one min. It is well documented that LTP (and also memory) is unstable during the induction period (Brashers-Krug et al. 1996, Shadmehr and Holcomb, 1997) before consolidation (or the establishment) of LTP. Asynchronous input from spontaneous activity may be a prime reason why the reproducibility was not better.

As reported in Table 3A, in a small percentage of SCTS trials, there was a decrease in spontaneous activity. One likely reason involves a prominent hypothesis that the level of postsynaptic intracellular calcium concentration ($[Ca^{2+}]_i$) largely determines the direction of synaptic modification. Depolarization of neural cells leads to in influx of calcium. This theory, commonly referred to as the sliding threshold theory, states that increases in $[Ca^{2+}]_i$ up to a certain level, causes the induction of synaptic depression, whereas going beyond this threshold causes the strengthening of the synapse. Included in this "sliding threshold" tenet, is the actual rate of calcium influx. The inclusion of the aspect of rate makes sense when one considers the ability of neurons to sequester $[Ca^{2+}]_i$ in internal compartments. For example, the nature of repetitive stimulation – single pulses, delivered at low frequencies

over a period of ~15min—would seem to cause a gradual rise in $[Ca^{2+}]_i$. This increase in calcium would be countered by the sequestration processes that are activated after the calcium elevation had begun. Thus, for any given period of time, the increase in $[Ca^{2+}]_i$ would be relatively modest. On the other hand, tetanic stimulation – short, high frequency stimulation trains—would tend to cause a rapid and large build-up of $[Ca^{2+}]_i$ within the cell(s) before the sequestration processes could be very effective. This sliding threshold concept can be applied to a variety of structures within the CNS (Hansel, et al. 1996; Cohen, et al. 1998; Yang, et al. 1999). Brief activation of excitatory circuitry has been shown to induce LTD in many brain areas, including the neocortex, striatum, hippocampus, and cerebellar cortex. In many of these preparations, the expression of LTD has required a minimum level of postsynaptic elevation of calcium, much like the requirements for the induction of LTP. Thus, in preparations susceptible to LTD as well as LTP, a tetanic stimulation pattern lacking the intensity for a large and rapid increase in $[Ca^{2+}]_i$ the target cell(s) (conditions favorable to LTP), could easily result in the induction of LTD (Artola and Singer, 1993). Stanton and Sejnowski (1989) mentioned evidence for heterosynaptic depression and how it can arise at inactive or weakly active synapses during the stimulation of a conditioning input. In addition, experiments where the extracellular calcium concentration was decreased, tetanic stimulation (which normally would have induced LTP) induced LTD. This was probably due to the reduction in amount of available calcium to enter the cell (Mulkey & Malenka 1992). However, Neveu and Zucker (1996), using photolysis of postsynaptic caged Ca^{2+} compounds, found no difference in thresholds for $[Ca^{2+}]_i$ for the expression of LTD versus LTP. But recently, Yang, et al. (1999), used a new

caged calcium compound in CA1 pyramidal cells to show that different postsynaptic $[Ca^{2+}]_i$ elevation patterns can be generated. These specific patterns signaled the direction of modification. Only LTP was triggered by a brief increase of $[Ca^{2+}]_i$ with relatively high magnitude, which corresponds to the $[Ca^{2+}]_i$ rise during tetanic stimulation, whereas, a prolonged modest rise of $[Ca^{2+}]_i$ reliably induced LTD.

The idea of simultaneous enhancement and depression of different synapses within a population of cells is supported by Scanziani, et al. (1996). They report that induction of LTP in one population of synapses can be associated with a modest depression at neighboring inactive synapses in the same population of cells. They also support the popular notion that bi-directional control of synaptic strength is important for the development of neuronal circuitry as well as information storage. Not only is there simultaneous bi-directional modification shortly after conditioning stimulation, but (in the dentate gyrus) there is a change in the amount of cell firing which is generated by a specified amount of synaptic current (Desmond and Levy, 1986). I have seen evidence of this type of response after many conditioning trials. After tetanic stimulation, most recording channels show an increase in the level of spontaneous activity but some channels actually show a decrease.

Similar results were obtained from repetitive stimulation trials. When the tetanic or conditioning stimulation is being applied, it is logical to assume that some synapses would already be active (and many would become active) during the application of three one-second trains at 100 Hz. The few synapses that are not active during the stimulation would tend to be depressed following the conditioning stimulation. In keeping with this same line

of thought, MCTS would tend to activate more synapses, resulting in a more global enhancement of spontaneous activity.

Glial contribution

The importance of glial cells to the overall health of neuronal network cultures has been well known for years. Briefly, the glial carpet provides structural support and acts as a compatible biological substrate for the neurons. Glial cells also produce various growth factors and other compounds that contribute to the health and viability of neurons. Thus in addition to contributing to the general health, viability and longevity to neurons in culture, glial cells also seem to contribute to the information processing and storage capabilities of neuronal networks (see Pfrieger and Barres, 1996, 1997).

One potential mechanism by which glial health and connectivity could influence network dynamics is calcium signaling. Calcium signals initiated in one or more glial (astrocytes) cells can be propagated in a spreading calcium wave across a glial carpet. This calcium wave promotes an exchange of information within glial networks. Neuronal activity can elicit the calcium signals in glia or the calcium signals that originate in glial cells may evoke electrical and calcium responses in adjacent neurons. Given these particulars, it is probable that glial calcium signals could integrate neuronal and glial compartments, thereby having a role in information processing from the level of neuronal networks to the brain (Deitmer, et al. 1998; Harris-White, et al., 1998; Giaume and McCarthy, 1996). These intercellular calcium signals can be initiated by a variety of stimuli including electrical, mechanical, and chemical stimuli, including neurotransmitters, neuromodulators, and hormones. Increases in $[Ca^{2+}]_i$ also display a variety of spatial and temporal patterns

These responses are the result of a series of molecular cascades effecting Ca^{2+} flux via the

extracellular space or intracellular stores. Furthermore, glial cells express various metabotropic receptors coupled to intracellular Ca^{2+} stores through the intracellular messenger inositol 1,4,5-triphosphate (IP_3) (Verkhratsky, et al., 1998).

With the aid of confocal microscopy and a Ca2+ indicator (fluo-3), Dani, et al. (1992) observed the capacity of synaptically released glutamate to trigger Ca^{2+} waves in astrocytes within organotypically cultured slices of rat hippocampus. They found that the latencies of these astrocytic Ca^{2+} waves were as short as two seconds and propagate both within and between astrocytes at velocities of 7-27 μm/s at 21 degrees C. The latency period (which is likely to be slightly shorter at 37° C) is well within the delay periods between the cessation of stimulation and the network response following conditioning stimulation in my stimulation trials. Thus, it is likely that calcium signaling played a role in some (perhaps all) of the delayed responses to HFS. These data are significant not only for this study but they have biological relevance because it illustrates the ability of astrocytic networks intermingled with neurons in their normal tissue relationships to respond dynamically to the firing of glutamatergic neuronal afferents. Gargan, et al. (1995), using immunofluorescence and electron microscopy, found that the preparations used in the studies presented contained 40% astrocytes, 9% oligodendrocytes, and 11% neurons.

Depression of Spontaneous Activity Following Tetanic Stimulation

Induction of associative LTD may be another reason for decreases in spontaneous activity following HFS (besides the potentiation of inhibitory circuitry). It has been widely reported that LTD occurs at synapses that are inactive when converging pathways are tetanized (McNaughton, et al., 1978; Levy and Steward 1979, 1983; White, et al., 1990). In

fact there were numerous times that HFS stimulation had different effects on different recording channels including a reduction in spontaneous activity. If the circuitry that was depressed happens to be (or has inputs to) a dominant circuit within the network, the net effect on spontaneous activity could result in network depression.

Planar electrode stimulation in neuronal networks is still largely unexplored, and the responses to the stimulation patterns are complex. Some of these complexities are expected because of the intricate structure and function of the network. For example, White, et al. (1990) reported that the associative interactions between inputs that lead to potentiation and depression is dependent on the amount of spatial overlap between inputs that converge on the same area of the dendrite. They also suggest that the threshold process is permissive for both LTP and LTD and that it can be restricted to local dendritic domains. There is also evidence of heterosynaptic interactions between LTP and LTD (Muller, et al., 1995). The experiments conducted by Muller, et al. (ibid) indicated that repeated induction of LTP or LTD on one group of afferents can actually reset synaptic efficacy at other nonactivated synapses. However, despite the complexities of the preparation used in this study, the percentage of expected responses to conditioning stimulation was similar to (or better than) the percentage of the successful induction of synaptic modification mechanisms obtained in similar studies using slice preparations with intracellular recordings (references cited earlier). My results, then, provide a basic template on which to build for other investigations that may fine-tune and exploit this type of stimulation in this system.

Repetitive Stimulation

There are generally two stimulation protocols to induce a depression of spontaneous activity in *in vitro* network preparations. One way is to tetanically stimulate inhibitory

circuitry in order to induce potentiation of those units. The enhanced firing of inhibitory neurons could lead to an overall decrease in the spontaneous activity of the network.

The other method of inducing depression of spontaneous activity in *in vitro* preparations is the delivery of single pulses at a frequency between 0.5-2 Hz (usually 1 Hz) for period of ~15 min. This is the most common stimulation protocol used to induce long-term depression (LTD) of synaptic transmission. Long-term depression, described by Bear and Abraham (1996), is a lasting decrease in synaptic efficacy that follows some types of electrical stimulation. They further distinguish between two main groups of LTD by noting that heterosynaptic LTD tends to occur at inactive synapses during tetanic stimulation of a converging synaptic input, while homosynaptic LTD is usually induced at synapses that are activated, usually via repetitive stimulation.

The number of single pulses delivered in a single episode (as inferred in the previous paragraph) is usually 900 (1 Hz for 15 min). This stimulation protocol, often referred to as low frequency stimulation (LFS), was the method used for this study. Mulkey and Malenka (1992) used LFS to induce LTD in CA1 pyramidal cells. They found that the form of LTD that was induced required activation of post-synaptic NMDA receptors, was synapse specific, and saturable. They also reported that the extracellular calcium concentration affected the induction of this synaptic modification. They found that removal of external Ca2+ prevented LTD altogether. Mulkey and Malenka reasoned that, because the effects of LTD were reversible, those effects were not due to the deterioration of individual synapses. Although in my study, the spontaneous activity was likely decreased by both methods, LFS of one or more channels was the only approach taken to induce the depression of network activity. The observation of the depression of spontaneous activity due to tetanic stimulation of inhibitory circuitry was not a predicted response.

Similar to the findings of Mulkey and Malenka, as well as several other researchers, that synaptic depression is reversible, I found that depression of spontaneous activity was also reversible. Tetanic stimulation delivered to the same channel(s) often reversed the effects of repetitive stimulation. The issue of reversibility of responses to stimulation will be dealt with in the following section.

Repeatability

The percentage of repetitive stimulation trials that resulted in a depression of network activity (35%) was less than the percentage of successes for tetanic stimulation (52%). Indeed, this is a stimulation protocol that may or may not work in certain areas of the central nervous system (CNS). Heynen et al. challenged this opinion in their 1996 publication by showing that homosynaptic LTD could be produced in the adult hippocampus *in vivo* and that it had all the properties attributed to the immature CA1 *in vitro*. They went on to show that both LTP and LTD were reversible modifications of the same Schaffer collateral synapses. They shunned the notions that LTD could only be induced in brain slices and that it was only of developmental importance; and went on to suggest that LTD and LTP may be equal partners in the mnemonic operations of hippocampal networks. Still, Burrette et al. (1997), using three different low frequency (0.5-1 Hz) stimulation protocols [single pulses, paired pulses (35-ms interpulse interval), and two-pulse bursts (5-ms interval) were unable to induce LTD at all in the hippocampal fiber pathway to the prefrontal cortex in the anesthetized rat. In fact, they found that a small-amplitude, persistent potentiation was induced. However, once LTP was induced, they were able to use the two-pulse burst protocol to depotentiate the pathway. Wagner and Alger (1996) define depotentiation as the response reduction that affects responses that have

been increased by the process of LTP, but not the basal responses affected by LTD. The issue of depotentiation will be addressed further in the next section. The success ratio of my LFS trials, although less than that of tetanic stimulation results, are still comparable to the percentage of depressed synapses versus trials in the literature (see previously listed references regarding LTD).

General comparisons between single and multiple channel stimulation were made earlier in this chapter. One specific difference between SCRS and MCRS was the magnitude of the depression. For example, application of MCRS has never caused a complete cessation of bursting activity across all channels. Whereas, SCRS on occasion, has led to the absence of bursting on one or more recording channels when prior to the stimulation episode, there was clear bursting activity present. There were 4 trials that showed a complete cessation of bursting activity on all channels following SCRS. Similar results were obtained by Torii, et al. (1997) while recording intracellularly from small EPSPs in rat visual cortex slices. They reported that LTD decreased the probability of transmitter release so strongly that some inputs became virtually silent.

A plausible explanation why MCRS does not seem to be as effective as SCRS at depressing network activity may again be the associativity factor of multichannel stimulation. This time associativity works against the desensitizing effect of repetitive stimulation. The synchronized inputs, though depressive in nature, have a component of enhancement that does not allow complete depression on the network level. Desensitization (which can result from synaptic depression) induced by repetitive stimulation on a behavioral level could be described as an adaptation to non-noxious stimuli from a single source.

Another possible explanation of differences between SCRS and MCRS is spreading depression. Fitzsimonds, et al. (1997) used triple whole-cell recordings to study the effects of spreading depression in simple networks of cultured hippocampal neurons. Their data suggests that the induction of LTD at glutamatergic synapses also brings about a back propagation of depression to input synapses on the dendrite of the presynaptic neuron. They also contend that this depression "also propagates laterally to divergent outputs of the presynaptic neuron and to convergent inputs on the postsynaptic neuron." They detected no forward propagation of depression to the output of the postsynaptic neuron. Their final determination was that activity-induced synaptic modification "is not restricted to the activated synapse, but selectively propagates throughout the neural network." Thus spreading depression could explain why SCRS was, in most respects, more effective than MCRS. The depressive effects of the stimulation spread outward through the network. Whereas, with MCRS, the different stimulation sites tended to be more disruptive—causing more collisions that hampered the fluidity of the spreading depression.

As was the case with HFS, the likely reason for the higher percentage of decreases following MCRS trials compared to SCRS trials, is the more global stimulation that multichannel stimulation provides. That is, the depression of many synapses simultaneously (via MCRS) should lead to a more network-wide decrease in spontaneous activity. Stimulation on single channels (like SCTS) resulted in a higher percentage of unexpected results (i.e. increases in spontaneous activity) than MCRS. This higher percentage of increases in activity is presumably due to the occasional depression of excitatory circuitry.

Another reason why the percentage of decreases in spontaneous activity following LFS is less than the percentage of increases in activity following HFS could be due (in

some cases) to the length of the stimulation episode. Unlike tetanic stimulation (with a total duration of 23 seconds), the repetitive stimulation episode lasted for 15 min. There have been occasions where a response to tetanic stimulation was clearly interrupted by the following train of pulses. If this interference can occur with such a short stimulation episode, then surely it is likely with an episode that is 40 times longer. As a matter-of-fact, there is evidence of changing network activity during the repetitive stimulation episode. It is generally assumed that as the activity is changing, the ongoing single pulses are reinforcing the conditioning stimulation. However, as varied and diverse as these preparations are, particularly when considering the different architecture of connectivity and how the response depends heavily on the stimulation site; it is not difficult to reason that in some cases the length of the stimulation episode actually antagonized the depressive effects of the conditioning stimulation. That is, the maximal effect of the repetitive stimulation could have, in some trials, been reached before the end of the stimulation episode, and continued stimulation "restimulated" the network. Nevertheless, data from these experiments have shown that standard repetitive stimulation parameters, particularly on single channels was quite effective.

Stimulus Histograms

Stimulus histograms were generated primarily in order to gain insight in to what was actually taking place during the 15 min stimulation episode. Spike rate data showed changes in spike rate during LFS, and I wanted to know if there was a change in the way the evoked action potentials were responding to the repetitive pulses. The secondary reason for the production of stimulus histograms was to address the question of whether or not there was any association between the change in evoked responses during stimulation and the

non-evoked, post-stimulus response (i.e. change in spontaneous activity). That is, were the units that had the greatest change in evoked firing patterns during LFS the same ones that showed the greatest change in spontaneous activity following LFS?

There was no relation between the way a unit responded during stimulation and its spontaneous activity following the stimulation. As a matter-of-fact, the number of units affected (as well as extent the units were affected) during LFS had no significant bearing on the number (or extent) of change in spontaneous activity following LFS. Although not significant, there did seem to be a tenuous relationship between the percentage of units that showed an association to the stimulus and the percentage of units that showed a reduction in spontaneous activity. This observation only suggests that the more units that respond to the stimulus during stimulation, the greater the reduction after stimulation.

