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Recovery of Rewarding Effectiveness of Lateral Hypothalamic Self-Stimulation Following Radiofrequency Lesions

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by
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Abstract

Following lesions that elevate frequency thresholds for medial forebrain bundle self-stimulation, thresholds often rapidly return toward pre-lesion levels. Why the stimulation's rewarding effect recovers has not been well understood. Small radiofrequency lesions were made through the stimulating electrode to explore some of the mechanisms that could underlie the functional recovery. By tracking post-lesion thresholds under different stimulation regimens, results of Experiment 1 indicated that the electrical stimulation itself did not promote the recovery. Experiment 2 studied the reaction of thresholds at bilateral stimulating electrodes following a unilateral lesion to determine whether a dopamine receptor supersensitivity or another distally-occurring compensation might underlie the recovery. Injection of apomorphine (0.5 mg/kg) after thresholds had returned to pre-lesion levels showed that dopamine receptor supersensitivity did not explain the recovery. As recovery took place at lesioned electrodes, thresholds at intact sites did not decrease, indicating that the recovery may not have been due to a post-lesion compensation occurring distal to the lesion site. In Experiment 3, regional post-lesion decreases in tissue resistance indicated that applying a lesion stimulus induced a change that altered resistance and accounted for threshold increases. Experiment 4 extended that investigation by comparing the effects of lesions on thresholds under constant-current and constant-voltage sources of stimulation. Post-lesion threshold increases seen under constant-current stimulation were not evident when voltage levels were not permitted to vary, indicating that when resistance was altered following a lesion, the effectiveness of the stimulation was reduced. Results of Experiment 5 showed that the refractoriness was not altered after

a lesion. A sustained increase in local potential summation was noted, indicating that the lesions affected cells close to the tip of the electrode more than those further away in the stimulation field. The post-lesion recovery of thresholds may be due to local changes in ionic concentration or the occurrence of edema in the tissue around the electrode that temporarily reduces resistance before normalizing over time.

General Introduction

Brain Stimulation Reward

In 1954, Olds and Milner discovered that direct electrical stimulation of the septal area and several other brain areas could evoke a powerful reinforcing or rewarding effect in animals. The strong behavioural effects of the electrical stimulation suggested that the behavioural process of positive reinforcement could be defined in neural terms. Rats readily press a lever to self-administer the stimulation, indicating that the stimulation acts like any other strong reward such as that produced by drugs of abuse (Wise, 1980). Many investigators believe that the neural circuitry involved in brain stimulation reward may also underlie natural biological rewards such as food, water and sex. As such, studying the neural circuitry underlying self-stimulation could allow a better understanding of the neural systems important in the mediation of reward and motivational processes in general, including self-administration behaviour and the rewarding actions of drugs of abuse.

Extensive mapping studies have found that stimulation of a number of sites along the medial forebrain bundle (MFB) is particularly effective in eliciting self-stimulation, as it is a location at which rats are easily trained and that reliably produces high rates of self-stimulation over a relatively long period of time. The MFB is a large collection of ascending and descending axons that interconnects forebrain and midbrain structures. Psychophysiological studies have determined that part of this collection includes small, myelinated fibers that directly link the lateral hypothalamus (LH) and ventral tegmental area (Bielajew & Shizgal, 1982; Shizgal et al., 1980). Although the origin of these directly activated axons is not yet clear, it appears that the reward signal activated by MFB

stimulation is conducted in a descending (or caudal) direction (Bielajew & Shizgal, 1986).

Lesions and MFB Reward

In combination with lesions, self-stimulation has been used extensively to explore the involvement of brain structures important to reward. Early studies compared the rate of lever-pressing at a single level of stimulation before and after a lesion as a measure of the stimulation's rewarding effect. Unfortunately, interpreting the effect of lesions on simple rate of response is difficult, as lesion-induced changes in the stimulation's rewarding effectiveness are confounded with changes in motor or performance capacity (Hodos & Valenstein, 1962).

A reliable measure of the rewarding effect of self-stimulation was introduced when Yeomans (1975) formulated a method that allows changes in reward to be inferred from shifts in frequency threshold. More recent studies using frequency threshold measurement can separate reward efficacy from performance capacity, dispelling much of the ambiguity inherent to the earlier rate research and allowing data to be analyzed quantitatively.

The finding that the direction of conduction along the reward pathway linking the LH and ventral tegmental area is likely rostrocaudal lead more recent investigators to focus on sites rostral to the LH that send descending projections to the ventral tegmental area. Studies have shown that the posterior MFB is important to self-stimulation reward, as lesions and knife cuts made at some anterior sites have led to decreases in the rewarding effectiveness of stimulation at more posterior sites. However, not all of the lesions or knife cuts made at these sites have resulted in a permanent degradation of the stimulation's rewarding effect. Instead, the lesions lead to transient threshold increases that diminished

over a few days. Because the rewarding effectiveness was not permanently decreased, some researchers have challenged the idea that a large proportion of directly activated reward fibers arise in forebrain areas rostral to the LH.

Anterior forebrain sites that send descending projections to the ventral tegmental area include the diagonal band of Broca/medial septal region, the lateral preoptic area, and the anterior lateral hypothalamus (ALH). Fibers that descend from the diagonal band of Broca/ medial septal region pass through the MFB to the level of the LH. Waraczynski (1988) made knife cuts that transsected many septal efferents, finding that thresholds increased slightly in 9 of the 12 rats. However, those threshold increases were still evident about two weeks later in only two of the animals. Because the knife cuts did not permanently or significantly decrease the stimulation's rewarding effect, Waraczynski concluded that cells in these forebrain areas likely do not contribute to those that are directly activated by MFB stimulation.

The lateral preoptic area gives rise to a descending projection to the ventral tegmental area. Janas and Stellar (1987) made knife cuts either side of the lateral preoptic area in seven rats. Although the anterior cuts made in three animals had no effect on MFB self-stimulation reward, slightly more posterior cuts led to a large sustained decrease in the stimulation's rewarding effectiveness in the other rats. However, when Waraczynski (1988) made 40 knife cuts at anterior MFB sites including the lateral preoptic area, she found that reward degradation was rarely permanent. Of the 10 rats that received knife cuts in the lateral preoptic area, substantial, long-lasting threshold increases for LH self-stimulation were evident in only four animals, with smaller increases persisting in two

others.

The lateral preoptic area and other anterior MFB sites send their descending projections through the ALH. Researchers have made lesions in the ALH, contending that damage of this area should degrade the stimulation's rewarding effect at more posterior sites. However, the results have not consistently supported this theory. In Waraczynski's (1988) study, knife cuts of the ALH resulted in a sustained decrease of the stimulation's rewarding effect in only 3 of the 8 animals. Stellar and Neeley (1982) made electrolytic lesions in eight rats, with four rats receiving anterior lesions and four others receiving lesions posterior to the stimulating electrode. The anterior lesions were considered ineffective, as thresholds remained unchanged in three of the rats while a small decrease in the stimulation's rewarding effect was seen in the other animal. Permanent threshold increases were seen in two of the rats that received lesions posterior to the stimulating electrode. Murray and Shizgal (1991) implanted stimulating electrodes at sites in the LH and ventral tegmental area in seven rats. Stable increases in threshold were seen following electrolytic lesions of the ALH at 6 of the 11 sites tested. The authors concluded that the exact placement of the lesion might explain why the rewarding effect of the stimulation was not permanently decreased at all of the sites, noting that the effective lesions damaged more lateral parts of the anterior MFB. However, although lesions were made more laterally in a subsequent study (Murray & Shizgal, 1996), substantial, long-lasting threshold increases were seen in only 4 of the 16 rats, with a small stable increase noted in one other.

Recovery of the Stimulation's Rewarding Effect

For some of the animals in these studies, the reduction of the stimulation's rewarding effect endured throughout the post-lesion testing period. Such long-lasting effects of a lesion imply permanent damage to fibers important to reward. However, other rats had threshold elevations that were transient, with at least partial recovery noted across time. Although some researchers have speculated as to why the threshold increases were only temporary, it is not yet clear why recovery was seen in these animals.

Waraczynski (1988) noted temporary threshold increases for LH stimulation following knife cuts made at various anterior sites. While 3 of the 8 animals had stable threshold increases, two others that received knife cuts in the ALH had large threshold increases that recovered to baseline levels within days. When she transsected lateral preoptic area fibers, thresholds for 4 of 10 rats increased and remained elevated for at least two weeks. However, thresholds for four others increased then returned to baseline levels. Recovery also occurred within days for these animals. Small temporary threshold increases were also seen in 7 of 9 animals following cuts to the diagonal band of Broca/medial septal region, with recovery complete within one week. Although Waraczynski considered the possibility that the transient effect might reflect functional recovery of the reward substrate, she concluded that the absence of permanent threshold increases indicated the unlikeliness that most of the reward-relevant fibers arise rostral to the LH.

In Stellar and Neeley's (1982) study, anterior MFB lesions led to a temporary reward degradation in one of four rats. Thresholds for this animal recovered to baseline levels within six days of the lesion. Two other animals also showed transient decreases in the

stimulation's rewarding effect, with thresholds recovering within two days of posterior MFB lesions. Because the posterior lesions had more effect than the anterior ones, the authors concluded that this descending pathway does not originate in the anterior forebrain.

Murray and Shizgal (1991) reported that elevated thresholds at 5 of 11 sites in the ventral tegmental area and LH decreased after electrolytic lesions of the anterior MFB. Recovery took place over four days to three weeks for these animals. In a subsequent study (Murray & Shizgal, 1996) the authors noted similar findings for 11 of 16 rats following lesions of the ALH, with much of the recovery occurring within 10 days. Lesions that resulted in transient increases damaged many of the same regions that were damaged by lesions that led to substantial, long-lasting threshold increases. However, the authors suggest that differences in the exact alignment of the lesion and stimulating electrode may account for the differences in the lesion's effect on thresholds.

Gallistel and his colleagues (1996) also noted a partial recovery of rewarding effectiveness of LH stimulation. Thresholds increased substantially in all four rats the day after an electrolytic lesion was made through the stimulating electrode. The threshold for one of the animals began to decrease nine days later, with the rewarding efficacy increasing by a factor of two over the next week. The authors suggested that the recovery may have been due to a regulatory neurophysiological process, such as the development of an enhanced receptor sensitivity.

A number of earlier studies using rate of self-stimulation as a behavioural measure also reported recovery following lesions. Although this method has been shown to be less

reliable than newer scaling techniques, the results provide qualitative support for later research using threshold measurement. Valenstein and Campbell (1966) implanted stimulating electrodes at septal sites in 24 rats, then made electrolytic lesions in the LH. They found that self-stimulation returned to near pre-lesion rates for 19 of the rats, with recovery complete within 7 to 10 days. As recovery occurred in most of the animals tested, they concluded that the MFB is not an essential route between structures in mediating self-stimulation.

Boyd and Gardner (1967) observed partial to complete recovery of self-stimulation in most of the animals tested. After small radiofrequency lesions made in the preoptic area and other sites near or adjacent to the MFB disrupted self-stimulation in the posterior LH, recovery was evident within one week in 29 of the 30 rats. Because self-stimulation was not permanently disrupted, the authors concluded that the lesioned sites were not essential to self-stimulation. Although no time frame was reported, Boyd and Celso (1970) also noted some recovery of self-stimulation rates. Following radiofrequency lesions in the ALH and preoptic areas, 6 of 11 rats showed at least partial recovery of septal self-stimulation. They suggested that the recovery indicated that the reward system may be capable of reorganization, with other parts of the system taking over the function of the damaged area.

In the studies mentioned above, a recovery of the stimulation's rewarding effectiveness was seen in 87 of the 131 rats/ sites tested. The transient effects of lesions on self-stimulation, then, occurs often and has been noted consistently throughout the literature. However, it remains unclear why the rewarding effect recovers in many rats.

Possible Mechanisms Underlying the Recovery

One possible mechanism that might explain the functional recovery is axonal regeneration. Although it is generally believed that in adult mammals the spontaneous regrowth of axons after injury is limited to the peripheral nervous system, a number of studies have shown that significant axonal regeneration in the central nervous system can occur. Foerster (1982) demonstrated that MFB neurons in adult rats regenerated spontaneously following mechanical knife cuts. Björkland and Lindvall (1979) reported that following chemical axotomy of hypothalamic sites in adult rats, axonal sprouts extended over several millimeters in the direction of pathways removed by the lesion. Other research has shown that severed spinal cord axons of mammals can regenerate in the presence of an applied electrical field (Borgens, Blight & McGinnis, 1987; Borgens et al., 1986). The authors conclude that the regrowth of axons was facilitated by the electrical field, and noted that the regrowth lead to the functional recovery of a sensory reflex. If axonal regeneration can explain the post-lesion recovery of rewarding effectiveness of electrical brain stimulation, with the regrowth enhanced by the stimulation itself, the findings of Borgens and his colleagues (1986) could be particularly relevant to the present study.

Given these findings, it seems possible that the recovery of thresholds for self-stimulation may be at least in part due to the growth and functional re-connection of lesioned axons. However, other mechanisms unrelated to regeneration may also be behind the recovery. One possible underlying mechanism is the compensatory neurophysiological process of denervation supersensitivity. When postsynaptic tissue is deprived of normal

fiber innervation, a heightened sensitivity to stimulation by naturally-occurring substances or drugs can occur. Thus, if a lesion interrupts fiber input and leads to the development of receptor supersensitivity, an increased efficacy in signals in surviving reward fibers could account for the recovery of the rewarding effect of electrical brain stimulation.

It is also possible that secondary effects induced by a lesion may contribute to the attenuation of the stimulation's rewarding effectiveness. Along with the destruction in the central zone of necrosis, a lesion will affect adjacent tissue in a number of ways, such as by inducing an extracellular edema (Broseta et al., 1988) or by changing the ionic concentration surrounding the cells (Edström, Ekström & Wiklund, 1995). Such artifacts resulting from the application of a lesion stimulus may lessen the effectiveness of the stimulation and lead to an elevated threshold before normalizing with time.

Tracking Post-Lesion Threshold Changes

Studying the post-lesion recovery of reward thresholds could clarify whether the stimulation's rewarding effectiveness returns when tissue normalizes following a lesion-induced artifact or is due to some sort of functional recovery. However, to be able to track that recovery small lesions must be made, as large lesions usually permanently elevate reward thresholds. Small lesions also allow thresholds to be measured beginning soon after the lesion, as small lesions will not affect all of the reward-relevant fibers in the stimulation field.

Lesions are usually placed distal to the stimulating electrode. Although this method is necessary for mapping studies, it does not always provide a high return in terms of a good alignment of the stimulating electrode and the lesion, particularly if the lesion is small.

However, if lesions are made through the stimulating electrode itself, a high return of animals is guaranteed, as we can be sure that fibers relevant to self-stimulation would always be directly affected by the lesion.

To study the recovery of reward thresholds, then, we made small radiofrequency lesions through the stimulating electrode. Changes in the rewarding effect of the stimulation were then measured beginning one day after the lesions were made.

Measuring Threshold Changes

We measured the recovery of the stimulation's rewarding effectiveness by changes in frequency threshold, as opposed to current threshold (the lowest stimulation current that will maintain a given rate of self-stimulation). With frequency threshold measurement, the extent of damage done to reward fibers by a lesion can be inferred by threshold increases. For instance, if the threshold doubles after the lesion (increasing from 25 to 50 Hz), the resulting ratio 50/25 implies that the lesion damaged half of the reward-relevant neurons in the stimulation field. In this way, measuring frequency thresholds before and after a lesion can scale the size of the lesion functionally. The functional size of a lesion cannot be estimated if current threshold is used as a measure. If a lesion damages reward fibers close to the stimulating electrode, a decrease in the rewarding effectiveness of the stimulation will be seen at low currents. However, if the current is increased, undamaged reward-relevant fibers will be recruited from an area further away from the electrode, and the functional effect of the lesion could be reduced.

Lesion Techniques

Of the various lesion methods employed as experimental techniques of physiological psychology and in cardiac and spinal cord research, direct current and radiofrequency lesion methods have been widely used. Direct current produces tissue damage due to a combination of effects, including forming gas bubbles, disrupting ions via polarization, and depositing metallic ions. Radiofrequency current induces damage by rapidly alternating polarity, generating high temperatures in the tissue surrounding the electrode tip. The electrode absorbs heat from the surrounding tissue, which is dissipated by conduction through the tissue and by convection of adjacent blood vessels (Pecson, Roth & Mark, 1969). As the rise in temperature decreases strongly with radius, the region of destruction remains close to the electrode tip (Aronow, 1960). Increasing the local temperature leads to progressively greater lesion diameter (Goldberg et al., 1996). However, when the local temperature is held constant, repeated lesions made at the same site increase the degree of coagulation without increasing the size of the lesion (Vinas et al., 1992).

Although the behavioural effects of radiofrequency lesions do not appear to differ from those of direct current lesions, we chose the radiofrequency lesion technique for this study for a number of reasons. First, using standard current parameters, anodal direct current lesions can be ineffective in damaging myelinated fiber tracts, while radiofrequency lesions are very effective for this purpose (Dicara, Weaver & Wolf, 1974). Second, lesions produced by radiofrequency are smaller and more homogeneous than those produced by direct current. Furthermore, following radiofrequency lesions the area adjacent to the central zone of necrosis contains significantly fewer inexcitable cells than following direct

current lesions (Ge et al., 1995). Finally, the depositing of iron ions during direct current lesions raises the concern that toxicity may persist after application of the lesion.

In this study lesions were made through the stimulating electrode so that subsequent changes at the lesion/stimulation site could be measured and tracked throughout the test period. The lesions had to be effective enough to substantially increase thresholds so that we could obtain reliable measures. At the same time, the lesions were deliberately small so that axons near the margin of the stimulation field survive. In pilot work with 15 electrodes we found that with a current of 50 μA, applying the radiofrequency signal for one second produced threshold elevations in a range of 0.26 to 0.42 $\log_{10}$ units above pre-lesion levels, with an average increase of 0.29 $\log_{10}$ units and a standard deviation of 0.14. Threshold increases in this range imply that approximately 45% to 62% of originally stimulated axons have been affected by the lesion. For three other animals with post-lesion threshold increases of less than 0.10 $\log_{10}$ units, a second application of radiofrequency lesioning stimulus of 1.4 s duration raised the average threshold increase to 0.34 $\log_{10}$ units with a standard deviation of 0.08 log.

Rationale

Understanding why some threshold increases are temporary could help to clarify results of studies noting a recovery of the stimulation's rewarding effect. To that end, the overall objective of this research is to determine the mechanism(s) responsible for the transient attenuation of rewarding effectiveness of electrical brain stimulation. To explore the possible factors responsible for the temporary increase in threshold, small radiofrequency lesions were made through stimulating electrodes implanted in the LH and

recovery was tracked beginning one day after lesions were made.

Experiments 1 through 5 examined a number of potential underlying factors. In Experiment 1 we studied the time course of threshold recovery, which has occurred as early as a few days after a lesion in some self-stimulation/lesion studies. We wondered if the recovery could be due to the regeneration of axons that made functional re-connections with target areas. Although it is generally believed that regeneration and functional recovery takes much longer than the that seen for reward thresholds in some studies, we considered the possibility that the electrical stimulation itself might be promoting the threshold recovery.

The time course of threshold recovery coincides with the amount of time necessary for the occurrence of an enhanced sensitivity of dopamine receptors. In Experiment 2 we looked at the possibility that denervation supersensitivity developed after a lesion, using the direct dopamine receptor agonist apomorphine. We also challenged the idea that some post-lesion compensation occurs distal to the lesion site and explains the recovery, by studying the reaction of thresholds at lesioned and unlesioned sites in animals implanted with bilateral electrodes.

As Experiment 2 showed no indication of a distal compensation, we turned our attention to the lesion site itself. In Experiment 3 we studied regional changes in tissue resistance at lesion sites, finding those changes correlated with threshold recovery. In Experiment 4 we extended that examination of the influence of this secondary lesion effect on reward thresholds, by comparing the effects of lesions on thresholds under constant-current versus constant-voltage stimulation pulses. In Experiment 5 we attempted to

obtain evidence of the lesion-induced artifact by following the recovery with post-stimulation excitability tests, to detect whether refractoriness is altered after a lesion.

Experiment 1: Effect of Stimulation on the Post-lesion Recovery of Reward Thresholds

This experiment investigated: (a) the time course necessary for reward thresholds to return to baseline levels following a small radiofrequency lesion, and (b) the possible influence of the electrical stimulation itself on that rate of recovery.

Introduction

We suggested that axonal regeneration and functional re-connection may underlie the recovery of rewarding effectiveness of electrical brain stimulation following a lesion. Severing an axon (axotomy) interrupts mechanisms that carry materials synthesized in the cell body to the axon terminals. Because the axon and synaptic terminals are deprived of their normal metabolic connection with the cell body, they degenerate. Degeneration first appears in the axon terminal within 24 hours of the lesion, with the synapses formed by the axon degenerating completely in about two weeks (Kelly, 1981).

Within hours of axotomy, regeneration begins. Severed axon stumps and intact collateral fibers sprout within two to three days and proliferate over the following two weeks (Ramon y Cajal, 1928). In the peripheral nervous system of adult mammals, the axons of regenerating neurons are capable of elongating over long distances (Sketelj, Bresjanac & Popović, 1989). This growth potential has been applied in research that has shown that regenerating central nervous system axons will grow along transplanted peripheral nerve segments (Aguayo et al., 1984; Richardson, McGuinness & Aguayo, 1980), Schwann cells (Kromer & Cornbrooks, 1985) or grafts that contain fetal central nervous system tissue (Harvey, Gan & Pauken, 1987; Wendt, Fagg & Cotman, 1981).

However, without such grafts, axonal growth appears to be limited to only a few millimeters after damage to the mature central nervous system (Ramon y Cajal, 1928; Schwab & Bartholdi, 1996). As such, it is generally believed that most central axons in adult mammals do not undergo extensive spontaneous regeneration following a lesion. This is thought to be due to the absence of Schwann cells in the central nervous system. In the peripheral nervous system, Schwann cells myelinate axons and promote regeneration by producing neurotrophic factors and cell-adhesion molecules. The growth of new axons is stimulated by the neurotrophic factors, and the cell-adhesion molecules provide paths along which regenerating axons grow. In the central nervous system, oligodendroglia myelinate axons. These cells do not stimulate or guide regrowth, and in fact release factors that actively block regeneration (Schwab & Bartholdi, 1996).

Other studies, however, indicate that differences in the capacity of certain damaged central nervous system axons may depend on the environment in which these axons are located, rather than on the type of cell itself (Björklund & Lindvall, 1979; David & Aguayo, 1981; Ramon y Cajal, 1928). As well, despite the consensus that neurons of the central nervous system are only capable of a limited regeneration without implanting biological materials to stimulate regrowth, a few studies have reported relatively extensive spontaneous regrowth of central axons. McQuarrie and Grafstein (1981) showed that outgrowth of the leading axon began about four days after a crush of the optic nerve in goldfish, proceeding at a rate of 0.3 to 0.4 mm/day. Björkland and Lindvall (1979) observed growth of lesioned axonal sprouts in the adult rat hypothalamus that extended over several millimeters, leading to a partial restoration of original fiber paths that had

been removed by chemical axotomy. Significant axonal regeneration in the adult rat brain following mechanical knife cuts has also been reported. Foerster (1982) showed that transsected MFB axons had regrown around lesion sites beginning three days after axotomy in 13% of the cases studied. The percentage of regenerating axons progressively increased over time, with growth noted in 24% of cases inspected six to eight days post-lesion. In brain sections examined 18 to 23 days after injury, axonal growth was seen in 68% of cases, with growth extending up to a few hundred micrometers from the end of the cutting device. Although the ultimate destinations of the new fibers were not determined in the study, Foerster suggested that the regenerating axons seemed to grow towards a reconstruction of severed pathways.

These results raise the possibility that spontaneous axonal regeneration can occur in the central nervous system of adult mammals. Such regeneration may lead to the appropriate reinnervation of target areas originally deprived by a lesion, which is necessary for functional recovery. If so, fiber regeneration and re-establishment of the original terminal connections could explain the functional recovery of reward thresholds that has been reported in various lesion studies.

This recovery may be facilitated by the electrical stimulation itself. Neural tissue responds to electrical stimulation with increases in the rate of growth and in the amount of branching of axons (Borgens, 1982). Self-stimulation promotes dendritic growth (Rao, Desiraju & Raju, 1993), considered to be a possible mechanism for recovery of function in learning (Kolb & Gibb, 1993). Borgens et al. (1986) reported that the application of an electrical field induced regeneration of severed dorsal column axons within the spinal cord

of adult mammals. This facilitated regeneration led to functional recovery of a sensory reflex, as severed fibers made relevant synaptic connections (Borgens et al., 1987). Thus it appears that electrical stimulation may promote the post-lesion regenerative process of axons. If so, such an enhanced axonal regeneration and subsequent receptor reinnervation could underlie the post-lesion recovery of the stimulation's rewarding effect.

In investigating this possibility it seems important to examine the length of time necessary for that recovery to take place. Although many studies have observed a transient increase in thresholds for self-stimulation following a lesion, a time course has not been substantiated. When reported at all, recovery has been noted to occur anywhere from within a few days to a few weeks of a lesion. Given such differing results, and because establishing a time course could help to elucidate the mechanism(s) underlying the recovery, this first study tracked the time it takes for thresholds to return to or near baseline levels following a small lesion. Because studies indicate that electrical stimulation may facilitate functional recovery, this study also examined whether or not the stimulation affects that time course. If the stimulation influences the rate of recovery following a lesion, there should be differences in the amount of threshold decrease seen between animals receiving different amounts of stimulation over a period of time.

To study the recovery, we collected baseline thresholds until they were stable, then made small radiofrequency lesions through the stimulating electrode. Thresholds were then tracked over a period of two weeks. To test whether the stimulation itself affected threshold recovery, groups of rats received different amounts of stimulation over the post-lesion test period. One group of rats received stimulation tests every day for two weeks,

another group was tested every other day, and a third group every fourth day. A fourth group received post-lesion stimulation tests only twice; once 24 hours after the lesion and again on the last test day.

Materials and Method

Subjects and Surgery

Twenty-seven male Long-Evans rats (supplied by Charles River Canada) weighed between 350 and 400 g at the time of surgery. The rats were housed individually in plastic cages (47 cm × 25 cm × 20 cm) in a climate-controlled animal care facility. Temperature was maintained at 21°C and a 24 hr cycle (12 hrs light and 12 hrs dark) was provided. The rats had unlimited access to food (Purina Rat Chow) and water.

Before surgery, all of the rats were given Atropine sulfate (0.05 mg sc) to control mucus secretions. They were then anaesthetized with sodium pentobarbitol (Somnotol; 65 mg/kg ip). Eleven of the rats required a supplemental dose of Somnotol, to a total of 100 mg/kg. Seven of the rats were given Xylazine (Rompun, 0.04 mg im) to supplement anaesthesia. To ensure the corneas would not dry out, a petroleum ointment was applied to the eyes. To prevent discomfort from the ear and incisor bars, a topical anaesthetic (Xylocaine; 2% lidocaine hydrochloride) was applied to the ear canals and behind the upper incisors. The rats were then placed in a stereotaxic apparatus, with the incisor bar adjusted to maintain a level skull position.

An incision was made in the scalp to expose bregma, and the fascia scraped away. With a dental burr, a hole was drilled for the electrode aimed at the LH; using coordinates of 2.0 mm behind bregma, 1.7 mm over from the midline, and 8.2 mm ventral from the

surface of the skull (based on Paxinos and Watson's (1986) atlas of the rat brain). The electrodes were made of 0.25 mm stainless steel wire soldered to gold plated plugs. The wire was insulated with 6 coats of enamel, then sharpened to expose the tip. Four 3 mm stainless steel screws were threaded into holes drilled into the skull. A 7 cm stainless steel wire soldered to a 5 mm stainless steel screw was wrapped around the four screws, serving as a current return. The electrode and current return assembly was anchored to the skull with acrylic dental cement to form a crown, covering the incised area. The edges of the incision were flushed with normal saline to rehydrate. At the end of surgery, normal saline (3 ml ip) was injected to guard against dehydration. The rats were allowed to recover for one week before being trained to press a lever to self-administer electrical stimulation.

Histology

When testing was complete, the rats were euthanized with overdoses of sodium pentobarbitol, then perfused intracardially with 0.9% saline followed by 10% formalin solutions. Electrodes were extracted before the brains were removed from the skulls. Brains were fixed in formalin until they were frozen, sliced into 60 μm coronal sections, mounted on glass slides, and stained with thionin. The locations of the electrode tips were determined by comparing sections with the illustrations in Paxinos and Watson's (1986) atlas of the rat brain (see Figures B-1 to B-4 in Appendix B for LH electrode placements).

Apparatus

Test cages had three wooden walls and a Plexiglas front panel of 51 cm in height, and a floor space of 27 cm × 36 cm. A Gerbrands test lever was positioned about 3 cm from

the cage floor, in the middle of one of the side walls.

Electrical stimulation was supplied by a constant-current generator through wire leads connected to a commutator mounted above each cage. The stimulation consisted of square, monophasic, cathodal pulses of 0.1 ms duration. The current was adjusted manually and monitored on an oscilloscope. A microcomputer implemented the programmed test parameters and recorded the rats' responses.

Threshold Measurement

The rewarding effectiveness of the electrical stimulation was measured using frequency threshold, which refers to the frequency of pulses (measured in Hz) in fixed trains just necessary to sustain criterion levels of self-stimulation. The stimuli were 100 μs cathodal current pulses, with one train, or series of consecutive pulses, administered each time the rat pressed the lever. The amount of time between the onset of the first and last pulses (train duration) was 500 ms.

Thirty second trials (discrete periods in which stimulation was available when the lever was pressed) were used. A response rate was obtained by dividing the number of times the lever was pressed during a trial by the duration of the trial.

A series of trials make up a sweep. The starting pulse frequency train was lowered in each subsequent trial until the frequency was too low to support lever pressing, ending the sweep. The test sessions were made up of 12 sweeps.

To calculate the threshold between responding and not responding for each sweep, the behavioural criterion used is one half of the maximum response rate obtained in each sweep. The pulse frequency that corresponds to that criterion is interpolated between two

consecutive trials with rates higher and lower than the criterion rate. Threshold, then, refers to the pulse frequency that corresponds to that criterion rate.

Training and Testing Procedures

One week after surgery, standard shaping techniques were used to train the rats to press a lever to self-administer electrical brain stimulation. In each training session, 12 sweeps of stimulation were offered. Each sweep consisted of 30 s trials. The sweeps began with three free priming pulses; the rat had to press the lever for further stimulation. The pulse frequency per train offered during the first 30 s trial was chosen such that the rat would respond near its maximal rate for at least two more trials. On subsequent trials, the pulse frequency was lowered in 0.05 $\log_{10}$ units, until the frequency was too low to elicit lever pressing. A current of 500 μA was used for both training and test sessions. When averaged thresholds were within 0.05 $\log_{10}$ units on three successive training days, testing began.

Testing took place at the same time (between 10:00 a.m. and 3:00 p.m.) each day. The test parameters were the same as for training, except that each sweep ended when there were five or fewer lever presses on two consecutive trials. Curves were cut at 12 stimulations. A threshold was determined for each sweep when at least three consecutive 30 s frequency trials were at or above this cut level. Log frequency thresholds were calculated for each test session, based on the pulse frequency that supported half-maximal rates of lever pressing. A baseline threshold was established for each rat when daily averaged thresholds varied less than 0.05 $\log_{10}$ units for five consecutive days.

Once baseline thresholds were established, each rat received a radiofrequency lesion for a duration of one second through the stimulating electrode. A current of 50 μA was used. As the animals were not anaesthetized for the procedure, post-lesion testing began 24 hours after the lesion was made (Day 1) and continued for a total of 13 days. Thresholds were measured under the same test parameters as those used in baseline testing. Following testing on Day 1, the rats were assigned to one of four groups: for the rest of the test period Group 1 (n = 8) was tested daily, Group 2 (n = 7) every other day, Group 3 (n = 8) every fourth day, and Group 4 (n = 4) was tested again only on Day 13.

Two things were considered in assigning the rats to groups: variability between animals in pre-lesion threshold, and variability between animals in the functional size of the lesion. Such differences could mask the rate and/or extent of recovery following the lesion.

Pre-lesion threshold variability between animals is generally due to differences in the precise placement of the electrode tip in the LH. From the five-day baseline, an average threshold was calculated for each rat. Based on this average, animals with low (20 to 25 Hz), medium (26 to 32 Hz) and high (more than 32 Hz) pre-lesion thresholds were then evenly dispersed among the four groups. This assured that threshold means were similar for all of the groups before the post-lesion test period.

The functional size of the lesion, or the amount of function lost due to the lesion, was determined as the difference between average pre-lesion threshold and the threshold measured on post-lesion Day 1. As lesions with identical parameters can result in very different initial threshold increases between animals, the rats were evenly assigned so that

each of the four groups was made up of animals with threshold increases considered small (0.1 to 0.2 $\log_{10}$ units), medium (0.21 to 0.3 $\log_{10}$ units) and large (0.31 $\log_{10}$ units or more). Because the possible masking effect of variance in both pre-lesion threshold and functional size of the lesion was controlled for, any substantial difference between the groups on specific test days would likely be due to the differing amounts of stimulation received.

Because reward thresholds can vary over time two criteria were set before post-lesion testing began. First, we determined that threshold increases of less than 0.1 $\log_{10}$ units on post-lesion Day 1 would be too small to clearly show an attenuating lesion effect and allow reliable tracking over the two-week test period. On post-lesion Day 1 thresholds had increased from between 0.1 $\log_{10}$ units and 0.7 $\log_{10}$ units above baseline levels in 18 of the 27 animals. As threshold elevations were less than 0.1 $\log_{10}$ units in the other nine rats at this time, these animals were re-lesioned for a duration of 1.4 seconds, after at least five consecutive days of stable thresholds. Twenty-four hours after re-lesioning, threshold increases for these rats ranged from between 0.1 $\log_{10}$ units and 0.4 $\log_{10}$ units. The second criterion was that recovery of the stimulation's rewarding effect would be considered complete when post-lesion thresholds returned to at least 0.1 $\log_{10}$ units of average pre-lesion levels.

Results

Thresholds were calculated for the five baseline test days and during the 13 post-lesion test days. Average thresholds for rats in Groups 1, 2 and 3 were compared on post-lesion Days 1, 5, 9 and 13, while thresholds for rats in all four groups were compared on

post-lesion Days 1 and 13.

Time Course for Recovery

Recovery of the rewarding effect of the stimulation was considered complete when thresholds had returned to within 0.1 $\log_{10}$ units of pre-lesion levels. On average, thresholds for animals in Groups 1, 2 and 3 met this criterion by post-lesion Day 5.

Group 1. Six of the eight rats that received daily testing had thresholds that returned to pre-lesion levels by Day 5. In the other two rats, thresholds remained elevated over baseline levels by 73% (rat #1255) and 104% (#1258) on the last test day. Some recovery was evident in those two animals; #1255 had an initial post-lesion threshold increase of 127%, and #1258 increased 392% above baseline average on post-lesion Day 1 (see Figures 1 and 2).

Group 2. In the group that was tested every second day, thresholds for five of the seven animals returned to pre-lesion levels by Day 5. From this group, one rat recovered by Day 9, and the other by Day 13 (see Figures 3 and 4).

Group 3. Five of the eight animals that received testing every fourth day had thresholds that returned to baseline levels by Day 5. Two others recovered by Day 9, and the other by Day 13 (see Figures 5 and 6).

Group 4. For the four rats that were tested only on Days 1 and 13, three recovered to pre-lesion levels and one (#1239) remained elevated by 74% on the last test day. This animal had an initial threshold increase 100% above baseline levels (see Figure 7).

Figures 1 and 2. Daily average pre- and post-lesion frequency thresholds for rats (n = 8) receiving electrical stimulation every day after a lesion (Group 1). The arrows indicate application of the lesion following testing on Day 0.

Figures 3 and 4. Daily average pre- and post-lesion frequency thresholds for rats (n = 7) receiving electrical stimulation every second day after a lesion (Group 2). The arrows indicate application of the lesion following testing on Day 0.

Figures 5 and 6. Daily average pre- and post-lesion frequency thresholds for rats (n = 8) receiving electrical stimulation every fourth day after a lesion (Group 3). The arrows indicate application of the lesion following testing on Day 0.

Figure 1

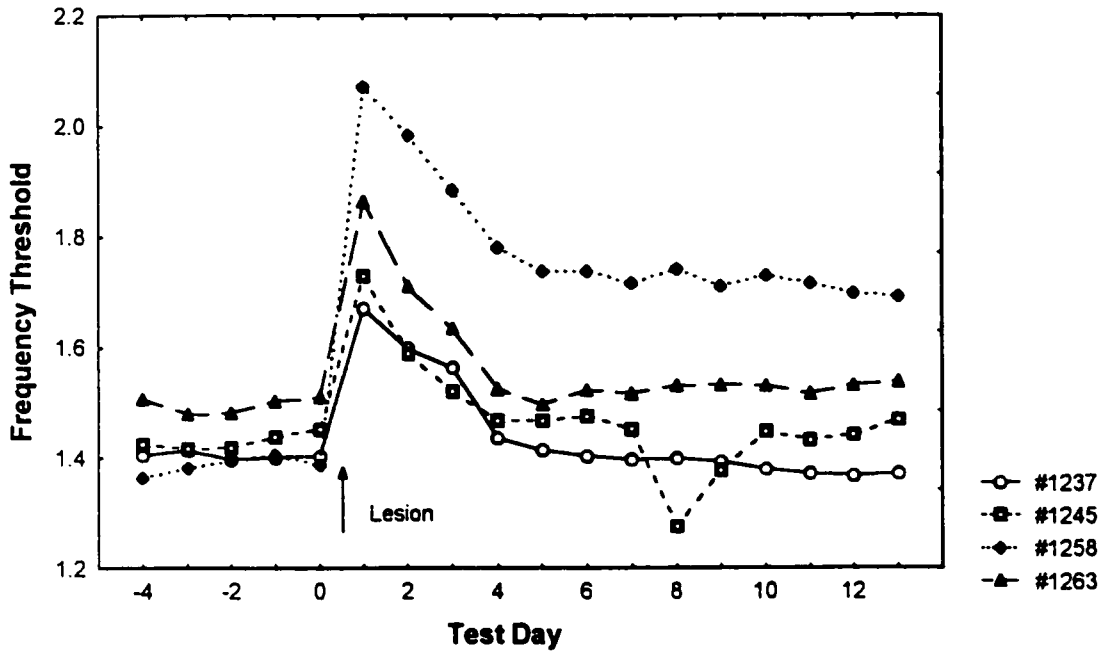


Figure 2

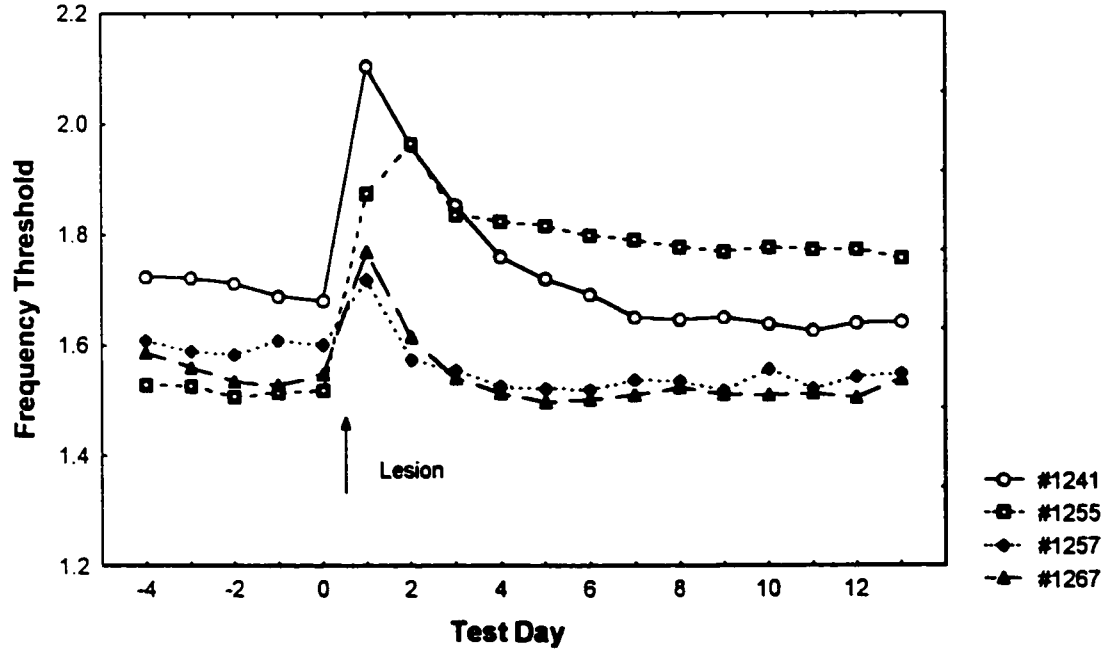


Figure 3

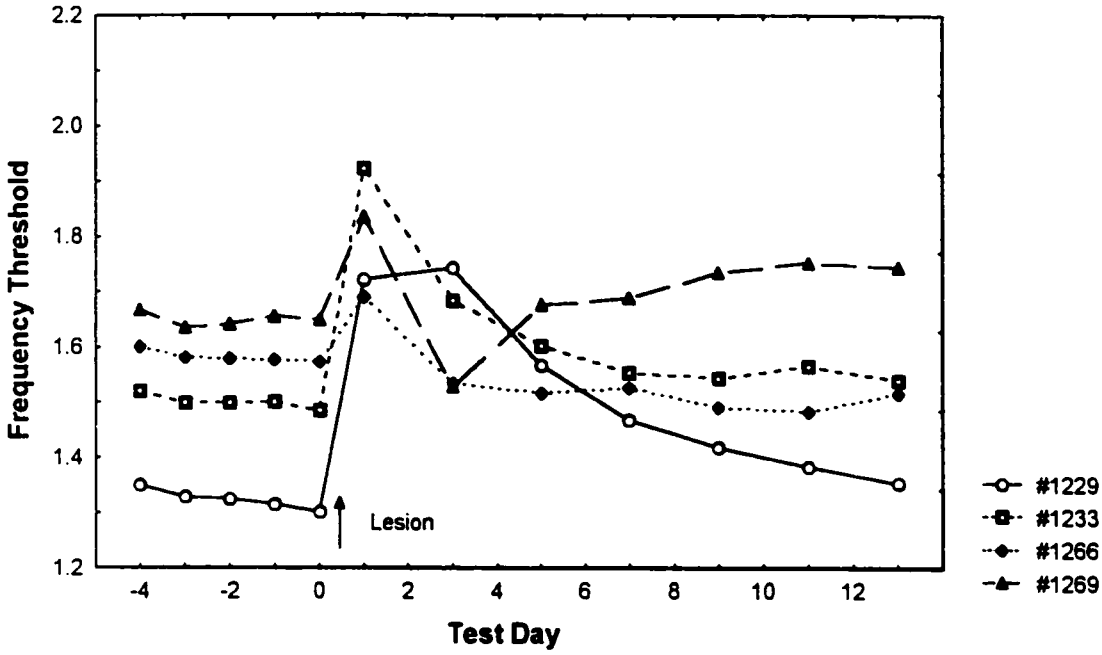


Figure 4

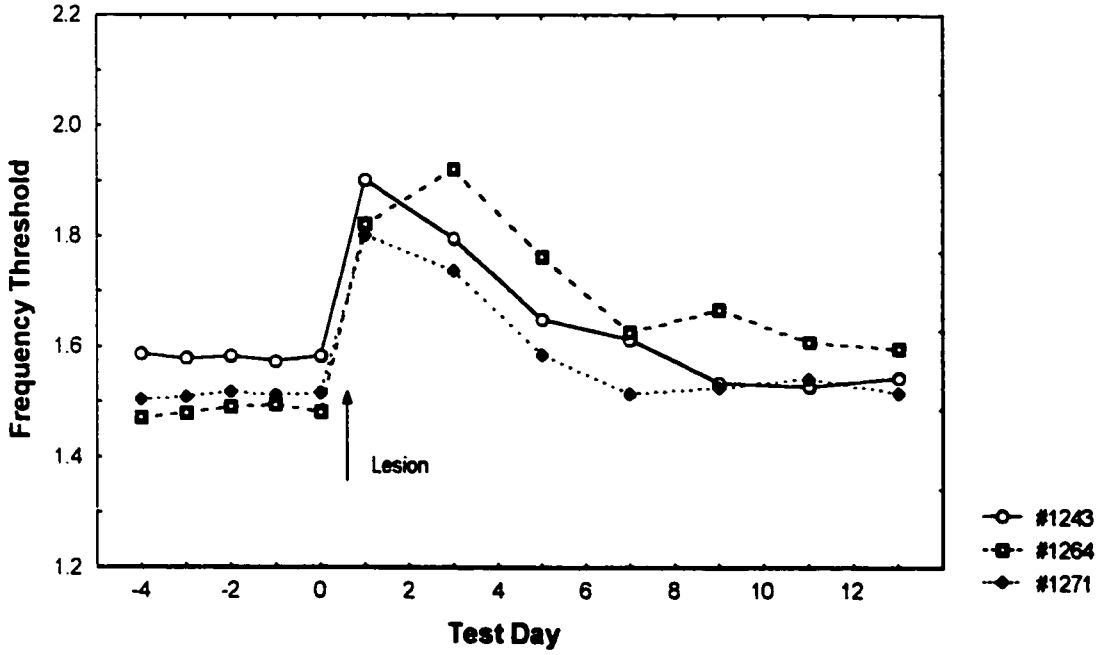


Figure 5

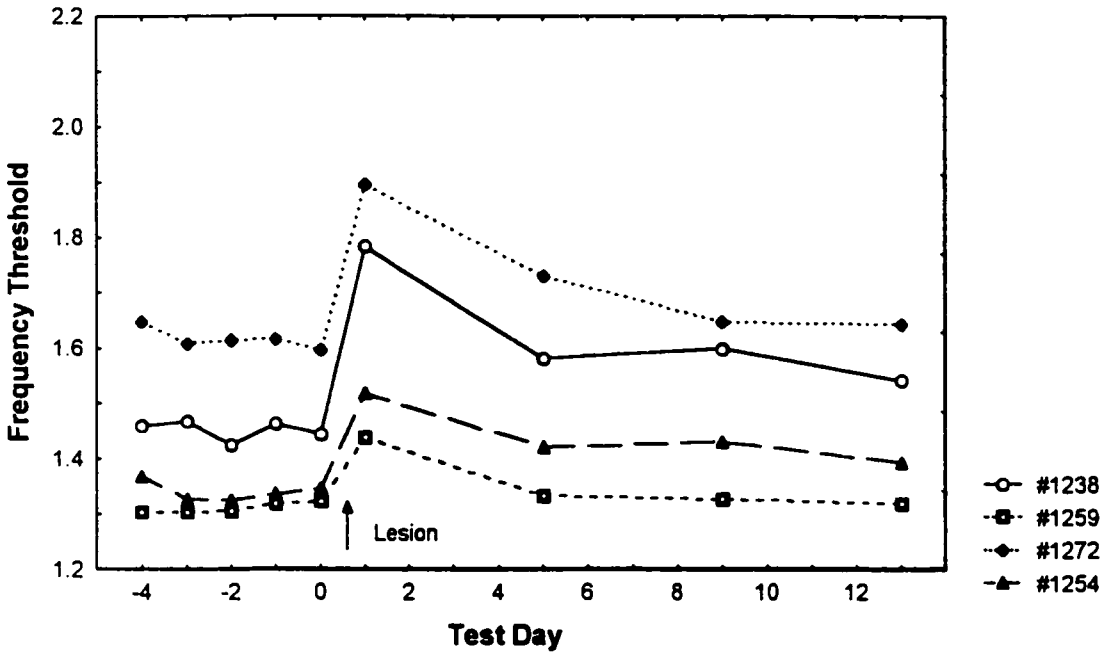


Figure 6

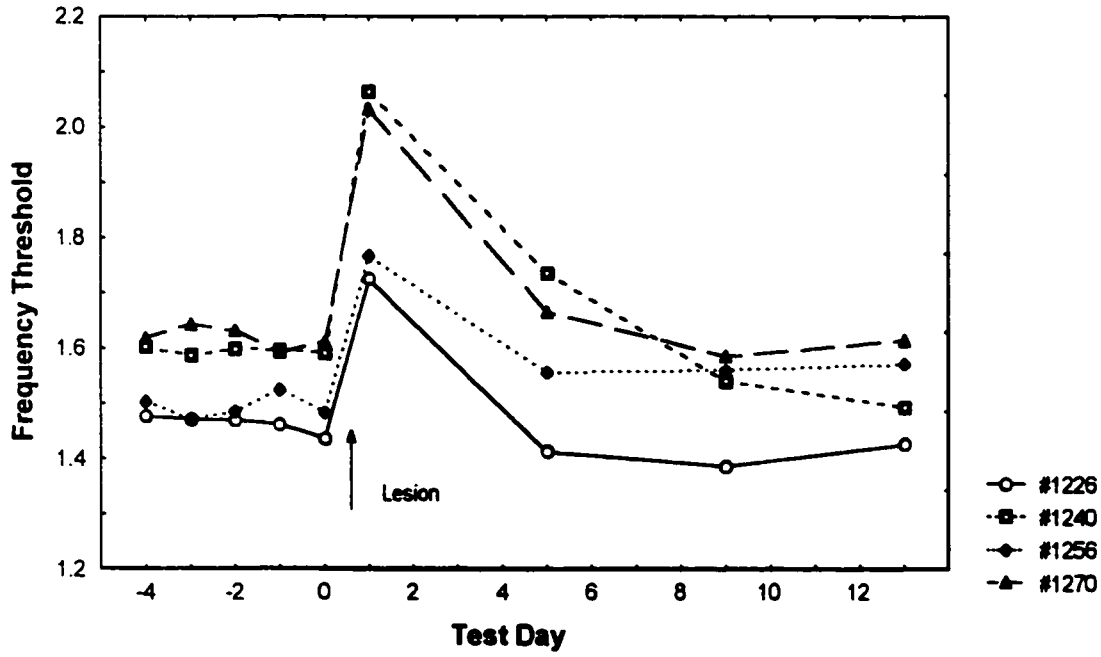


Figure 7

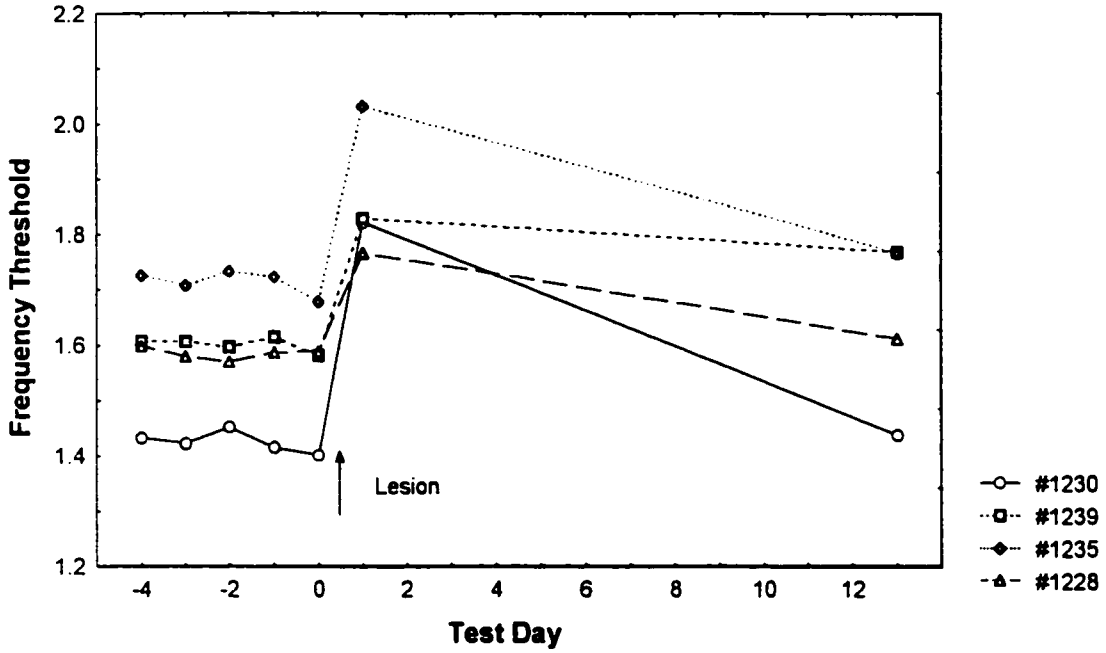


Figure 7. Daily average pre- and post-lesion frequency thresholds for 4 rats receiving electrical stimulation only twice after a lesion (Group 4). The arrow indicates application of the lesion following testing on Day 0.

To summarize, thresholds from rats in three of the four groups can be compared on post-lesion Days 1, 5, 9 and 13. Of the 23 rats, 16 had thresholds that recovered within five days of the lesion. For these rats, the lesion had increased thresholds 26% to 162% above pre-lesion levels. Three other rats recovered by Day 9. Thresholds for these animals had increased by 93%, 150% and 195% one day after the lesion. Two more rats recovered by Day 13, and had initial post-lesion threshold increases 118% and 120% above pre-lesion levels. Two other animals (with initial post-lesion increases 127% and 392% above baseline levels) had thresholds that remained elevated at the end of the test period.

Effect of the Stimulation

Data from Groups 1, 2 and 3 were included in a statistical analysis (N = 23). A within-subjects repeated measures analysis of variance (ANOVA) was performed on the dependent variable test day. The independent variable represented amount of stimulation offered per group over the test period (every day, every other day, and every fourth day). The ANOVA was conducted using STATISTICA.

As shown in Table 1, the ANOVA procedures determined a significant main effect of post-lesion test day, $F(2,47) = 60.59$, $p < .00$, but not of group, $F(2,20) = .52$, $p < .60$. There was no significant interaction of the two factors, $F(8,79) = .45$, $p < .89$.

Table 1

Analysis of Variance Summary Table

Source	df	SS	MS	F
Between Subjects	22	1.43		
Groups	2	0.07	0.04	0.52
Ss w/in groups	20	1.36	0.07	
Within Subjects	57	1.81		
Day	2**	1.35	0.34	60.59*
D x G	8	0.02	0.00	0.45
D x Ss w/in groups	47**	0.44	0.01	
Total	79	3.24		

*$p < .01$

** adjusted df (Greenhouse/Geisser)

Discussion

The statistical analysis of the data resulted in a significant main effect of test day. It is likely that this effect reflects the large differences between pre-lesion and post-lesion Day 1 thresholds across all of the groups. This result was expected, as pilot work showed that thresholds increase substantially and usually peak 24 hours after a lesion. Only those animals with a threshold increase above baseline levels of at least 0.1 $\log_{10}$ units were subsequently tested.

Differences in recovery that are due to the amount of stimulation received would likely be indicated by a significant interaction of a particular group on a specific post-lesion test day. However, results of the analysis fail to show an interaction of the variables; group means did not differ significantly on any of the test days following the lesion.

The analysis also did not indicate a significant main effect of group. Along with the absence of an interaction between any group on any post-lesion test day, this result likely reflects the fact that mean group thresholds were similar before and after the lesions were made. Each of the groups was made up of animals with low, medium and high pre-lesion thresholds. Those baseline thresholds were compared to thresholds collected on post-lesion Day 1 to determine the functional size of the lesions. Rats with small, medium and large functional lesion sizes were then evenly dispersed among the groups.

Results of the analysis, then, do not indicate an influence of amount of electrical stimulation on the recovery of reward thresholds following a lesion. Regardless of the amount of stimulation received, all of the animals showed the same post-lesion pattern, with initial threshold elevations immediately following a lesion and subsequent decreases

over time. Thresholds recovered within five days of the lesion in 16 of the 23 rats, with full recovery observed in 19 of the animals within nine days. Thus, for most of the rats, a time course of about one week was observed for reward threshold recovery.

Although it appears that differences in the extent of threshold recovery are not due to the amount of stimulation received, it is less clear why a few of the rats did not recover to pre-lesion threshold levels by the end of the two-week test period. As it seems possible that recovery from a large lesion effect may take longer than from a smaller lesion effect, we examined the data to see whether a potential relationship exists between recovery and functional lesion size.

For the 23 animals in Groups 1, 2 and 3, six had functional lesion sizes that were considered small, six were medium, and eleven were large. All of the rats with small and medium lesion sizes had recovered within about one week of the lesion. Seven of the animals with large lesion effects also recovered within this amount of time, and two others recovered within two weeks of the lesion. Two rats with large lesion effects had thresholds that remained elevated at the end of two weeks.

The fact that only those rats with large lesion effects did not recover by the end of the test period could lead to the natural conclusion that recovery may require a longer period of time in animals with large functional lesion sizes. However, that explanation cannot account for the fact that 7 of the 11 animals with large lesion sizes recovered in about one week. As well, the threshold for one rat in Group 4 also remained elevated at the end of the test. This animal had a medium functional lesion size.

A sustained threshold elevation is generally assumed to indicate permanent damage to the fibers that support self-stimulation. However, that assumption was not confirmed by a histological examination of the lesion site. As shown in Appendix B, a thinning of cells is evident in some, but not all of the rats, regardless of the size of the functional lesion. For the seven rats with small functional lesions (Figure B-6), fiber thinning is apparent in two animals (#1254 and #1257). Both animals recovered fully by post-lesion Day 5. For the seven rats with medium functional lesions (Figure B-7), a thinning of cells is seen in three cases (#1237, #1267, and #1271). Thresholds for all three animals recovered completely by Day 5. For the 13 rats with large functional lesions (Figure B-8), cell thinning is clear in six animals. Recovery was complete for four of these rats by post-lesion Day 5 (#1238 and #1270) or Day 9 (#1229 and #1264).

Although considerable recovery was seen in the other two rats (#1255 and #1258) in the week following the lesion, thresholds of these rats remained elevated above baseline levels two weeks after the lesions were made. To see whether or not the thresholds would remain high over time, we continued testing these rats for three more months. The threshold for #1258 was elevated 104% over pre-lesion levels at the start of long-term testing. As little further decrease was seen over the next three months, it seems likely that the lesion caused permanent fiber damage in this animal. A histological examination of the lesion site showed a thinning of fibers near the tip of the electrode track (Figure B-8). In contrast, #1255 showed a gradual recovery to baseline levels, three months after the lesion was made. The threshold for this animal was 73% higher than pre-lesion levels at the start of long-term testing. For this rat, then, it seems that fibers relevant to self-stimulation were

only temporarily affected by the lesion. However, what appears to be a thinning of cells near the electrode track is evident in an examination of the lesion site (Figure B-8). If that thinning represents permanent fiber damage to reward-relevant fibers, why this rat's threshold recovered over time is unclear.

These conflicting results might be explained in part by the fact that, although the functional size of the lesion was large in both animals, it was considerably larger in #1258, with a functional loss of 94% of baseline reward effectiveness noted on post-lesion Day 1, as compared to a deficit of 56% for #1255. The greater deficit seen in #1258 -- the only rat with a threshold increase of this magnitude -- may have resulted in the fact that this animal's recovery was not complete. It seems likely that the rewarding effectiveness of the stimulation is permanently attenuated following a lesion that induces a very large functional deficit.

It is not clear why lesions made using identical parameters can result in very different functional deficits between animals. We considered the possibility that these variations might be related to differences in the functional location of the stimulating/lesion electrode, which is inferred from each animal's pre-lesion threshold. The average pre-lesion threshold for #1258 (considered low) was more than 0.10 $\log_{10}$ units lower than that of #1255 (considered high). A lower pre-lesion threshold indicates that the tip of the stimulating electrode -- and therefore the lesion -- is more centered on neurons that support self-stimulation. With such a central location, the lesion is likely to affect an area of more densely packed fibers, potentially damaging more fibers within the core of the stimulation field and having less of an effect on the more sparsely populated fibers in the

periphery of the field. Conversely, a less centrally located lesion would probably affect more fibers at the periphery of the distribution of densely packed fibers, thus sparing more of those fibers. In this way, it seems possible that the lesion may have damaged more of the reward relevant fibers in #1258 and caused a permanent threshold increase.

However, a Pearson product-moment correlation does not indicate a significant association between the functional location of the electrode and the functional size of the lesion. Across all of the animals, a correlation coefficient of -0.181 was determined. This value was not significant at $p < .05$ (one-tailed test; $df = 21$).

Thus it seems that the functional deficit produced by a lesion is not well predicted by the functional location of the stimulating/lesion electrode. Given these results, despite long-term test results indicating that the lesion caused permanent damage to reward fibers for #1258, why this animal -- and only this animal -- sustained a permanent threshold increase remains unclear.

This study examined the transient effect of lesions on thresholds for self-stimulation and determined a general time course for the recovery of rewarding effectiveness of the stimulation following a small radiofrequency lesion. For most of the animals, thresholds returned to pre-lesion levels within about one week of the lesion. As the recovery occurred at a similar rate in each group of animals, regardless of the amount of stimulation received, it seems clear that the electrical stimulation itself does not influence the speed or degree of reward threshold recovery.

It is not clear whether the post-lesion recovery observed in this study occurred because of axonal regeneration that led to functional recovery. Following damage to

axons, the regenerating fibers must reconnect to appropriate target receptors for recovery of function to take place. Although a few studies have noted axonal regeneration following central nervous system injury, most have not determined whether that fiber regrowth led to functional recovery. Foerster (1982) noted substantial axonal regeneration following MFB knife cuts, with fibers beginning to grow around lesion sites three days later. In some cases, the regrowth had extended a few hundred micrometers 23 days later. However, the ultimate destinations of the fiber growth were not determined in this study. For successful nerve regeneration, it is believed that axons have to extend for distances of millimeters to centimeters (Forman et al., 1980; McQuarrie & Grafstein, 1981; Smith & Skene, 1997). Björkland and Lindvall (1978) noted initial axonal sprouting in the hypothalamus five days after chemical lesions were made, with proliferation of the sprouts evident during the next two weeks. However, the forward growth that lead to partial restoration of the original fiber paths and terminal networks was not seen until three to six months post-lesion. In one study that reported substantial central nervous system axonal regrowth that led to recovery of function in adult mammals, the functional recovery of a muscle reflex was first apparent 56 to 100 days after spinal cord hemisection (Borgens et al., 1987).

Although axonal regeneration in the central nervous system may result in appropriate re-connections that enable recovery of function, the time course for the rate of regrowth noted in the above studies is at odds with the results of this study. Functional recovery was complete in most of the rats in this study within about one week of the lesion. Although regeneration begins within hours of axotomy, the first signs of sprouting of

lesioned axons are not evident for a few days. The regrowth then proliferates over the following weeks. To account for the recovery of function seen in this study, the lesioned axons would have had to rapidly regrow and make functional synaptic connections within days of the lesions.

Although axonal regeneration remains a possible explanation for the recovery of thresholds following a lesion, it seems important to investigate other mechanism(s) that may underlie the recovery, as an understanding of the transient effects of lesions on reward thresholds could help to clarify results of self-stimulation/lesion studies that attempt to map pathways important to reward.

Experiment 2: Denervation Supersensitivity and Recovery of Reward Thresholds

In Experiment 2 we tested the possibility that an enhanced dopamine receptor supersensitivity or another compensation occurs away from the site of stimulation and underlies the recovery of reward thresholds. We also investigated the possibility that the recovery may instead occur regionally.

Introduction

Denervation Supersensitivity and Compensatory Mechanisms

When an axon is severed, receptors deprived of normal fiber input can compensate for the deafferentation by becoming more sensitive. When those supersensitive receptors are stimulated, an enhanced neural response occurs. It has been suggested that the development of a denervation supersensitivity may account for the recovery of reward thresholds following a lesion (Gallistel et al., 1996).

Much of the research investigating denervation supersensitivity has used selective chemical lesions to denervate nigrostriatal pathways and deplete dopamine levels on one side of the brain. When a lesion unilaterally denervates this pathway, rats predictably show an asymmetrical behavioural deficit, such as a turning or circling toward the lesioned side. This ipsilateral rotation is believed to indicate the dominance of the undamaged hemisphere, as the turning behaviour is reinforced in response to dopamine-releasing drugs like amphetamine.

Animals with more severe lesions can recover from the behavioural asymmetry within one to three weeks (Fornaguera et al., 1994; Schwarting et al., 1991). This recovery

occurs because postsynaptic receptors on the lesioned side have become supersensitive, a development that appears within 10 days of a lesion (Zigmond et al., 1990).

Behavioural recovery occurs within two weeks following more moderate lesions that do not lead to the development of postsynaptic receptor supersensitivity (Fornaguera et al., 1993; 1994; Schwarting & Huston, 1997). The behavioural recovery accompanies the normalization of extracellular dopamine in the lesioned striatum. The presynaptic regulatory changes that lead to the normalization include increases in dopamine biosynthesis, metabolism and release (Casteñeda, Whishaw & Robinson, 1990; Robinson & Whishaw, 1988; Robinson et al., 1994).

Apomorphine, a direct dopamine receptor agonist, has been used to determine the presence of receptor supersensitivity following moderate to severe lesions. If changes in receptor sensitivity have occurred, apomorphine's direct stimulation will induce behavioural asymmetries on the lesioned side. Following recovery from moderate to severe lesions, the drug-induced asymmetries reflect an enhanced sensitivity of postsynaptic receptors (Ungerstedt, 1971). In animals that have recovered from smaller lesions, apomorphine induces asymmetries that are believed to reflect an up-regulation (Pucak & Grace, 1991) or changes in the function or sensitivity (Fornaguera et al., 1993) of presynaptic autoreceptors that control the release of dopamine.