One factor that may confound the relationship between stimulus histograms and change in spontaneous activity following a repetitive stimulation episode is the delay in response following LFS. A consolidation period may need to take place—units affected undergo modification of synapses and then their firing rate doesn't necessarily change but less transmitter is released per action potential so that the response by postsynaptic units change.

I also wanted to know if there was any relationship between the relative number of channels stimulated and the number of units associated with the stimulus pulse. In other words, was there a difference in stimulus histogram profiles between SCRS and MCRS. Likely due to the small sample size there was no significant difference found between these two groups. While not statistically significant, there seemed to be a higher percentage of units correlated to the stimulus pulse when stimulating on single channels than when stimulating on multiple channels. This observation, taken with the observation regarding the

relative percentage of units associated with the stimuli, coincides with earlier findings that SCRS is generally more effective than MCRS. Future experiments may yet reveal more conclusive answers to the secondary questions involving responses during LFS. Nevertheless, one of the primary questions of whether recorded units within the network were responding during the 15 minute stimulation episode was answered. This question was important because in the midst of ongoing spontaneous activity, evoked responses were not nearly as obvious during LFS as they were was during HFS. The other primary question of whether there was a change in the firing pattern during repetitive stimulation was also important, because an increase (or decrease) in firing during LFS does not necessarily mean that the change in spike rate is a direct result of the stimulus pulse. One would need to determine how action potentials are correlated in time with the LFS pulses as well how that relationship changed over time.

Reversal of Effects

Under certain conditions, a repetitive stimulation pattern was delivered to the same stimulus electrode(s) to which the previous tetanic stimulation pattern was delivered. Conversely, under the same general conditions, a tetanic stimulation followed a repetitive stimulation. The conditions for the application of the opposing stimulation were as follows: there was a clear and fairly robust response to the initial stimulation; and the stability and duration of the response was such that there was minimal fluctuation and no noticeable decay in the response to the initial stimulation. The purpose of the opposing stimulation was to reverse the effect of the previous stimulation pattern. These conditions were put in place to ward off the likelihood that the effect of the initial stimulation was beginning to decay on its own, rather than the opposing stimulation reversing the trend of spontaneous

activity. Although total durations were not determined for many of the longer effects following tetanic and repetitive stimulation, without the deliverance of the opposing stimulation before the decay of the effect of the initial stimulation, the question of reversibility of the effect would not have been able to be addressed.

Several different groups have demonstrated the reversibility of LTP by LFS and the reversal of LTD via HFS (Mulkey and Malenka, 1992; O'Dell and Kandel, 1994; Stäubli and Chun, 1996; Holland and Wagner, 1998). It has also been reported that LFS can heterosynaptically reverse previously induced LTP by the induction of LTD in a different pathway. In addition, LTD was also reversed by the heterosynaptic induction of LTP via HFS. These findings suggest that induction of LTP or LTD on one group of afferents can reset synaptic efficacy at other nonactivated synapses (Muller, et al., 1995; Doyere et al., 1997).

Because of the lack of sustained, potent responses, there were not many opportunities to reverse the effects of the initial stimulation. Because of this circumstance, opposing stimulation trials that did not meet the above conditions were included in this section in order to determine if the response to the opposing stimulation was greater than the response to the initial stimulation. In other words, the opposing stimulation was delivered in spite of the lack of a clear response to the initial stimulus to see if there was a bias or propensity toward the alteration of activity in a particular direction (enhancement or depression of spontaneous activity).

Interpretation of empirical data indicated that the effect of the opposing stimulation tended to mirror the effect of the prior stimulation. When the duration of the response to a particular stimulation was relatively long, the duration of the opposing stimulation was comparable to the length of the previous stimulation. One must note that the total duration

of the initial response (and often the response to the opposing stimulation) was never recorded due to the fact that the opposing stimulation was delivered while the response was still ongoing. Often, the total duration of the opposing stimulation was not recorded because the opposing stimulus pattern (i.e. a repeat of the initial stimulus pattern) was again delivered for the purpose of reversing the effect of the opposing stimulation. This back and forth procedure was carried out until the response of the network to the two stimulus patterns diminished to a level that there was no longer a clear a response from the network to the conditioning stimulation. In most cases, the responses to the stimuli diminished rapidly after the first opposing stimulation. There were only two occasions when there was a response to the repeated initial stimulus (following the opposing stimulus), and there were no recorded responses to the second opposing stimulus (following the repeated initial stimulus).

Despite these limitations, eight responses with a duration of over 20 min to the initial stimulus were recorded. Of these trials, only one of the responses to the opposing stimulus failed to last longer than 20 min (and the opposing stimulation that failed to last longer than 20 min is one that was a second opposing stimulus). That is, 86% of the responses to the opposing stimulation lasted for longer than 20 min. These data suggest that when a stimulation electrode(s) is found that, when stimulated, is capable of altering spontaneous network activity for a significant amount of time, the opposing stimulation is just as effective at long-term modification of network activity.

If one were to suggest that a stimulation site that causes a long-term change in activity within the network corresponds to a key unit(s) [whether it is at the input (dendrite) or output (axon) or a particular conglomeration of processes within the network], then one might surmise that this unit(s) is an influential, or dominant unit within the network as a

whole, because stimulation at this one point causes a network-wide response that lasts for over 20 min, regardless of the type of stimulation. Therefore, based on this notion along with data collected from other trials, I have set forth what I consider to be optimal conditions for clear, long-term alteration of spontaneous activity: (1) stimulation must be at a site(s) within the network that will respond to electrical stimulation; (2) this site must also be a key site within the network for influencing the spontaneous activity of the entire network; (3) the level of spontaneous activity must be such that there is an allowance for increases and decreases in spontaneous activity (dynamic range); (4) the timing of the stimulation must be "correct" –timing can be critical to the outcome of the response. All four criteria need to be met in order to elicit the expected response. The first two items may seem redundant, but the key stimulation site(s) within the network may not be near a stimulation electrode, and there are many sites that respond to electrical stimuli that do not produce long-term effects following conditioning stimulation. The first and third items are things that the experimenter can establish by trial and observation respectively. However, the second and fourth items are conditions that the experimenter either does not know beforehand and/or is unable to detect or control. The key sites, for the most part are hard-wired, and stimulating electrodes, although there is a choice among any of the 64, are fixed. And as for the timing component, several researchers have demonstrated that at the time of the stimulation, the level of membrane depolarization alone can be a crucial factor in determining the nature of the response (Artola, et al., 1990; Hirano, 1991; Huerta and Lisman 1995; Ngezahayo, et al., 2000). These researchers and others have shown that the polarity of the shift in synaptic weights may depend heavily on the level of ongoing synaptic activity. Although there was often no perceived periodic fluctuations in the spontaneous activity, there are likely to be underlying fluctuations in subthreshold activity (Hutcheon, et al., 1996; Lampl and Yarom,

1993). Consequently, much of the subthreshold activity gives rise to suprathreshold action potentials, and one never knows when one of these spontaneous spikes (or bursts) will arrive to influence the response to stimulation. This issue of unpredictable spiking activity brings us back the concept of associativity and synchronous versus asynchronous activity.

Evaluation of empirical data also suggested that when the magnitude of the initial effect was large, the likelihood of reversing the effect was increased. In contrast, if the overall change in spontaneous activity following the initial stimulation episode was relatively small; the probability of reversing the effect was diminished.

Lastly, if there was no response to the initial stimulation, there tended not to be a response to the opposing stimulation. These results not only suggest that the effect of a particular type of stimulation pattern can be reversed by the opposing stimulation pattern, but that the initial change in spontaneous activity was caused by the initial stimulation. A common method employed in scientific investigation is to show that if a certain response can be blocked or reversed, then the response itself was real and not just happenstance. That is, in an effort to prove cause and effect, the effect must be susceptible to nullification by a method or action that is considered to be the opposite of, or blocks the effect of the initial action.

One could argue that the changes seen following opposing stimulation are due to spontaneous decay and not the opposing stimulation. However, the relatively rapid response to the stimulation (which was much faster than spontaneous decay); the changes in stimulus histograms and scatter plots (during LFS—which show that the stimulation is having an effect over a relatively short amount of time); and the change in MEAP values all suggest that the reversal in spontaneous activity was caused by the opposing stimulation and not a decay of the effect of the initial stimulation. In addition, the change in spontaneous

activity variables often surpassed the baseline level of activity following the opposing stimulation. Decay of the effect would normally just return to baseline levels.

In general terms, repetitive stimulation was not as effective at changing the level of spontaneous activity as tetanic stimulation. For example, using the same stimulation electrodes, when an initial LFS episode had no noticeable effect on network activity, and a subsequent HFS resulted in an elevation of spontaneous activity; then a subsequent episode of LFS produced a noticeable depotentiation of the elevated bursting activity. While I did notice this seemingly odd effect, I did not give it much attention. However, others have reported this phenomenon. Staubli and Lynch (1990) stimulated the Schaffer collateral/commissural system of the hippocampus and recorded from the stratum radiatum in adult rats. One of the results from these experiments was that LFS applied prior to induction of LTP had no lasting effects on evoked responses; nor did it affect responses to a control stimulating electrode in those cases in which LFS reversed LTP. Doyle, et al. (1997) saw similar results in *in vivo* studies of anaesthetized rats. Bramham and Srebro (1987) obtained, different, but related results. They found that LTP evoked by high-frequency stimulation was larger and generally reached peak magnitude faster when it followed low-frequency stimulation. These results indicate that LFS, while having no noticeable effect on evoked activity (and in my case—spontaneous activity), may have somehow acted to lower the threshold of activation for the subsequent HFS. These examples support the theory that LTD and depotentiation are separate mechanisms and that depotentiation may be a resetting device only.

The "subliminal priming" effect that LFS seemed to have for the following HFS was not reciprocal. That is, if initially there was no response to HFS, there tended not to be a response to LFS. The fact that a prior stimulation (which does not have to be a

"conditioning" stimulus) often affects the response to a subsequent conditioning stimulation falls within the realm of metaplasticity. Metaplasticity is a rather new term that may be used to describe a group of relatively old phenomena. The priming effect of prior stimulation, paired-pulse facilitation, synaptic activity, the sliding threshold theory, synchronous (or associative) versus asynchronous activity, and several other effects have been accepted by neuroscientists for quite some time, and all can be considered to be examples of metaplasticity. The effects listed above are examples of how the capacity of synapses to undergo modification is itself influenced by a substantial amount of activity-dependent variation. In other words, the recent activity of a neuron or synapse can be a major factor in its future response, even if there was no direct synaptic effect or response to the prior activity (Goussakov, et al., 2000; Moody et al., 1999; Tompa and Friedrich, 1998; Wang and Wagner, 1999). Metaplasticity, a concept introduced by Abraham, Bear, and Tate (Abraham and Bear, 1996; Abraham and Tate, 1997) [but also see Deisseroth, et al., 1995?] was derived from the Bienstock, Cooper and Munro model (or BCM theory) of experience-dependent synaptic plasticity (Bienstock, et al. 1982). Often referred to as "higher-order" plasticity, metaplasticity may provide a way for synapses to integrate a response across temporally spaced events of activity, and maintain synapses within a dynamic functional range by preventing saturation of LTP and LTD. It may be that metaplasticity basically adds a temporal aspect to neuromodulation (see articles by Hille, 1992; Lopez and Brown, 1992; Katz and Frost, 1996).

In light of this description of metaplasticity, one could conclude that neuromodulation and metaplasticity played prominent roles in the type of results collected in many of the stimulation trials in my experiments. As stated earlier, the primary reason I did not do more test-pulse experiments was because of the potential alteration of ongoing

spontaneous activity. As more and more researchers try to explain the effects of responses to stimulation on a network level, particularly when running experiments close to physiological conditions, when various enzymatic proteins and cellular components are active, the understanding of a higher-order of plasticity will be critical.

Burst Duration

Scatter plots of spontaneous activity intervals were made in order to depict the reversal of mean burst duration (MBD) following the application of the opposing stimulation. These graphs clearly show that the nature (or polarity) of the spontaneous activity (enhanced or depressed) depended upon the type of stimulation pattern delivered to the network, and that this effect was reversed by the opposing stimulation.

Tetanic stimulation tended to increase the MBD and repetitive stimulation tended to decrease MBD. Thus the increase in MBD tended to match the overall increase in spontaneous activity. Likewise decreases in MBD coincided with decreases in burst rate and spike rate. This trend continued even when conditioning stimulation was delivered to inhibitory circuitry. That is, when inhibitory circuitry was stimulated, HFS resulted in a decrease in the MBD and LFS delivered to the same channel produced an increase in MBD. This transposition of the effect that conditioning stimulation had on the MBD indicates that the direction of modification of the spontaneous activity, and not the stimulation pattern per se, influenced the mean duration of individual bursts. This influence of MBD may also indicate that the effect of network spontaneous activity and not stimulation modifies the way spikes are packaged into bursts.

The increase in MBD could be an artifact of the system. Often more than one unit was recorded for a single recording channel. Thus spikes from different units arriving in

close temporal proximity to one another may be counted as bursts on single channels. The general increase in firing rates following HFS may lead to more overlap between spiking and bursting which the computer would count as longer bursts. Yet work by Maeda et al. (1998) confirms that the number of spikes per burst tends to increase following HFS.

One other observation involving network dynamics following conditioning stimulation that may provide more insight to some of the general mechanisms involved was the order of change in spontaneous activity variables. A great majority of the time, the burst rate and spike rate changed at roughly the same time. However, there were a few instances when the change in spike rate preceded the change in burst rate. Because this difference in onset of change rarely happened, I chose to omit it from the Results chapter. However, taken with the data from burst duration, one may surmise that the increase in spike traffic throughout the network may activate a chain of events that bring about an increase in the burst rate and/or the burst duration in order to "handle" the increase in spike traffic. Some of the factors that may be involved in the activation of these mechanisms during tetanic stimulation may be the release of colocalized neuroactive peptides, changes in membrane thresholds, activation of different receptor populations (including metabotropic receptors), as well as an increase in internal calcium concentration which would in turn trigger a cascade of events including the release of more calcium from calcium stores.

Following LFS, the general reduction in overall spike may prompt a decrease in the burst rate and MBD in order to package the spikes into bursts that can be detected by the postsynaptic units. Several researchers have shown that responses to individual spikes are highly variable and that spikes packaged in bursts are more likely to be detected (Arieli, et al., 1996; Eggermont and Smith, 1996). For example, Muller, et al. (1997), using a whole cell configuration of the patch-clamp technique, obtained simultaneous recordings from cell

pairs. They found that the probabilty that an excitatory event is transmitted to another excitatory unit with the outcome being an above-threshold stimulation was only one in three to four. Even paired action potentials are more likely to produce a postsynaptic response than a single action potential (Smetters and Zador, 1996). In his review, Lisman (1997) cited work revealing the unreliability of central synapses when it comes to signaling the arrival of single action potentials generated presynaptically to the postsynaptic neuron. He then went on to note that brief (<25 ms) high frequency bursts are reliably signaled due to the facilitation of transmitter release. Lisman then proposes that these types of synapses may be seen as filters that transmit bursts, while filtering out single spikes. He also reviews evidence that these bursts are important to synaptic plasticity and information processing particularly in the hippocampus. Finally, he puts forth the notion that the best stimulus for exciting a cell is coincident bursts.

Identified Problems

One major problem with this study is that the cultures can be seen as "black boxes"—one can input information (stimulation patterns) and one can expect to get information out (spontaneous activity—whether it is changed or not); but exactly what has happened before, during and after the stimulation with specific cells within the network (with the exception of data from stimulus histograms) is not known.

Even if the circuitry were able to be determined with low density cultures, exactly which cells were responding to stimulation and more importantly, in what direction was the flow of information, would not be known. Without this information it is difficult to accurately predict whether or not a change in activity will take place.

Another obvious drawback with these preparations is the fact that the mean ratio of excitatory to inhibitory units is not known; much less the actual ratios of neuronal cell types. However, one can speculate that there are a significant number of inhibitory cells within these preparations. Although no analysis has been performed to determine a typical ratio of cell types per culture, there is some evidence (data not presented) that the dominant cell type in at least some of these preparations are GABA-ergic and/or glycine-ergic. One indicator is the instances when visual examination of the culture indicates a viable, healthy preparation, yet very little spontaneous activity is evident. This suggests that inhibitory circuitry is dominating the level of spontaneous activity of the culture. Another other indicator is the pharmacological evidence. The addition of bicuculline or strychnine alone can drastically alter the spontaneous activity of the network. That is, once this inhibitory "control" is removed, the culture is free to exhibit its inherent oscillatory behavior. Once the network reaches this state, it is difficult to alter the mode of activity via electrical stimulation. And finally, the low responsiveness to exploratory test pulses, implies that inhibitory circuitry is suppressing the responsiveness of the unit(s) to electrical stimuli.