Apomorphine and Self-Stimulation

Dopamine and the mesolimbic dopaminergic system are strongly implicated in the rewarding effects of electrical brain stimulation. One component of this system, the ventral tegmental area, is the source of ascending dopaminergic fibers that terminate in the

nucleus accumbens and other limbic/cortical areas. Stimulation of rewarding sites in the ventral tegmental area cause enhanced dopamine release in the nucleus accumbens (Fiorino et al., 1993). Autoradiographic mapping studies (Gallistel, 1983; Yadin, Guarini & Gallistel, 1983) have shown that the ventral tegmental area is activated during self-stimulation of either the anterior or posterior MFB. Pharmacological studies further support the involvement of dopamine in MFB self-stimulation. With its dopamine-releasing effect, amphetamine will decrease an animal's reward threshold, while selective dopamine receptor blockers pimozide and (+)-butaclamol attenuate the stimulation's rewarding effect (Fouriezos, Hansson & Wise, 1978). Together, the above findings indicate an important role for dopamine and the mesolimbic pathway in the process of brain stimulation reward.

Apomorphine has been used in studies that examine the role of dopamine in reward produced by electrical stimulation of various areas of the MFB. Fouriezos and Francis (1992) showed that the influence of apomorphine on self-stimulation depends on time and dose. Self-stimulation thresholds were recorded over a two-hour period following injection of a range of doses of apomorphine. At low doses (<0.1 mg/kg) the rewarding effect of brain stimulation was decreased. The authors suggest that the inhibition of reward may be due to a reduced presynaptic level of dopamine following the activation of presynaptic autoreceptors. With higher doses (0.3 and 1.0 mg/kg), thresholds initially increased (presynaptic effect), then dropped below (pre-injection) baseline levels, indicating a facilitation of the reinforcing effect of the stimulation that is thought to be due to apomorphine's direct agonism of postsynaptic receptors.

Fouriezos and Francis (1992) examined the effect of apomorphine on self-stimulation in intact animals. In the present study, we used apomorphine to determine whether an enhanced sensitivity develops in residual neurons spared by a unilateral lesion. If so, this change in receptor sensitivity might explain the post-lesion recovery of the stimulation's rewarding effectiveness. To examine this possibility, apomorphine was given to rats implanted with stimulating electrodes in both lateral hypothalami so that thresholds could be measured at both sites. As drug effects are theoretically the same at both sites, the bilateral preparation allows within-animals comparisons to be made. We measured thresholds daily at both electrodes, before and after a lesion was made through one of the electrodes. To see whether changes in receptor sensitivity had developed, apomorphine (0.5 mg/kg) was injected after thresholds had recovered to pre-lesion levels. We expected the systemic effect of apomorphine to enhance the stimulation's rewarding effect at both electrode sites. However, if receptors on the lesioned side had become supersensitive, we expected that apomorphine would affect thresholds at that site more than those at the intact electrode. As such, after recovery was complete, a larger threshold decrease at the lesioned site compared to that at the unlesioned electrode following apomorphine could indicate an enhanced receptor sensitivity that could account for the recovery of the stimulation's rewarding effectiveness.

Materials and Method

Six rats (#1236, #1246, #1318, #1319, #1324 and #1327) were implanted with bilateral electrodes in the LH (see Figure B-9 in Appendix B). The rats weighed between 325 and 415 g at the time of surgery. All were given Atropine sulfate (0.05 mg sc) to

control mucus secretions prior to being anaesthetized with sodium pentobarbitol (65 mg/kg ip). As four of the rats (#1236, #1318, #1324 and #1327) still reacted to tail pinch or noise, this standard dose was supplemented up to a maximum of 100 mg/kg. Bupivicaine, a topical anaesthetic, was applied to the incisions. Otherwise, the surgery, housing conditions, and histology were the same as described in Experiment 1. The same apparatus and training methods were also used.

Procedure

There were three procedural differences between Experiments 1 and 2, all involving the bilateral implants. The first difference was that we adjusted the currents before baseline testing so that the thresholds at both electrodes using single pulses were matched (within 0.1 $\log_{10}$ units).

The second procedural difference was that, along with sweeps of single pulses, sets of double pulses were offered during baseline testing, so that we could obtain summation estimates between the two electrodes. Daily baseline tests were made up of 18 stimulation sweeps: seven sweeps of single stimulation pulse trains at each electrode, and four sets of trains of double pulses between the electrodes. The first pulse train of each train of double pulses was alternated between electrodes, with an interval of 5.0 ms separating each train. Thus, single-pulse thresholds at both electrodes were obtained separately from thresholds for double-pulse stimulation and recorded daily throughout baseline testing.

The third difference between Experiments 1 and 2 was that electrical stimulation was applied in alternating sweeps to allow thresholds at both electrodes to be recorded. When thresholds at both electrodes were stable, a radiofrequency lesion of 1.4 s duration was

applied through one of the stimulating electrodes. Testing resumed 24 hours later and thresholds for single pulse stimulation at both electrodes were recorded daily for 15 days following the lesion. For all non-injection test, the pulse frequencies decreased by 0.05 $\log_{10}$ steps every 10 seconds. The behavioural criterion was a constant cut level of five stimulations. On injection test days, pulse frequencies were decreased in 0.10 $\log_{10}$ steps.

Injection Tests

Each of four rats (#1318, #1319, #1324 and #1327) received an intraperitoneal injection of either Apomorphine HCl dissolved at a concentration of 0.5 mg/ml in a vehicle of ascorbic acid, or the ascorbic acid vehicle (0.5 mg/ml) on four separate test days, before and after the lesion was made. For each animal, the order of injection (drug or vehicle) was determined with a toss of a coin. Thus, apomorphine doses of 0.5 mg/kg were injected twice: once four or seven days before the lesion was made (pre-lesion injection) and again 10 or 13 days after the lesion (post-lesion injection). Immediately following each apomorphine or vehicle injection, thresholds at both electrodes were recorded for one hour. Each injection test day was followed by at least two non-injection test days.

Results

Effects of Pre-Lesion Apomorphine

Following the pre-lesion injection of apomorphine, thresholds at both electrodes increased for approximately five minutes for all four of the rats. Stereotypy, a repetitive behavioural disruption induced by higher apomorphine doses, was evident in three of the animals. When responding resumed, thresholds decreased to below pre-injection levels. At their lowest points, thresholds were 92% (#1318), 50% (#1327) and 42% (#1319)

below the previous day's averages. Overall, these thresholds remained low for about 20 minutes before gradually returning to pre-injection levels by the end of the test hour. Thresholds for the other rat (#1324) could not be calculated for 15 minutes following the initial threshold increase immediately after injection, as the rat continued responding to progressively weaker stimulation trains, at which point the program reset to begin another sweep. When thresholds at both electrodes could again be recorded they were as much as 135% lower than those recorded the previous day, until they returned to pre-injection levels by the end of the test period.

The pre-lesion injection data, then, demonstrate the influence of a higher dose of apomorphine on response for stimulation, with initial inhibitory effects followed by a facilitation of the stimulation's rewarding effect, as indicated by decreased thresholds at both electrodes in all four rats (see Figures 8 to 11).

Figures 8 to 11. Pre-lesion effects of apomorphine (0.5 mg/kg) on frequency thresholds at bilateral electrodes. The arrows indicate the time of injection (0 minutes). The number in the top left corner identifies the subject.

Figure 8

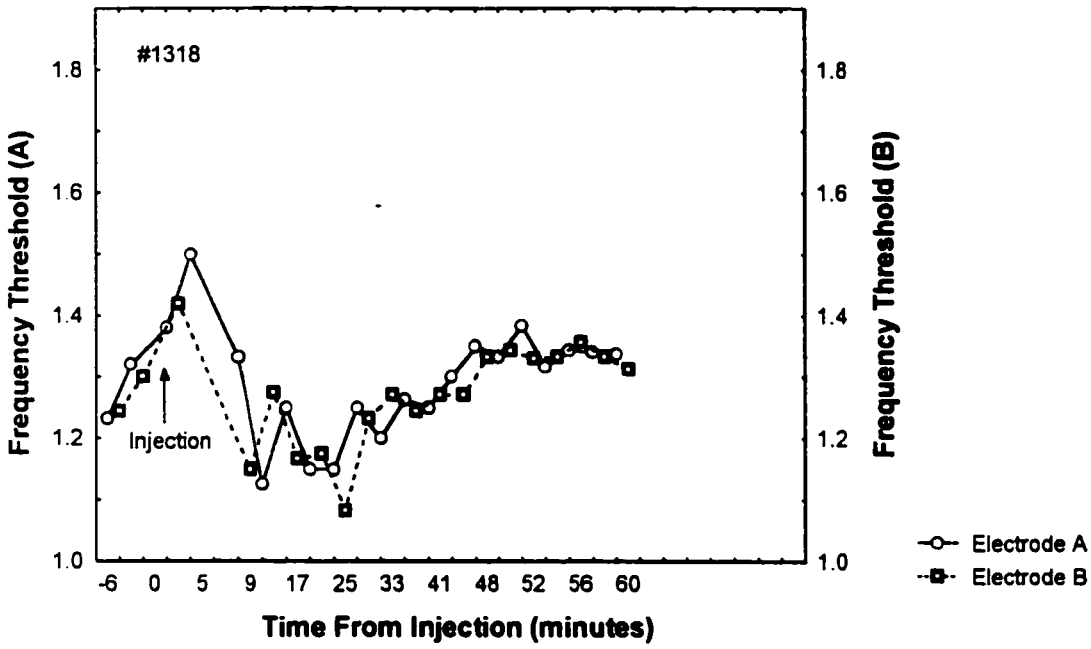


Figure 9

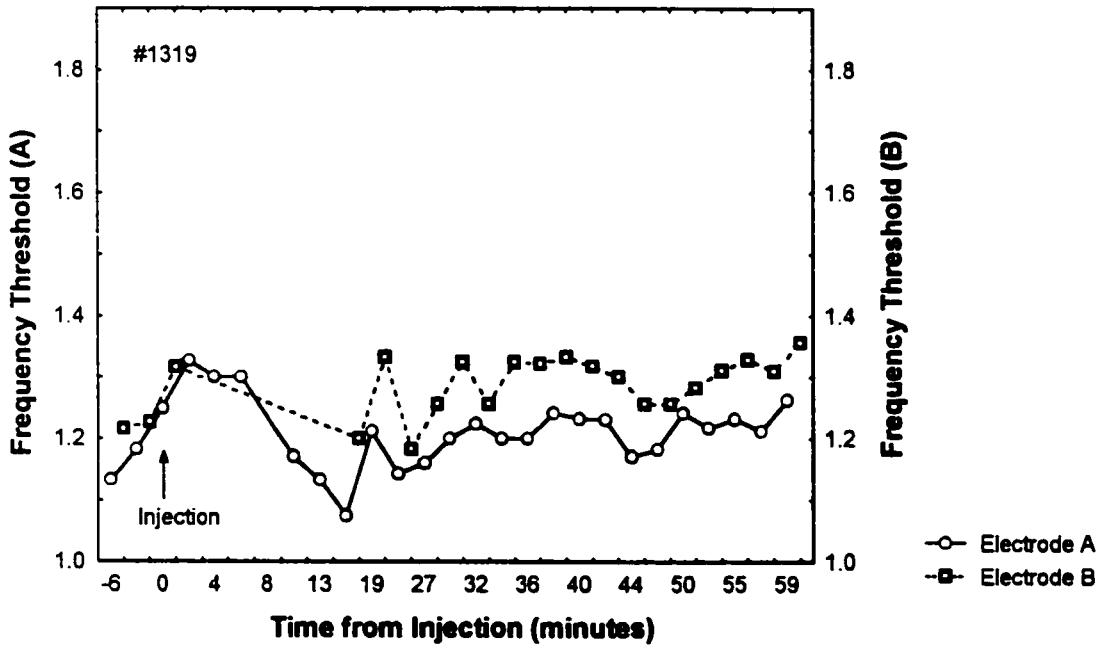


Figure 10

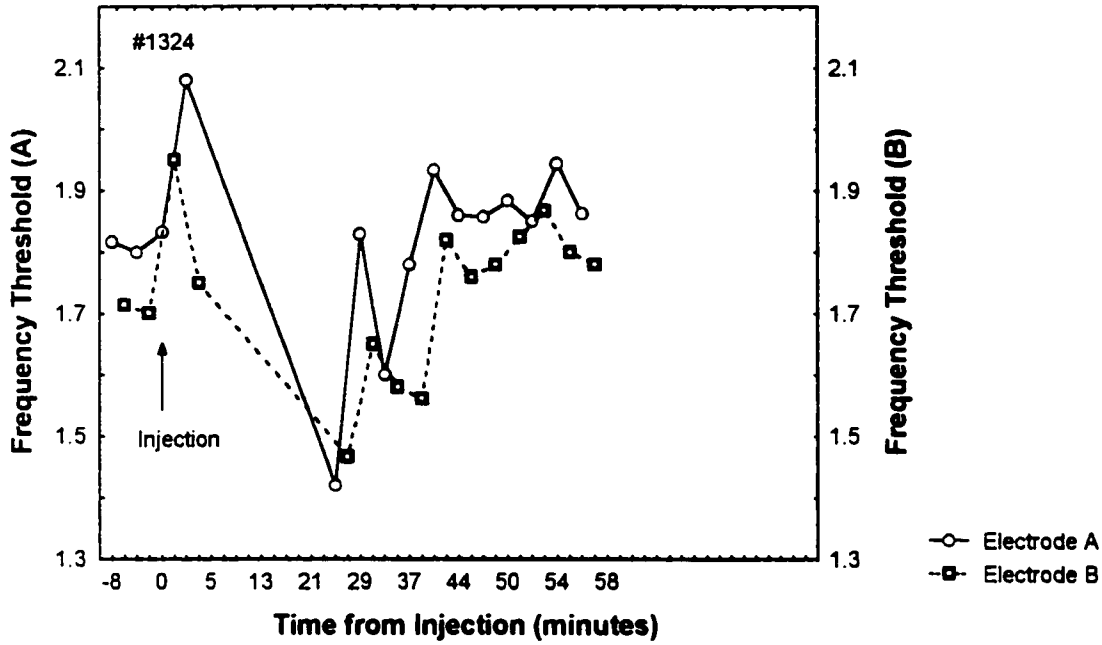
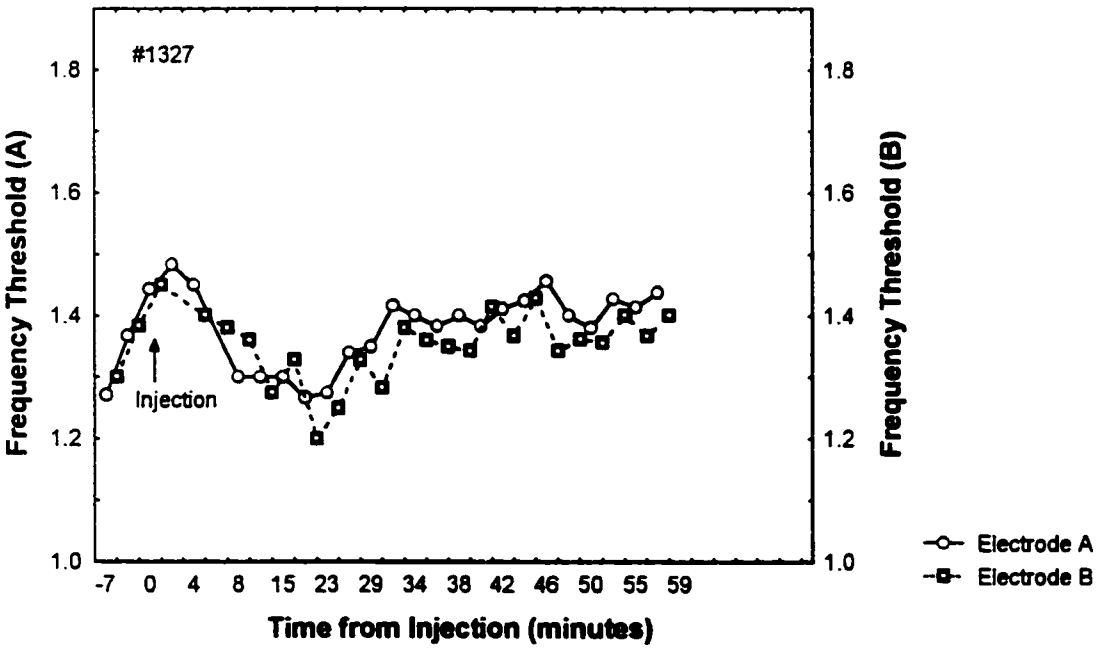


Figure 11



Effect of Lesion on Thresholds at Bilateral Sites

Lesioned Sites: At both electrodes thresholds of six rats were tracked for about two weeks after lesions were made. For all of the animals thresholds at the lesioned sites increased between 0.32 to 0.5 $\log_{10}$ units over baseline levels by the second post-lesion day. For five of the rats thresholds decreased to within 0.1 $\log_{10}$ units of pre-lesion levels by the fifth day after the lesion, where they remained for the duration of the testing period. For the other rat (#1246) thresholds at the lesioned electrode decreased to within 0.1 $\log_{10}$ units by the eleventh post-lesion day.

Intact sites: Thresholds at the unlesioned sites did not change from pre-lesion levels for four of the six rats. Rats #1318 and #1236 showed slight threshold increases (less than 0.1 $\log_{10}$ units) at the unlesioned electrode after thresholds at the lesioned sites had recovered. The thresholds decreased again to pre-lesion levels by post-lesion Day 8 for #1236, while thresholds remained elevated for the rest of the test period for #1318 (see Figures 12 to 17).

Figures 12 and 13. Pre- and post-lesion frequency thresholds at lesioned and unlesioned electrodes. The circles joined by solid lines represent daily average thresholds at the lesioned electrode, while the squares joined by dotted lines represent daily average thresholds at the unlesioned electrode. The arrows indicate application of the lesion following testing on Day 0. The number in the top left corner identifies the subject.

Figure 12

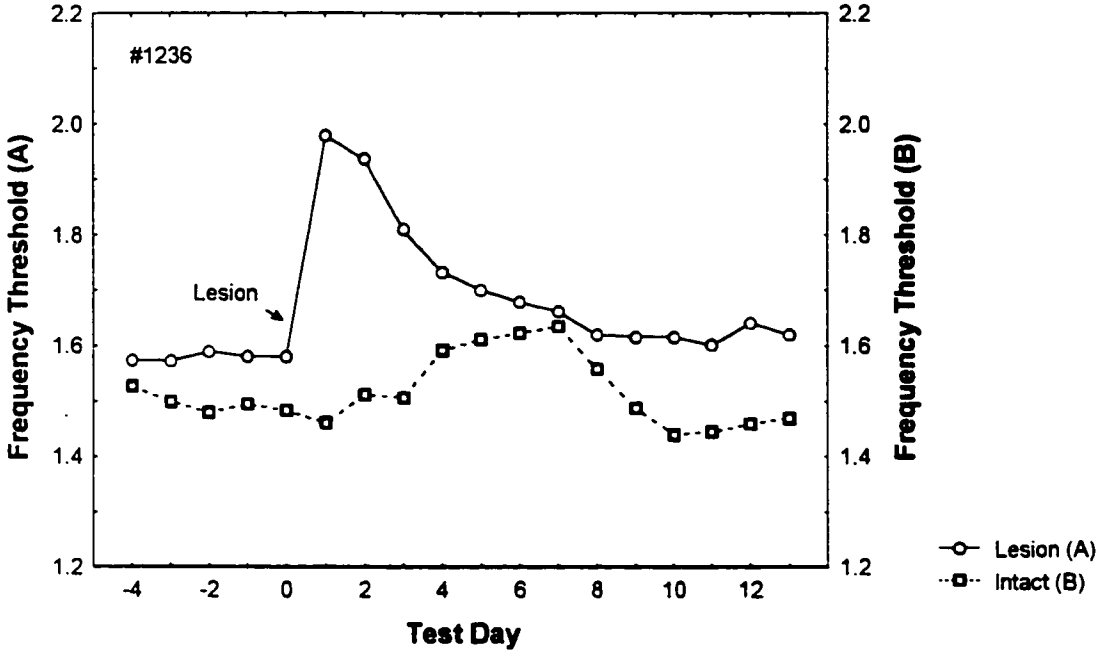
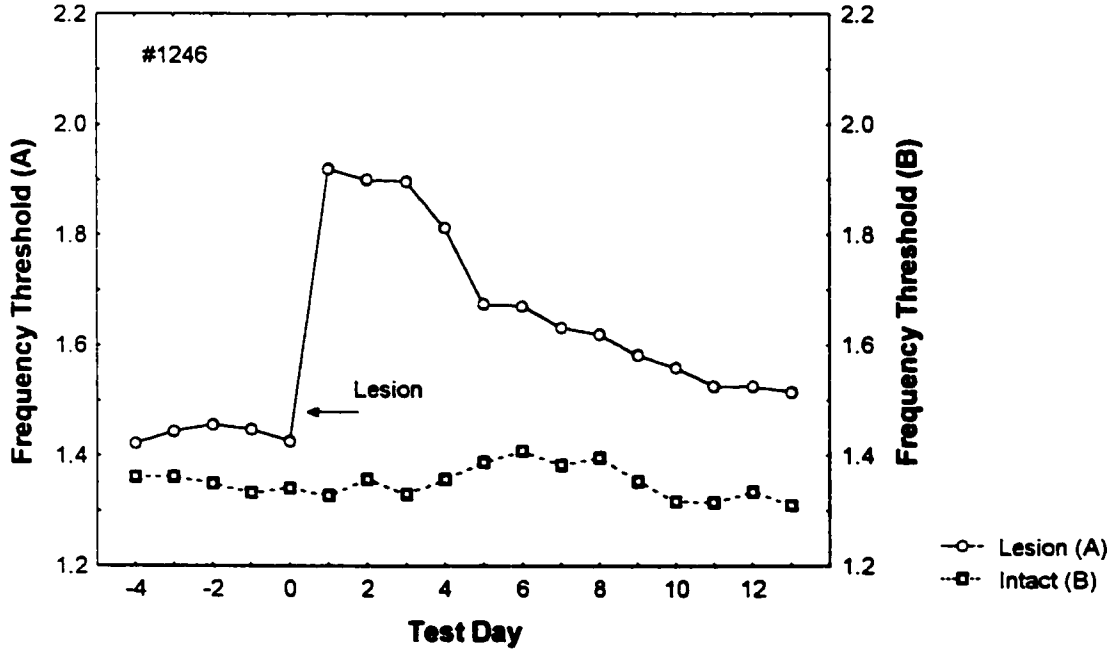


Figure 13



Figures 14 to 17. Pre- and post-lesion frequency thresholds at lesioned and unlesioned electrode sites on non-injection test days. The arrows indicate pre- and post-lesion apomorphine injection test days, and application of the lesion following testing on Day 0. The number in the top left corner identifies the subject. Circles joined by solid lines represent thresholds recorded at electrode A, while squares joined by dotted lines represent thresholds recorded at electrode B. Lesions were made through electrode A for rats #1319 and #1324, and through electrode B for rats #1318 and #1327.

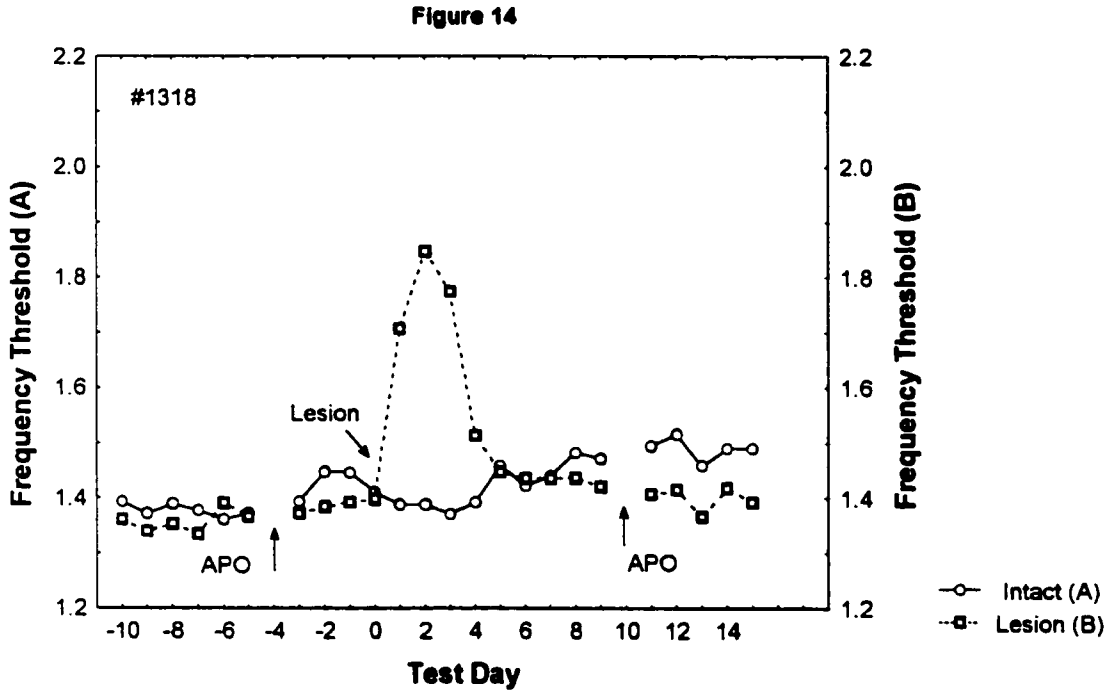


Figure 15

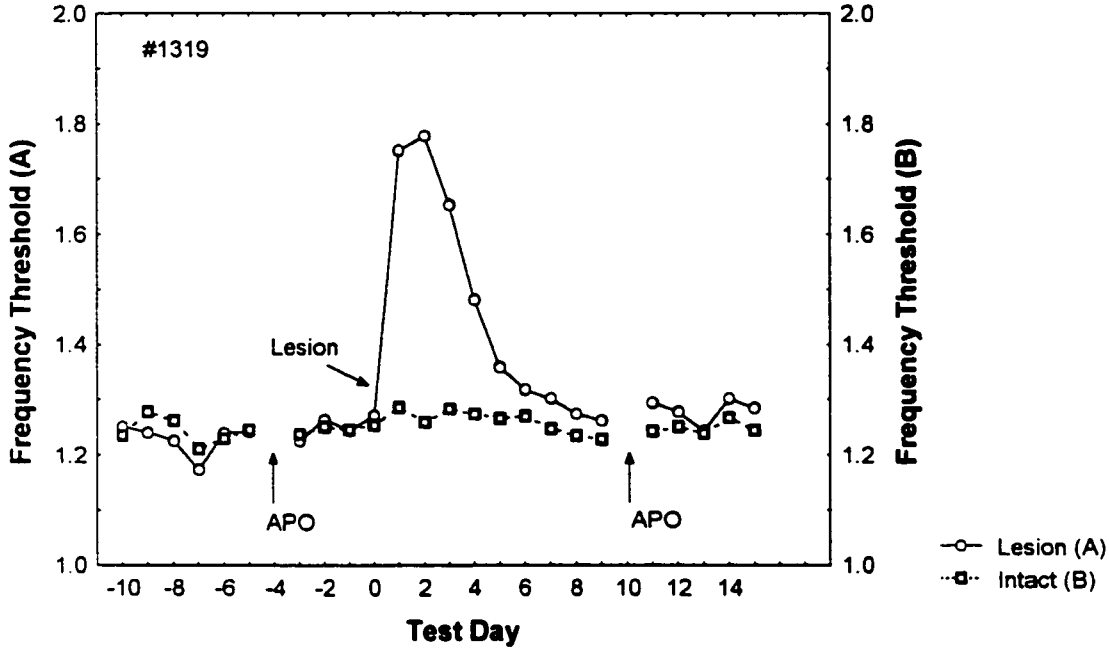


Figure 16

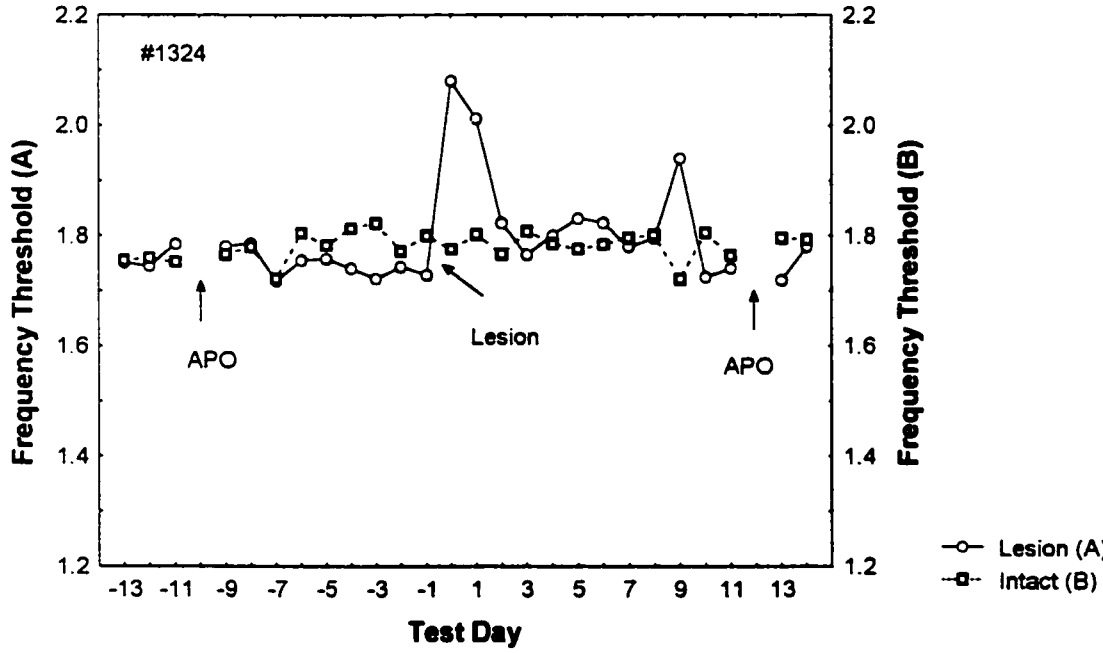
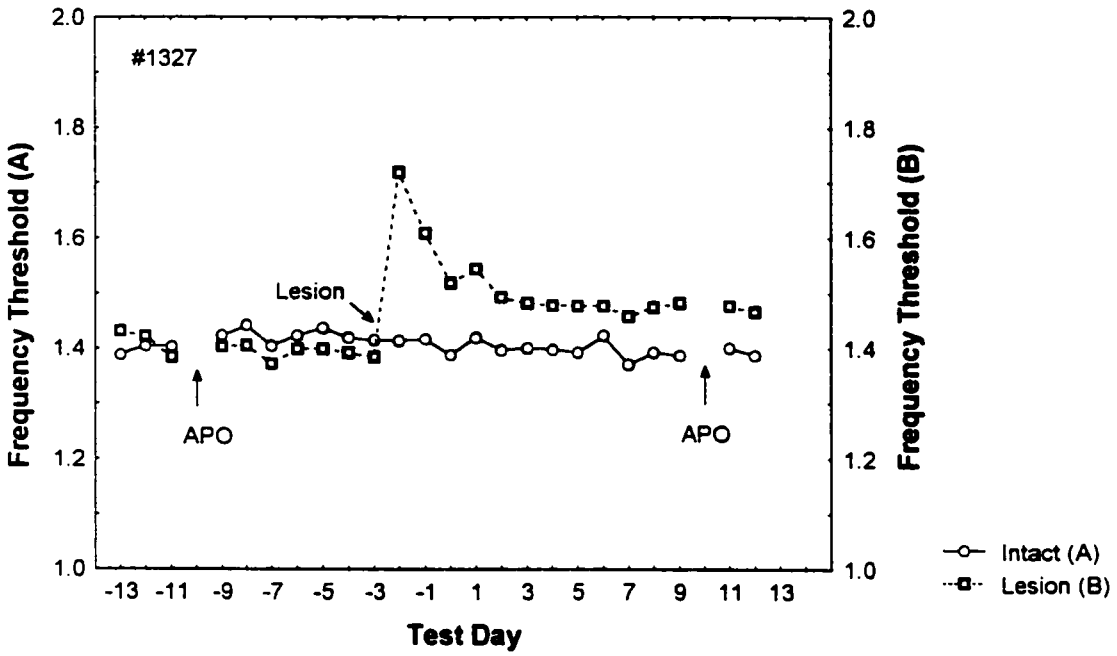


Figure 17



As shown in Table 2, results of repeated measures t tests indicate significant differences between thresholds measured for the first five post-lesion days at the unlesioned electrode and those measured at the lesioned electrode for five of the six rats.

Table 2

Comparison of Thresholds at Lesioned and Unlesioned Sites

Subject	t value
#1236	3.62*
#1246	8.80**
#1318	2.93*
#1319	4.15*
#1324	1.68
#1327	4.56*

*$p < .05$; one-tailed test; $df = 4$
**$p < .01$

Effects of Post-Lesion Apomorphine

To see whether a dopamine receptor supersensitivity had developed, the animals received a second injection of apomorphine 10 or 13 days after the lesion was made, once thresholds had returned to pre-lesion levels. Rat #1318 did not respond to stimulation at either electrode for 5 to 10 minutes following apomorphine. When responding began thresholds at both sites decreased slightly below pre-injection levels for the duration of the test. At most, thresholds at the unlesioned electrode were 13% lower than those recorded the previous day, while thresholds at the lesioned site decreased by 7% (see Figure 18).

Figure 18

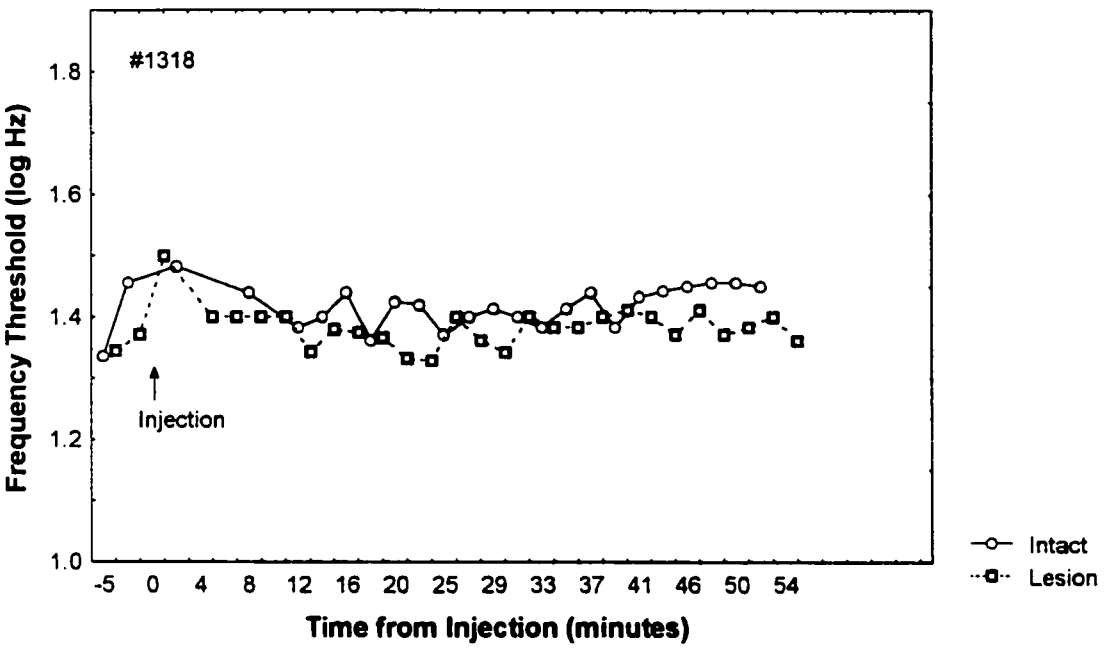


Figure 18. Post-lesion effects of apomorphine (0.5 mg/kg) on frequency thresholds at lesioned and unlesioned electrode sites for rat #1318. Circles joined by solid lines represent thresholds recorded at the unlesioned electrode, while squares joined by dotted lines represent thresholds recorded at the lesioned electrode. The arrow indicates the time of injection (0 minutes).

Following apomorphine, thresholds for two other animals (#1319 and #1327) did not decrease from the previous day's averages at either electrode. Stereotypy was evident throughout the first half of the test period, with no response to the stimulation at either electrode observed in either rat. When responding resumed thresholds similar to those recorded on the previous day were seen at both electrodes for these two animals (see Figures 19 and 20). For #1324 thresholds at the lesioned site decreased by up to 20% throughout the test hour, while thresholds at the unlesioned site did not on average differ from those recorded on the previous day (see Figure 21).

Figures 19 to 21. Post-lesion effects of apomorphine (0.5 mg/kg) on frequency thresholds at lesioned and unlesioned electrode sites. Circles joined by solid lines represent thresholds recorded at electrode A, while squares joined by dotted lines represent thresholds recorded at electrode B. The arrows indicate the time of injection (0 minutes). The number in the top left corner identifies the subject. Lesions were made through electrode A for rats #1319 and #1324, and through electrode B for rat #1327.

Figure 19

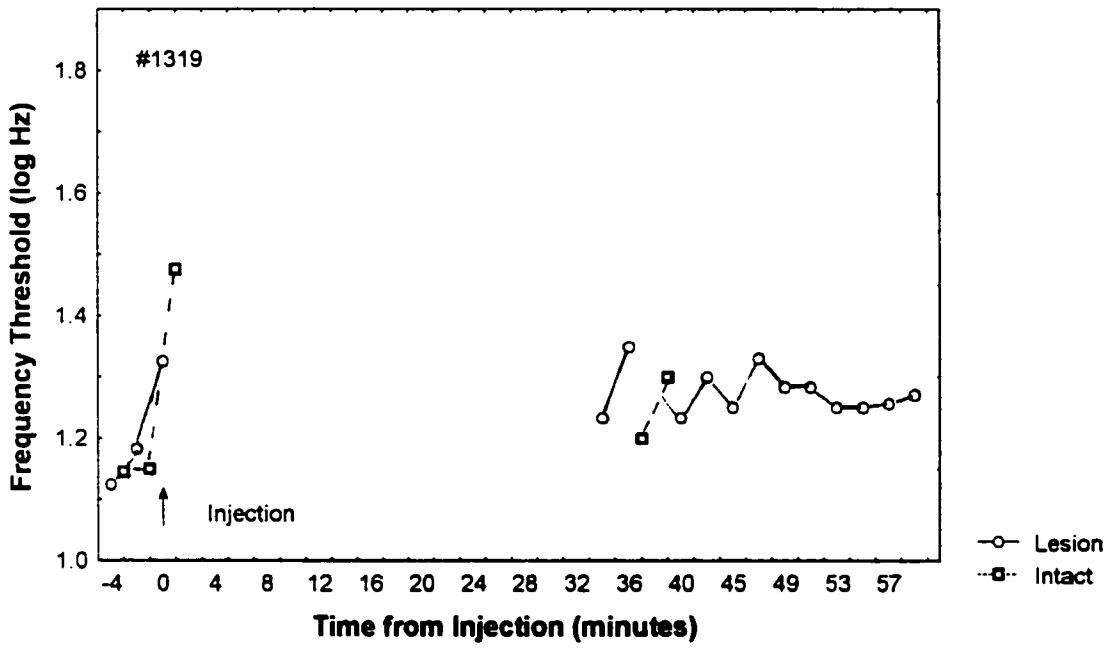


Figure 20

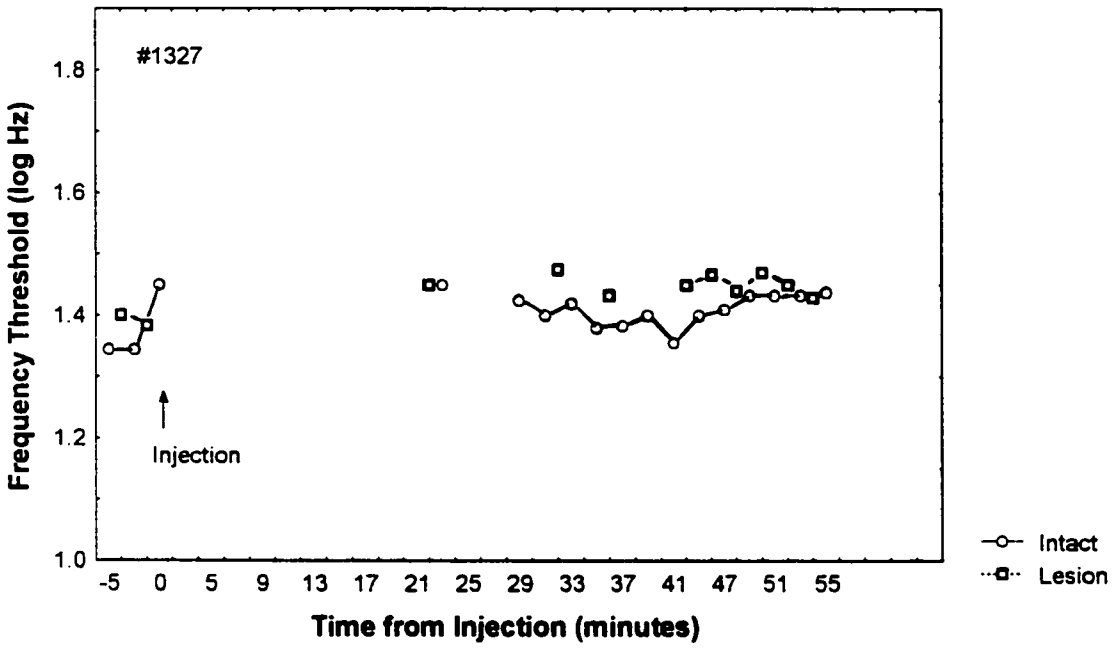
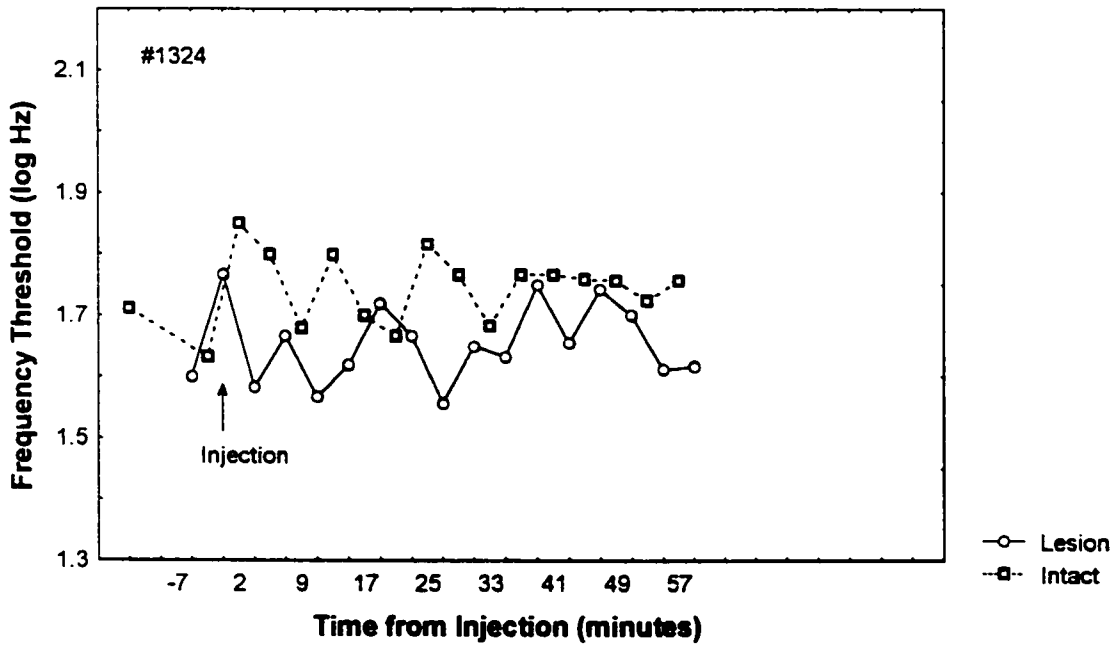


Figure 21



Discussion

Response to Apomorphine

The post-lesion apomorphine injection was given to detect the possible occurrence of a dopamine receptor supersensitivity that could account for the recovery of reward thresholds following a lesion. An enhanced receptor sensitivity could be indicated by substantially different threshold decreases between lesioned and unlesioned sites. However, three of the four animals responded similarly to the stimulation at both electrodes. It seems improbable that the small threshold difference between electrodes (at most a 20% decrease at one electrode compared to 10% at the other electrode) seen in the other rat (#1324) is due to the occurrence of denervation supersensitivity. This rat recovered from a lesion that elevated the threshold by 0.4 $\log_{10}$ units, a 151% increase over baseline average. With such a large lesion effect, apomorphine would have resulted in a big difference in thresholds between the lesioned and unlesioned sides, if the recovery had been due to denervation supersensitivity. Instead, the threshold difference was slight. Given these results, the post-lesion injection test data do not clearly indicate that apomorphine facilitated thresholds at lesioned sites more than those at unlesioned sites. Because the drug's effects were similar at both electrodes, we can conclude that an enhanced dopamine receptor sensitivity cannot account for the recovery seen in these animals.

Tracking Thresholds at Bilateral Sites

In support of this conclusion, data from the non-injection tests show that for four of six rats, thresholds at the unlesioned electrode remained unchanged as thresholds at the

lesioned electrode recovered after a lesion. This is particularly relevant given the possibility that the electrodes stimulate neurons in homologous structures.

If stimulation sites are directly linked or share common fibers, post-lesion effects at one site could also have effects at the other site. Collision studies have provided evidence of a direct anatomical and functional link between ipsilateral MFB self-stimulation sites (Bielajew & Shizgal, 1982; Bielajew, Thrasher & Fouriezos, 1987). Research using tracing methods (Pritzel, Huston & Buscher, 1983) and a number of lesion studies suggest that MFB reward neurons also send collaterals to the opposite hemisphere (Buscher et al., 1989; Colle & Wise, 1987; Huston, Grimm & Ornstein, 1984). A recent study (Malette & Miliaressis, 1995) tested this theory using moveable electrodes implanted in LH and ventral tegmental sites. Possible connectivity between the sites was explored by delivering pairs of pulses through the bilaterally placed electrodes. At shorter intervals between pulse pairs, elevated thresholds were seen with many of the electrode placements, indicating a collision-like effect between those sites. Although it was not concluded that direct anatomical links exist for those sites, the authors suggest that for each bilateral placement, the two electrodes may have excited branches of the same neuron. In over 60% of the contralateral sites tested, indications of interhemispheric MFB connections were detected, further supporting the view that MFB reward neurons branch contralaterally.

The paired-pulse stimulation technique can also be used to estimate summation, a measure of the combined rewarding effect of double pulses. Summation has been demonstrated between a number of contralateral LH stimulation sites (Bielajew & Shizgal, 1980; 1986; Shizgal et al., 1980; Malette & Miliaressis, 1995; Walker & Fouriezos,

1995), indicating that the stimulation excites branches of neurons that converge at a common site. To detect summation between two sites, a larger interval between pulse pairs is used, thus avoiding collision. When the combined rewarding effect between the electrodes is no better than that seen with only one electrode, thresholds do not change, indicating that no summation has occurred. With high summation the resulting lower thresholds indicate an enhancement in the combined double pulse rewarding effect, and it is inferred that the stimulation is converging at target areas shared by both sites.

In the present study, before any injections were given or lesions made, we estimated summation between each animal's bilateral electrodes. Thresholds for single-pulse stimulation at each electrode were compared to thresholds for paired-pulse stimulation. To calculate the effectiveness (E) of paired-pulse stimulation, the paired-pulse collision method formulated by Shizgal et al. (1980) was used:

$$E = \frac{\frac{DP}{FT_L} - 1}{\frac{FT_L}{FT_H}}$$

where DP is the mean threshold recorded under double pulse conditions, FT_L is the lower threshold for the single pulse condition, and FT_H is the higher threshold for the single pulse condition.

For all of the animals, pre-lesion thresholds for paired-pulse stimulation at both electrodes at an interpair interval of 5.0 ms were lower than thresholds at each electrode alone, indicating summation between the bilateral sites. Summation estimates ranged from 0.21 to 0.30 for these bilateral placements, indicating that paired-pulse stimulation was

21% to 30% more effective than single pulses alone.

For all six animals, then, the combined rewarding effect of paired pulses was better than for single pulses alone, indicating that these bilateral electrode placements integrate their reward signals. If reward summation is produced in target cells immediately post-synaptic to the directly-activated axons, a unilateral lesion would affect a route to those shared cells (see Figure 22a).

Figure 22a

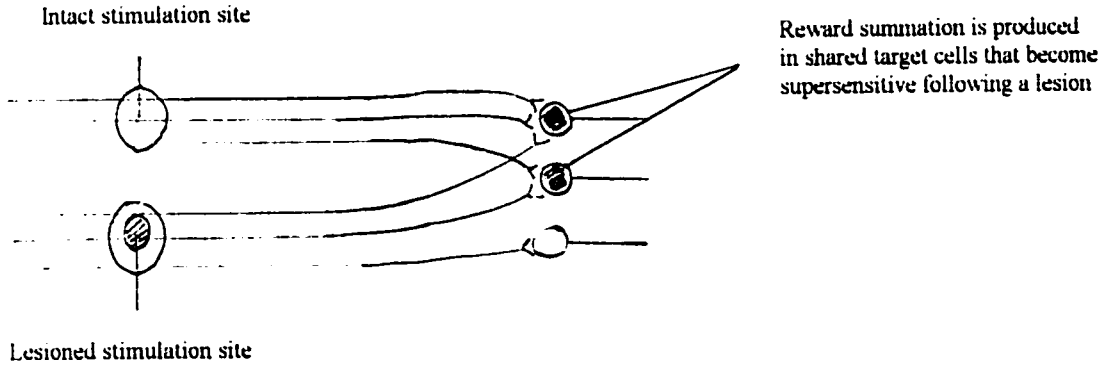


Figure 22a. Representation of bilateral electrode placements with shared target cells immediately post-synaptic to the directly-activated fibers. A unilateral lesion would likely affect a route to the target cells common to both electrode sites.

If shared target cells at the next synapse had become more sensitive, thresholds should have decreased at the intact sites, at least in proportion to the summation measured between the electrodes, paralleling the recovery of thresholds at the lesioned sites. However, thresholds at the unlesioned electrodes did not decrease in any of the animals. In fact, thresholds rose slightly at the unlesioned electrode in two of the six rats, after

thresholds at the lesioned electrode had recovered to pre-lesion levels.

It is possible, however, that activity could converge well beyond the cells immediately postsynaptic to the directly activated axons, as shown in Figure 22b).

Figure 22b

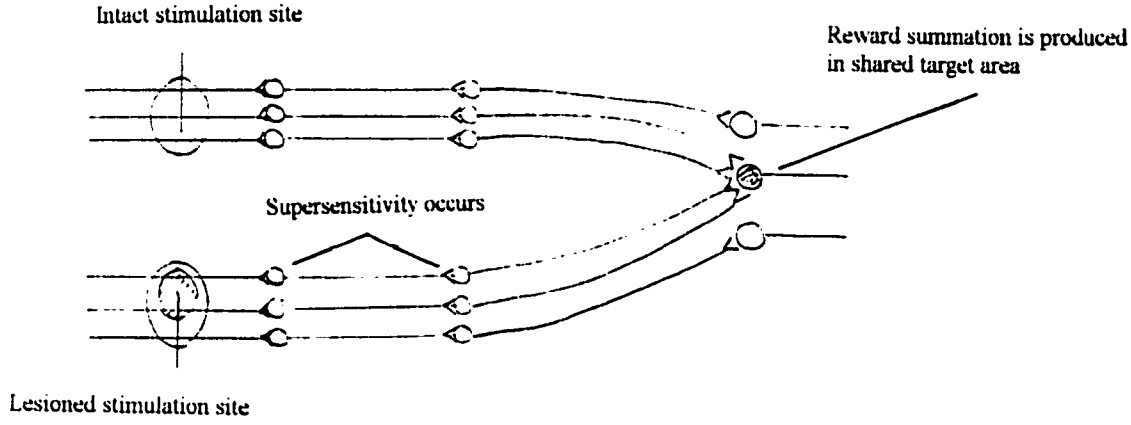


Figure 22b. Representation of bilateral electrode placements with shared target cells not immediately postsynaptic to the directly activated fibers. A supersensitivity following a unilateral lesion would not affect thresholds at the contralateral electrode.

If so, a compensation like receptor supersensitivity occurring in the lesioned hemisphere could act prior to the point at which the hemispheres' inputs converge to produce reward summation. In that case, thresholds at the intact site would not be affected by that compensation.

Anatomic Location of the Recovery

Based on the results of apomorphine injections on thresholds following a unilateral lesion, it seems clear that a dopamine receptor supersensitivity cannot be behind the recovery of reward thresholds. Consistent with this conclusion, and going beyond just dopamine, is the fact that thresholds at the unlesioned electrodes remained unaffected as those at the lesioned sites recovered. If a post-lesion compensation occurred in cells immediately postsynaptic to the directly stimulated fibers, thresholds would have decreased at the intact electrode as those at the lesioned electrode recovered. Because thresholds at unlesioned electrodes were not facilitated despite evidence that the two sites integrate their reward signals, this observation challenges the idea that some compensation occurs at the next synapse and underlies the recovery. However, even with high reward summation between the two sites, thresholds at the intact electrode would not be affected if a distal post-lesion compensation occurred prior to the point of convergence of activity.

Although it is possible that a distal non-dopaminergic compensation could account for the recovery of reward thresholds, it is also possible that thresholds recover when something in the vicinity of the lesion changes over time. For instance, applying a lesion stimulus can induce secondary effects that may lead to local changes that temporarily affect reward thresholds before normalizing over time. Thus the attenuation and recovery of the stimulation's rewarding effect could be occurring regionally, possibly right at the point of stimulation.

Experiment 3: Post-Lesion Changes in Tissue Resistance and Reward Thresholds

The results of Experiment 2 are consistent with the premise that the attenuating effect of a small lesion is not due to a systematic change affecting the entire brain, and that recovery of the stimulation's rewarding effect is not due to a compensatory dopaminergic sensitivity that occurs distal to the lesion. The attenuating effect and its recovery may be occurring locally, in an area around the tip of the stimulating/lesion electrode. This experiment tested whether an artifact due to the application of a lesion stimulus through the stimulating electrode influences reward thresholds.

Introduction

Central nervous system injury is followed by an inflammatory response that begins within hours and peaks within days (Dusart & Schwab, 1994). This response can include the development of an extracellular edema that could lead to an abnormal electrolyte environment, compression of the tissue (Beggs & Waggener, 1979), and a reduction of blood flow due to a decreased vascular resistance (Schwab & Bartholdi, 1996). Edema is maximal in the first few days (Broseta et al., 1988), with vascular inflammation peaking between one and three days after injury (Schoenfeld & Hamilton, 1977). If edema forms at the electrode tip following a lesion, the swelling due to excessive accumulation of fluid could decrease the effectiveness of subsequent stimulation. More of the current would find low resistance shunts through the interstitial fluid. As less current would flow through stimulated axons, fewer reward-relevant neurons would be fired, increasing the threshold.

A lesion would also likely lead to changes in the ionic concentration in the extracellular space at and around the site of injury. Even small variations in the concentration of certain ions in the interstitial fluid can disturb neuronal excitability and synaptic transmission (Schwab & Bartholdi, 1996). Studies investigating pathophysiological responses to spinal cord trauma show that extracellular potassium (K^+) levels are acutely elevated following injury, with a loss of this ion from the damaged tissue (Eidelberg, Sullivan & Brigham, 1975; Young & Koreh, 1986). The immediate increase of extracellular K^+ following injury could affect the threshold of excitation of cells in the vicinity of the lesion, by contributing to a block in impulse conduction (Stokes, Fox & Hollinden, 1985). The injury-induced increase of extracellular K^+ may occur when neuronal support cells such as astrocytes are affected by a lesion. Astrocytes take up the excess K^+ that accumulates in the extracellular space during high rates of neuron activity or when an axon is damaged, redistributing it around active neurons to maintain neuronal electrical functions (Edström et al., 1995). By storing the K^+ the astrocytes protect neurons from the effects of high concentrations of these cations. If these support cells are incapacitated by a lesion the excess K^+ may not be taken up, leading to a change in the resting membrane potential of the neurons (Feldman, Meyer & Quenzer, 1997).

If applying a lesion stimulus induces an extracellular edema or disturbs the ionic balance around the electrode, either effect is likely to be reflected in a change in the usual resistance of the tissue in the vicinity of the lesion. A decreased resistance, or opposition to current flow in the tissue, would likely result in a reduced effectiveness of the stimulation current as fewer fibers important to reward are excited. As such, it seems

possible that threshold increases following a lesion could in part occur because of such a drop in tissue resistance.

Resistance in an electric circuit can be calculated using Ohm's Law ($R = E/I$), where R is resistance, E is voltage and I is current. Applying this law to this study, tissue resistance can be estimated from stimulation voltage values read directly from an oscilloscope, using a constant-current generator that maintains the intensity of the current via feedback through the electric circuit. To maintain this constant current when changes are detected in the resistance of the tissue around the electrode, the generator adjusts voltage output (Mundl, 1980). Thus current does not change even if resistance is altered. With current defended against a drop in tissue resistance following a lesion, any reduction in resistance would be indicated by lower post-lesion voltage values.

In work done in conjunction with this study, Grandmaître (1997) measured post-lesion changes within the stimulation field, using a second (probe) electrode implanted about 0.5 mm from the stimulating/lesion electrode. She recorded the local voltage at the stimulating electrode and the probe electrode, using the difference to determine the voltage within the stimulation field. This value was compared to the voltage recorded between the stimulating/lesion electrode and the current return attached to skull screws, the preparation generally used in self-stimulation studies. As the values were nearly identical we were assured of an accurate measurement of local voltage in this experiment, which uses only the stimulating/lesion electrode and a current return attached to skull screws.

If applying a lesion stimulus induces an artifact that leads to a lowered tissue resistance, then, that drop in resistance would be indicated by a decrease in voltage values. Thus we recorded voltage values with the current maintained at a constant level to get an estimate of tissue resistance around the electrode, before and after the lesions were made. While voltage was recorded thresholds were also measured before and as they recovered following a lesion. If post-lesion changes in threshold and tissue resistance show a reciprocal relationship before and after the lesion, an altered tissue resistance could account for the post-lesion reduction of the stimulation's rewarding effect.

Materials and Method

The housing conditions, apparatus, training methods and histology were the same as for Experiment 1.

Subjects and Surgery

Ten Long-Evans rats were implanted with electrodes aimed at the LH (see Figures B-10 and B-11 in Appendix B). Six of the rats required a supplemental intramuscular injection of Xylazine (20 mg/ml) during surgery, with doses ranging from 0.04 to 0.07 mg. The surgical procedure was the same as that of Experiment 1 for all of the animals, except that each of four rats (#1285, #1292, #1294 and #1299) was implanted with a single electrode, while the other six rats (#1275, #1276, #1277, #1280, #1282 and #1284) were implanted with modified electrodes, made of two single electrodes attached together approximately 0.5 mm apart. One of these served as the stimulating electrode, while the other (probe) was used with the stimulating electrode to determine a value for voltage within the stimulation field.

Procedure

Current was set at 500 μA for the training and test sessions. Baseline test parameters were the same as for Experiment 1, except that pulse frequencies decreased in 0.05 $\log_{10}$ unit steps every 10 seconds. Curves were cut at five stimulations.

While thresholds were measured at the stimulating electrode, voltage readings were also recorded. For the animals with modified electrode implants, voltage values were taken from the stimulating and probe electrodes, acquiring two voltage values per sweep. For the rats with single electrode implants, voltage values were taken from the stimulating electrode and the current return attached to the skull screws. For all of the animals, voltage values were read directly from an oscilloscope.

When baseline thresholds were stable, radiofrequency lesions of 1.4 s duration were made through the stimulating electrode, using a current of 50 μA. Beginning 24 hours after the lesion, we recorded thresholds and voltage values daily for 13 days, under the same parameters as for baseline testing.

Results

Thresholds for all 10 rats were elevated 24 hours after a lesion was made, with increases ranging from 20% to 290% above pre-lesion levels. Decreases in measures of tissue resistance were also seen on post-lesion Day 1, in a range from 1800 Ω to 4080 Ω (see Figures 23 to 32).

Figures 23 to 32. Pre- and post-lesion frequency thresholds (Hz) and measures of tissue resistance (Ω). Circles joined by solid lines represent average daily thresholds, while squares joined by dotted lines represent average daily resistance measures. The arrows indicate application of the lesion. The number in the top left corner identifies the subject (n = 10).