This problem is not unique to this researcher. As noted by Daya and Chauvet (1999), the properties due to the location of neurons, synapses, and possibly even synaptic channels, in neuronal networks are still unknown. They go on to suggest that the relative positions of the units within the network, as well as the interconnections between them, are of importance in the learning process. Daya and Chauvet also pointed out how the hierarchical structure of a network tends to influence its activity. For example, the flow of information at the neuronal level (i.e. action potential output) is affected by the synaptic efficacy of its connections. This idea was the primary focus of the experiments presented here. They also noted that the circuitry of the system involves varying propagation

velocities along different fibers, and that these different delays tend to stabilize the dynamics of the network.

Connors and Regehr (1996) used computer modeling of neurons of varying morphology to show that the cell's firing patterns, based solely on its intrinsic properties and not its synaptic connections, are as varied and stereotyped as its dendritic patterns. In other words, the morphology of a neuron may profoundly affect its firing patterns (also see Ternaux, et al., 1992). This information, taken in conjunction with the fact that dissociated cells plated on a substrate that allows for growth and development in a two-dimensional environment (three dimensional to a much lesser extent) adds several levels of complexity to the preparation, and indeed to the systematic analysis and assessment (quantification and reproducibility) of these types of studies.

One of the most prominent drawbacks with the preparations used in this study is the diverse architecture that accompanies each culture. Inasmuch as this trait is in some respects a liability, it could also be considered to be a strength. After all, if emergent properties are discovered in preparations with inconsistent hard-wiring, then the validity of the properties are reinforced. Moreover, as Requin, et al. (1988) pointed out, even in structurally defined networks there is still a functional heterogeneity.

The investigation of the assessment of changes in synaptic modification on spontaneous activity has proven to be a difficult task; in part because of the lack of established methodology from which to build. Hopefully, this study will serve as an aid for others that may choose to investigate this area. Even though there was little groundwork done in this area, and little attention has been given to these particular types of studies (which is often considered to be somewhat risky), the primary motivating factor for my

decision to undertake these studies can be summed in the direct quote from P. B. Medawar (1979):

> It can be said with complete confidence that any scientist of any age who wants to make important discoveries must study important problems. Dull or piffling problems yield dull or piffling answers. It is not enough that a problem should be "interesting"--almost any problem is interesting if it is studied in sufficient depth....no, the problem must be such that it matters what the answer is-- whether to science generally or to mankind.

It may be my own singular opinion that these types of experiments are important to the better understanding of network dynamics, information processing, and learning and memory processes. However, one will never know the significance of one's findings unless or until those experiments are performed and the results are published.

Suggested Improvements

Experiments were also undertaken to investigate the feasibility of constant current stimulation. While this type of stimulation would have probably yielded better responses and thus, more reproducible results, the electrical noise and feedback that was generated at intensities needed to evoke responses was much too large to record any biological data. Future experiments with simultaneous (i.e. intracellular and extracellular) recordings would likely yield better results. Constant current stimulation via an intracellular electrode would not only allow for more reliable stimulation tactics, but would provide valuable information regarding the relative location of the stimulated unit within the network. This type of stimulation would also be more specific. That is, only a single unit would be stimulated.

Thus, the effect that single unit stimulation has on the network could be addressed. By the same token, the effect of spontaneous network activity on single units could also be studied.

Calcium imaging and/or voltage-sensitive dyes probably would have been an acceptable solution to the problem of not knowing which units within the network are participating in the responses to stimulation. At the very least, we could have obtained a gross determination of how global the response was. This type of imaging not only would have been helpful during stimulation episodes to determine which cells are being stimulated and which cells are in good synaptic contact with those that are being stimulated, but the imaging system would have reinforced the decision of which cells to stimulate in the first place during the exploratory test pulse phase of the experiment. In addition, calcium imaging might have been instrumental in determining when the responsiveness of a unit starts to change to the same stimulus (especially during long stimulation episodes).

Kawaguchi, et al. (1996) has shown that optical recording of neuronal networks can yield meaningful results. Using a 128-channel optical recording apparatus and an absorptive voltage-sensitive dye, they were able to visualize responses to pulse stimuli, analyze synaptic delay, and observe synaptic potentiation.

As far as the experimental environment is concerned, more well defined preparations should prove to be beneficial. This improved definition not only includes a reasonable estimate of the cell types and network architecture, but also includes using defined media, at least for the duration of the experiment. In addition, better measuring techniques should be employed. Since the completion of this study, upgrades have already improved the data collection and analysis procedures. Hardware and software upgrades now allow for single unit recording and analysis as well as the analysis of bursting activity.

Obviously, the next step in this series of experiments is to couple the same type of experiments with imaging experiments---especially timed with the test-pulse experiments to see the decay of facilitation. What also needs to be done in the future is to monitor the EPSPs of one or more units that respond to test pulses before or after HFS or LFS to confirm the induction of LTP/LTD while simultaneously monitoring the spontaneous activity of (or the dominant units within) the network (also see Jimbo, et al. 1998). It is likely that there will be several cases when LTP/LTD is induced, but that no noticeable change in spontaneous network activity has taken place.

Summary

Electrical stimulation of neural tissue has been a method employed by scientists for many years. In recent history, the bulk of experiments involving patterned electrical stimulation of isolated, self-contained networks have focused on the induction of synaptic modification. In this study, the same type of conditioning electrical stimulation normally used to induce synaptic modification was used to induce changes in spontaneous activity.

Many experiments aimed at the induction of plasticity mechanisms (e.g. LTP or LTD) use test pulses to verify whether or not LTP or LTD was induced. Test pulses are delivered to naïve pathways before the conditioning stimulation and again after the stimulation to determine if an enhancement (LTP) or depression (LTD) of synaptic transmission was induced by the conditioning stimulation. I delivered test pulses to the same stimulation channel(s) before and after conditioning stimulation for the same purpose.

Tetanic stimulation was found to induce an enhancement of spontaneous network activity with a success ratio comparable to published findings involving synaptic modification experiments. In like manner, repetitive stimulation was shown to decrease the

level of spontaneous activity at or above the percentages reported by researchers investigating synaptic plasticity. In addition, the opposing stimulation, delivered to the same stimulation channel(s) following the change in spontaneous activity after the initial stimulation, reliably reversed the effect of the initial stimulus.

The results from test pulse experiments provided convincing evidence that synaptic modification had taken place following conditioning stimulation (both HFS and LFS). Seventy-five percent (33out of 44) of all the test stimulation trials resulted in a grand MEAP value change of 20% or more (in either direction) following conditioning stimulation. Thus, test pulse experiments indicated that some synaptic modification had taken place within the network even if not manifested at the level of spontaneous activity. In addition, on occasions where there was a decrease in spontaneous activity following HFS, or an increase in activity after LFS, the change in MEAPs often indicated that the modification of inhibitory circuitry had taken place.

These studies are the first to show that stimulation patterns typically used for the induction of synaptic plasticity mechanisms can indeed be used to modify spontaneous activity in primary spinal cord networks; and are among the first of its type in the general areas of network electrophysiology and synaptic plasticity studies.

Behavioral studies involving the use of electrical stimulation to change spontaneous activity have dealt largely with the activation of working memory. It is conceivable that the results from some of the stimulation trials in this study demonstrated a type of "artificial working memory." The results from these experiments should provide a foundation upon which others can build in this relatively new area of network electrophysiology. The results may also provide a different prospective on how information is processed in neuronal network preparations.

The very idea that evidence of storage mechanisms can be demonstrated in dissociated networks complements the fact that storage mechanisms (e.g. LTP/LTD) can be found in several different areas of the CNS. Moreover, the idea that a few configurations of circuitry (as in slice preparations and *in vivo* experiments) is necessary for plasticity mechanisms (LTP/LTD) to occur needs to be challenged.

CHAPTER V
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APPENDIX

Figure A-1. Spinal cord tissue on multimicroelectrode plates.

A. Hoffman modulation optics of neurites crossing electrode.

Experiment: CS9. Magnification: 100X

B-D. Photographs of live neurons on MMEP.

Experiment: CS69. Magnification: 40X

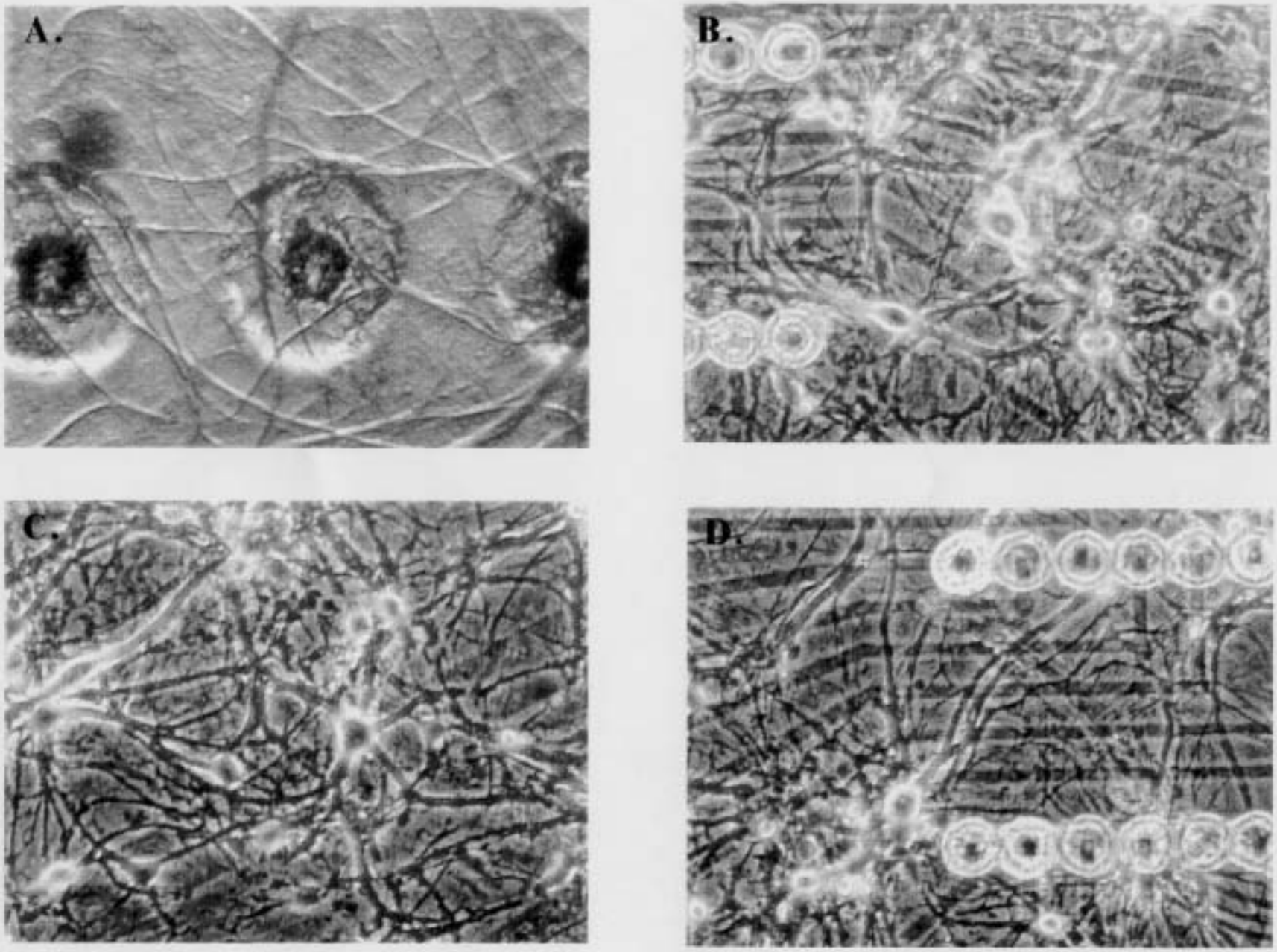

A.
B.
C.
D.

Figure A-2. Photographs of spontaneous activity recorded on cathode ray oscilloscopes.

A.	Experiment: C-62 (8/4/95)	Rec. Chnl: 62	Units: 3
	SNRs: 14:1, 10:1, and 5:1	Sweep speed: 0.5 ms/div	Gain: 1 V/div
B.	Experiment: C-153b (3/9/97)	Rec. Chnl: 33	Units: 2
	SNRs: 17:1 and 8:1	Sweep speed: 50 ms/div	Gain: 1 V/div
C.	Experiment: C-77 (10/25/95)	Rec. Chnl: 12	Units: 2
	SNRs: 9:1 and 3:1	Sweep speed: 2 ms/div	Gain: 5 V/div
D.	Experiment: C-77 (10/2595)	Rec. Chnl: 33	Units: 1
	SNR: 12:1	Sweep speed: 2 ms/div	Gain: 1 V/div

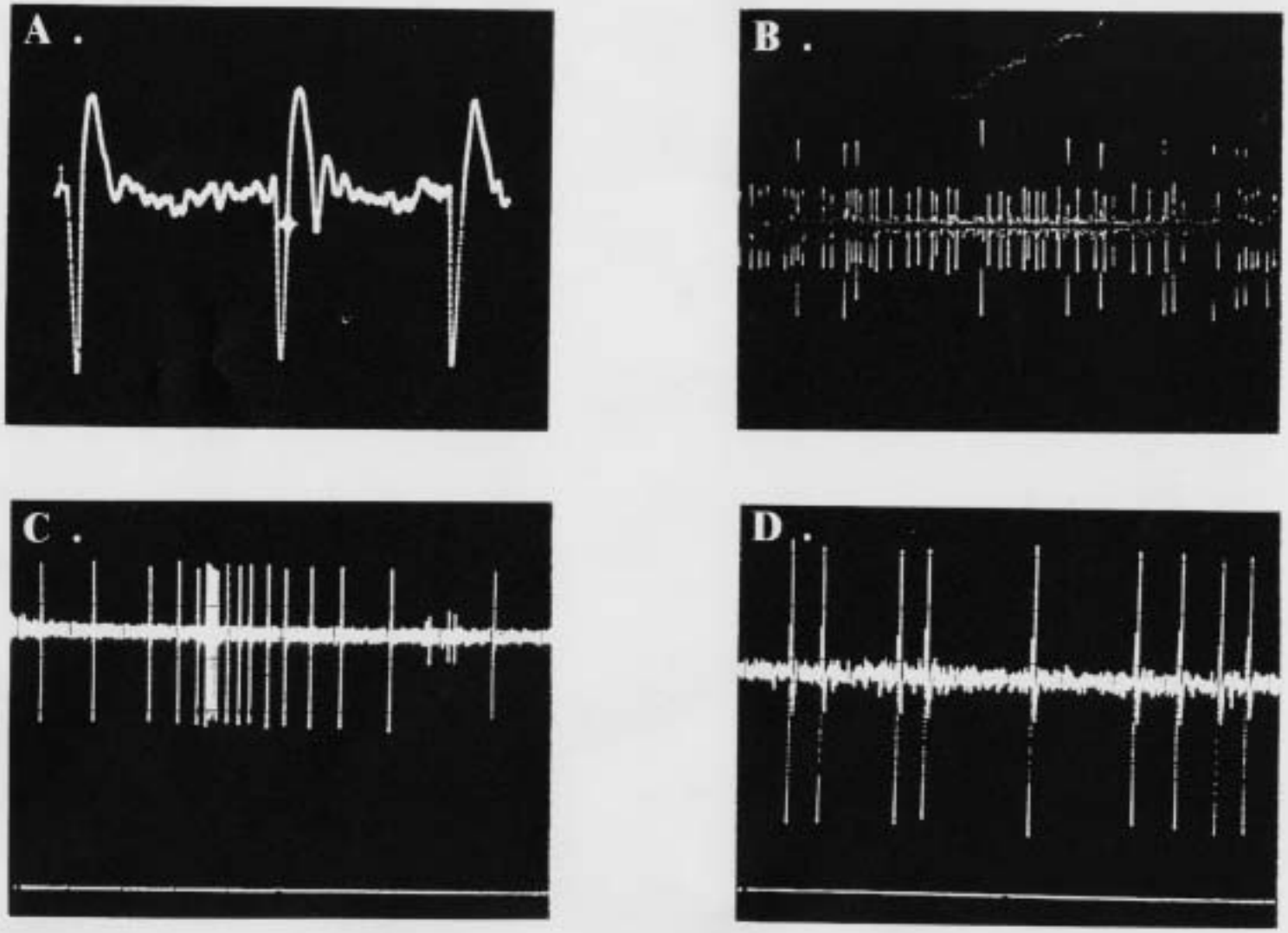
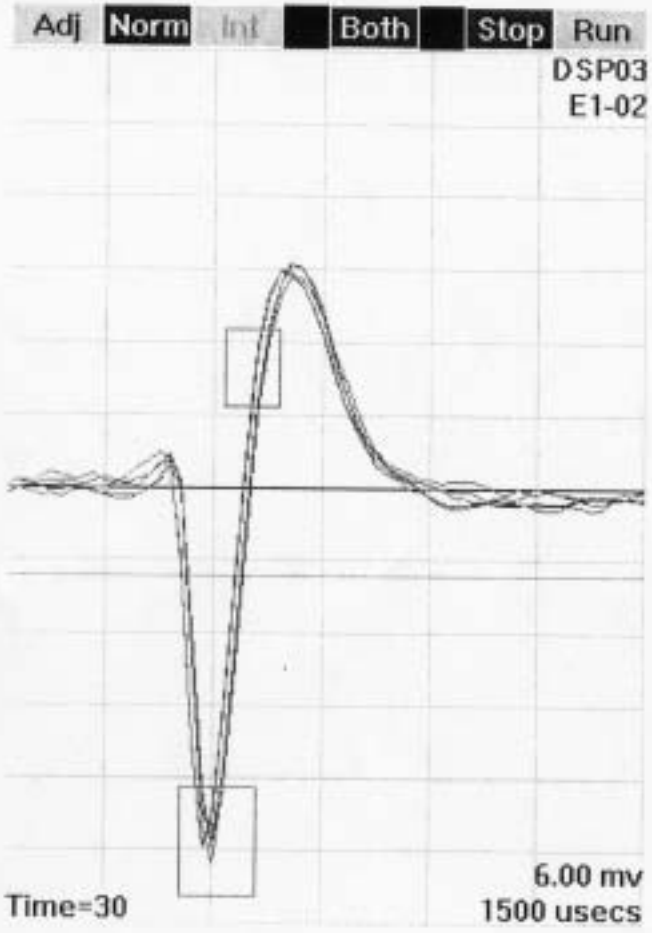

A .
B .
C .
D .

Figure A-3. Waveforms recorded by the MNAP system.

A. Example of discriminated action potentials. The signal must pass through both boxes in order to be counted as an action potential on DSP1a. Other boxes may be added to capture spikes on the same recording channel. Subsequent spikes would be designated DSP1b, DSP1c, etc. Digital stripchart of discriminated spikes can be generated by the MNAP program. Examples are shown in figure 10E and F.

B. Example of two discriminated waveforms on a single recording channel. The darker trace is the profile of the stimulus. This type of trace was used in the stimulus histograms as the reference spike, because stimulus histograms were generated post hoc. The lighter trace is likely an artifact.

A.


Adj
Norm
Int
Both
Stop
Run
DSP03
E1-02
Time=30
6.00 mv
1500 usecs

B.

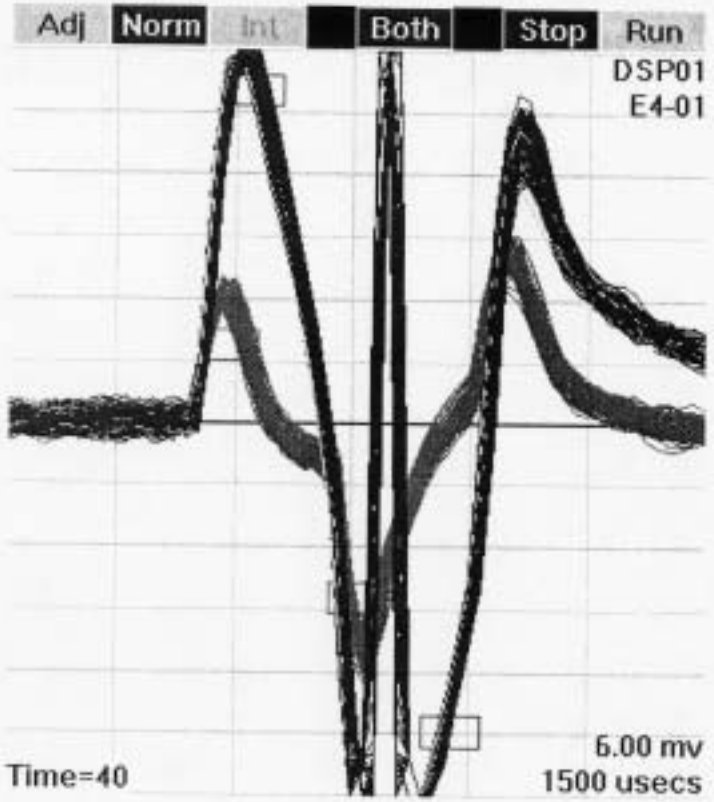
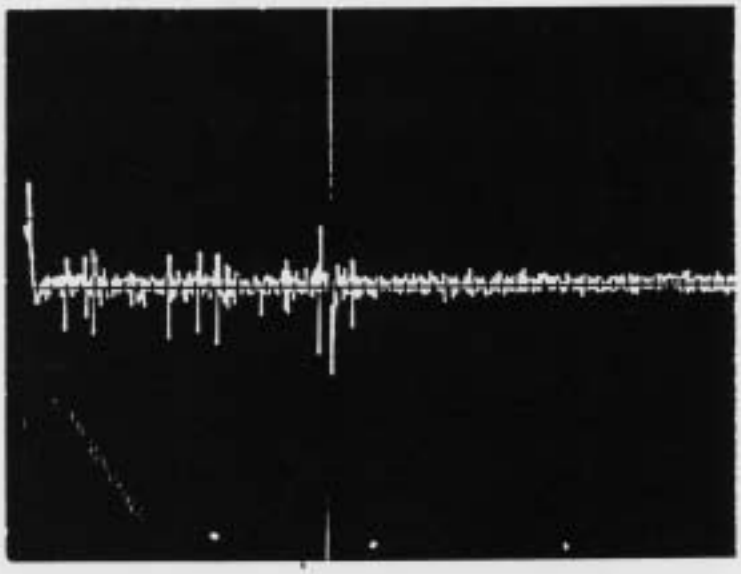
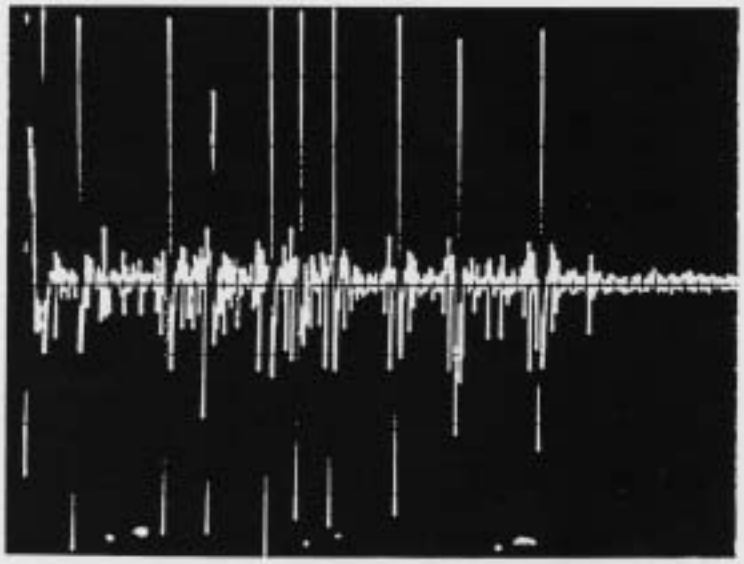

Adj
Norm
Int
Both
Stop
Run
DSP01
E4-01
Time=40
6.00 mv
1500 usecs

Figure A-4. Responses of single channels to different stimulus voltages. A) One large action potential was recorded on channel 29 after a 3 V single, biphasic pulse (duration = 300 μs) was applied to channel 32 (CS87-Plexon system). B) Eleven large action potentials were recorded after a 6.5 V pulse on the same channel. There are at least 2 other units on this recording channel. [Gain = 5 V/div, sweep = 10 ms/div]

A.



B.



Stimulus response curve of stimulus channel.

Several trials were carried out in which evoked action potentials were recorded from the stimulus channel using the Plexon preamplifiers. The results from one of these trials are shown in Figure A-6. Failure at lower voltages must occur. Currents do not always flow the same way, and glial cells may affect current flow as well due to swelling and changes in local ion concentrations. Most of the experiments involving the counting of mean evoked action potentials (MEAPs) were carried out in conjunction with conditioning stimulation trials.

There are perhaps two reasons why there was a 1:1 ratio of pulse to action potential: 1) this was a single-unit recording channel, 2) the electrode was far enough away from the axon hillock so that only a single action potential could be elicited. Although the signal-to-noise ratio for channel 36 was 1.5:1, this was a channel that provoked a network response when stimulated.

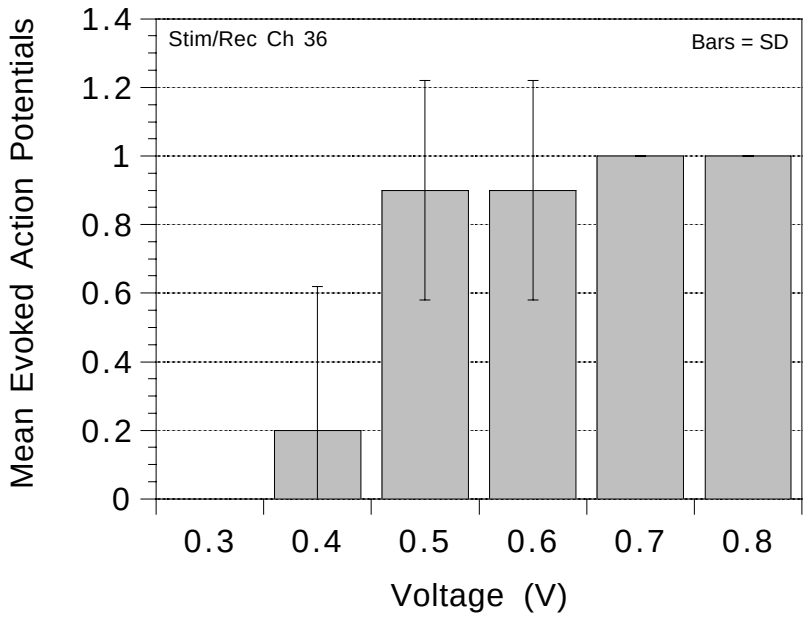


Figure A-5. Mean evoked action potentials as a function of stimulus voltage using the Plexon system. Single pulses were delivered to a single electrode. Responses to the test pulses were recorded on the same electrode. All action potentials recorded within 100 ms following each pulse were counted and averaged.

The ratio of evoked action potentials increased with the stimulus voltage until the ratio of evoked action potentials to stimuli was 1:1.

Time interval between pulses: ~30 s.

Pulse parameters: 300 µs/phase, biphasic.

Data set: 10 pulses per episode of a specific stimulation voltage.

Episode interval: 20 min.

Spiking activity during 100 Hz stimulation

It is reasonable to assume that in order for 100 Hz stimulation to be effective, the biological response must be able to follow the pace of the stimulus pulses within the stimulus trains. Figures A-6A, B, C are examples of spontaneous activity, evoked activity during 20 Hz stimulation, and 100 Hz stimulation. In this experiment (CS171), there was a large unit (listed as 9:1) on recording channel 3. Channel 2, the adjacent stimulus channel, listed units at 6:1, 15:1 SNRs (in that order--an indication that the larger unit does not fire very often). In the first panel (showing spontaneous activity), the large unit did not fire. During 20 Hz stimulation, the action potential from this unit was likely masked (or entrained) with the stimulus pulse. However, there was one isolated action potential with a ~8:1 (7.8) SNR (see arrow). During 100 Hz stimulation, several isolated action potentials [~10:1 (9.8:1)] were observed between most of the stimulus pulses, and one was seen [~8:1 (8.2:1)] 115 ms after the cessation of the pulse train. Based on these observations, one can conclude that this large unit fires preferentially during a stimulus pulse (or pulse train) or shortly thereafter.

These data clearly show that spiking activity was not inhibited during tetanic stimulation. In fact, because the number of action potentials per unit time was dramatically increased, the action potentials seen between stimulus pulses are apparently evoked responses during high frequency stimulation.

Figure A-6. Spiking activity during high frequency stimulation. Data collected from digital oscilloscope. Thus, amplitude of some signals may vary due to sampling process. Gain = 500 mV/div for all traces.

A. Spontaneous activity on recording channel 3 (C-171). (Oscilloscope sweep speed = 25 ms/div)

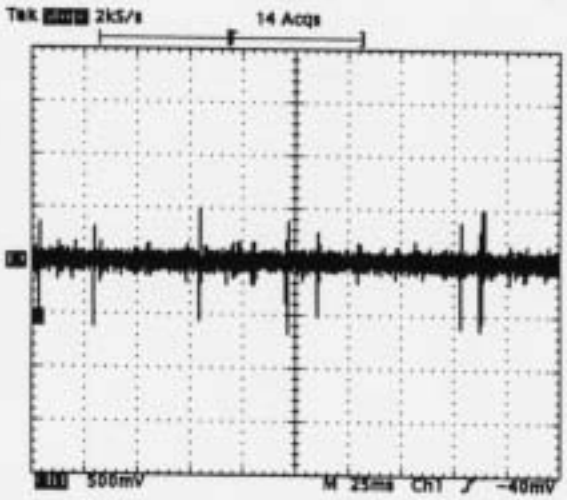
B. Oscilloscope trace before and during 20 Hz stimulation. Notice increase in number of spontaneously occurring action potentials (including 8:1 SNR unit). There may be an even larger unit (~12:1) entrained with the stimulus (or it may be an artifact). This was train number 12 in a series of 20 delivered @ 20 Hz. Therefore the increase in number of action potentials may be due in part to prior stimulation as well as a larger time window (sweep speed = 100 ms/div).

C. Part 1 of 100 Hz stimulus train. Several short bursts of action potentials (largest ~10:1) were observed between most of the stimulus pulses.

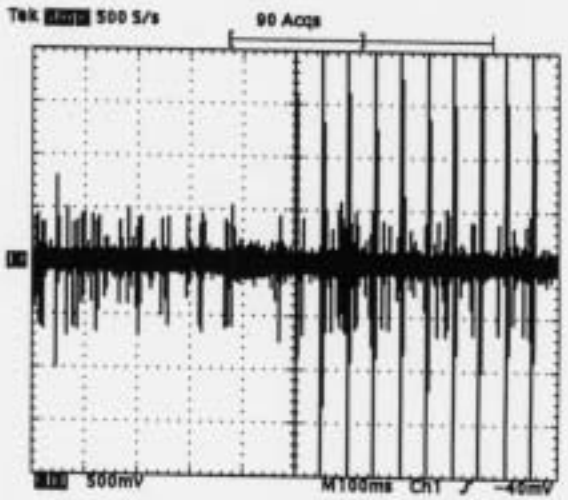
D. Part 2 of the same 100 Hz stimulus train. The short bursts of action potentials continued throughout the train and 3 more bursts are seen (followed by a single 8:1 SNR action potential) the after cessation of the pulse train. [100 Hz parameters were the same throughout this study.]

Based on these observations, one can conclude that this large unit fires preferentially during a stimulus pulse (or pulse train) or shortly thereafter.

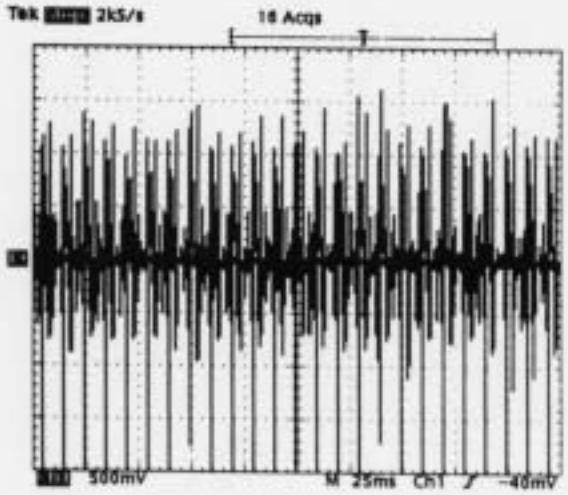
A.


Tek 2kS/s
14 Acqs
500mV
M 25ms Ch1 -40mV

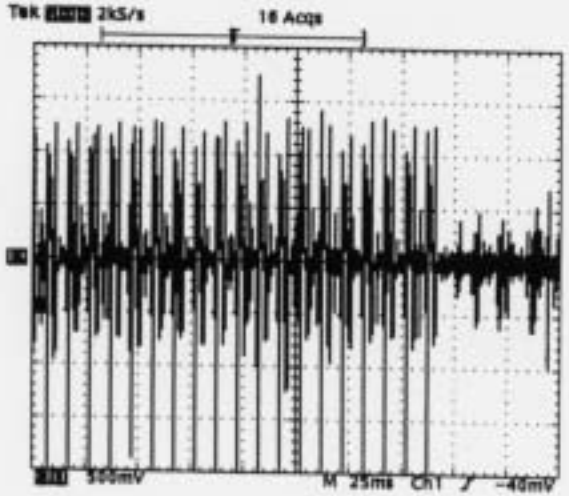
B.


Tek 500 S/s
90 Acqs
500mV
M100ms Ch1 -40mV

C.


Tek 2kS/s
16 Acqs
500mV
M 25ms Ch1 -40mV

D.