Figure 23

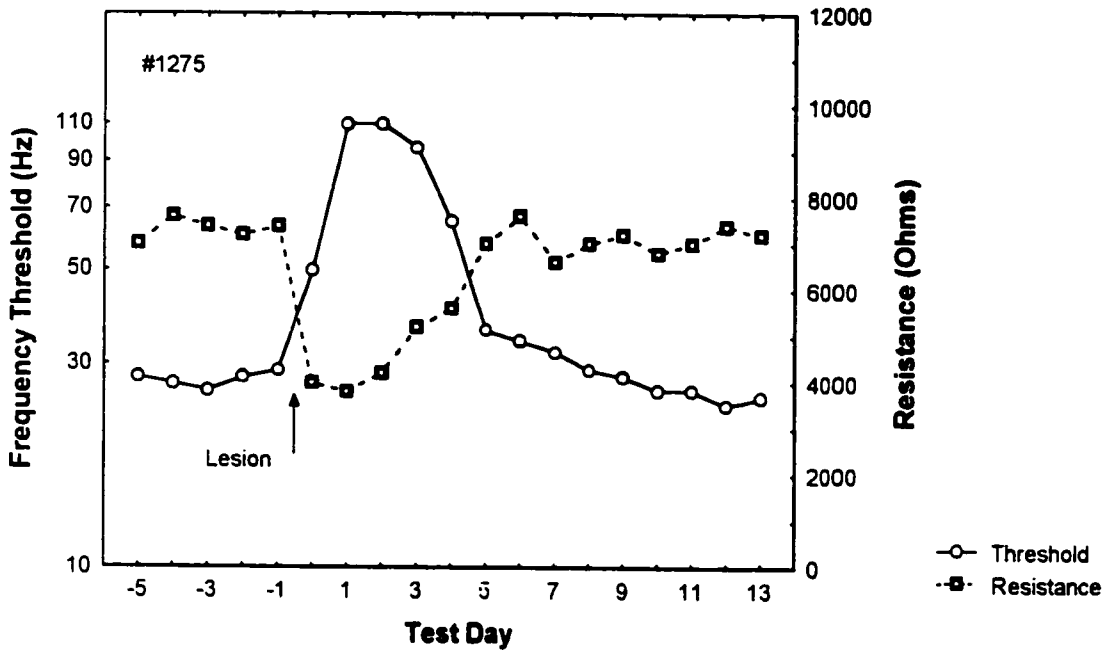


Figure 24

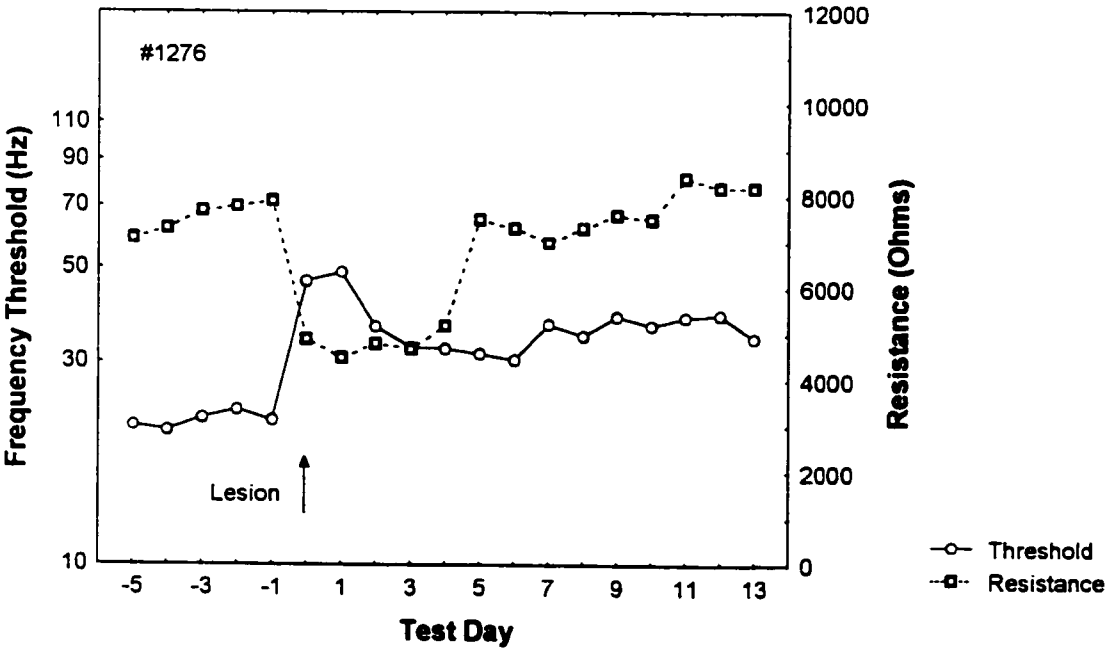


Figure 25

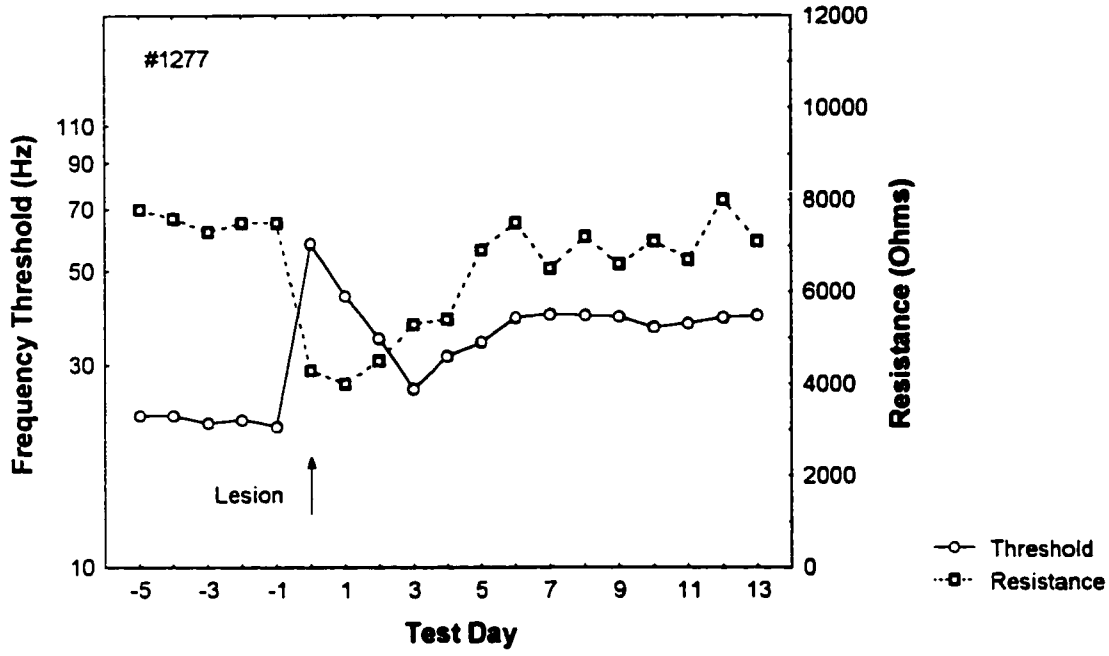


Figure 26

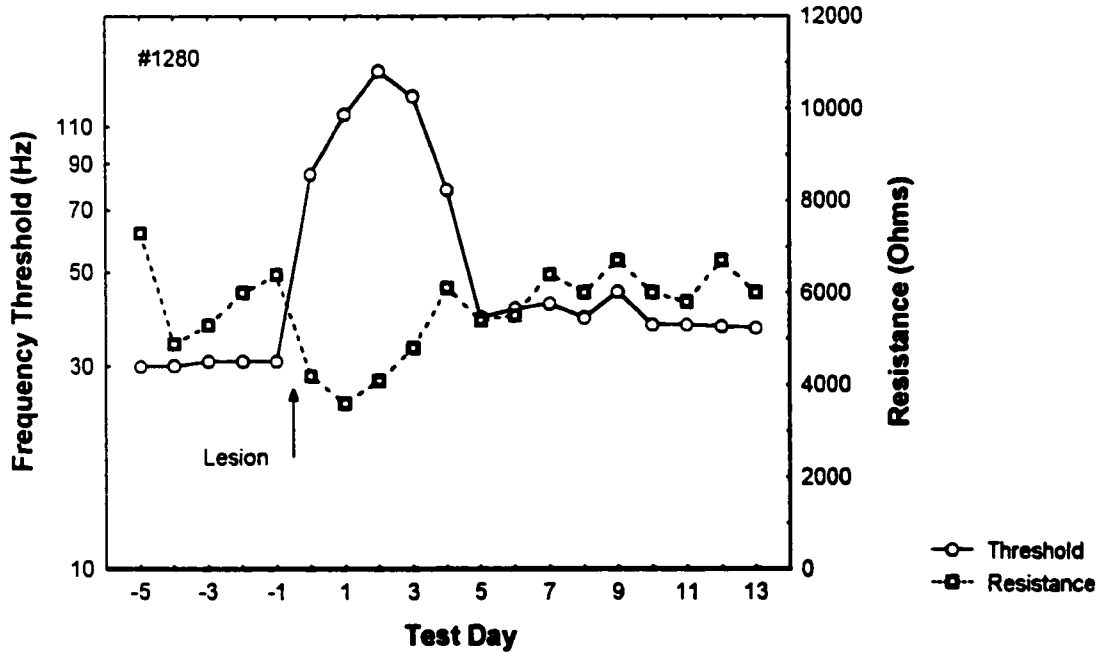


Figure 27

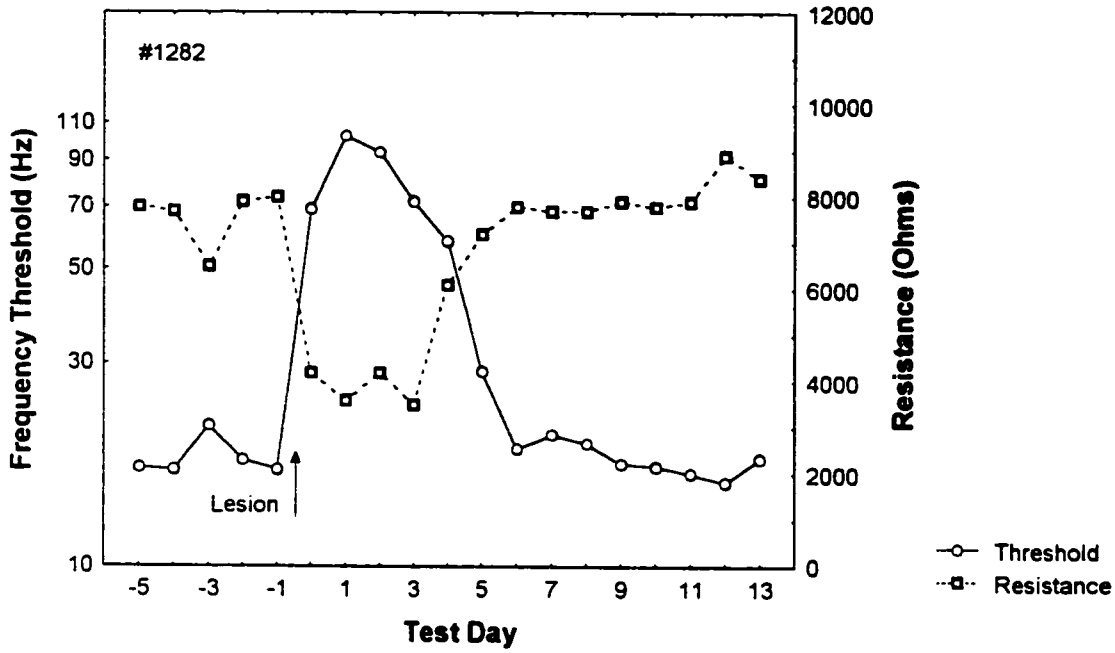


Figure 28

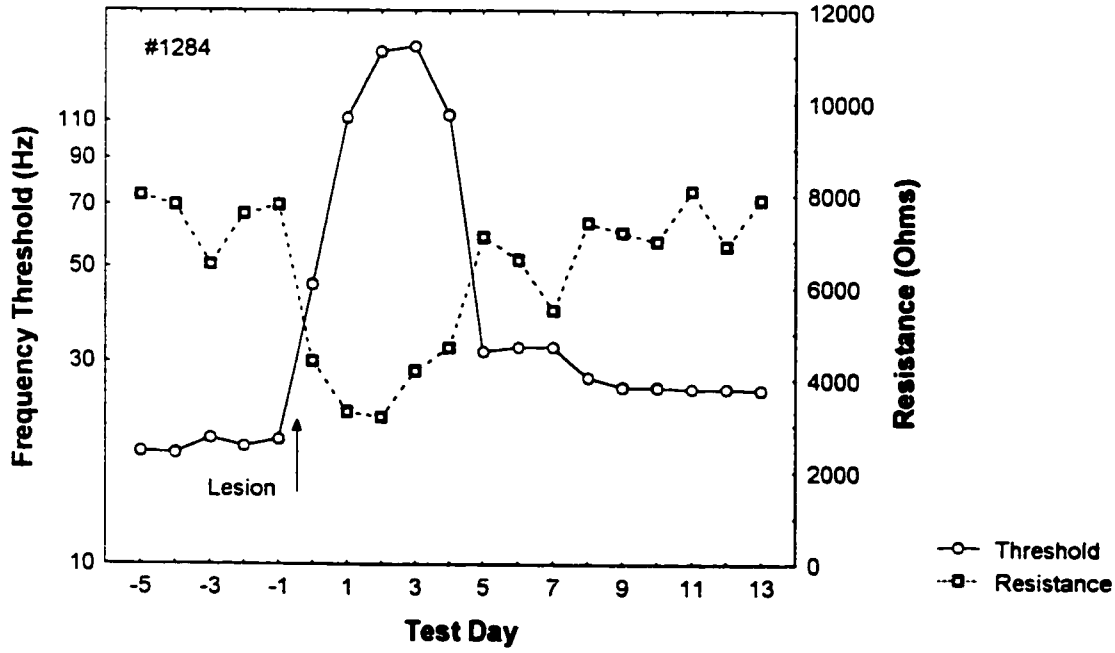


Figure 29

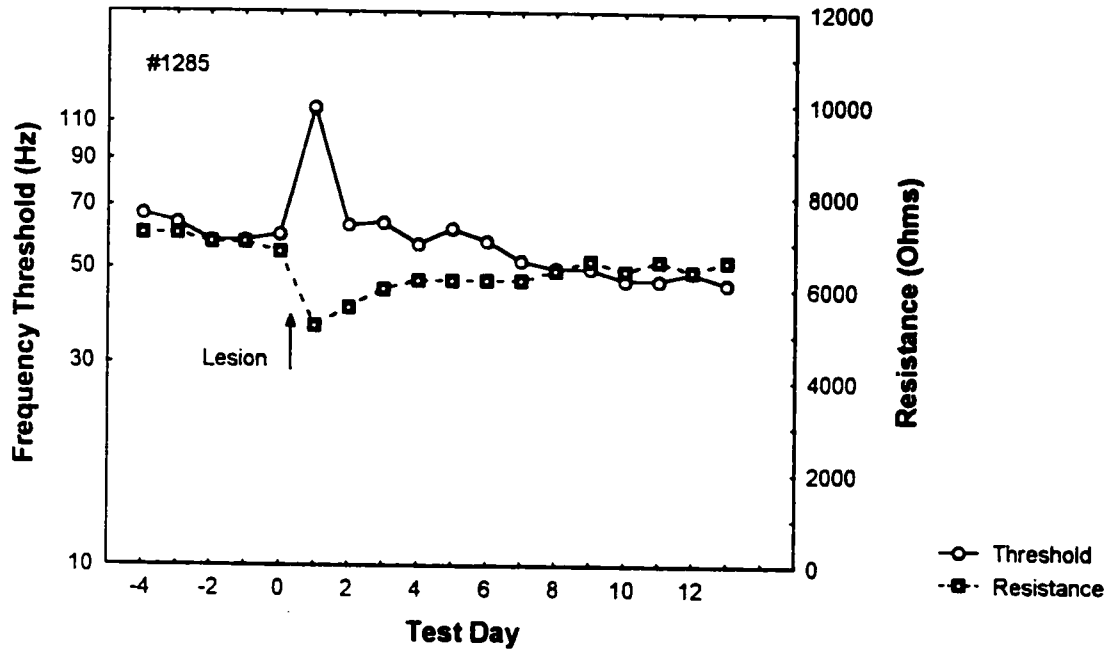


Figure 30

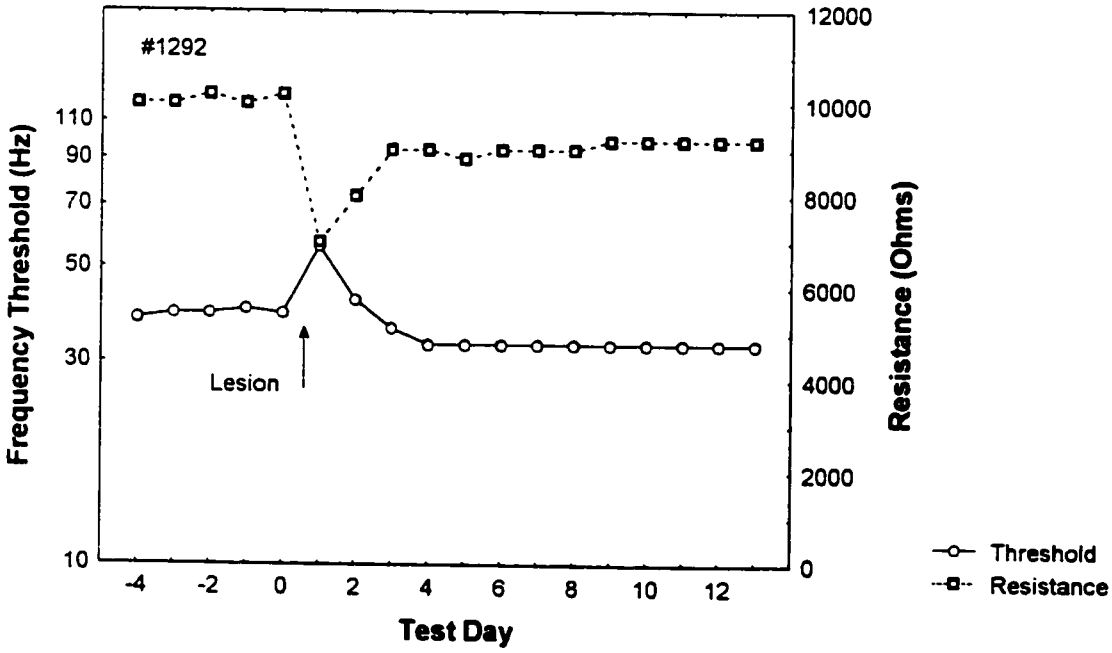


Figure 31

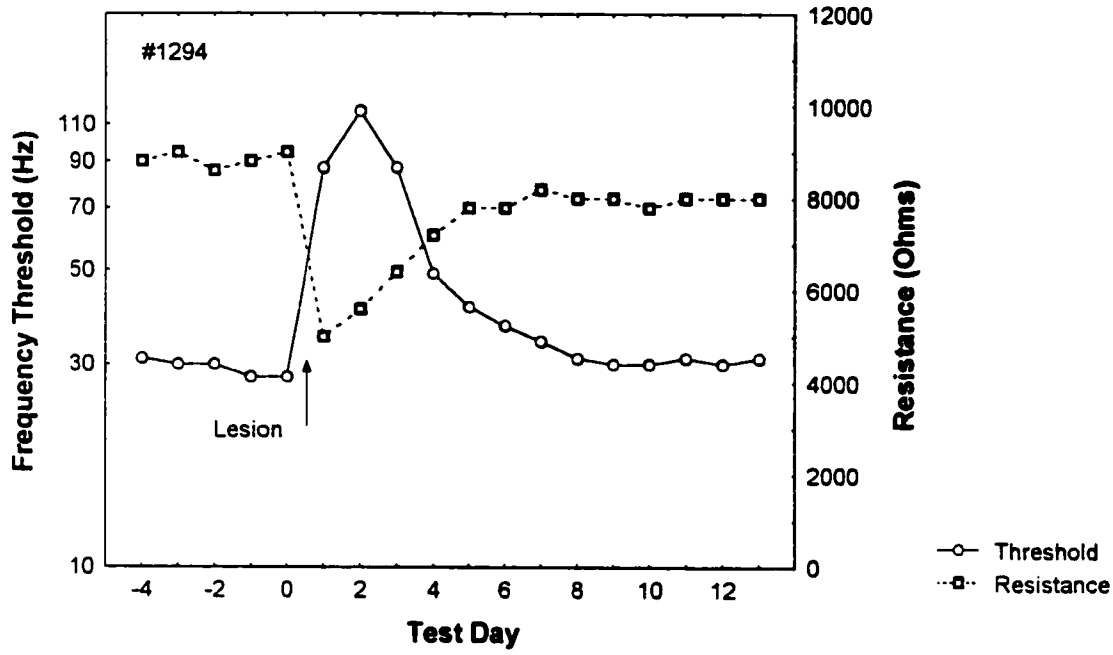
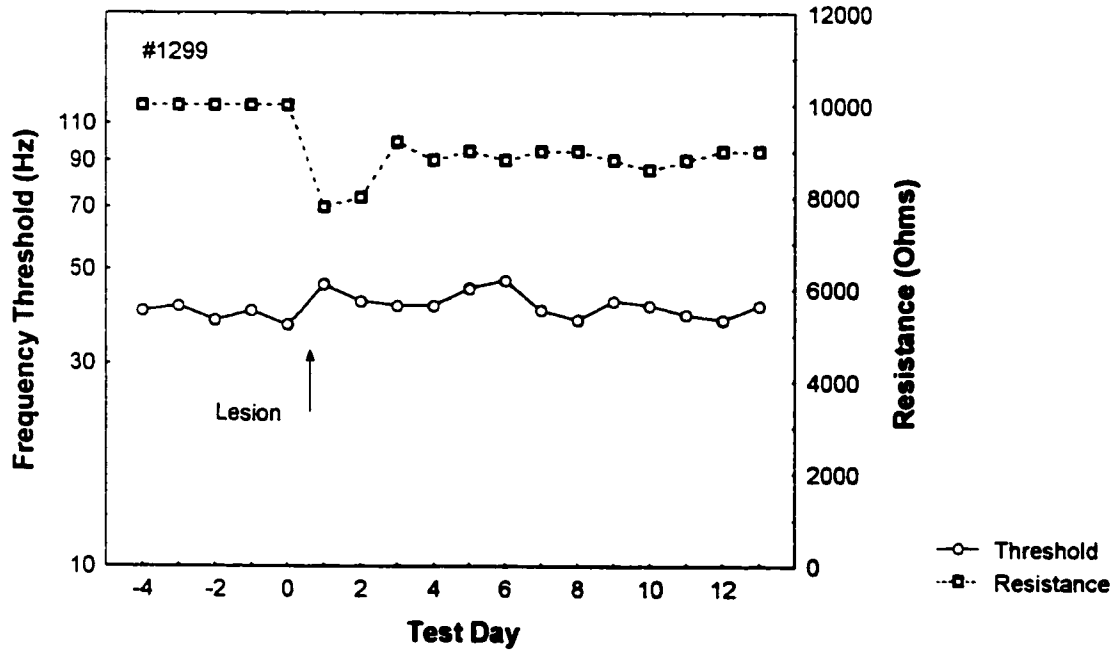


Figure 32



Results of repeated measures t tests show that across all of the rats, post-lesion Day 1 threshold and resistance values differ significantly from pre-lesion levels (see Table 3).

Table 3

Changes in Measures of Threshold and Resistance

Measure	t value
Threshold	-5.70*
Resistance	-12.08*

*$p < .01$; one-tailed test; $df = 9$

On average, thresholds and resistance values returned to or near baseline levels by the end of the test period. As shown in Table 4, repeated measures t tests show that neither measure is significantly different from pre-lesion levels on post-lesion Days 9 to 13.

Table 4

Differences in Measures of Threshold and Resistance

Measure	t value
Threshold	-1.29
Resistance	-1.41

*$p < .05$; one-tailed test; $df = 9$

For each rat, Pearson product-moment correlation coefficients were calculated to determine whether a significant relationship exists between measures of threshold and resistance throughout the test period. Statistically significant relationships were found for 9 of the 10 rats (see Table 5).

Table 5

Correlations Between Measures of Threshold and Resistance

Subject	t value	Pearson r
#1275	-8.20**	-0.89**
#1276	-1.80	-0.40
#1277	-2.23*	-0.48*
#1280	-4.01**	-0.70**
#1282	-13.40**	-0.96**
#1284	-8.67**	-0.90**
#1285	-4.06**	-0.76**
#1292	-4.33**	-0.78**
#1294	-9.62**	-0.92**
#1299	-2.61*	-0.55*

*$p < .05$; one-tailed test; $df = 17$
**$p < .01$

Discussion

When a small lesion was made through a stimulating electrode, changes in reward thresholds were accompanied by changes in local tissue resistance. Post-lesion thresholds temporarily increased before returning to or near pre-lesion levels within about one week. As thresholds increased after the lesion, measures of tissue resistance at the electrode site decreased, then returned to or near pre-lesion values within about a week. For most of the animals, the changes in threshold and resistance were strongly associated. Data from only one (#1276) of the rats did not show a relationship that was statistically significant. In examining the data for this animal we found that, while measures of resistance returned to

pre-lesion levels, the threshold remained somewhat elevated at the end of testing. This sustained threshold increase likely accounts for the absence of a significant association between threshold and resistance for this rat.

Applying a lesion stimulus can induce an extracellular edema that could be responsible for the decreased tissue resistance. Researchers investigating conditions associated with ischemic brain lesions note that two different but coexisting types of edema can occur following even slight cerebral ischemia (for a review see Kimelberg, 1995). Extracellular edema can occur with damage to local blood vessels that leads to an influx of solutes and water into the surrounding brain area (Kimelberg, 1995). This type of edema is associated with a widening of extracellular spaces and has been observed following brain lesions in gerbils (Crockard et al., 1980; Garcia, Klatzo, Archer & Lossinsky, 1981) and cats (Schuier & Hossmann, 1980). In response to the movement of solutes and water, astrocytic swelling is also seen with this form of edema.

A second type of edema that can occur with various kinds of brain injury is a consequence of energy failure. It involves a shift of extracellular fluid into the intracellular compartment and mainly affects astrocytes. The intracellular swelling can occur when a sudden loss of potassium from injured cells leads to massive depolarization of astrocytes in the vicinity of the lesion. Astrocyte depolarization in turn opens voltage-sensitive anion channels in the cells, leading to passive potassium and chloride flux into astrocytes (Sweeney, Yager, Walz & Juurlink, 1995). This gain of electrolytes leads to astrocyte swelling and can result in a narrowing of the extracellular space (Kimmelberg, 1995). Thus, astrocytic swelling can occur in response to increased extracellular potassium, and

the changes of extracellular space that occur with brain injury are closely coupled with the loss and recovery of ion homeostasis (Hanson & Olson, 1980).

The occurrence of edema is by measuring cortical impedance or resistance, which is a function of extracellular space (Crockard et al., 1980). Cortical impedance can increase following an ischemic insult that induces intracellular edema, indicating that intracellular swelling can narrow the extracellular space (Williams, Gunn & Gluckman, 1991). However, if there is coexisting extracellular edema, the extracellular space can remain enlarged despite astrocytic swelling until the fluid dissipates during the first week after the injury (Kimelberg, 1995). It seems likely that the widened extracellular space would result in a decreased tissue resistance. Resistance would decrease again as the extracellular fluid normalized over time.

It is possible, then, that an extracellular edema and/or a change in ionic concentration due to the application of a lesion stimulus could lead to a drop in the local tissue resistance. Whatever the nature of the artifact, the resulting deceased resistance could reduce the effectiveness of subsequent stimulation. For instance, the lower post-lesion resistance may lead to a lower voltage gradient of neurons in the stimulation field. A lower voltage gradient would result in fewer reward-relevant cells being stimulated per pulse, and an increase in reward threshold would be seen. The effects of the artifact are transient; thresholds recover as the stimulation becomes more and more rewarding, and measures of tissue resistance rise again toward pre-lesion levels within approximately one week of the lesion. Although further research is necessary before the exact nature of the lesion-induced artifact can be known, results from this study indicate that the attenuation of the

stimulation's rewarding effectiveness may at least in part occur as a result of an temporarily altered tissue resistance in the vicinity of the lesion.

Experiment 4: Constant-Current versus Constant-Voltage Stimulation

In this experiment we extended the exploration of a post-lesion decrease in tissue resistance by comparing the effects of constant-current and constant-voltage stimulation on reward thresholds.

Introduction

In Experiment 3, we noted that tissue resistance in the stimulation field is altered following a small radiofrequency lesion made through the stimulating electrode. Within 24 hours of the lesion measures of resistance are substantially lower than pre-lesion levels. Concurrent to the drop in resistance thresholds for self-stimulation increase, indicating a reduction in the rewarding effectiveness of the stimulation. This attenuation is temporary; within days of the lesion thresholds decrease while measures of tissue resistance increase toward pre-lesion levels.

When a drop in resistance is detected in an electric circuit, a constant-current (CC) generator will automatically apply lower pulse voltages to defend the current against the change in resistance. Applied to this study, although the same current will flow through the tissue the lower voltages will stimulate fewer neurons per pulse, requiring greater frequency thresholds. However, a constant-voltage (CV) source of stimulation would ensure that pulse voltage remains the same, regardless of changes in resistance. While a CC pulse generator would reduce its voltage in the face of a reduced resistance, a CV unit would maintain its voltage and therefore deliver a more consistent field of stimulation before and after a lesion. As such, if the rewarding effect of the stimulation is attenuated

because of a drop in resistance, threshold increases following a lesion may be smaller when voltage levels are held constant.

To determine whether a decreased tissue resistance is responsible for reducing the rewarding effectiveness of the stimulation, we recorded thresholds daily before and after lesions were made, and compared them under two test conditions: a CC source of stimulation and a CV source of stimulation. We expected that post-lesion threshold increases would be seen under CC stimulation. However, if a lower post-lesion tissue resistance is responsible for those threshold increases, comparatively lower thresholds should be observed when pulse voltage is held constant.

Materials and Method

Housing conditions, surgery, training methods and histology were the same as for Experiment 1 (see Figure B-11 in Appendix B for LH electrode placements). Test parameters were also the same, except that throughout the five-day baseline and post-lesion tests, each of four rats (#1285, #1292, #1294 and #1299) was tested twice daily; using two stimulators.

In the first daily test, voltage was recorded for each rat from the stimulating electrode and the current return attached to skull screws while thresholds were recorded under CC stimulation. In the second daily test, voltage was held at that baseline level while thresholds were recorded using a CV unit. When baseline thresholds were stable under both stimulators, each rat received a radiofrequency lesion of 50 μA for a duration of 1.4 s through the stimulating electrode. Post-lesion testing began 24 hours later and continued for 13 days.

Results

Thresholds recorded under CC stimulation were compared to those recorded under CV stimulation for each rat. Under CC, post-lesion thresholds increased in a range from 20 to 200% from pre-lesion levels on Day 1. Concurrent decreases in voltage values were observed for all four animals. Over the next week thresholds and voltage values returned to or near pre-lesion levels (see Figures 33 to 36).

Under CV no increase in threshold was observed on Day 1 for three of the four animals. Thresholds remained at these levels throughout the test period. For the other rat (#1294), a threshold increase of 14% was noted under CV on post-lesion Day 1, which increased on Day 2 to 36% over baseline values. This threshold decreased to pre-lesion levels within a few days. Under CC this animal had a substantial threshold increase by post-lesion Day 2 that peaked at 320% over pre-lesion levels

Figures 33 to 36. Pre- and post-lesion frequency thresholds under constant-current (CC) and constant-voltage (CV) sources of stimulation. The circles joined by solid lines represent thresholds recorded under CC, while the squares joined by dotted lines represent thresholds recorded under CV. The arrows indicate application of the lesion following testing on Day 0. The number in the top left corner identifies the subject (n = 4).

Figure 33

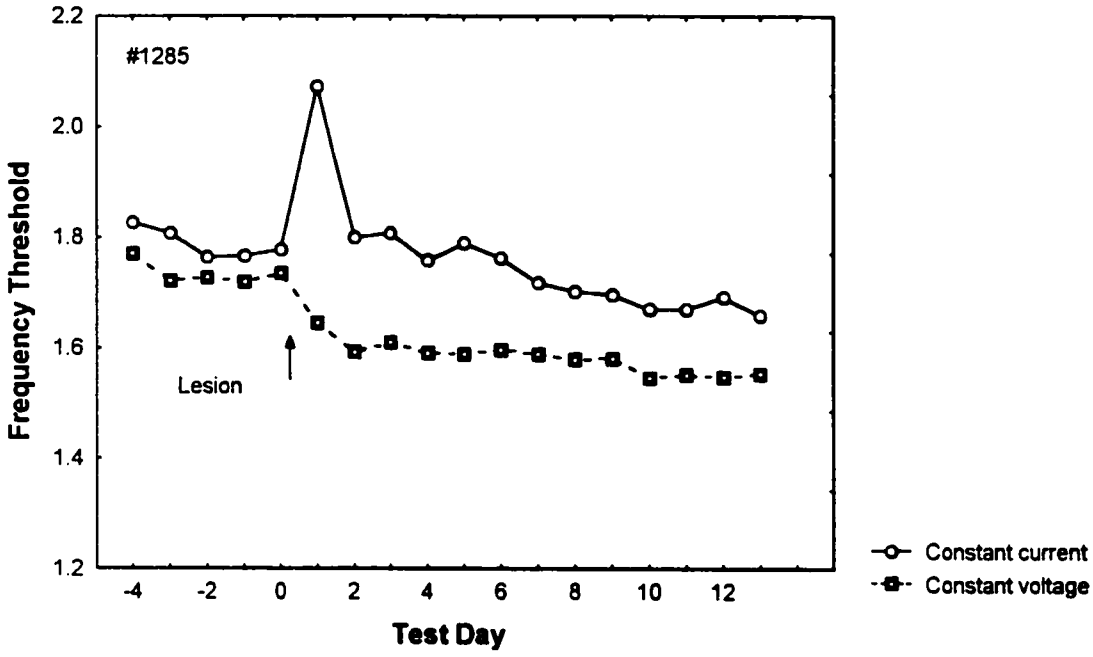


Figure 34

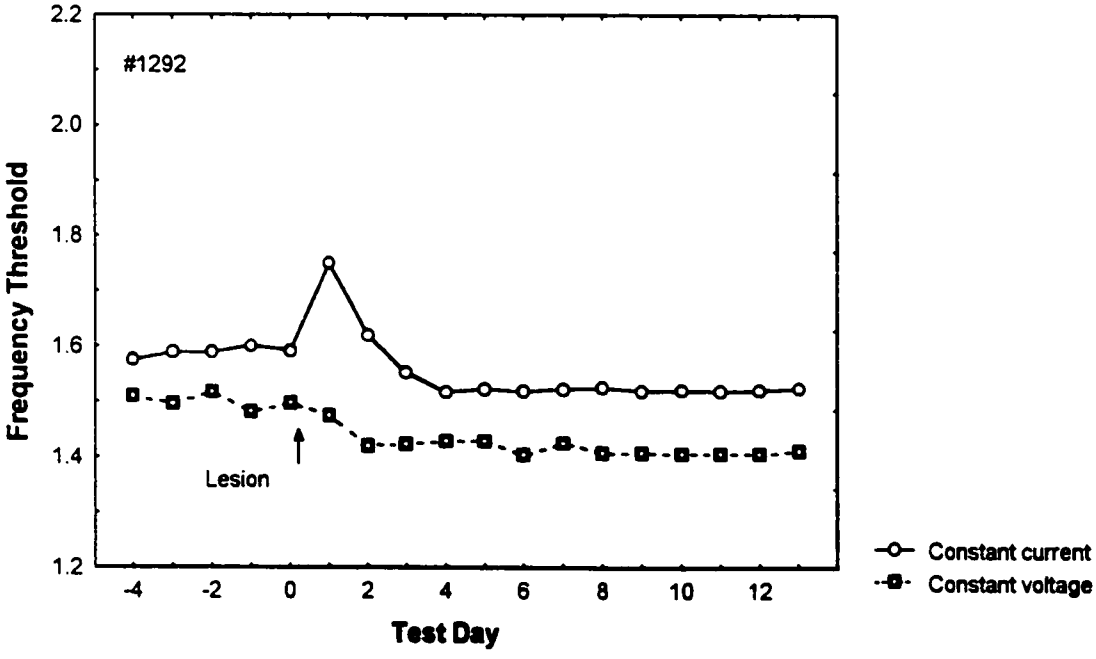


Figure 35

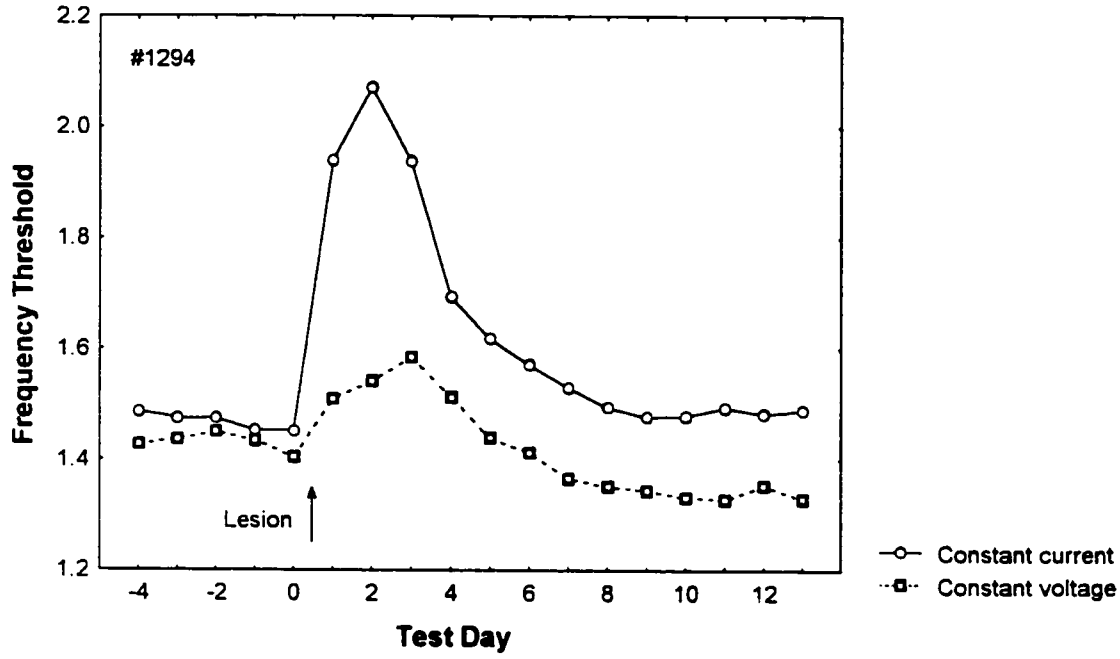
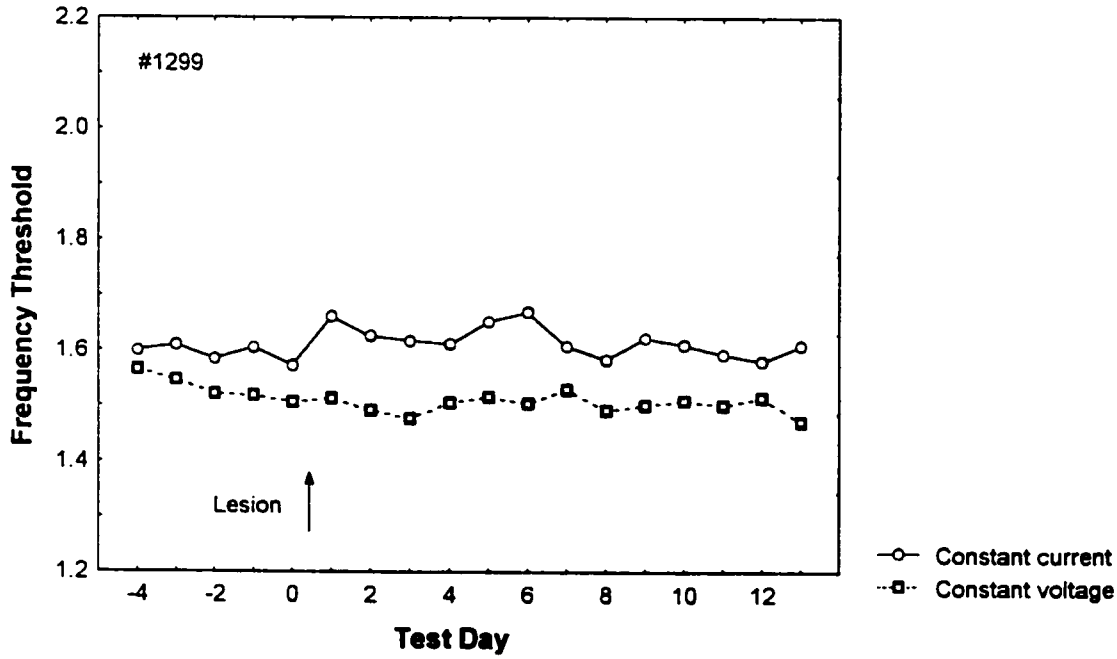


Figure 36



Discussion

Following the lesion, thresholds recorded under constant-current stimulation initially increased then decreased toward pre-lesion levels over the next week. However, when the animals were tested the same day with the voltage output held constant, no elevation in threshold was seen for three of the four animals. The threshold increase seen in the other rat was much smaller than the substantial elevation noted under the first test condition.