Tek 2kS/s
16 Acqs
500mV
M 25ms Ch1 -40mV

Evoked Responses Following Tetanic Stimulation

In four different experiments, evoked responses to tetanic stimulation were measured by using integrated responses resulting from all activity recorded by each channel (Fig. A-8 shows how activity is integrated). The area-under-the-curve was determined by counting square millimeter segments under the integrated profile. Because ten seconds was the time interval between each tetanic stimulus, ten seconds of activity was measured after each of the three 1-second, 100 Hz trains. There was no standard pattern of response to tetanic stimulation (see Figs A-9 and A-10). In some cases the first train evoked the largest response, in others, the second or third train evoked the largest response. As expected, the response profile from different stimulus channels tended to be different (Fig. A-10). Evoked responses following multichannel tetanic stimulation were not greater than responses from single channel stimulation. The number of recording channels responding to MCTS was also not more than those responding to SCTS.

Figure A-7. The five steps in real time data processing used to convert data.

1. **Data Acquisition**

14 Channels of analog data can be sampled at 30,000Hz each. Signals are synchronized throuugh a pair of Sample&Hold boards. The analog data is then converted to digital information through a central 12-bit A/D. The combined sampling rate is 0.840 Mbytes per second.

2. **Matched AC Filter and DC Offset Adjustment**

AC line related noise is removed by subtracting a template of the line noise from the signal. The template is generated by an exponentially weighted average, and DC offsets from the A/D are represented in thiis average. The entire operation requires an arithmetic shift, an increment, and two adds.

3. **Rectification and Compression**

Information on each channel is compressed by recording the maximum absolute value from the 8 samples contained within each 1/3 ms/bin. This operation results in a 10:1 compression.

4. **Activity Detection by an Adaptive Threshold**

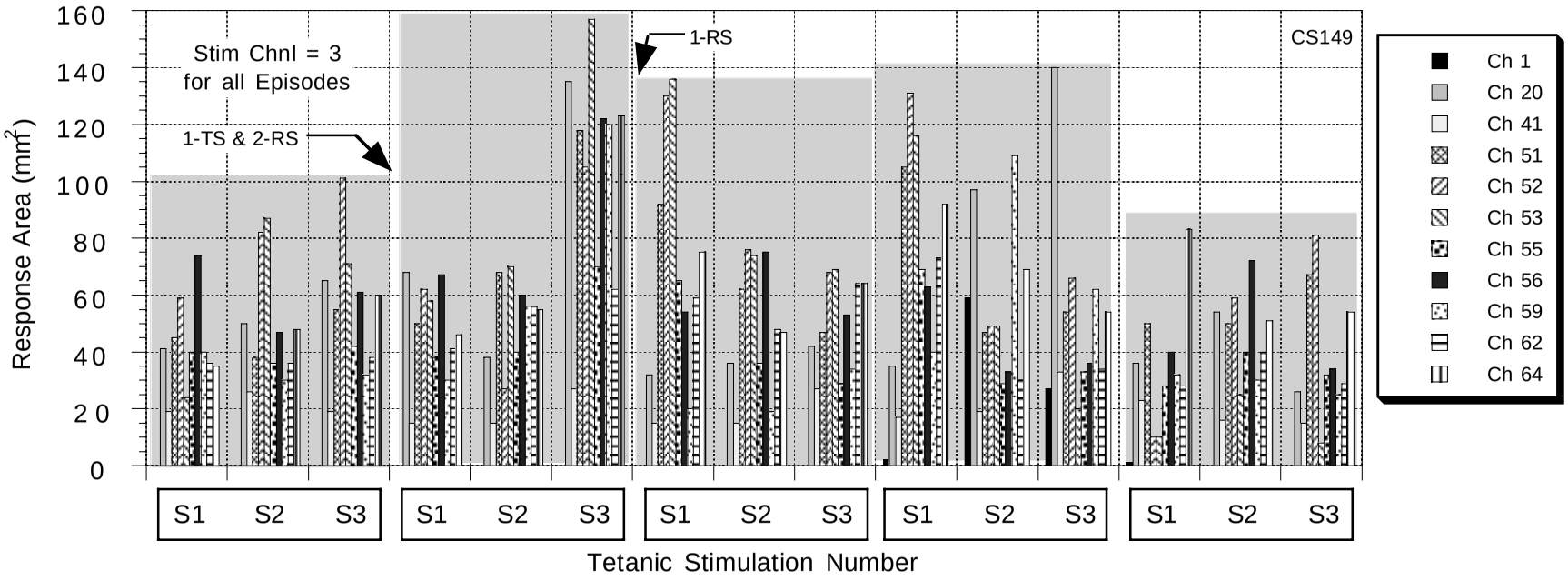
The compressed data are used to estimate the noise standard deviation. A signal threshold is established at 4 noise standard deviations. Activity in any 1/3 ms bin above this threshold will be detected by this system. 15 values are packed into a word for an additional 15:1 compression.

5. **Integration**

Integration is performed by a resistor / capacitor network. Such an R/C network is modeled to mimic integrated activity by the computer software using empirically derived leakage constants.

Figure A-8. Response area under integrated curve during tetanic stimulation.
A. Tetanic stimulation was delivered to channel 3 for all episodes shown. Arrows show other stimulation episodes between SCTS. The SCTS not shown did not have the standard parameters used throughout this study. Therefore, the response is not shown here. The last three stimulations were delivered in succession. Time intervals between SCTS episodes were 10-15 min.

Responses for each SCTS were recorded on 11 different channels. Bars represent area under integrated activity curve for 10 s immediately following tetanic stimulation event (10 s was the interval between each 100 Hz train; see Figs. 17B and 18B).


160
140
120
100
80
60
40
20
0
Response Area (mm²)
Stim Chnl = 3
for all Episodes
1-TS & 2-RS
1-RS
CS149
S1
S2
S3
Tetanic Stimulation Number
Ch 1
Ch 20
Ch 41
Ch 51
Ch 52
Ch 53
Ch 55
Ch 56
Ch 59
Ch 62
Ch 64

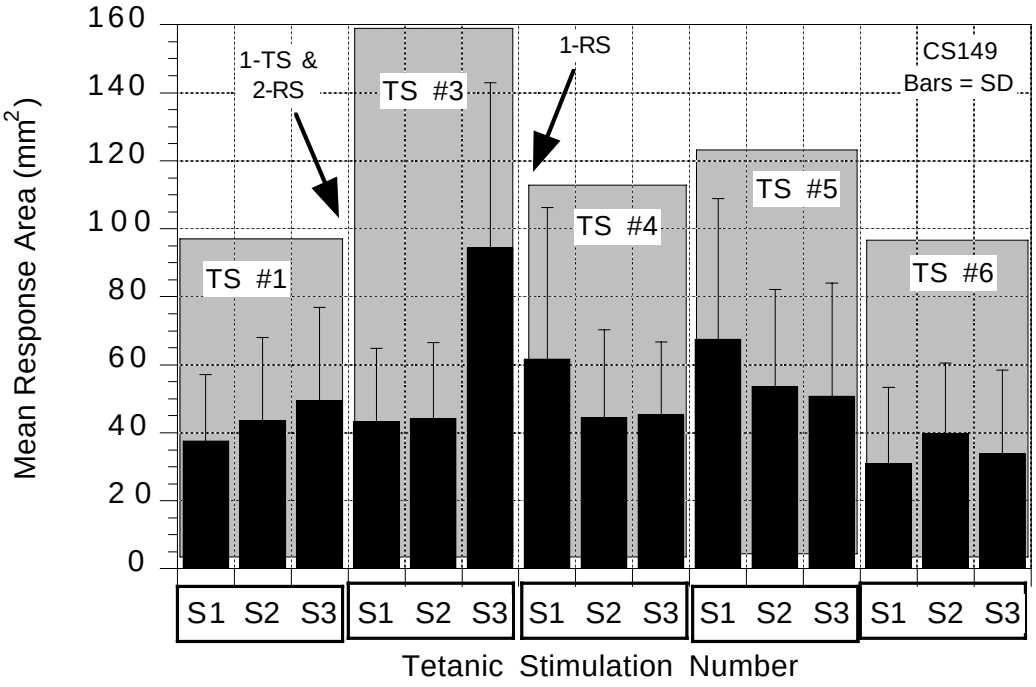


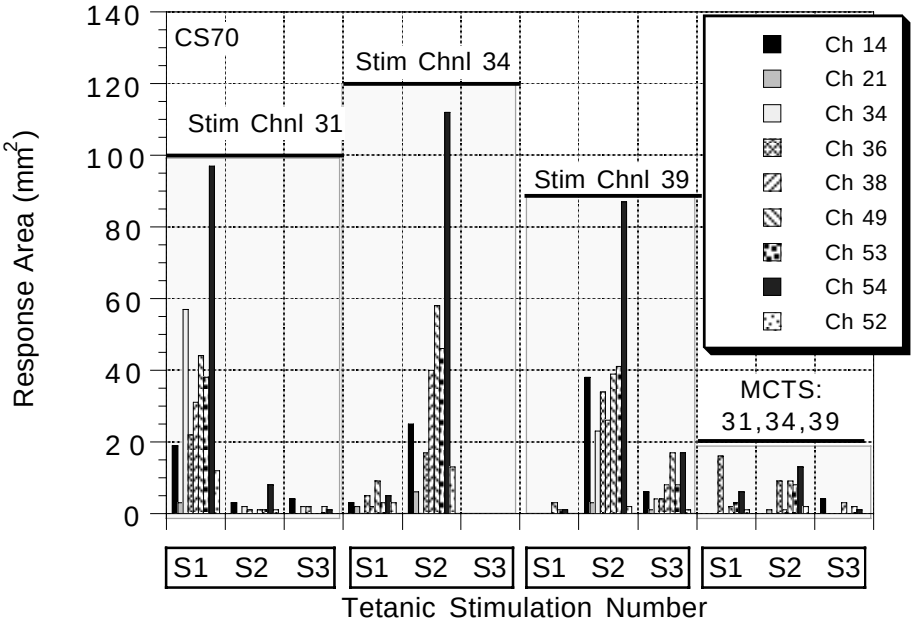
Figure A-8B. Mean channel response area during tetanic stimulation episode. Values from individual recording channels were averaged to get a mean network response for each tetanic stimulation event.

Figure A-9. Response areas of single and multiple channel stimulation

A. Tetanic stimulation was delivered to three separate stimulus channels sequentially and then all channels simultaneously. Responses for each were recorded on nine different channels. Bars represent area under integrated activity curve for 10 s immediately following tetanic stimulation event (10 s was the interval between each 100 Hz train).

B. Mean response area during tetanic stimulation episode. Values from individual recording channels (from above) were averaged to get a mean network response for each tetanic stimulation event. No particular pattern or trend emerged. Simultaneous stimulation on all three channels produced a lesser response than any of the responses from single channel stimulation.

A.


CS70
Stim Chnl 31
Stim Chnl 34
Stim Chnl 39
MCTS:
31,34,39
Ch 14
Ch 21
Ch 34
Ch 36
Ch 38
Ch 49
Ch 53
Ch 54
Ch 52
Response Area (mm2)
0
20
40
60
80
100
120
140
S1 S2 S3
S1 S2 S3
S1 S2 S3
S1 S2 S3
Tetanic Stimulation Number

B.

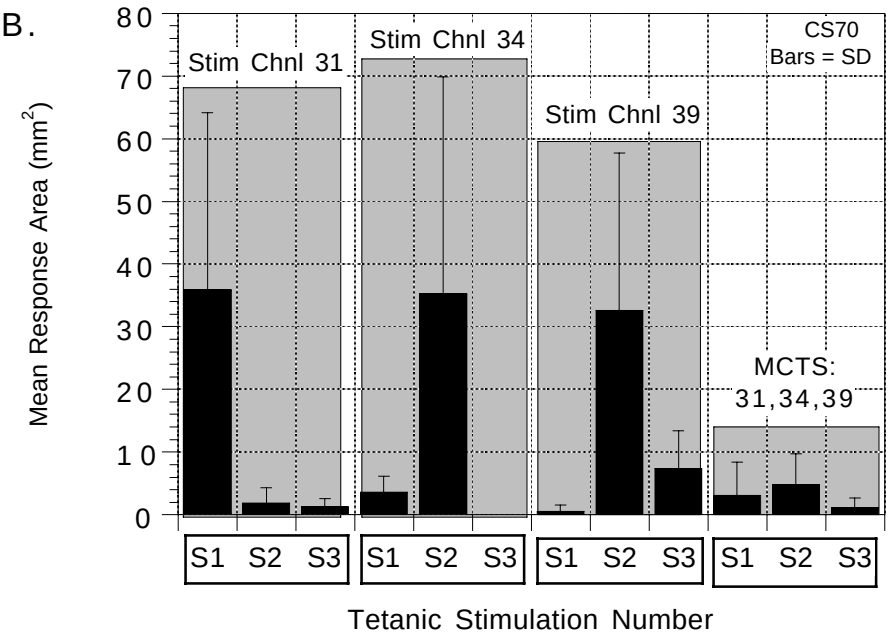
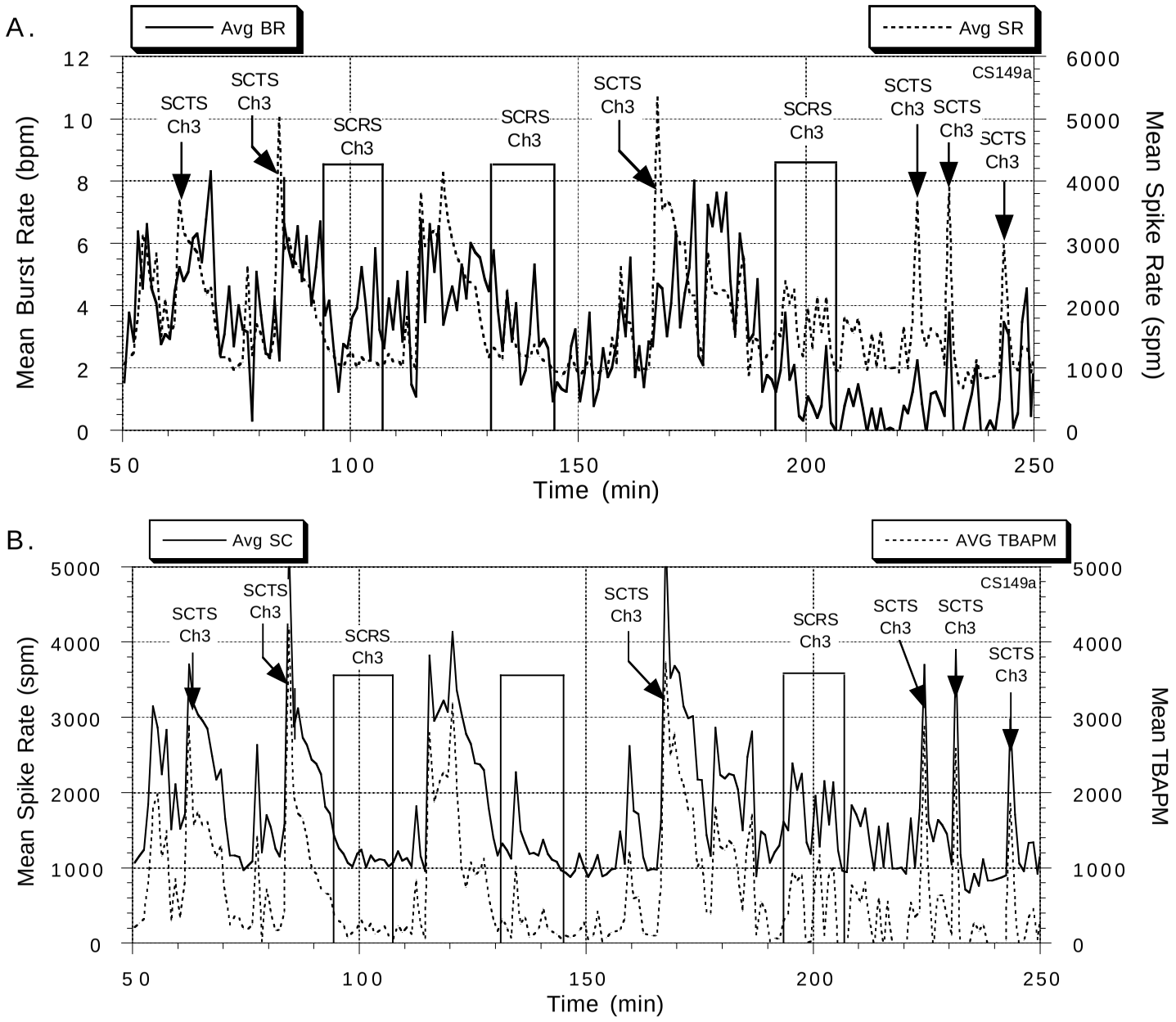

Stim Chnl 31
Stim Chnl 34
Stim Chnl 39
CS70
Bars = SD
MCTS:
31,34,39
Mean Response Area (mm2)
0
10
20
30
40
50
60
70
80
S1 S2 S3
S1 S2 S3
S1 S2 S3
S1 S2 S3
Tetanic Stimulation Number

Figure A-10. Direct comparison of spike rates and TBAPM.