The comparatively higher thresholds measured under constant-current support the premise that a change in the resistance of the tissue in the vicinity of the lesion is responsible for altering reward thresholds. To compensate for the decreased resistance that occurs following the application of a lesion stimulus, the constant-current generator automatically lowers voltage output to maintain the current. A reduction in the electrode voltage gradient results in a smaller effective radius of excitation, as the lower pulse voltages no longer stimulate neurons further away from the electrode tip. Thus, post-lesion frequency thresholds increase as a lower pulse voltage excites fewer neurons per pulse.

Comparatively lower thresholds were seen when voltage output was held constant. Keeping pulse voltage constant ensures that the effective radius of excitation is not reduced after a lesion. However, the lower thresholds seen under constant-voltage stimulation are likely due to more than a restoration of the stimulation field. Applying Ohm's Law, maintaining constant pulse voltages in the face of a reduced resistance would also automatically increase the current. As the spread of activation around the tip of an electrode is a function of current, the increased current would extend the effective radius

of excitation. This would recruit more axons in the fringe around the lesion. Thus it is possible that thresholds under constant-voltage stimulation were lower partly because the increased current reached previously unstimulated and undamaged axons. This explanation could account for the fact that post-lesion thresholds actually decreased under constant-voltage stimulation for two of the rats (see Figures 33 and 34). Given this it seems likely that the lower thresholds seen under constant-voltage stimulation result from both maintaining pulse voltage and recruiting previously unstimulated and undamaged fibers in the fringe around the lesion.

Measuring thresholds under constant-current and constant-voltage conditions reinforces the conclusion that resistance decreases in the vicinity of a lesion. The decreased resistance results in a temporary reduction of the rewarding effect of the stimulation. Thresholds recover as tissue resistance normalizes again over time.

It is not yet clear what precipitates the change in tissue resistance, as such an alteration could conceivably result from a number of lesion-induced conditions. An extracellular edema and/or a disturbance in ionic concentration in the vicinity of the lesion could lower tissue resistance. Further research could determine that precipitating factor. Although the exact nature of the artifact remains unknown, the results of the present study support those of Experiment 3, indicating that the temporary post-lesion attenuation of the stimulation's rewarding effectiveness can be attributed to a transient decrease in tissue resistance in the stimulation field. This finding may help to clarify results of other studies noting a partial post-lesion recovery of reward thresholds. Although for those animals thresholds remained elevated at the end of testing, some of the stimulation's rewarding

effectiveness returned. That partial recovery likely occurs as the resistance of the tissue normalizes.

Experiment 5: Post-Lesion Changes in Neural Excitability

In Experiment 4 we showed that the rewarding effect of electrical brain stimulation decreases when an artifact due to the application of a lesion stimulus results in a drop in tissue resistance at the lesion site. An extracellular edema and/or a disturbance in ionic concentration may be responsible for altering tissue resistance. This experiment attempted to obtain evidence of that artifact by following the recovery with post-stimulation excitability tests.

Introduction

A radiofrequency lesion can precipitate the release of large quantities of potassium ions (K^+) into the extracellular space. Cellular electrophysiological abnormalities can follow, including a decreased resting membrane potential, decreased action potential amplitude, and decreased action potential duration from 6 to 8 mm from the lesion site (Ge et al., 1995). If such an ionic disturbance is responsible for reward threshold increases following a lesion, it is possible, then, that the shape and duration of action potentials of reward-relevant neurons would also be affected. Changes in neural excitability could be determined by examining the effect of a lesion on the refractoriness of neurons in the stimulation field, and on the occurrence of local potential summation.

Refractoriness

The absolute refractory period of neurons is the time interval following the onset of an action potential, when the axon is unresponsive to stimuli for a brief period of time. This time period coincides with the falling phase of the action potential, caused by K^+

efflux (Koester, 1981). Refractory periods of neurons in the stimulation field can be examined by administering trains of paired pulses through an electrode. A conditioning (C) pulse is followed by a test (T) pulse, with the time interval between pulse onsets (C-T interval) varied. If the C-T interval is longer than the refractory period, the effectiveness of both pulses is maximal. With maximal effectiveness the number of action potentials is doubled, and thresholds for paired-pulse stimulation are lower than those for single-pulse stimulation. However, if the pulse pairs are too closely spaced, only the C pulse evokes action potentials, and the pulse pairs will be no more effective than single pulses.

Using scaling methods formulated by Yeomans (1975), studies have found that in reward fibers of the LH, recovery from refractoriness begins at a C-T interval of 0.4 ms and continues to increase until 1.2 ms (Bielajew et al., 1981; 1987; Yeomans, 1975; 1979). However, a lesion could affect that recovery of excitability; a post-lesion increase in external K^+ concentration can reduce the rate of membrane repolarization, potentially altering a neuron's refractory period. For instance, if refractoriness is prolonged, fewer action potentials would be generated with subsequent stimulation, and higher thresholds would be observed. Thus, a change in refractoriness following a lesion could suggest an altered ionic concentration.

Local Potential Summation

Local potential summation (LPS) can also contribute to post-stimulation excitability changes. With C and T pulses of equal current, each C pulse will excite neurons in the area around the tip of the stimulating electrode. Outside of this area, in the fringe of the stimulation field, the current might elicit axons to near-threshold points of depolarization.

If a T pulse arrived before this local potential died away, the combined depolarizing effect of the two pulses can add to produce an action potential. Thus, at C-T intervals within the refractory period, LPS between subthreshold C and T pulses can occur, but the C-T interval must be very small (less than 0.4 ms) (Yeomans, 1979).

Local potential summation can only occur in the fibers in the fringe of the stimulation field, where current from both pulses is near threshold. Typically, this LPS declines rapidly before the end of the refractory period. However, LPS could change after a lesion is made through the stimulating electrode. The lesion would predominantly affect fibers closer to the electrode tip, leaving a greater proportion of the excitable population in the fringe of the field. If that occurred, an increase in LPS should be observed following a lesion, as a higher percentage of fringe fibers relative to core fibers would be stimulated.

If an ionic imbalance due to the application of a lesion stimulus is responsible for reward threshold increases, then the refractory periods of neurons in the stimulation field may change. As well, if a lesion has more of an effect on cells closer to the electrode tip and less of an effect further away, the effectiveness of LPS could increase. Either change in typical excitability would be indicated in a comparison of pre- and post-lesion refractory curves, which plot the effectiveness of paired-pulse stimulation as a function of C-T interval. Thus, we measured thresholds for single-pulse and paired-pulse stimulation and estimated the refractoriness of neurons in the stimulation field before and after the lesions were made. We then compared pre- and post-lesion refractory curves to see whether a lesion: (a) alters the refractory periods of neurons in the stimulation field, and (b) increases the LPS of stimulated neurons.

Materials and Method

The housing conditions, surgery, apparatus, training methods and histology were the same as for Experiment 1. All of the rats' electrodes were implanted in the LH (see Figure B-12 in Appendix B). When baseline thresholds were stable, each of four rats (#1287, #1289, #1295 and #1296) was given a radiofrequency lesion of 50 μA for a duration of 1.4 s through the stimulating electrode. Testing began 24 hours after the lesion, continued daily for five days, then every second day until post-lesion Day 13.

The test parameters were the same for baseline and post-lesion testing, with current fixed at 500 μA. The number of self-administered stimulation trains of 500 ms duration per 10 s trial was recorded for two pulse conditions; single-pulse and paired-pulse. Each sweep ended when there were five or fewer lever presses on two consecutive trials. Each of two patterns of sweeps began with one single-pulse sweep that was followed by paired-pulse sweeps with different C-T intervals. The C-T intervals tested were 0.16, 0.25, 0.4, 0.63, 1.0, 1.6, 2.5, and 4.0 ms. The first pattern of paired-pulse sweeps began with pulse pairs separated by the shortest C-T interval (0.16 ms), and the second one began with pulse pairs separated by the longest C-T interval (4.0 ms). The order of the two patterns was then reversed.

It should be noted that while a stronger T- pulse is generally used to measure the absolute refractory period, this experiment used C- and T-pulses of equal strength. As researchers have shown that the relative refractory period contributes very little to neural excitability (Bielajew & Shizgal, 1982; Yeomans, 1979), the contribution of the absolute refractory period would likely be shown using equal pulses.

Results

Before and after a lesion was made, thresholds for single-pulse and paired-pulse stimulation were measured and refractoriness of neurons in the stimulation field was estimated. Thresholds for single-pulse stimulation increased in a range of 32% to 145% from baseline levels for all four rats 24 hours after the lesion. As shown in Figure 37, those thresholds decreased toward pre-lesion levels for all of the animals within about one week of the lesion.

Figure 37

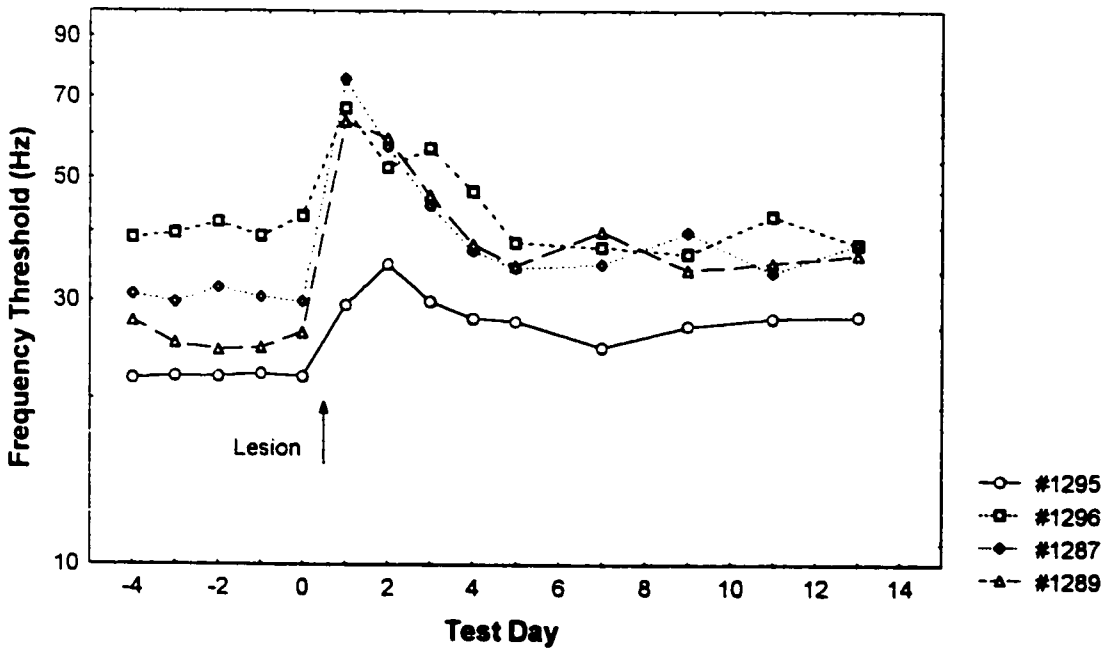


Figure 37. Pre- and post-lesion frequency thresholds (Hz) for 4 rats receiving single-pulse stimulation. The arrow indicates application of the lesion following testing on Day 0.

Thresholds for paired-pulse stimulation at each C-T interval tested also increased 24 hours after the lesion, then decreased within about one week post-lesion. The effectiveness (E) of paired-pulse stimulation was calculated by Yeomans's (1975) formula:

$$E = \frac{FT_{SP}}{FT_{C\text{-}T}} - 1$$

where FT_{SP} is the frequency threshold for the single-pulse condition, and $FT_{C\text{-}T}$ is the frequency threshold for a given C-T interval.

Refractoriness

Mean pre- and post-lesion refractory data was plotted across all C-T intervals (see Figures 38 to 46). To see whether refractoriness was changed after a lesion, the data was examined at C-T intervals between 0.4 and 1.0 ms, when recovery of excitability typically occurs. A post-lesion refractory curve shift to the right at the rising phase of the curve would indicate that refractoriness was extended. However, no lateral shift was seen at the rising phase of the post-lesion refractory curve, indicating no change in paired-pulse effectiveness at these intervals.

Figures 38 to 46. Mean (n = 4) pre- and post-lesion effectiveness of paired-pulse stimulation at different C-T intervals. The circles joined by a solid line represent pre-lesion data points, while the squares joined by a dotted line represent post-lesion data points. The post-lesion test day is identified in the top left corner.

Figure 38

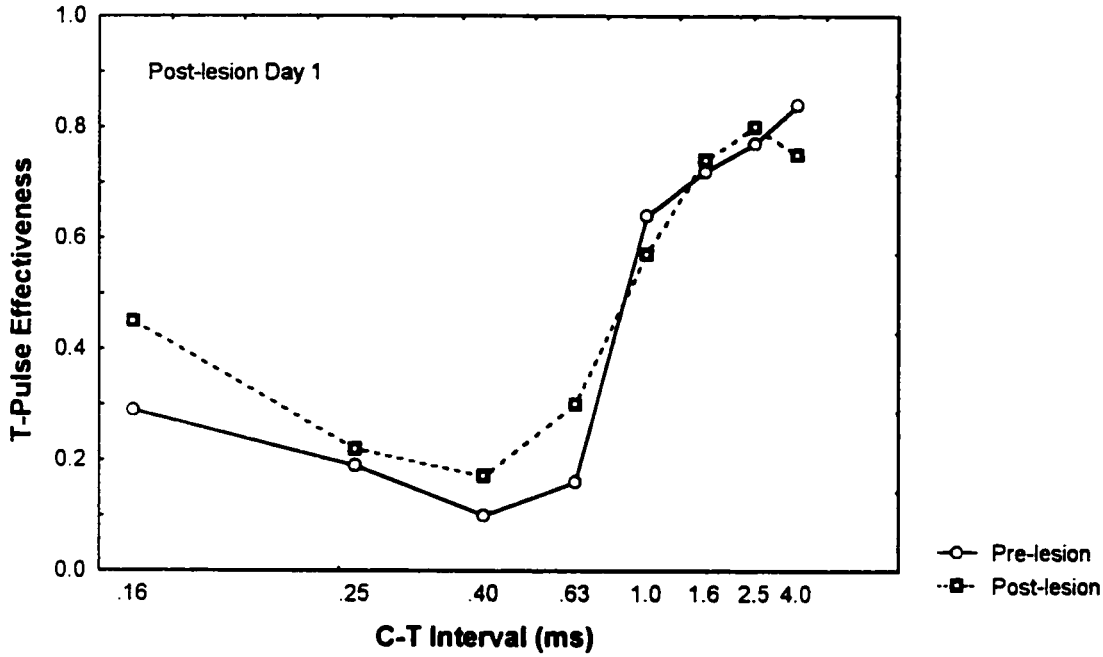


Figure 39

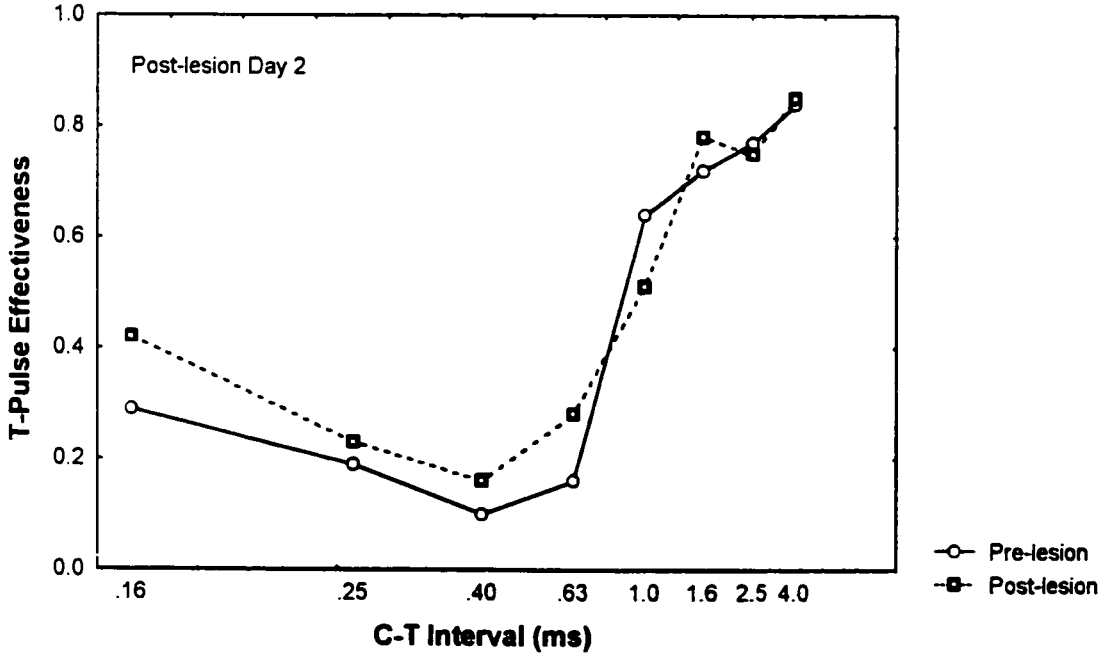


Figure 40

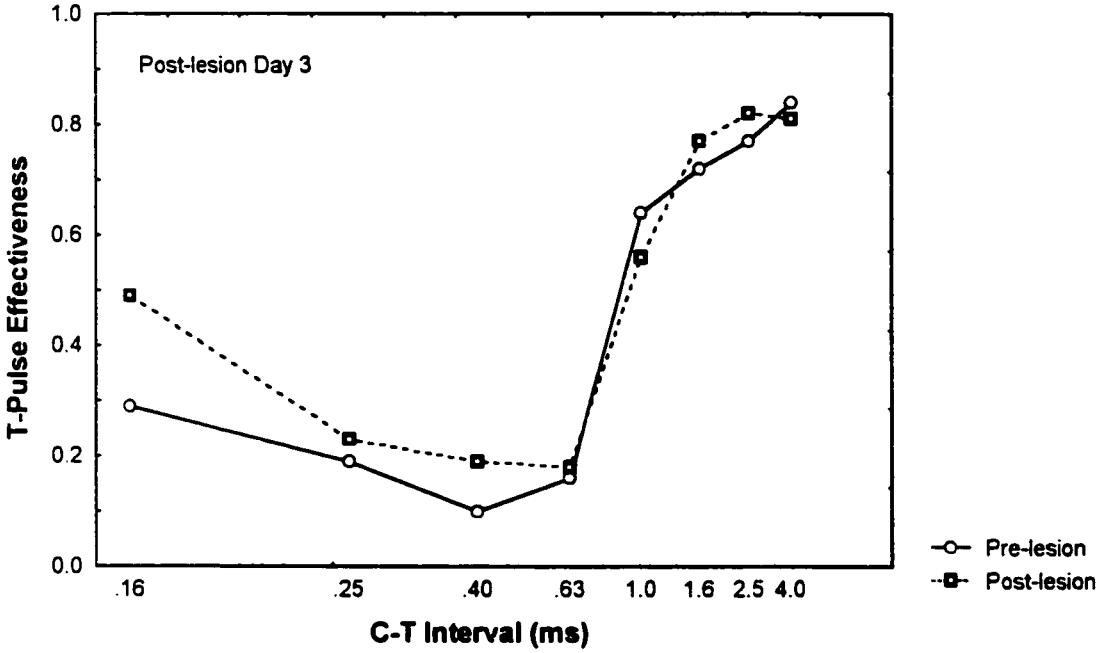


Figure 41

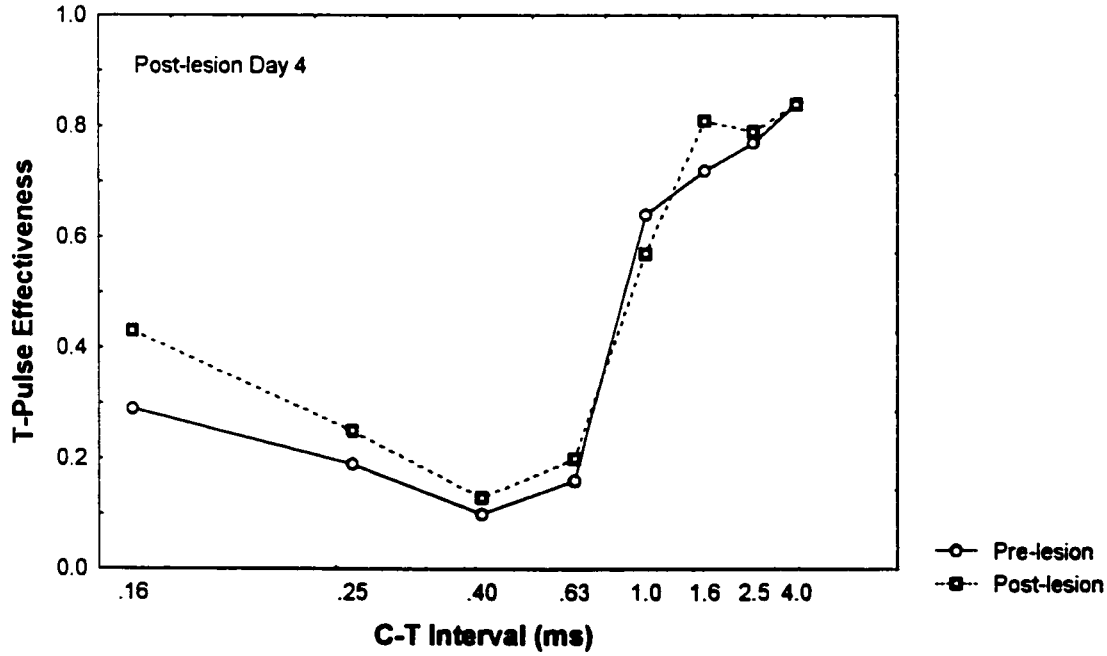


Figure 42

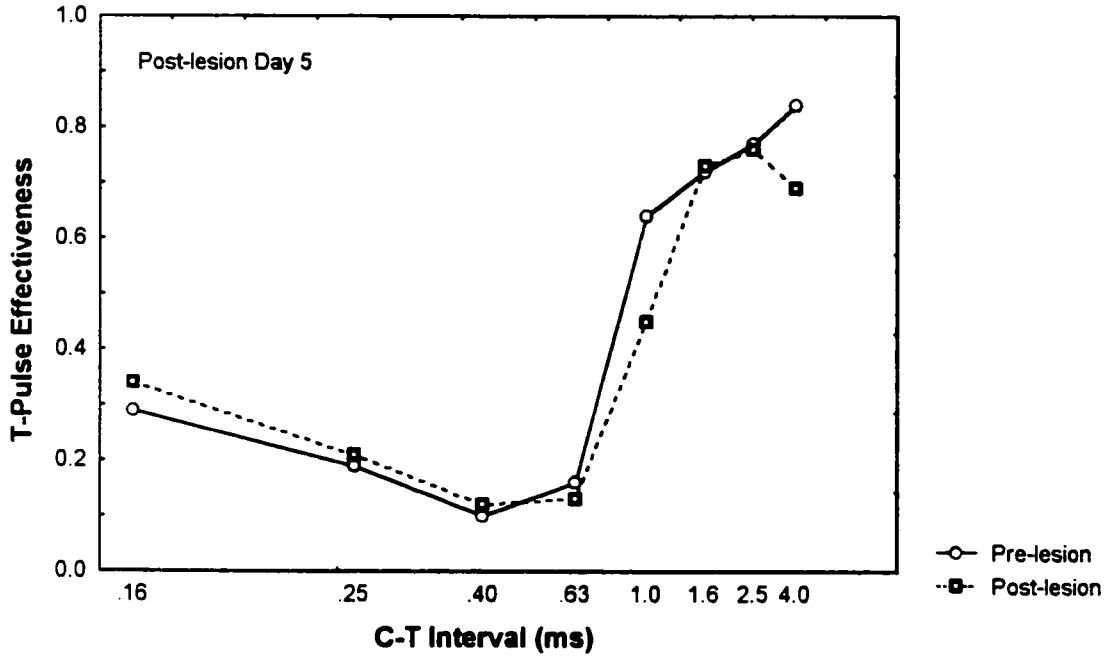


Figure 43

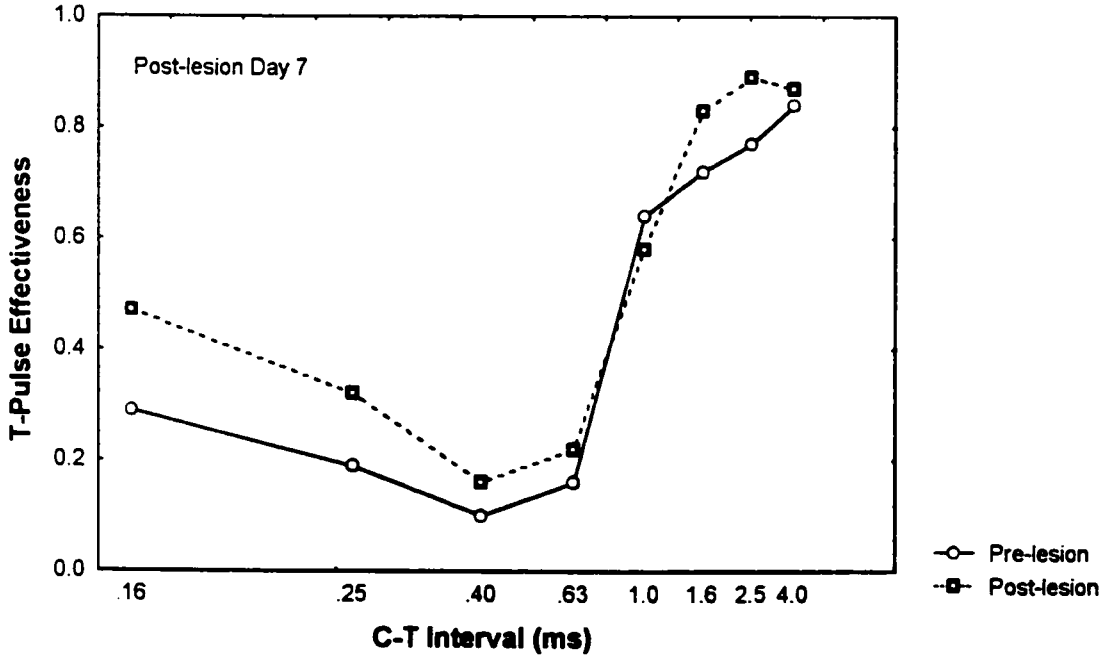


Figure 44

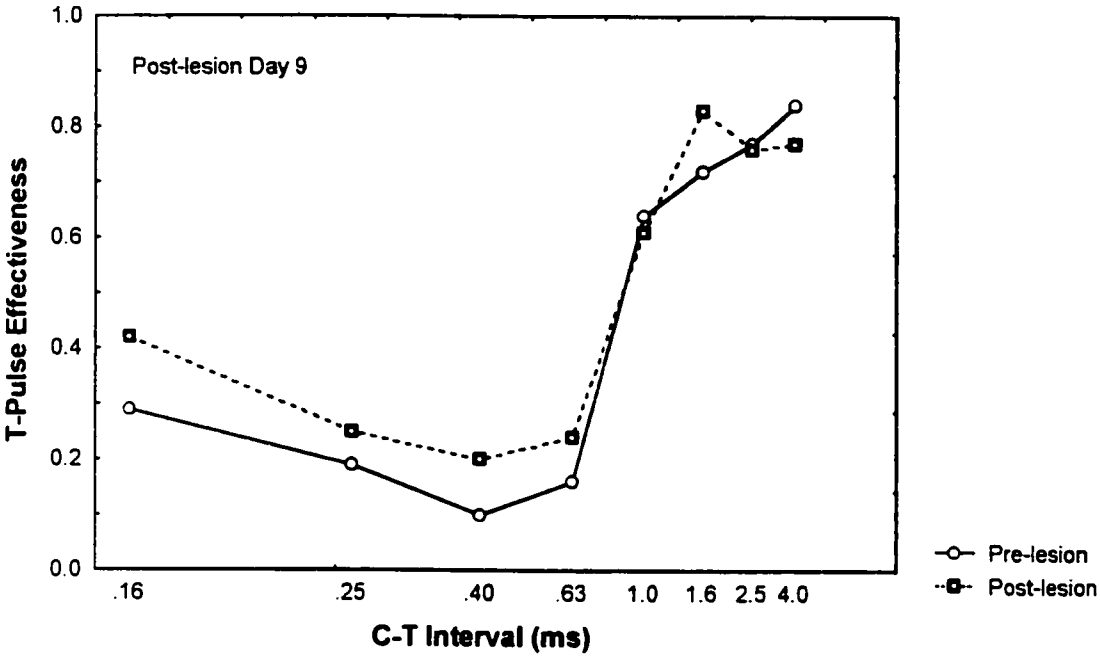


Figure 45

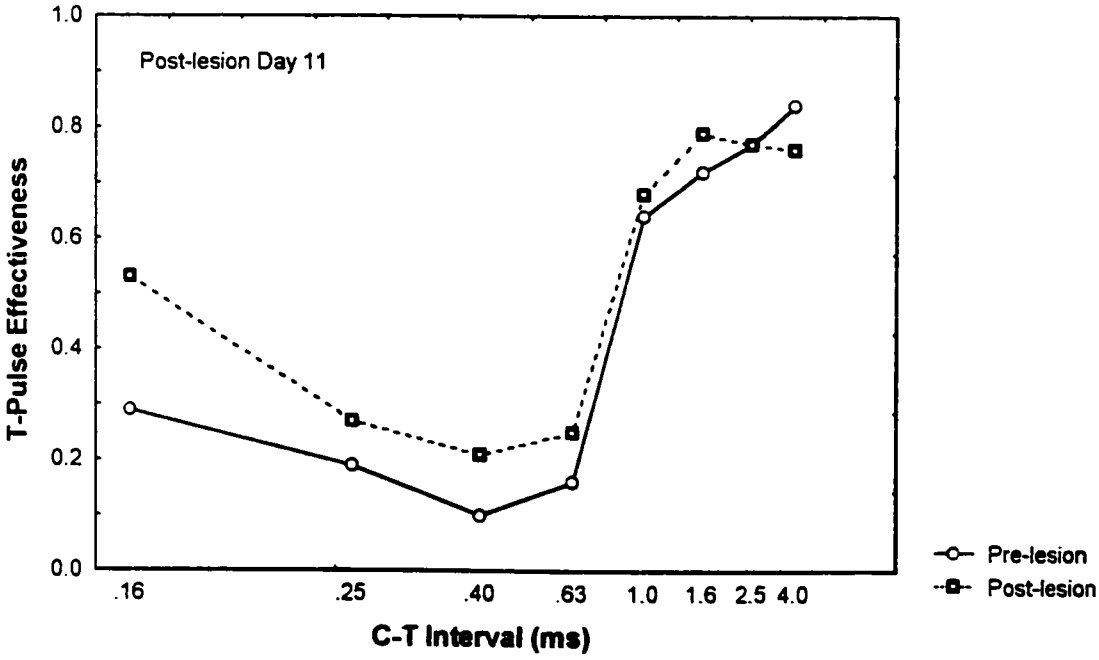
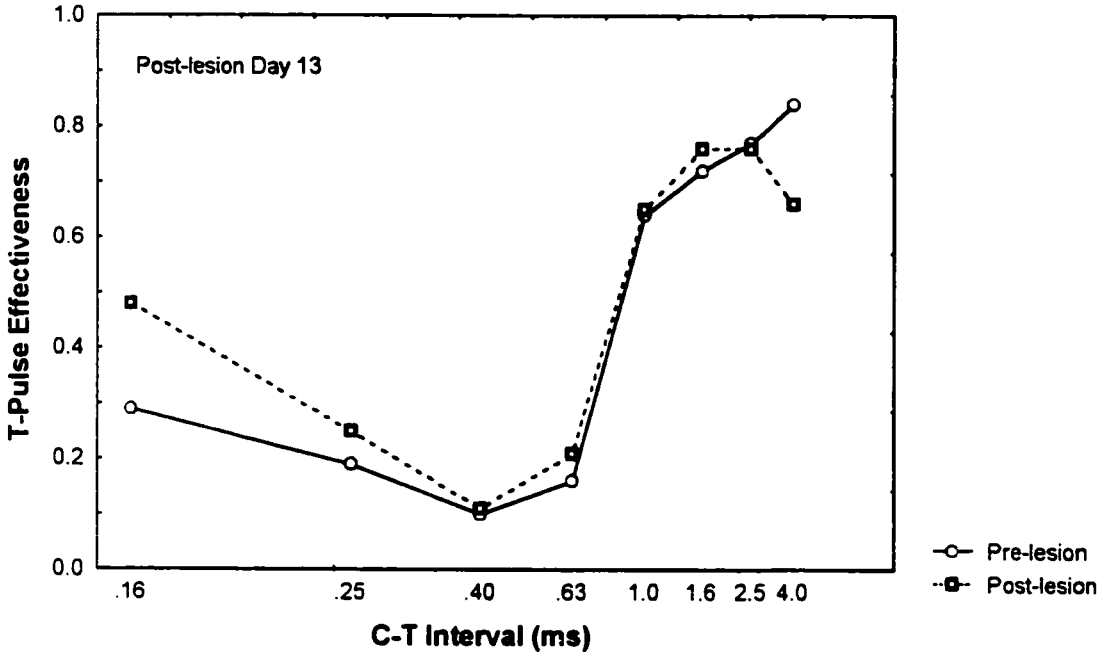


Figure 46



Local Potential Summation

As LPS typically contributes to excitability at very small C-T intervals, pre- and post-lesion refractory data were examined at 0.16 and 0.25 ms intervals. A change in the shape of the post-lesion refractory curve at 0.16 ms was evident, indicating an increase in LPS effectiveness at that interval (refer to Figures 38 to 46). Pre-lesion refractory data and post-lesion refractory data from the first and last three test days were also plotted across all C-T intervals for each rat (see Appendix A).

Repeated-measures *t* tests compared the effectiveness of paired-pulse stimulation across all of the animals before and after the lesion, at the two smallest C-T intervals (0.16 and 0.25 ms). Results of the *t* tests indicate that the increase in paired-pulse effectiveness was significant at 0.16 ms on post-lesion Days 1 and 13 (see Table 6).

Table 6

Post-Lesion Effectiveness of Paired-Pulse Stimulation

C-T Interval	t value					
	Day 1	**Day 2**	**Day 3**	**Day 9**	**Day 11**	**Day 13**
0.16 ms	-3.51*	-2.51	-1.81	-1.26	-2.41	-3.23*
0.25 ms	-0.37	-0.77	-1.21	-0.67	-1.29	-0.84

*$p < .05$; one-tailed test; $df = 5$

Discussion

We suggested that an artifact of a lesion stimulus like an extracellular edema or a disturbed ionic balance could be responsible for increasing reward thresholds. A change in the concentration of ions could alter a neuron's refractory period, as the rate of membrane repolarization could be reduced, affecting the recovery of excitability. However, although the lesion increased thresholds by as much as 145%, the refractory period did not change. Although this argues against the idea of an ionic imbalance, it is possible that applying a lesion stimulus for such a short period of time simply would not change the refractoriness of neurons in the stimulation field.

In this study, refractoriness was estimated using paired pulses of equal strength. Although applying a stronger T pulse is the appropriate test for measuring the contribution of the absolute refractory period to excitability, the equal pulses used in this study are likely showing that contribution. Researchers have shown that with stronger T pulses, the relative refractory period contributes very little to neural excitability at all pulse pair intervals (Bielajew & Shizgal, 1982; Yeomans, 1979).

One day after the lesions were made, the effectiveness of paired-pulse stimulation increased at 0.16 ms, the shortest C-T interval tested. As LPS typically occurs at pulse intervals of less than 0.4 ms, this change in excitability is likely due to an increase in the effectiveness of LPS. The increased effectiveness of LPS was not only observed soon after the lesion when thresholds were highest, but was maintained at the end of the test period. As LPS can only occur in fibers located at the edge of the stimulation field, the increased LPS effectiveness indicates that the lesion predominantly affected fibers very near to the tip of the electrode, leaving more of the excitable population in the fringe of the field. If fibers around the electrode tip remain affected by the lesion, a greater proportion of fibers in the fringe of the field relative to core fibers would continue to be reached by the stimulation. This richer distribution of fibers would maintain an enhanced effectiveness of LPS.

Thus the sustained increase in LPS effectiveness likely indicates permanent damage to fibers near the tip of the electrode. A histological examination of the lesion site lends some support to this conclusion. As shown in Figure B-12 in Appendix B, a thinning of fibers in the area around the tip of the electrode tracks is evident for two of the rats (#1289 and #1295). For these animals, LPS effectiveness remained higher at the end of the test period. Consistent with this indication of permanent damage is the fact that although thresholds decreased over the week following the lesions, they remained somewhat elevated in three of the four animals at the end of the test period. Results of repeated-measures t tests indicate that thresholds over the last four test days (post-lesion Days 7, 9, 11 and 13) were significantly higher than pre-lesion levels for three of the four rats (see Table 7).

Table 7

Pre- and Post-lesion Threshold Differences

Subject	t value
#1287	-3.37*
#1289	-20.37*
#1295	-7.76*
#1296	-1.49

*$p < .05$; one-tailed test; $df = 3$

Although thresholds remained somewhat elevated in three of the four rats at the end of the test period, indicating the likelihood of permanent damage, some recovery was evident in all of the animals within the first week. We considered the idea that the recovery might occur as collateral fibers sprout following the injury and take over some of the lost function. Studies supporting plasticity of the CNS have shown that adaptive changes in neural tissue adjacent to damaged areas can lead to a recovery of function lost due to injury (Nudo & Friel, 1999; Nudo, Jenkins, Merzenich, Prejean & Grenda, 1992). However, our LPS findings do not support that explanation. If threshold recovery was due to a sprouting of adjacent fibers, LPS should have increased in the first week as recovery took place, as collateral sprouting would result in even more fringe fiber stimulation versus core fiber stimulation. Instead, after the initial increase seen on post-lesion Day 1, LPS effectiveness decreased while thresholds for single-pulse stimulation recovered. Thus it seems unlikely that the partial recovery of thresholds seen in this study was due to collateral sprouting. It seems more likely that the recovery occurred as local tissue

resistance normalized over time.

In Experiments 3 and 4 we showed that after a lesion elevates reward thresholds, a decrease in threshold is probably due to recovery from an artifact of the lesion stimulus that alters the resistance of the tissue around the electrode. In this experiment we showed that the lesion-induced artifact did not change the refractory period of neurons in the stimulation field. Although this finding argues against the idea that an ionic imbalance occurred, it is possible that an ionic change could take place but not have an obvious effect on the refractoriness of those neurons. Whether or not an ionic change occurred, however, the stimulation's rewarding effectiveness could be attenuated when applying a lesion stimulus induces an extracellular edema. The normalization of an extracellular edema may well account for the recovery of thresholds seen in this study.

Overview

When a lesion increases the threshold for self-stimulation, it is concluded that the rewarding effect of the stimulation has been decreased. Thresholds often remain elevated throughout post-lesion testing, indicating permanent damage to reward-relevant fibers. However, at least some of the stimulation's rewarding effectiveness can recover after a lesion, with thresholds decreasing toward pre-lesion levels over time. Why this recovery occurs has not been well understood.

We suggested that the recovery may be due to the regeneration of axons. In the central nervous system, axonal regeneration begins within hours of axotomy, and axonal sprouts will proliferate rapidly. Within three days, the regrowth can extend around the ends of the lesions. Foerster (1982) showed that severed MFB fibers extended 300 to 400 μm around lesion sites within 18 days. The orientation of the regrowth appeared to be towards a reconstruction of the fiber tracts that had been interrupted by the knife cuts. Björklund and Lindvall (1979) found that fibers in the LH had extended a few millimeters to partially reconnect target cells three to six months after chemical axotomy.

Severed axons must extend and reconnect to appropriate target cells for function to recover. Borgens and his colleagues (1986; 1987) showed that functional recovery of a sensory reflex occurred when severed axons in the spinal cord made relevant synaptic connections. They also found that the regeneration was facilitated with the application of an electrical field, with behavioural recovery first seen 56 days after the lesions were made. The distance of the axonal growth was not reported.

These studies show that damaged central nervous system fibers can regenerate and extend at least a few millimeters to re-connect to appropriate target areas, enabling a functional recovery. Thus we thought that axonal regeneration and functional re-connection might explain the post-lesion recovery of reward thresholds. Although the functional recovery noted by Borgens et al. (1987) did not occur before about two months after the lesion, we considered the possibility that the distance necessary for the regrowth of axons in that study may have been much greater than that required in the present study. If so, axonal regeneration and functional re-connection could be a viable explanation for reward threshold recovery, particularly if the electrical stimulation used to measure thresholds actually promoted that recovery.

However, the results of Experiment 1 showed no indication that the electrical stimulation itself influenced the rate of recovery. Thresholds for most of the animals returned to pre-lesion levels within just over one week. Although a few of the rats did not fully recover, with thresholds remaining elevated at the end of the test period, at least some of the stimulation's rewarding effectiveness returned for all of the animals within this time frame.

We then tested the possibility that the functional recovery of reward thresholds could be due to another process that occurs distal to the stimulation site, like the development of an enhanced dopamine receptor sensitivity. Denervation supersensitivity is thought to account for the recovery of various behavioural deficits that follow a unilateral lesion. The behavioural recovery and the development of receptor supersensitivity occur within one to two weeks of the lesion, a time frame similar to that seen for the recovery of reward

thresholds. Thus we used apomorphine in Experiment 2 to test for supersensitivity of dopamine receptors. We also challenged the idea that reward thresholds recover because of another distally-occurring compensation, whatever the neurotransmitter, by studying the reaction of unlesioned and lesioned sites in unilaterally lesioned bilateral preparations.

The results of the apomorphine injections indicate that dopamine denervation supersensitivity did not account for the return of thresholds to baseline following the lesions. Consistent with this, and going beyond just dopamine, we noted that thresholds at unlesioned electrodes remained unchanged while those at lesioned sites recovered. If any sort of compensation occurred in cells immediately postsynaptic to the lesion site, thresholds at both electrodes should have been affected, given evidence of the existence of shared targets between the sites. It is possible that activity converges well beyond the cells immediately postsynaptic to the directly stimulated axons. If so, a distal compensation cannot be ruled out. On the other hand, the recovery may well occur regionally, possibly involving a restoration right at the lesion itself.

In Experiment 3 we studied regional changes in resistance at lesion sites, finding that measures of tissue resistance in the stimulation field initially drop, then gradually return to pre-lesion levels. The drop in tissue resistance mirrors the increase in reward thresholds seen right after a lesion, with both measures recovering at a similar pace over the next week. We concluded that some artifact that occurs when a lesion stimulus is applied alters the resistance of the tissue. The drop in resistance lowers the effectiveness of the stimulation, resulting in higher thresholds.

The stimulation's effectiveness is decreased when the pulse generator lowers voltage output to compensate a drop in resistance. As such, we expected to see a smaller attenuating effect if pulse voltage was not allowed to vary. To that end, we compared post-lesion threshold changes daily under two sources of stimulation in Experiment 4. Under constant-current stimulation, thresholds increased substantially over pre-lesion levels. However, when constant voltage pulses were used the same day with the same rats, that functional lesion effect was not seen. We concluded that a lesion-induced decrease in resistance around the electrode is responsible for attenuating the rewarding effect of the stimulation. The stimulation's rewarding effectiveness returns as the tissue resistance normalizes over time.

Applying a lesion stimulus can result in a local extracellular edema and/or an imbalance in ionic concentration. Either of these artifacts could alter tissue resistance. To see whether evidence for an artifact could be obtained, we followed the recovery of thresholds with post-stimulation excitability tests, considering that the refractoriness of neurons may change following a lesion. Experiment 5 indicated that the refractory period of neurons in the stimulation field did not change after a lesion. This argues against the idea that an ionic imbalance is responsible for reducing tissue resistance. However, it is possible that a change in the ionic concentration in the vicinity of the lesion occurred but had no obvious effect on the absolute refractory period. The post-stimulation excitability tests did show that changes occurred in LPS following the lesion. Those changes were sustained at the end of the test period, indicating that the lesion caused long-term or permanent damage to fibers near the tip of the electrode, where LPS occurs.

Consistent with this conclusion, thresholds for single-pulse stimulation remained somewhat elevated at the end of the test period.

This study, then, examined some possible mechanisms that could underlie the post-lesion recovery of the rewarding effectiveness of electrical brain stimulation. Our results show that reward thresholds increase when an artifact due to the application of a lesion stimulus results in a drop in tissue resistance near the electrode. Within days of a small lesion, thresholds recover as the resistance normalizes. The post-lesion drop in tissue resistance results in a reduced effectiveness of the stimulation current, with less transmembrane potential of neurons in the stimulation field accounting for the increases in reward thresholds.

Although it is not clear what precipitates the drop in tissue resistance, we considered two possible events that can occur with the application of a lesion stimulus. First, an extracellular edema may be induced following even slight damage to local blood vessels. The efflux of solutes and water from the damaged vessels results in a widening of the local extracellular space that would decrease tissue resistance in the area. The extracellular space can remain enlarged until the fluid disperses during the first week or two after the injury. Tissue resistance would normalize as the extracellular edema dissipates over time.

Second, the effectiveness of the stimulation current can be reduced when a lesion induces a local ionic change. Even small variations in the concentration of certain ions in the interstitial fluid can disturb cell excitability and synaptic transmission. While any ion change could affect the transmission of action potentials, it seems possible that post-lesion changes in potassium levels could play an important role. Specifically, a high concentration

of potassium in extracellular fluid would be expected to decrease the excitability of cells in the area. Results of a study by Huxley and Stämpfli (1951) provide indirect support for this idea, showing that raising the potassium concentration of a solution surrounding myelinated nerve fibers caused a decrease in both the resting membrane potential and the overshoot (excess action potential beyond the resting potential), blocking impulse conduction.

When neurons are injured potassium is lost from the damaged tissue, leading to acutely elevated extracellular levels of this ion (Eidelberg et al., 1975; Young & Koreh, 1986). Even without neuronal injury, however, extracellular potassium levels could increase with damage to astrocytes, which may be more susceptible to the effects of a small lesion. Astrocytes take up and store potassium, protecting neurons from the effects of high concentrations of these cations (Edström et al., 1995). With astrocyte damage, the released potassium would not likely be taken up. The excess potassium would likely result in a partial hyperpolarization of cells near the lesion. The high levels of extracellular potassium would also likely result in a decrease in local tissue resistance, as more charge carriers would now surround the cells. Both results would contribute to a block in the generation and conduction of action potentials.

Thus a lesion can induce edema and increase the concentration of potassium in the extracellular space, both of which would lower tissue resistance that would reduce the effectiveness of stimulation. We have shown that the rewarding effectiveness is altered in the area around a lesion. With a lesion made through the stimulating electrode, increases in reward thresholds indicate an interference with the generation of action potentials.

However, given that a drop in tissue resistance would occur wherever the lesion was placed, this finding is relevant to researchers studying the effects of distal lesions on thresholds for self-stimulation. Because tissue resistance is decreased in an area around the lesion, reward thresholds at a stimulation site distal to the lesion would increase if both sites shared common reward-relevant fibers that were directly affected by the drop in resistance. Thus, with distal lesion/stimulation sites, a reduced tissue resistance could interrupt the conduction of action potentials traveling along a reward pathway and increase reward thresholds.

Our findings can help to clarify results of lesion/self-stimulation studies attempting to map pathways important to reward. When a distal lesion results in permanent threshold increases but partial recovery is seen, it can be concluded that the lesion interrupted some but not all of the fibers in the pathway that supports self-stimulation. The threshold is permanently increased because of the cut fibers, while the partial recovery occurs because remaining fibers in the vicinity of the lesion are temporarily affected by the decreased resistance due to application of the lesion stimulus.

Although permanent threshold increases can clearly indicate anatomically connected lesion/stimulation sites, mapping information can also be obtained from animals with lesion effects that are wholly transient. When the rewarding effectiveness of the stimulation is only temporarily attenuated by a distal lesion, it is clear that the lesion must be close enough to affect some of the reward cells in the pathway, as thresholds for self-stimulation increase. As the lesion is slightly offside, no permanent damage is done to fibers. Over time the tissue normalizes and thresholds recover. Thus with temporary threshold

increases it can be concluded that the reward pathway must run by the edge of the lesion. A slight realignment of the lesion placement could result in permanent threshold increases at distal stimulation sites, indicating permanent damage to fibers common to both sites.

Understanding that transient threshold increases indicate that the lesion is placed very near a pathway important to reward may help researchers in future attempts to map the neural circuitry involved in reward. However, this information is also important to past researchers that have reported transient threshold increases following a lesion. Because the locations of the lesions are known from histology, a re-assessment of the data may help to clarify results and guide future research.

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Appendix A

Pre- and Post-Lesion Effectiveness of Paired-Pulse Stimulation

Figures A-1 to A-8. Pre- and post-lesion effectiveness of paired-pulse stimulation. Post-lesion effectiveness of pulse pairs is plotted across C-T intervals on post-lesion Days 1, 2 and 3, and on Days 9, 11 and 13 for each rat (n = 4). The post-lesion (PL) test days are identified in the top left corner of each figure, while the number in the bottom right corner identifies the subject. Circles joined by solid lines represent average pre-lesion data points.

Figure A-1

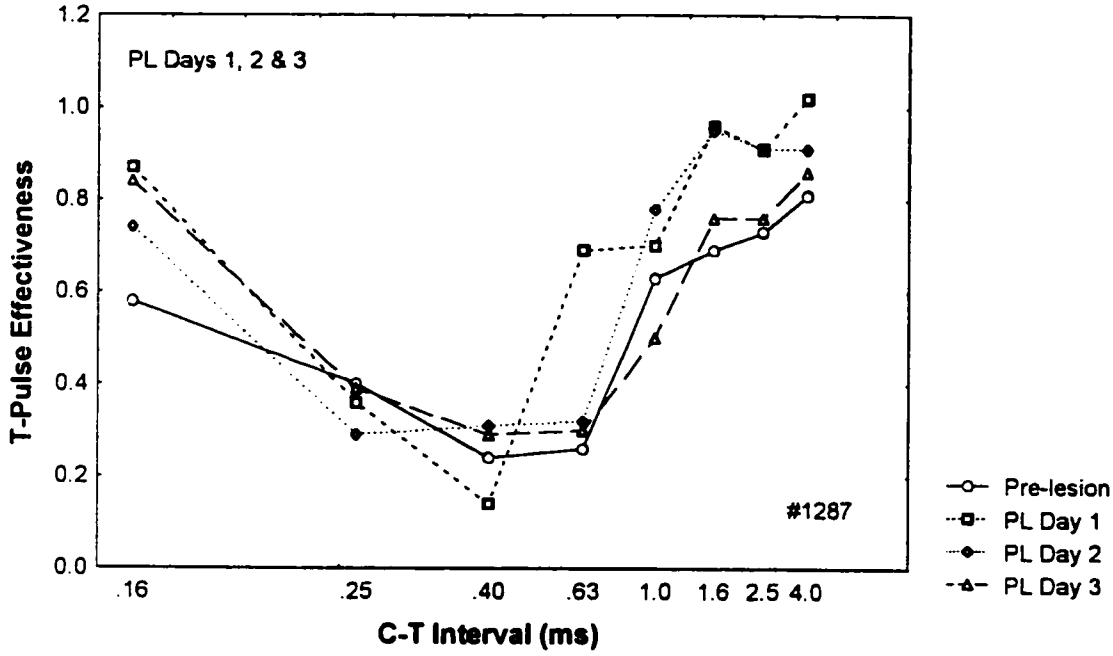


Figure A-2

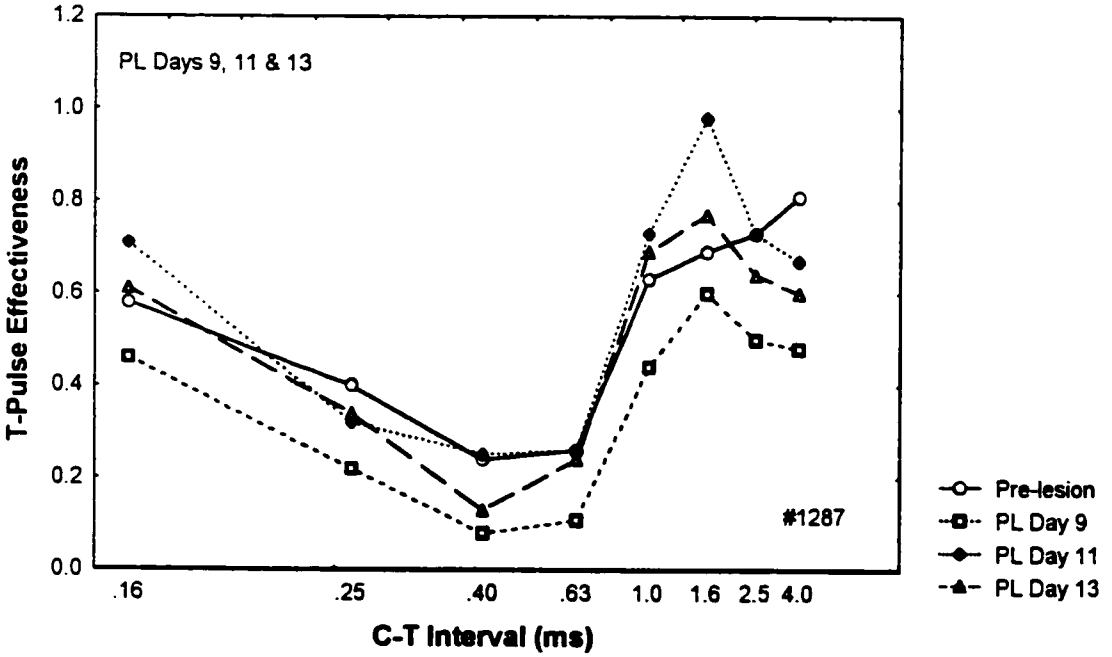


Figure A-3

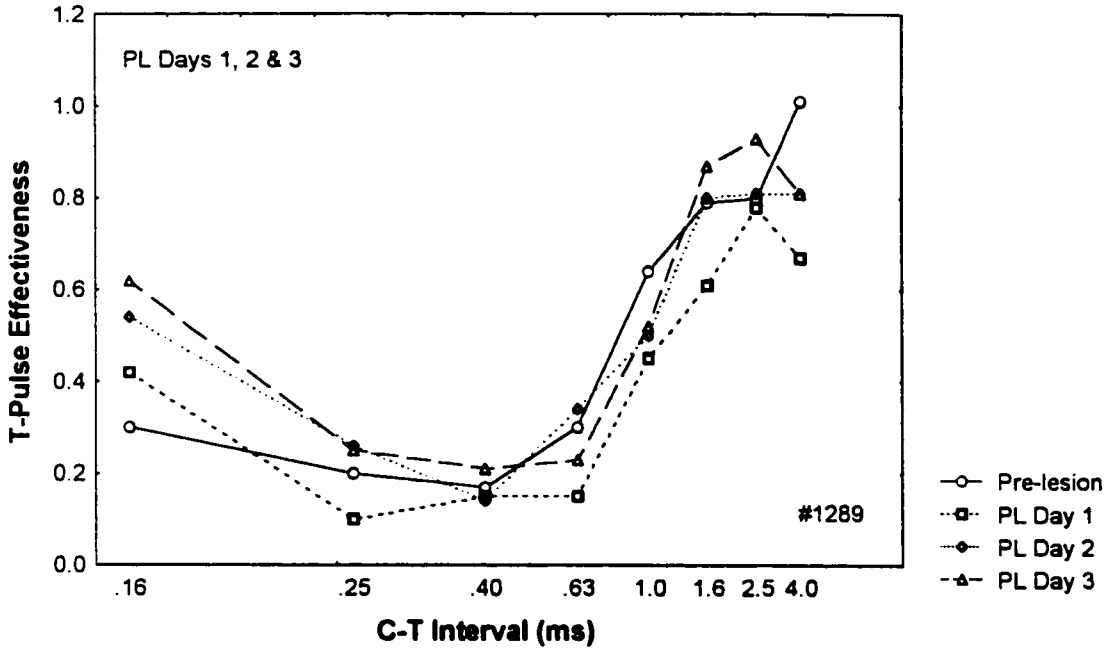
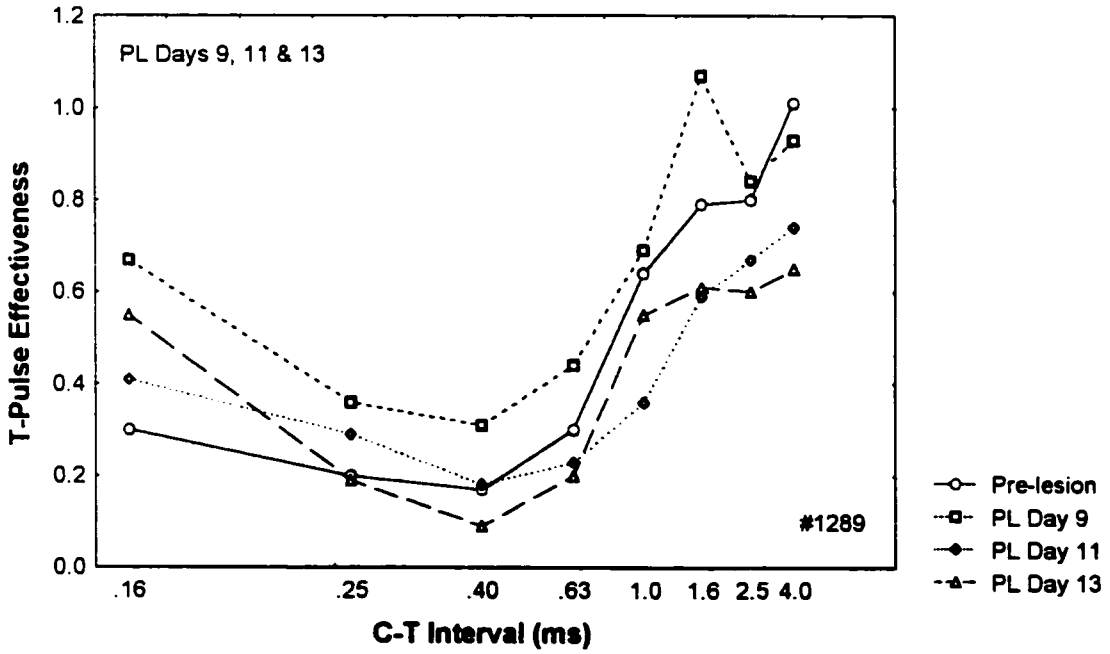


Figure A-4



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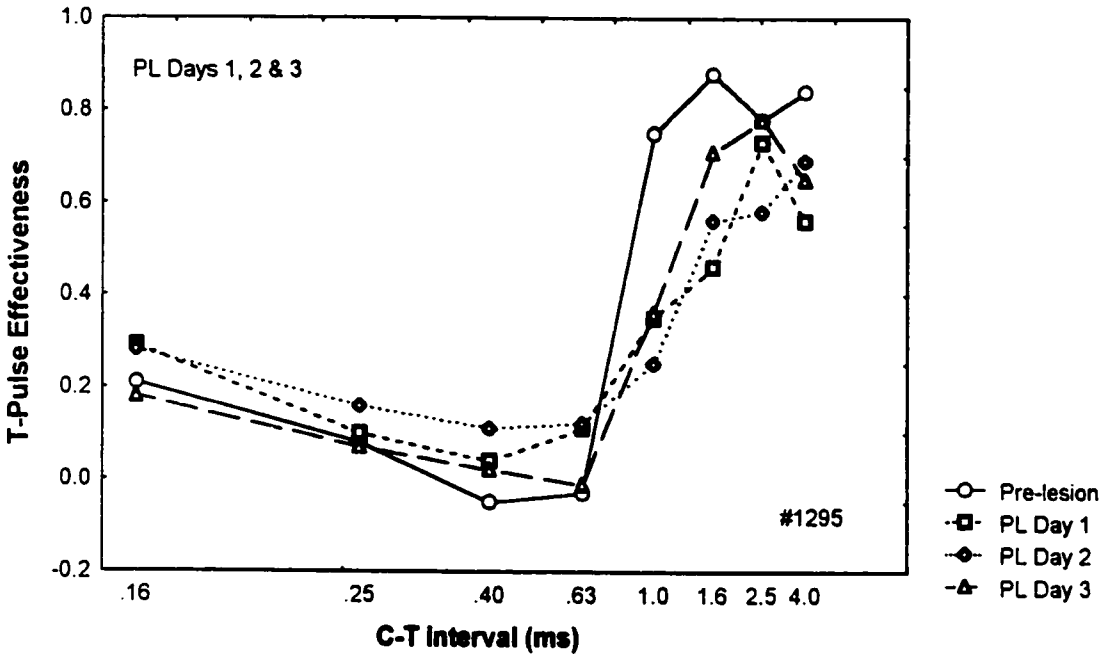