A. Comparison of mean burst rate and mean spike rate shows some differences in relative rates of activity on a minute to minute basis. In some instances the change in burst rate seems to follow the change in spike rate after some delay.

B. The changes in calculated TBAPM is synchronized with the changes of the mean spike rate.


A.
Avg BR
Avg SR
Mean Burst Rate (bpm)
Mean Spike Rate (spm)
Time (min)
SCTS Ch3
SCRS Ch3
CS149a
B.
Avg SC
AVG TBAPM
Mean Spike Rate (spm)
Mean TBAPM
Time (min)

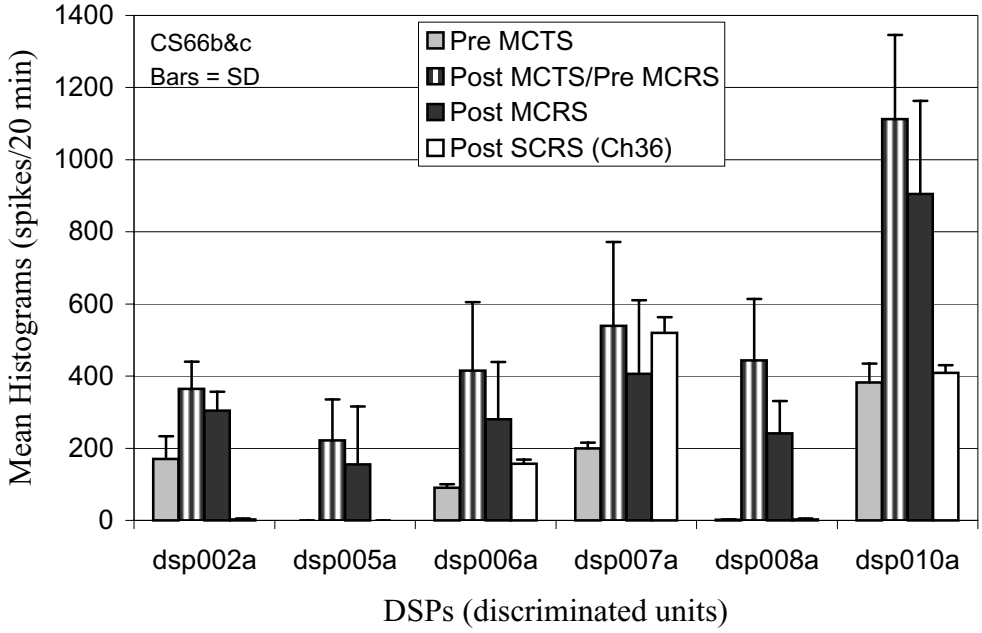
Single unit responses to HFS and LFS.

In this particular experiment (C-66), tetanic stimulation on single channels was attempted before MCTS was tried (data shown in Fig. 34). Multichannel tetanic stimulation was very successful compared to the SCTS attempts on the three channels separately. Nevertheless, comparison of the three channels alone revealed that SCTS on channel 36 was the most effective of the three. So following the failed attempt to fully reverse the effect of MCTS by initiating the opposing stimulation (LFS) on the same channels, SCRS on channel 36 was delivered. As one can see, LFS on the same three channels as MCTS clearly reversed the effect with some, but not all units. However, SCRS on channel 36 resulted in a statistically significant reversal with all but one unit. In this instance, MCRS was clearly not as effective as SCRS. The results of this trial are consistent with other data that suggests that overall, SCRS is more effective than MCRS.

Figure A-11. Reversal of effect via multi-channel stimulation: single unit data.
Mean firing rates during four 20 min time intervals prior to MCTS (Pre MCTS), after MCTS, but before MCRS (Post MCTS/Pre MCRS), after MCRS (Post MCRS), and after SCRS (Post SCRS). The intervals represent means of 20 min samples of spontaneous spiking activity taken during the maximum period of the effect and/or when the activity had leveled off or stabilized. Of the 15 discriminated units for this experiment, only twelve are shown. Three units were omitted because none of the mean values for the time bins exceeded 20 spikes/20 min (i.e. units with all mean spike frequencies at or below 1 spike/min were filtered out). Unpaired student t tests were run on each activity interval for each recording unit, and on the 20 data points for each activity interval comparing pre- versus post stimulation segments (degrees of freedom = 38 for each test).

A. Histograms of mean action potentials for discriminated units (e.g. dsp002a). Following MCTS on channels 36, 42, and 60, there was a significant increase in the mean number of action potentials for every unit depicted. Following MCRS on the same three channels, there was a reduction in the spike production for every unit (although only two showed a decrease that was significantly different). Following LFS on channel 36, every unit except one showed a statistically significant difference not only between the Post SCRS and Post MCTS, but also between the activity following MCRS and activity following SCRS.

B. Histograms of units with lower spike frequencies. The units represented in this panel had intervals that passed the 1 spike/min filter, yet the scale for this graph is one order of magnitude below that of panel A. Despite the difference in overall spike output, the trend was the same. Every unit showed a significant difference in mean spike rates (including unit 3b that showed a reduction in spike rate) following MCTS. Unit 3b was also the only unit that showed an increase in mean spike rate following MCRS, followed by an even larger increase after SCRS (number above error bar is the y-axis value--this bar was cut off to maintain the scale so the smaller means would not disappear), thus indicating a reversal of the effect of the previous stimulation, which is consistent with most of the other recording units. All of the other units (except 12a) showed some level of reduction following MCRS (the data for SCRS for unit 12a was not collected). As in the panel above, SCRS depressed activity to near or below Pre MCTS levels.


CS66b&c
Bars = SD
Pre MCTS
Post MCTS/Pre MCRS
Post MCRS
Post SCRS (Ch36)
Mean Histograms (spikes/20 min)
1400
1200
1000
800
600
400
200
0
dsp002a
dsp005a
dsp006a
dsp007a
dsp008a
dsp010a
DSPs (discriminated units)

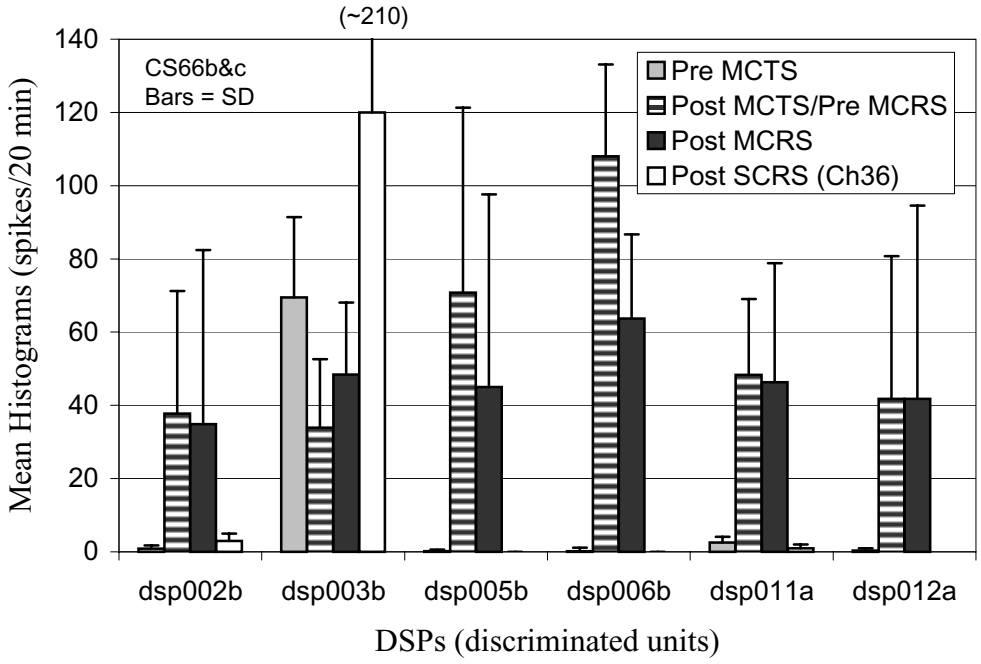
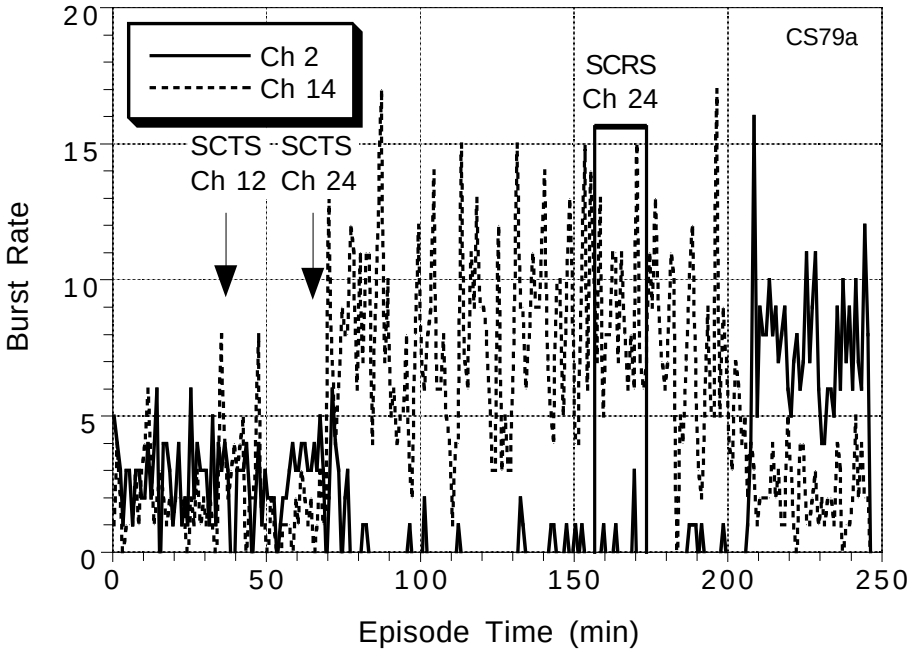

(~210)
CS66b&c
Bars = SD
Pre MCTS
Post MCTS/Pre MCRS
Post MCRS
Post SCRS (Ch36)
Mean Histograms (spikes/20 min)
140
120
100
80
60
40
20
0
dsp002b
dsp003b
dsp005b
dsp006b
dsp011a
dsp012a
DSPs (discriminated units)

Figure A-12. Differing responses to the same stimulation pattern. Tetanic and repetitive stimulation on the same recording channel produced opposite responses on different recording channels.

A. Following SCTS on channel 24, the burst rate on channel 14 (dotted line) was elevated until the SCRS on the same channel (following a delay period). The SCTS on channel 24 caused a reduction in the burst rate on channel 2 (solid line) that lasted until the SCRS (following the same delay period), in which the burst rate was elevated to a level above that of the native state.

B. Graph of activity of the same episode. Following SCTS, the burst rate decreased on channel 3 (solid line), while increasing slightly on channel 60 (dotted line). Repetitive stimulation had no lasting effect on the burst rate recorded on either channel.

A.


CS79a
Ch 2
Ch 14
SCRS
Ch 24
SCTS
Ch 12
SCTS
Ch 24
Burst Rate
Episode Time (min)
0
5
10
15
20
0
50
100
150
200
250

B.

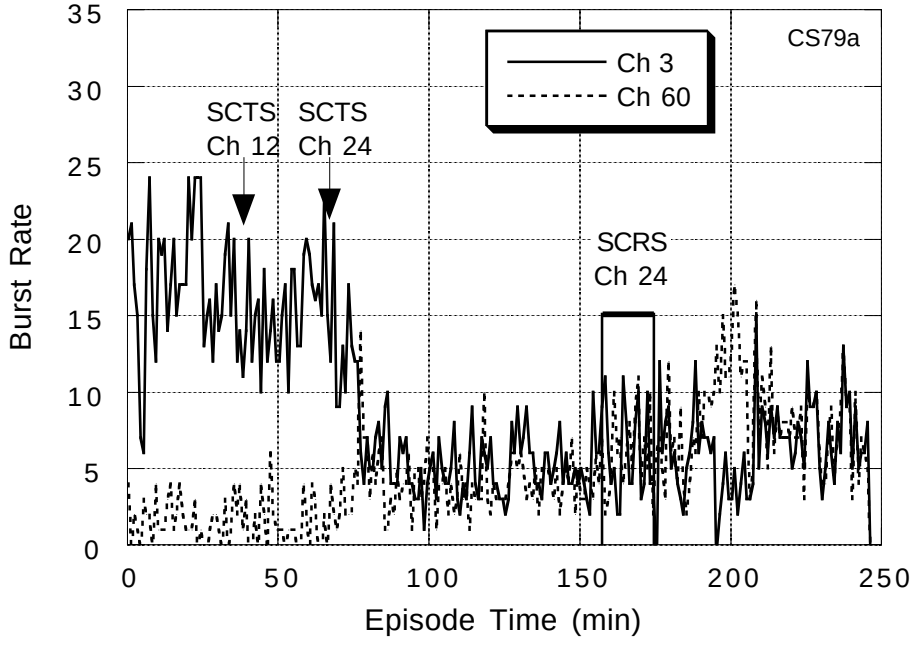

CS79a
Ch 3
Ch 60
SCTS
Ch 12
SCTS
Ch 24
SCRS
Ch 24
Burst Rate
Episode Time (min)
0
5
10
15
20
25
30
35
0
50
100
150
200
250

Figure A-13. Reversal of burst duration on single recording channels.
There were four experiments in which clear, sustained responses followed the conditioning stimulation and this same response was in turn reversed by the opposing stimulation (see Table 9). The response of the network (scatter graph of channel means) was shown in Figure 58. Scatter graphs of 4 individual recording channels from that same experiment are shown in Figure A-11.

Open circles represent 15 min of spontaneous activity prior to SCRS. Crosses represent both the activity interval following SCRS, and the same activity interval prior to SCTS on the same stimulus channel. Closed circles represent the 16 min interval following SCTS.

Repetitive stimulation shortened the mean duration of bursts on all recording channels shown. Following SCTS, the mean burst duration of bursts were increased.

A. Channel 1

B. Channel 20

C. Channel 54

D. Channel 59

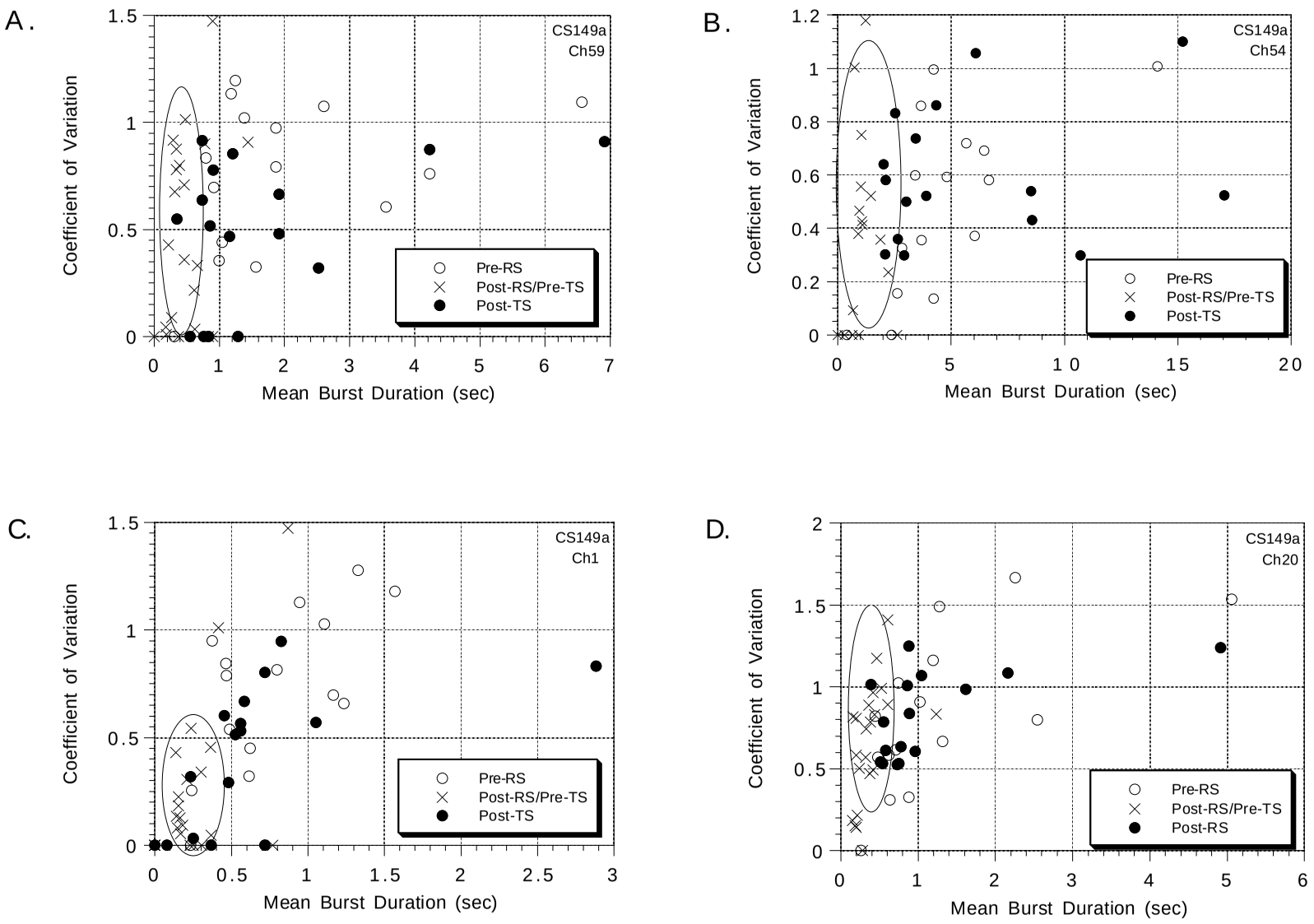

A.
CS149a
Ch59
Coefficient of Variation
Mean Burst Duration (sec)
Pre-RS
Post-RS/Pre-TS
Post-TS
B.
CS149a
Ch54
Coefficient of Variation
Mean Burst Duration (sec)
Pre-RS
Post-RS/Pre-TS
Post-TS
C.
CS149a
Ch1
Coefficient of Variation
Mean Burst Duration (sec)
Pre-RS
Post-RS/Pre-TS
Post-TS
D.
CS149a
Ch20
Coefficient of Variation
Mean Burst Duration (sec)
Pre-RS
Post-RS/Pre-TS
Post-RS

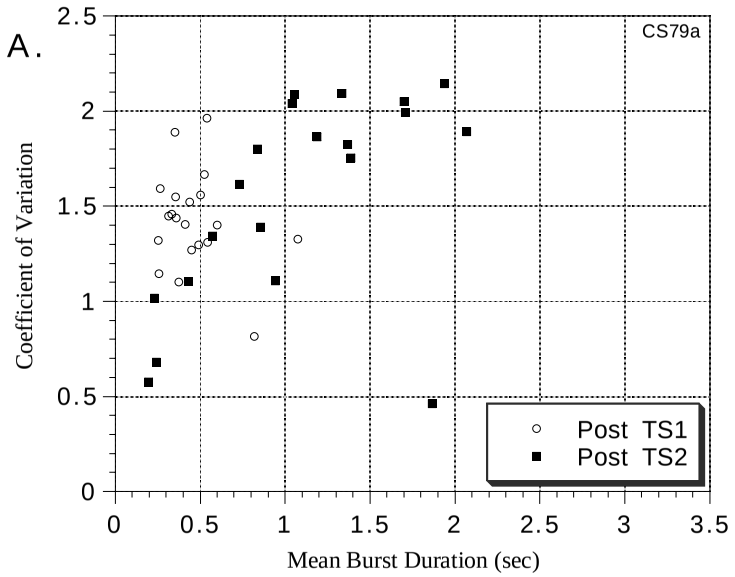
Figure A-14. Scatter graphs of changes in mean burst duration: Network data. Example of delayed shifts in mean burst duration (MBD) following patterned stimulation. [Please see Fig. 58 for the burst and spike rates for this experiment.] Network responses (means of 13 channels) of each 20 min interval are shown. Unpaired student t test were used to determine whether or not there was a difference between the mean burst duration intervals (38 degrees of freedom for all tests).

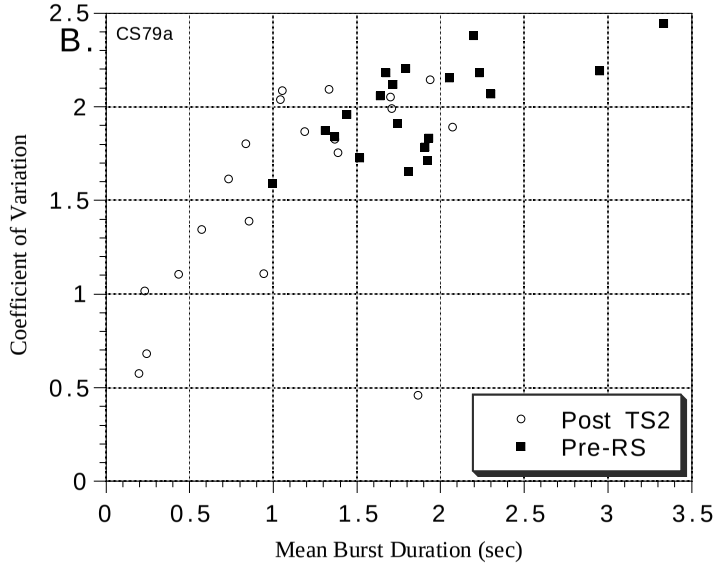
A. Following the second SCTS (this time on channel 24), the MBD increased significantly ($P < 0.001$).

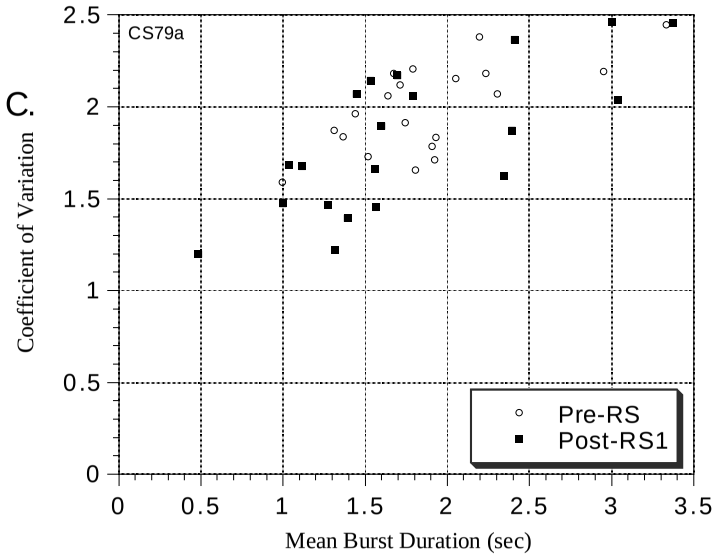
B. The first 20 min immediately following SCTS was compared to the 20 min interval immediately preceding SCRS on the same channel (no stimulation between these two intervals). There was a significant increase in MBD ($P < 0.001$).

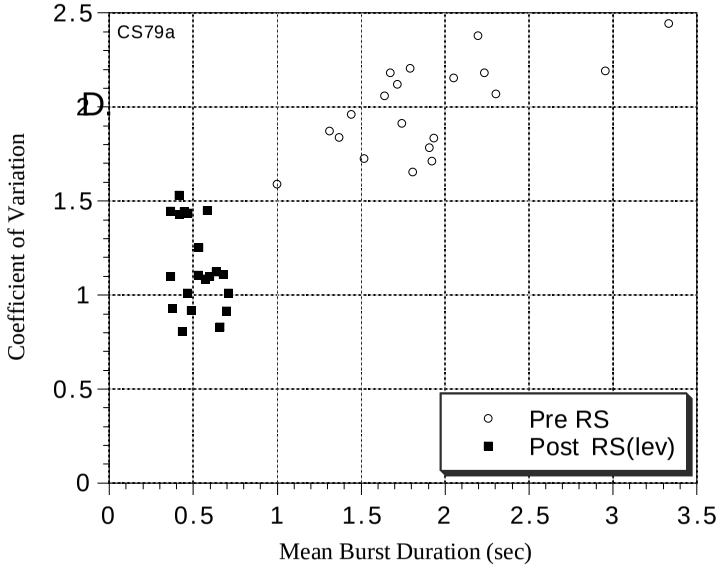
C. There was no significant change in MBD between the interval immediately before SCRS, and the interval immediately following SCRS ($P = .5562$).

D. There was a significant ($P < 0.001$) reduction in MBD shown between the 20 min activity interval immediately following SCRS and the 20 interval at the end of the experimental episode.


A.
CS79a
Coefficient of Variation
Mean Burst Duration (sec)
Post TS1
Post TS2


B.
CS79a
Coefficient of Variation
Mean Burst Duration (sec)
Post TS2
Pre-RS


C.
CS79a
Coefficient of Variation
Mean Burst Duration (sec)
Pre-RS
Post-RS1


D.
CS79a
Coefficient of Variation
Mean Burst Duration (sec)
Pre RS
Post RS(lev)

Recording action potentials on four different recording channels.

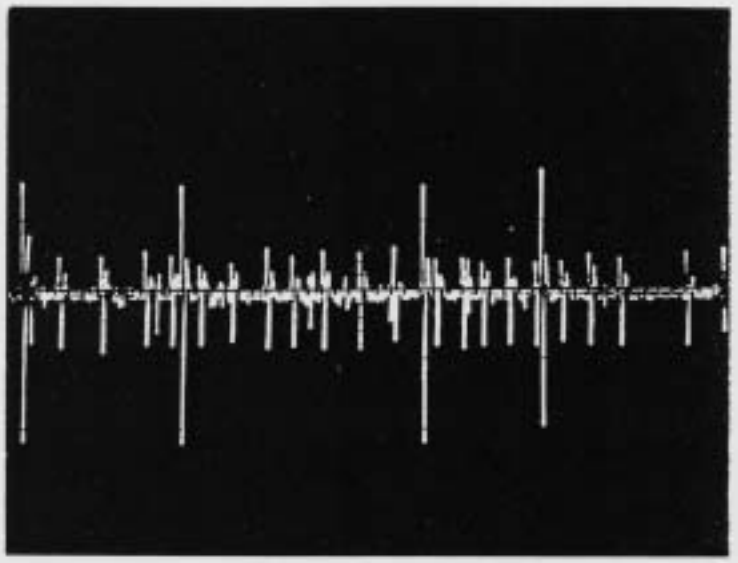
In an effort to evaluate a more global response to single pulses, recording channels (with adequate SNRs) separate from the stimulus channel(s) were selected to sample network responses to single pulses using the SMU preamplifiers. The percentage of evoked activity depended primarily on the preparation, stimulus and recording sites, stimulus intensity, and responsiveness of unit(s) to stimulation. There was also the possibility that spontaneous action potentials would be counted among the evoked action potentials.

Figure A-15. Example of MEAP recordings. For test pulse stimulation experiments, evoked action potentials on four separate recording channels were counted. Following a 0.4 V (300 μs) stimulus (Ch 54-C-149), action potentials were counted for 100 ms.

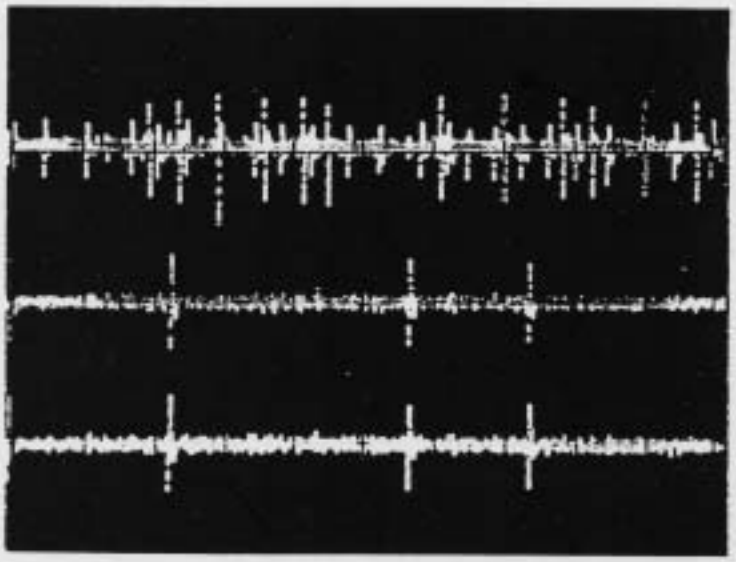
A. Recording channel 3. At least two different units can be detected.

B. Recording channels 1, 52, 55. Activity from the same unit can be seen on the last two channels. [Gain = 1 V/div.]

A.



B.



Activation of inhibitory circuitry

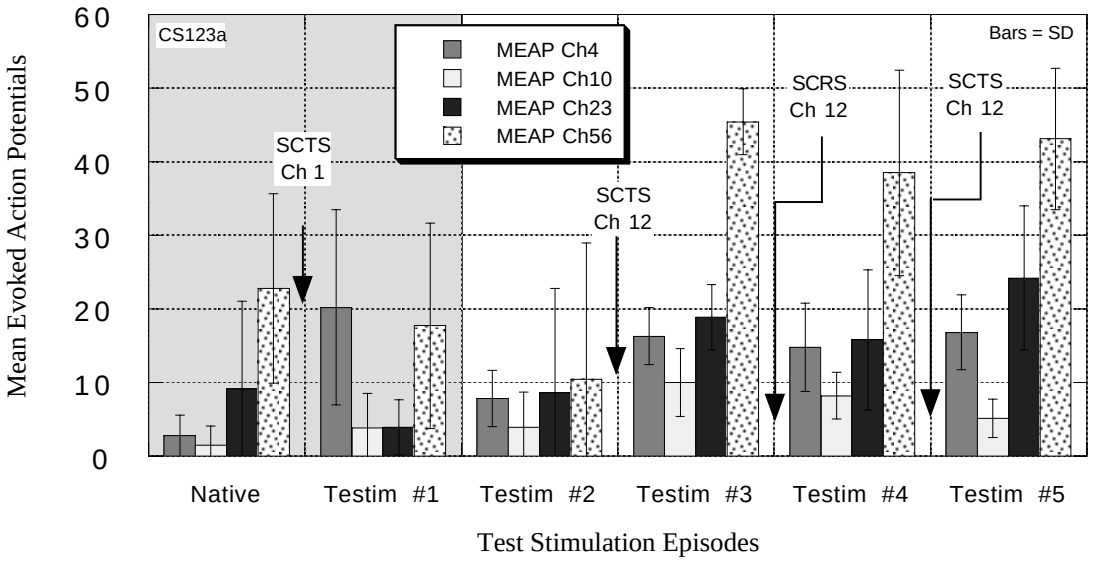
In some preparations, inhibitory circuitry was activated by conditioning stimulation. Figure 59 was presented as an example of such an occurrence. The MEAP values from that same experiment are shown as confirmation of that assertion. Even though there was a sharp decrease in the burst and spike rate following SCTS on channel 12, the MEAP value for all 4 recording channels showed an increase, indicating an enhancement of the responses to test pulses. In addition, although there was an increase in the burst and spike rates following SCRS on the same channel, the MEAP values for all 4 recording channels decreased, indicating a reduction in responses to test pulses.

Figure A-16. Test stimulation of inhibitory circuits.

A. Mean evoked action potentials of four recording channels. Ten single test pulses were delivered to the stimulus channels [1 (shaded area) and 12 (unshaded area) respectively]. The number of action potentials were counted on four traces and averaged. Bars = SD

B. Grand mean (GM) of MEAPs. Grand means of the four means calculated for each recording channel were calculated and plotted for a more global representation of how the conditioning stimulation affected the evoked responses to test pulses. Following stimulation on channel 1, there was a 21% increase in the GM of MEAPs. The next test stimulation (testim) episode revealed a decay of the effect. Following the SCTS on channel 12, there was a significant increase (by 195%) in the GM of MEAPs. Subsequent SCRS on the same channel only yielded a slight (15%) decrease in the GM value. The final HFS on channel 12 resulted in a modest (15%) increase that made the final GM value close to the initial value following HFS on channel 12.

A.


CS123a
Bars = SD
MEAP Ch4
MEAP Ch10
MEAP Ch23
MEAP Ch56
SCTS Ch 1
SCTS Ch 12
SCRS Ch 12
SCTS Ch 12
Mean Evoked Action Potentials
0
10
20
30
40
50
60
Native
Testim #1
Testim #2
Testim #3
Testim #4
Testim #5
Test Stimulation Episodes

B.

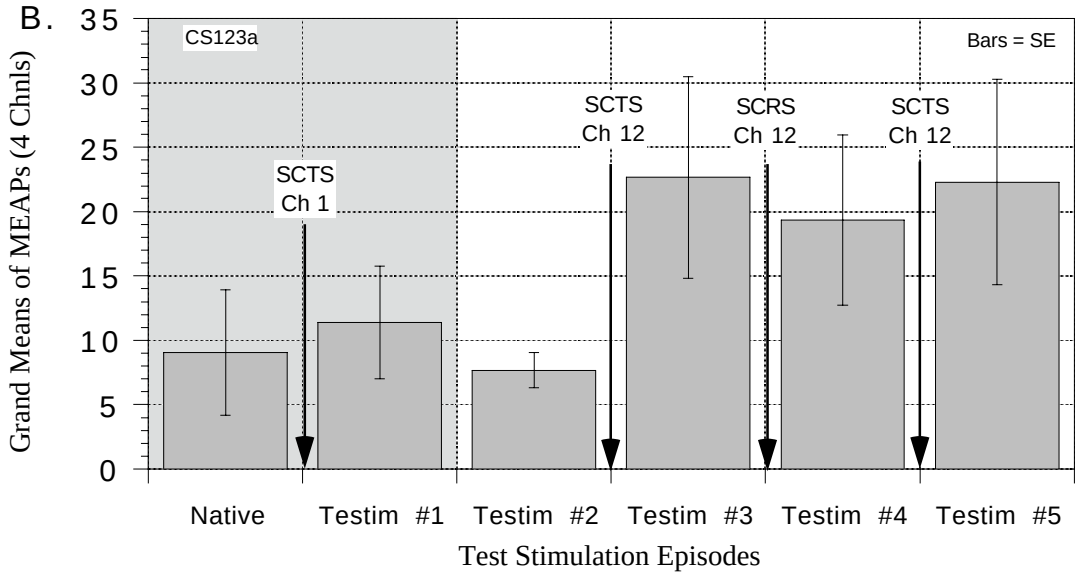

CS123a
Bars = SE
SCTS Ch 1
SCTS Ch 12
SCRS Ch 12
SCTS Ch 12
Grand Means of MEAPs (4 Chnls)
0
5
10
15
20
25
30
35
Native
Testim #1
Testim #2
Testim #3
Testim #4
Testim #5
Test Stimulation Episodes

Table A-1. Data set for test pulse experiments.

Testim Data Set

Expt	Date	Age (d)	SNR	Electrodes Active (%)	% Electrodes Stimulatable	Teststim Episodes
123	8/20/96	60	3.9	66	40	4
124	9/4/96	102	2.6	51	45	6
126	9/11/96	81	2.1	55	53	1
(128)	10/9/96	74	5.4	50	6	1
130	10/16/96	75	3.8	40	39	1
131	10/23/96	68	3.2	40	30	1
132	10/30/96	41	4.7	19	38	2
133	11/6/96	134	4.4	92	65	5
135	11/13/96	43	4.3	88	39	5
136*	12/11/96	68	4.2	41	39	(S-R data)
147	1/29/97	68	1.8	59	20	1
149	2/5/97	60	4.5	78	70	2
150	2/12/97	40	2.6	44	49	9
154	3/26/97	50	3.0	45	47	6
170*	6/24/97	87	3.3	93	34	(S-R data)
(14)	**Means**	70	3.6	57.4	40.9	(44)

Stimulus response curves of MEAP values using SMU amplifiers.

For this study, constant voltage pulses were used because the variables required for network stimulation were not known. Yet, because most of the types of stimulation used in these types of studies are delivered with constant current (although through a penetrating electrode), I tested the use of constant current in single pulse trials in order to determine if the responses would be more reliable.

Earlier stimulus response trials (like the one shown in Fig. A-6) involved a single stimulus/recording channel. This was not possible with constant current stimulation due to increased noise levels. A stimulus response curve was generated using constant voltage (Fig. A-17A). The amount of voltage applied was randomized in order to minimize the effect of the previous voltage. Using the same technique, a stimulus-response experiment using constant current was performed (Fig. A-17B). The results were very similar to the expected results for constant voltage experiments. At 10 μA, there was too much noise introduced into the system once the channel was activated for stimulation. In addition to the noise problem, constant current stimulation presented a more pressing problem. With the MMEP fabrication methods used between 1990 and 1995, each electrode impedance was different and may have ranged in value from 1-4 MΩ. A constant current pulse would have, therefore, created different voltages at the electrode and possibly achieve electrolysis. To avoid inadvertent electrolysis (with lethal consequences to cells or cell processes on or near the electrode crater), constant voltages were used.

Because the primary focus of this project was aimed at changes that occurred after tetanic or repetitive stimulation, other test-pulse/dose-response experiments were performed before and after HFS or LFS.

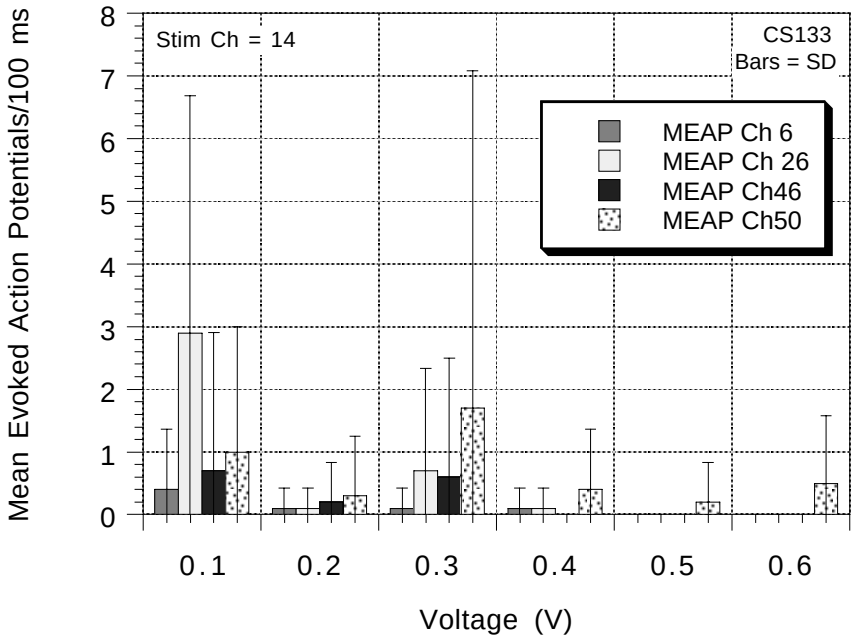
Figure A-17. Stimulus-Response curves of constant voltage and constant current.

A. Stimulus-response curve of constant voltage using SMU system. Four different recording channels (6, 26, 46, 50) were monitored for evoked action potentials immediately following single pulses delivered to channel 14. All action potentials recorded 100 ms following pulse were counted. Dose-response curve shows a primarily negative relationship to increasing voltage. The bars shown at 0.1 V likely represent mostly spontaneously firing units.

Possible explanations for the inverse relationship: 1) the unit(s) stimulated on channel 14 was a dominant inhibitory unit within the network, 2) the unit on channel 14 triggered the activation of inhibitory circuitry within the network. Desensitization may not explain the effect because of the speculated number of synapses involved in the response.

B. Stimulus-response curve of constant current. Four different recording channels (8, 36, 44, 53) were monitored for evoked action potentials immediately following single pulses delivered to channel 46 (counting procedure same as previously noted). Mean evoked action potentials (10 pulses/episode) are shown for 3 of the 4 recording channels. Data from channel 44 was not recorded while in the constant current mode due to an excessive amount of noise on that channel. Test episodes using constant voltage were performed before and after the stimulus-response experiment. The order of pulse intensities were randomized in an effort to minimize the effect of previous stimulations. Sequence was as follows: 0.6 V, 6 μA, 2 μA, 4 μA, 8 μA, 0 μA, 10 μA, 0.6 V (10 μA responses were not recorded because of excess noise).

A.


Stim Ch = 14
CS133
Bars = SD
MEAP Ch 6
MEAP Ch 26
MEAP Ch46
MEAP Ch50
Mean Evoked Action Potentials/100 ms
0
1
2
3
4
5
6
7
8
0.1
0.2
0.3
0.4
0.5
0.6
Voltage (V)

B.

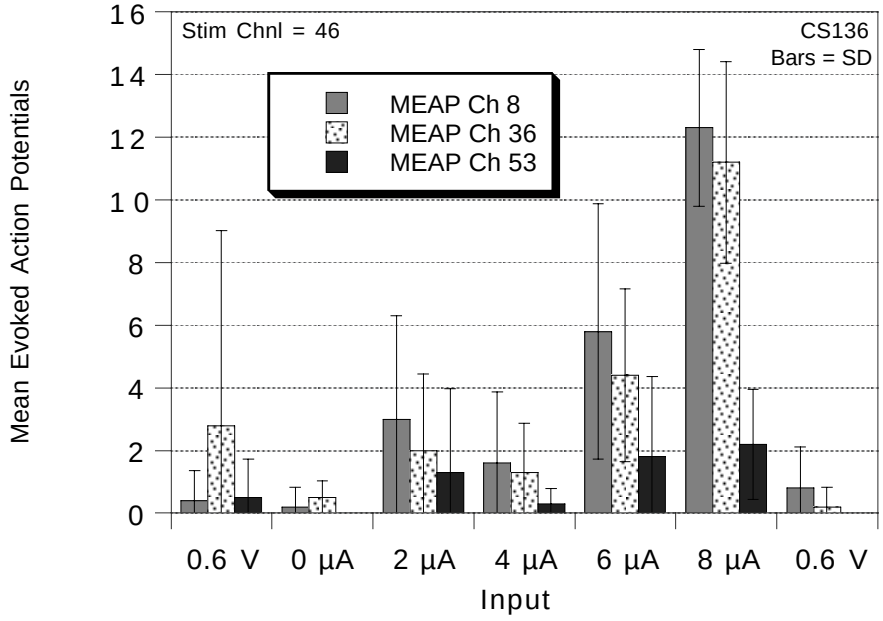

Stim Chnl = 46
CS136
Bars = SD
MEAP Ch 8
MEAP Ch 36
MEAP Ch 53
Mean Evoked Action Potentials
0
2
4
6
8
10
12
14
16
0.6 V
0 µA
2 µA
4 µA
6 µA
8 µA
0.6 V
Input

Figure A-18. Example of grand mean data for quantification purposes.

For comparisons of pre- versus post stimulation changes in spontaneous activity variables, channel means (e.g. burst rate per min, averaged across all recording channels) were averaged across activity intervals (usually 20 min). Some of these values were given in the legend of some graphs where percent change was quoted. Values from all experiments were used to calculate the grand means of percent increase shown in Tables 4, 7, and 9. Some values for this particular experiment were not included in the calculation of grand mean values in Table 4 because of the infinity values generated by using zeros in the calculations.

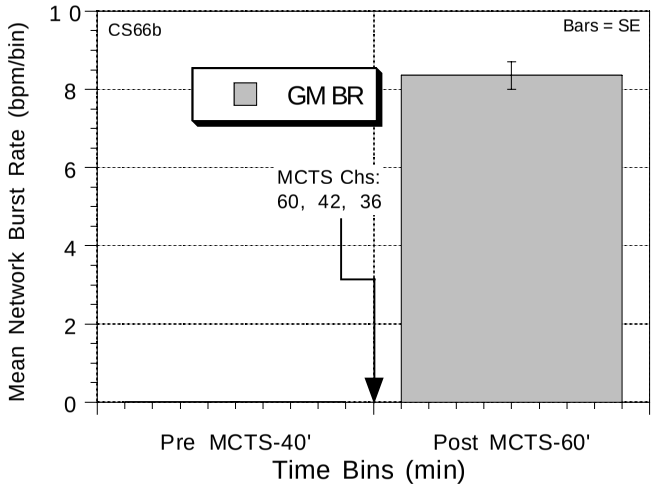
A. Grand mean of burst rate (pre-MCTS vs post-MCTS).

B. Grand mean of spike rate.

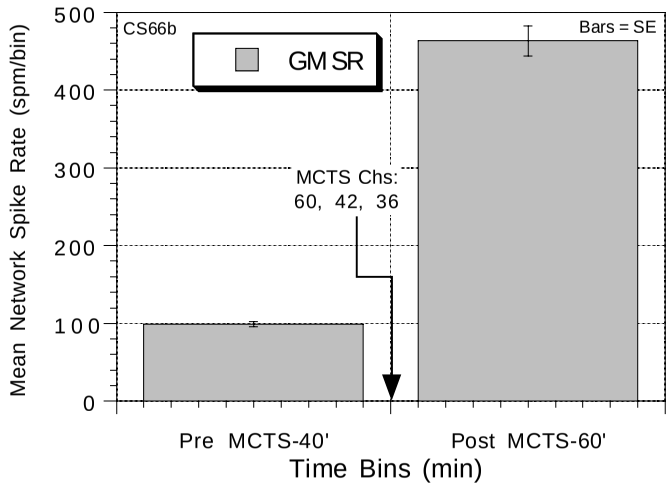
C. Grand mean of mean burst duration.

D. Grand mean of total burst area per minute.

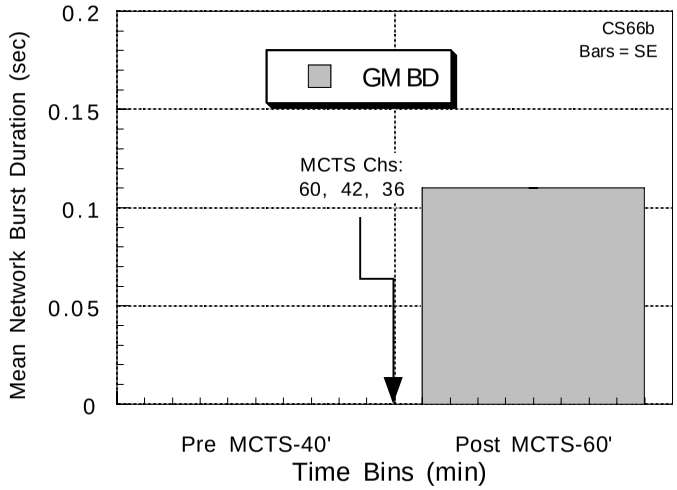
A.


CS66b
Bars = SE
GM BR
MCTS Chs:
60, 42, 36
Mean Network Burst Rate (bpm/bin)
10
8
6
4
2
0
Pre MCTS-40'
Post MCTS-60'
Time Bins (min)

B.


CS66b
Bars = SE
GM SR
MCTS Chs:
60, 42, 36
Mean Network Spike Rate (spm/bin)
500
400
300
200
100
0
Pre MCTS-40'
Post MCTS-60'
Time Bins (min)

C.


CS66b
Bars = SE
GM BD
MCTS Chs:
60, 42, 36
Mean Network Burst Duration (sec)
0.2
0.15
0.1
0.05
0
Pre MCTS-40'
Post MCTS-60'
Time Bins (min)

D.

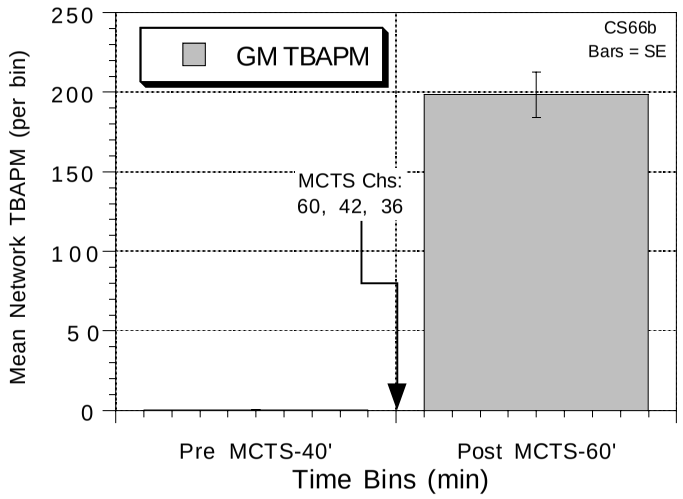

CS66b
Bars = SE
GM TBAPM
MCTS Chs:
60, 42, 36
Mean Network TBAPM (per bin)
250
200
150
100
50
0
Pre MCTS-40'
Post MCTS-60'
Time Bins (min)

Table A-2. Glossary of Terms

TERM	Explanation of Meaning
TIME	
Interval	Spontaneous Activity time intervals:15-20 min (usually 20 min)
Episode	Stimulation Episode (HFS: ~30 s, LFS: ~15 min)
Bin	Usually 1 ms to 1 min (used in calculation and/or display of activity rates.
STIMULATION	
HFS	High Frequency Stimulation (synonymous with SCTS and MCTS)
SCTS	Single Channel Tetanic Stimulation
MCTS	Multi-Channel Tetanic Stimulation
LFS	Low Frequency Stimulation (synonymous with SCRS and MCRS)
SCRS	Single Channel Repetitive Stimulation
MCRS	Multi-Channel Repetitive Stimulation
RESPONSES	[Short-term = less than 15 min; Long-term = greater than 15 min]
STSAP	Short-Term Spontaneous Activity Potentiation
LTSAP	Long-Term Spontaneous Activity Potentiation
STSAD	Short-Term Spontaneous Activity Depression
LTSAD	Long-Term Spontaneous Activity Depression
MISCELLANEOUS	
MEAPs	Mean Evoked Action Potentials
GM MEAPs	Grand Mean of MEAPs
BD	Burst Duration
MBD	Mean Burst Duration
BR	Burst Rate
SR	Spike Rate
TBAPM	Total Burst Area Per Minute