Figure A-6

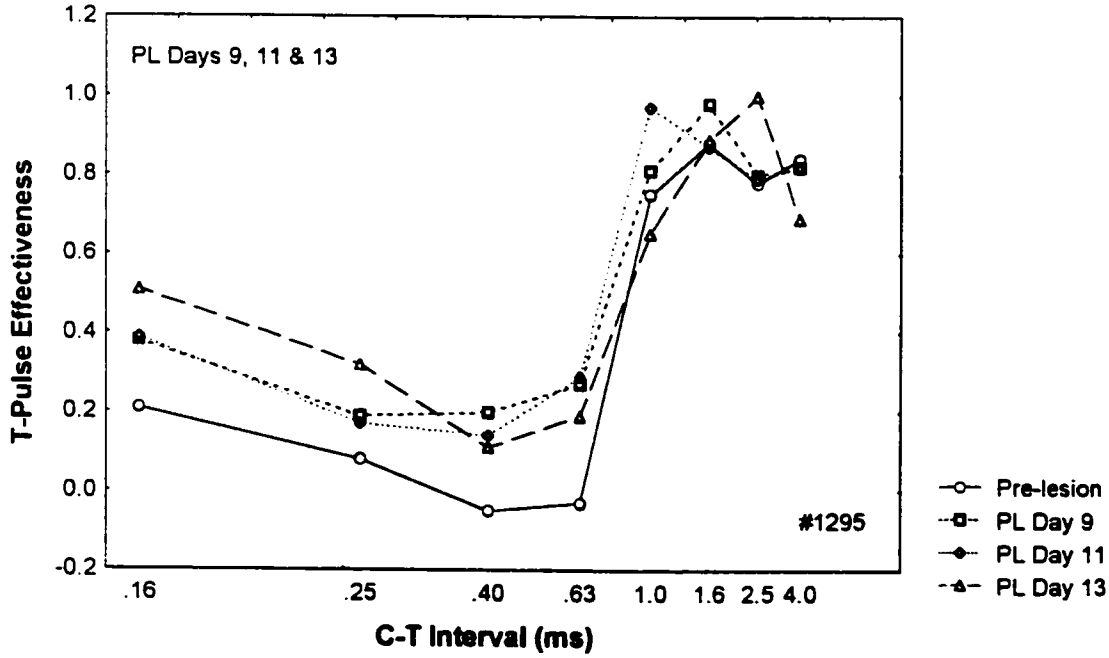


Figure A-7

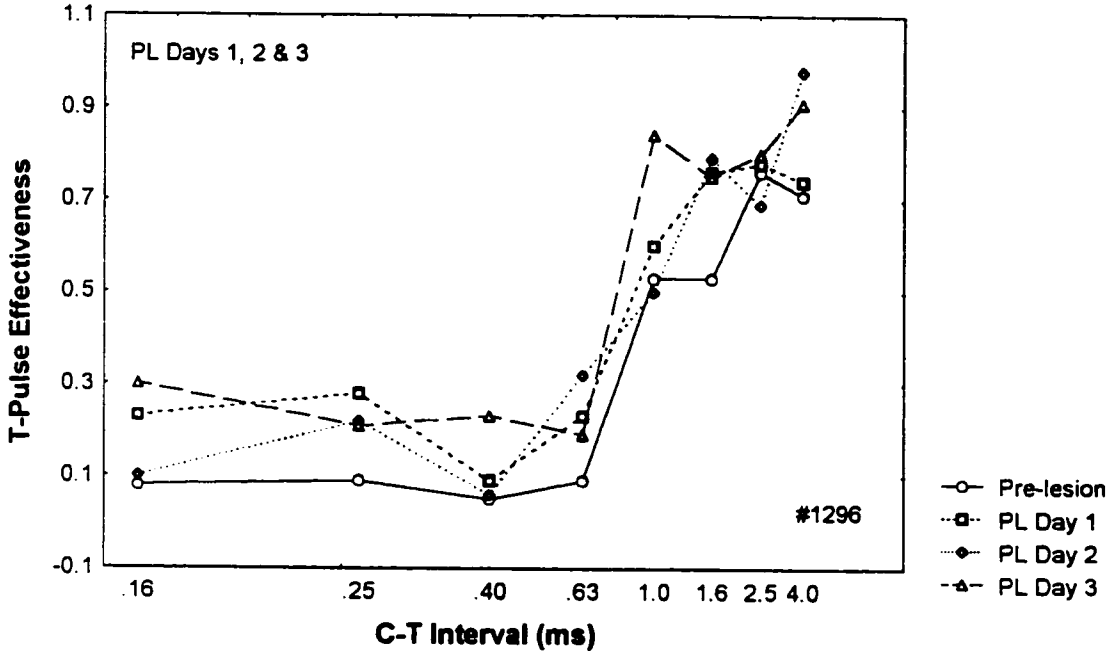
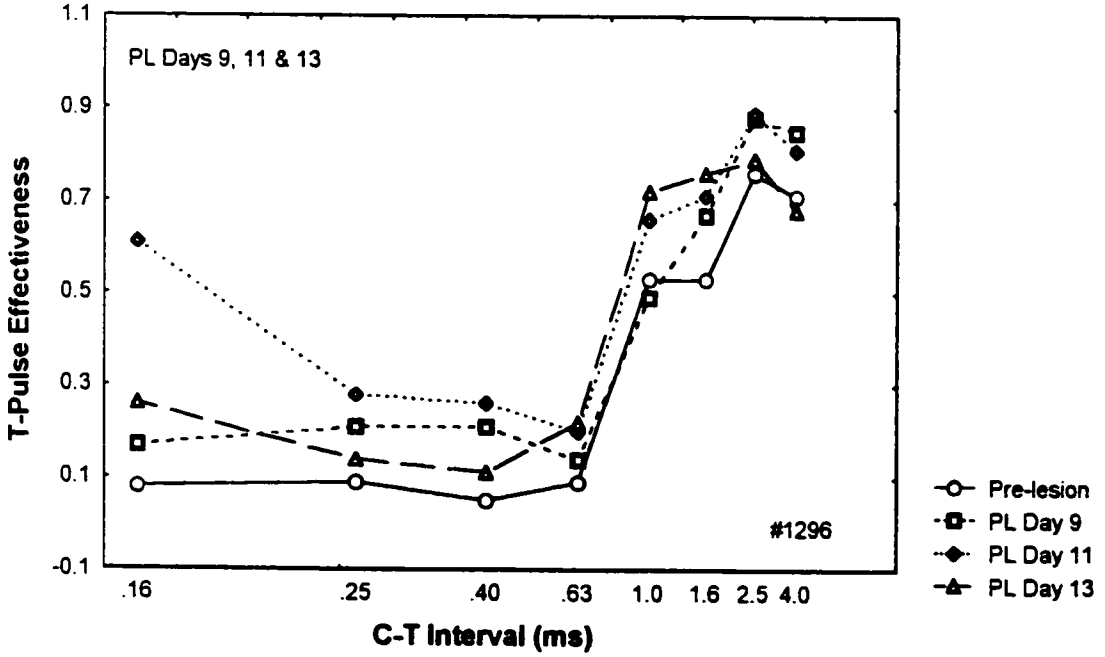


Figure A-8



Appendix B

Photomicrographs of Stimulating Electrode Tip/Lesion Sites

Experiment 1

Figure B-1. Photomicrographs of coronal sections showing LH lesion/stimulating electrode tip locations for 8 rats tested every day post-lesion. Numbers in the upper left corners identify the rats. Bar = 0.5 mm.

Figure B-2. LH lesion/stimulating electrode tip locations for 7 rats tested every other day post-lesion. Numbers in the upper left corners identify the rats. Bar = 0.5 mm.

Figure B-3. LH lesion/stimulating electrode tip locations for 8 rats tested every fourth day post-lesion. Numbers in the upper left corners identify the rats. Bar = 0.5 mm.

Figure B-4. LH lesion/stimulating electrode tip locations for 4 rats tested only on post-lesion days 1 and 13. Numbers in the upper left corners identify the rats. Bar = 0.5 mm.

Figure B-5. LH stimulating electrode tip locations for 2 rats without lesions. Numbers in the upper left corners identify the rats. A magnification of each electrode track is shown in the right column. Bar = 0.2 mm.

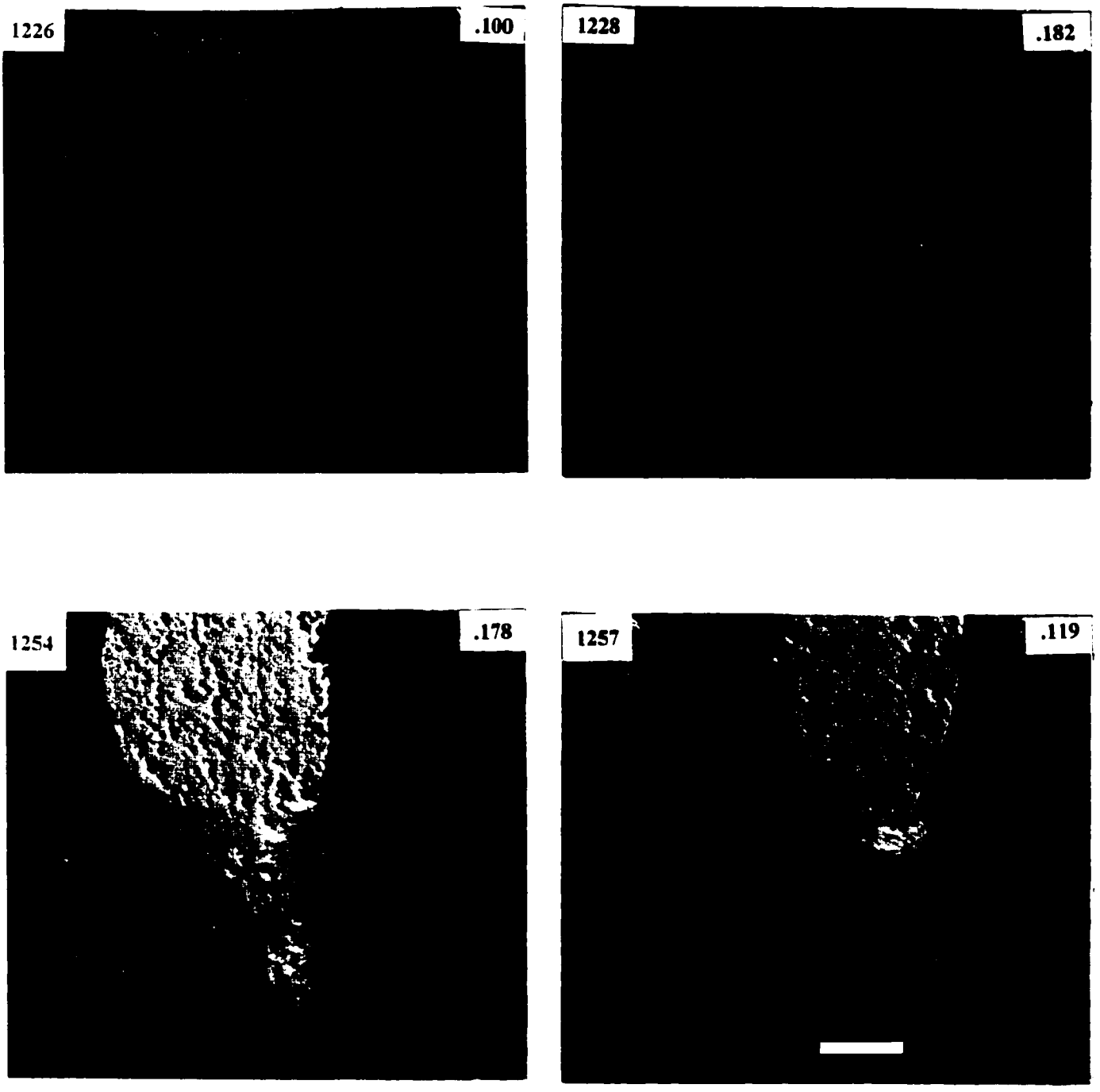
Figure B-6. Magnification of lesion/stimulating electrode tracks for 7 rats with small functional lesion sizes. Numbers in the upper right corners show the functional lesion sizes in log units. Numbers in the upper left corners identify the rats. Bar = 0.2 mm.

Figure B-7. Magnification of lesion/stimulating electrode tracks for 7 rats with medium functional lesion sizes. Numbers in the upper right corners show the functional lesion sizes in log units. Numbers in the upper left corners identify the rats. Bar = 0.2 mm.

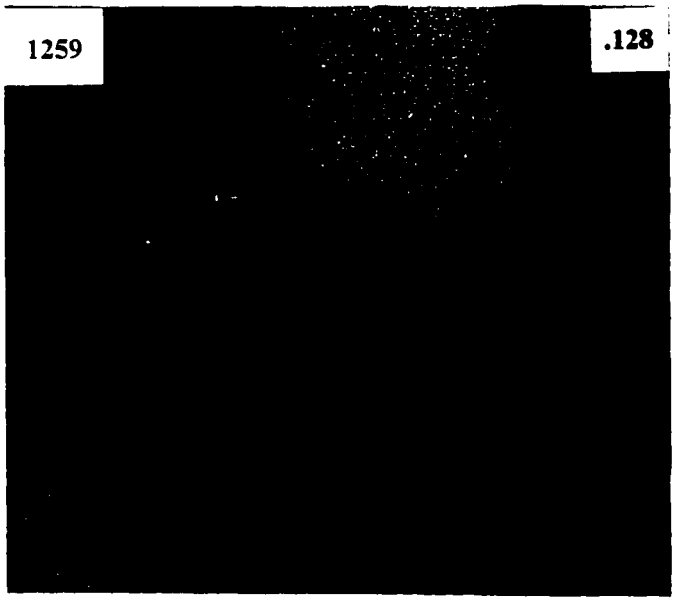
Figure B-8. Magnification of lesion/stimulating electrode tracks for 13 rats with large functional lesion sizes. Numbers in the upper right corners show the functional lesion sizes in log units. Numbers in the upper left corners identify the rats. Bar = 0.2 mm.

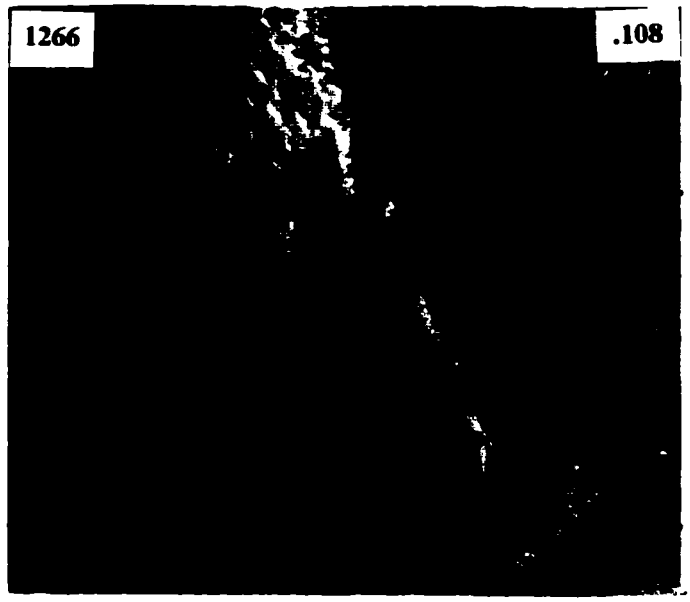
Small Functional Lesion

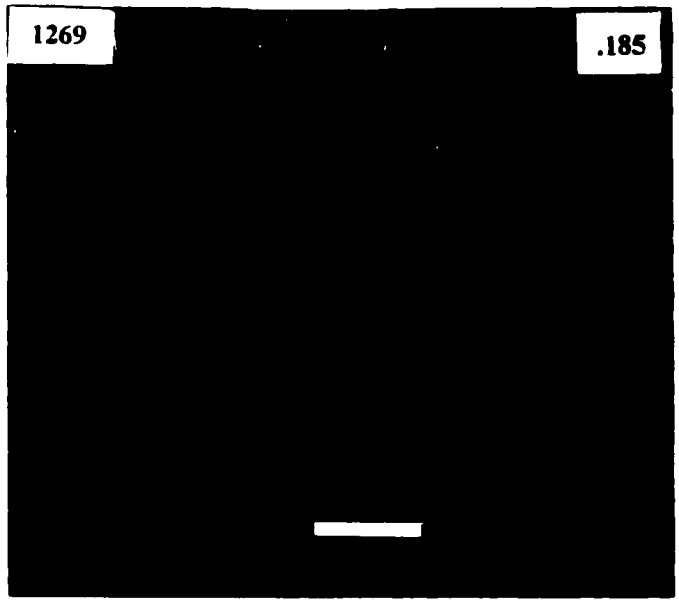
Figure B-6



Small Functional Lesion

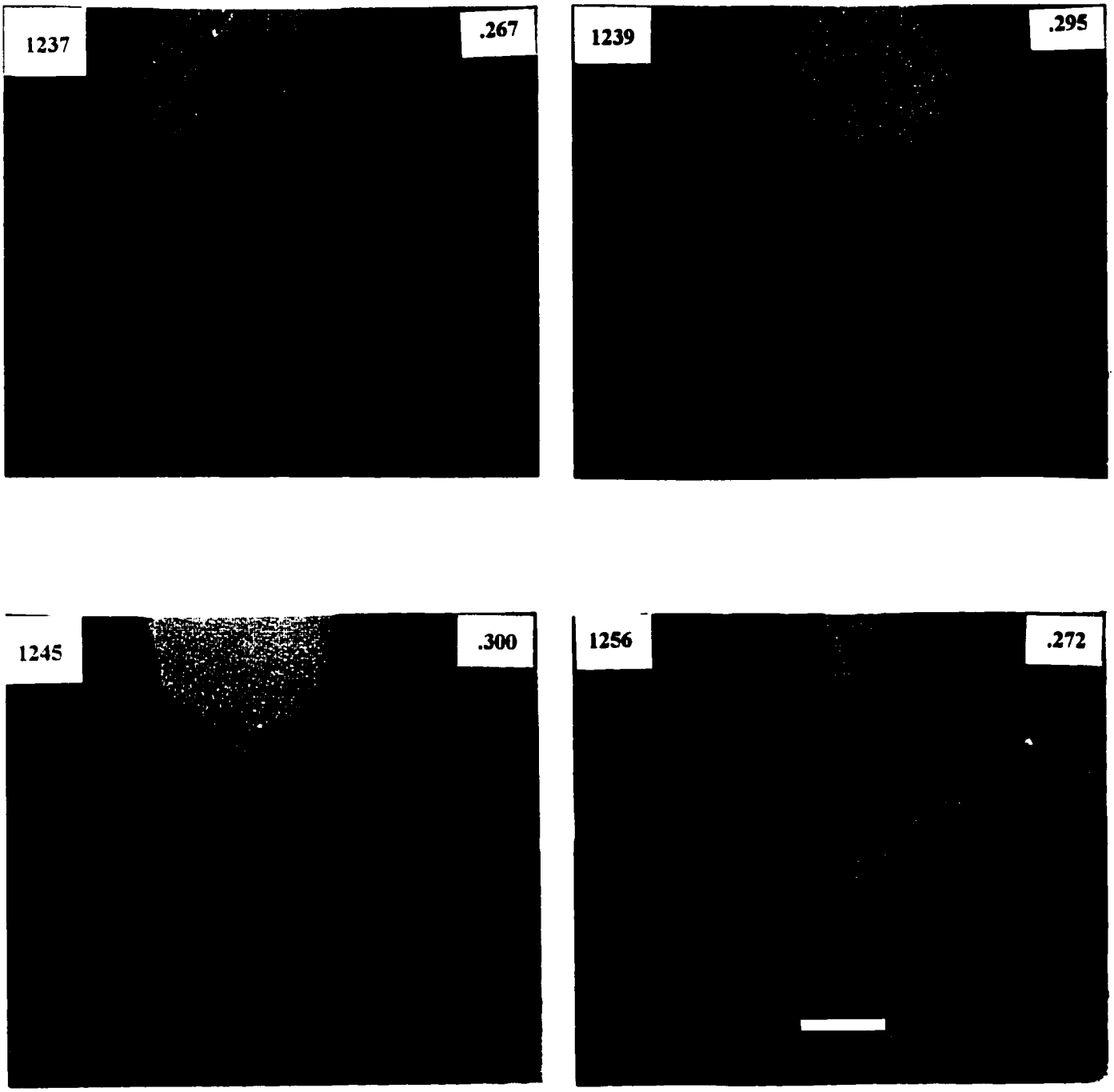




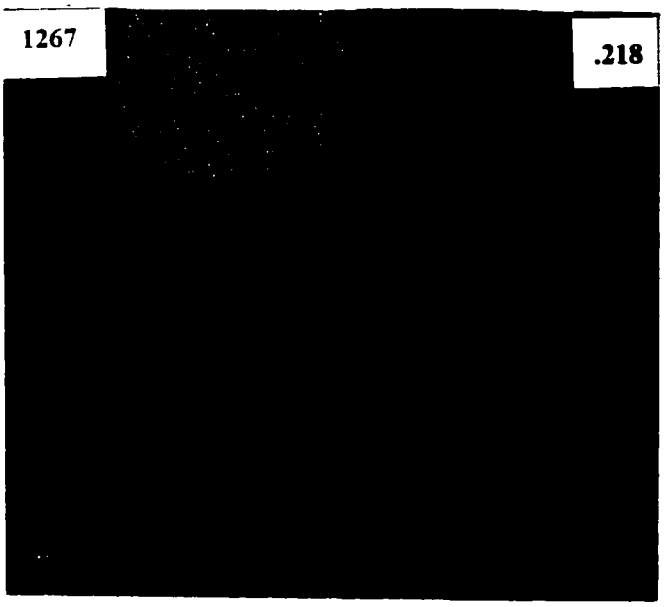


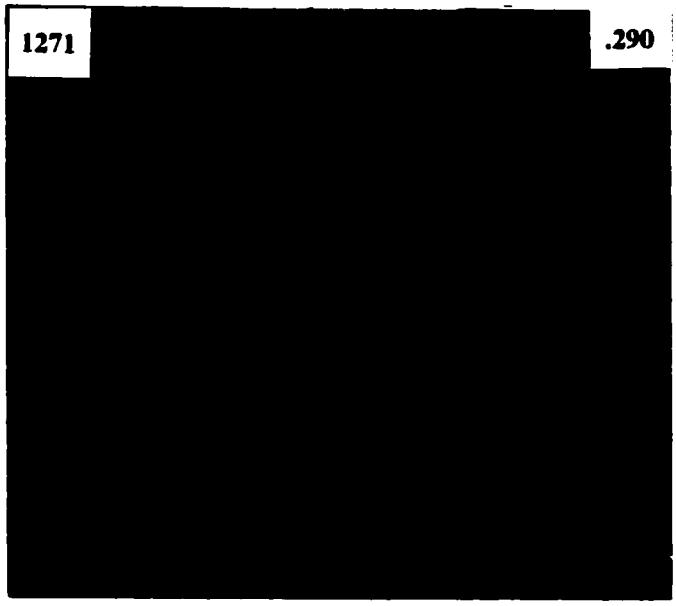
Medium Functional Lesion

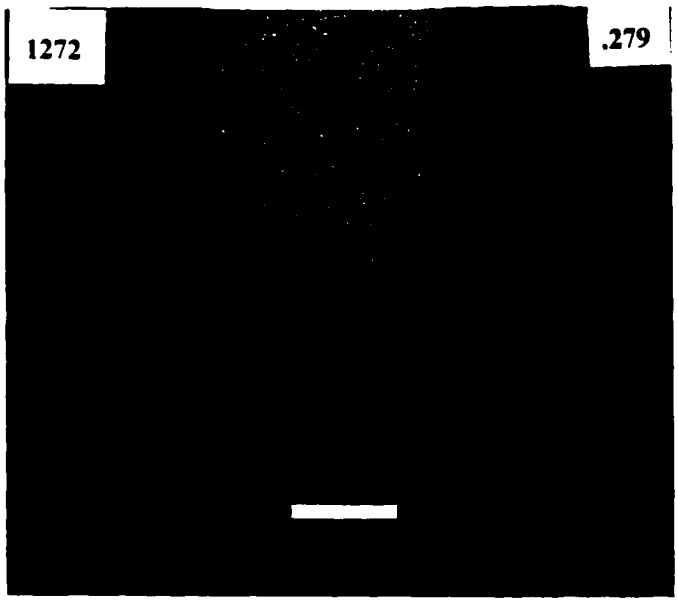
Figure B-7



Medium Functional Lesion

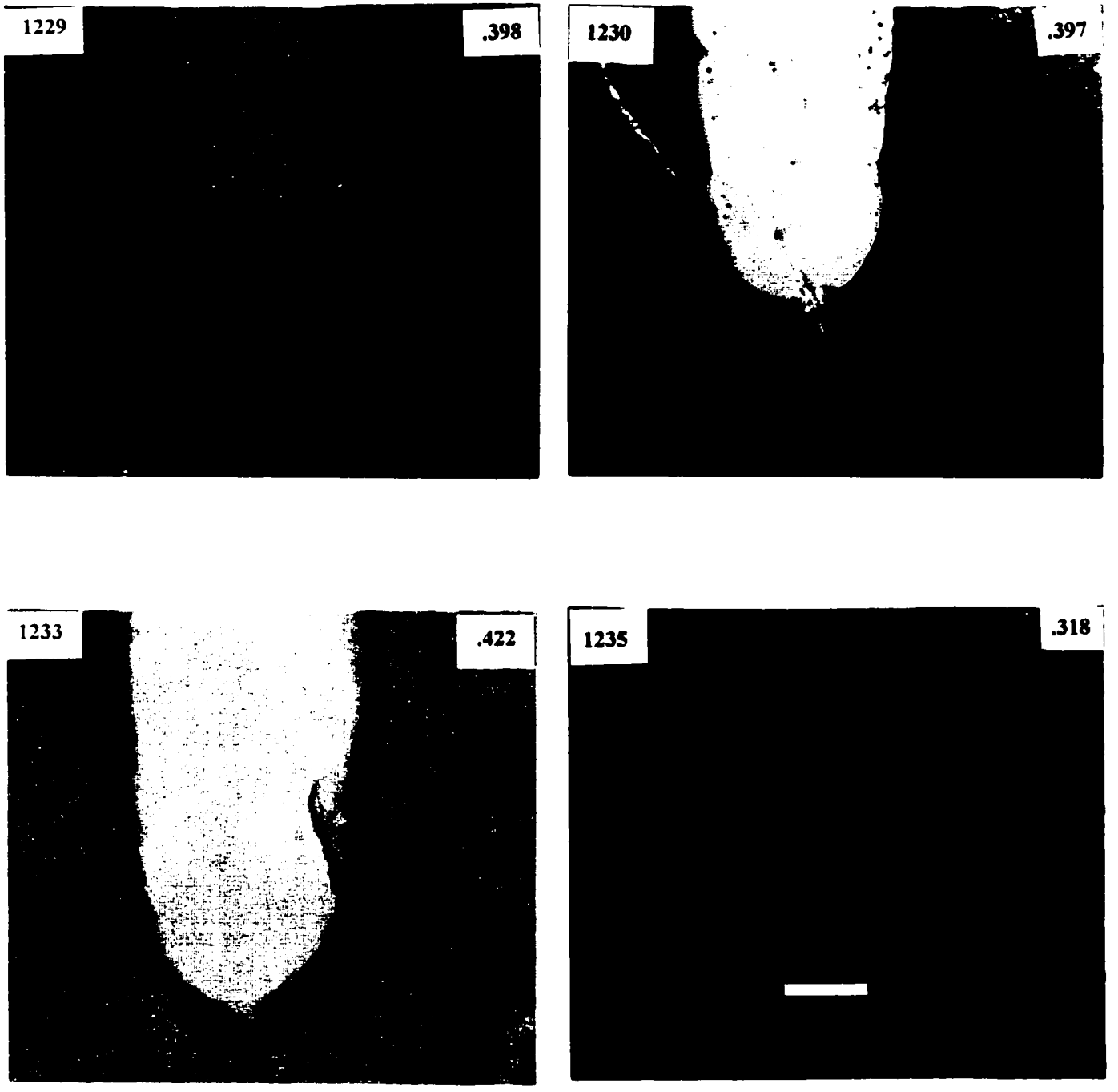




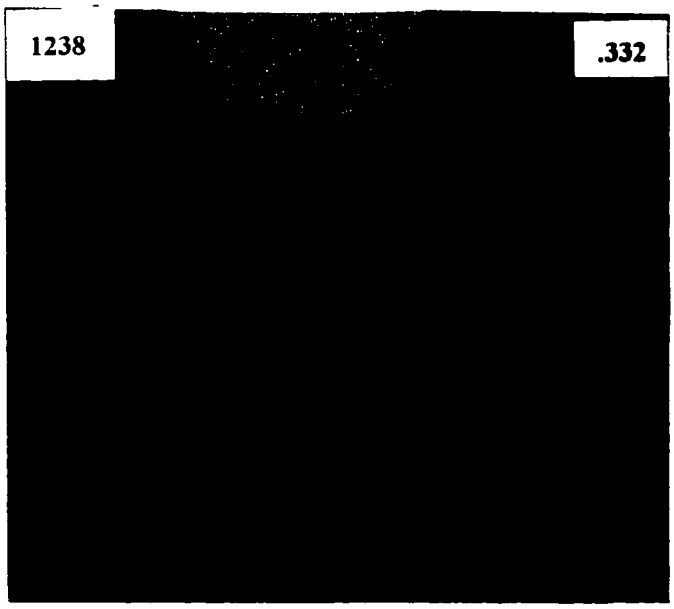


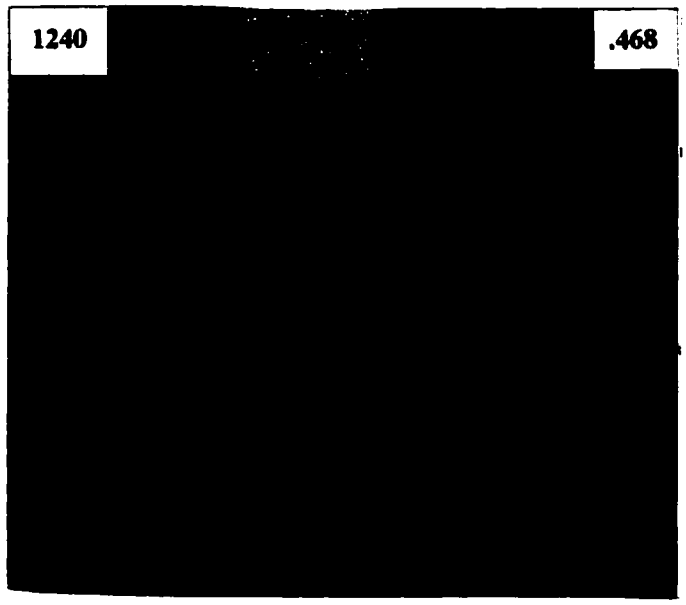
Large Functional Lesion

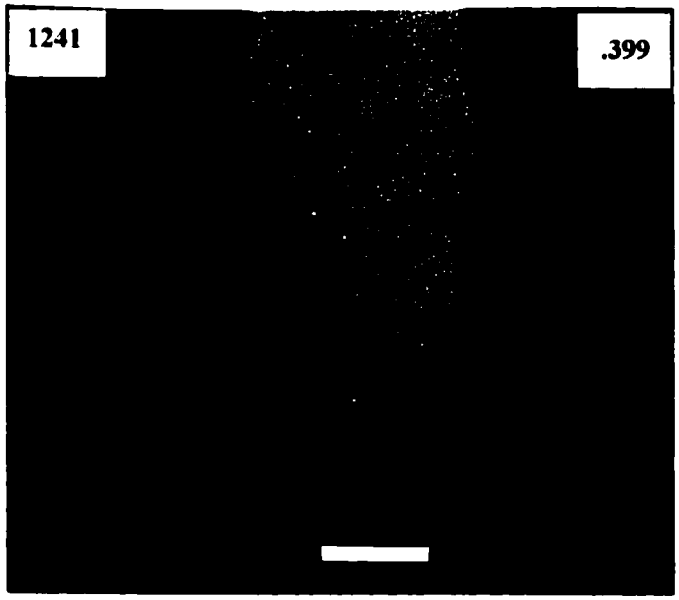
Figure B-8



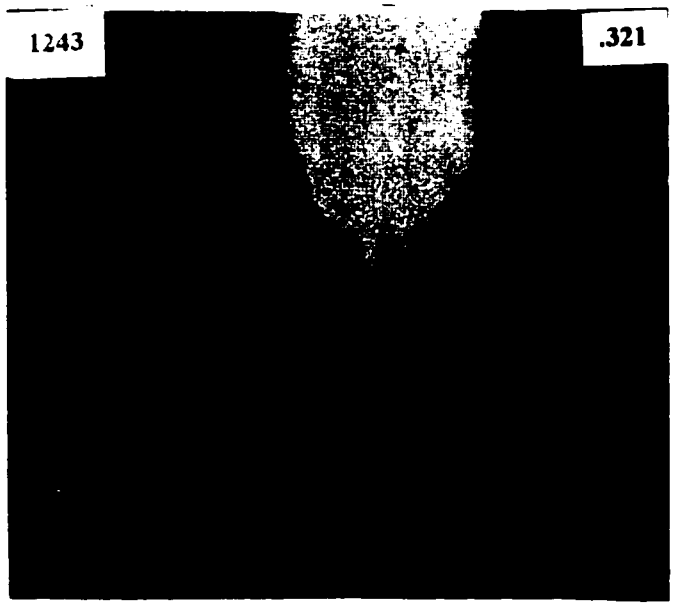
Large Functional Lesion

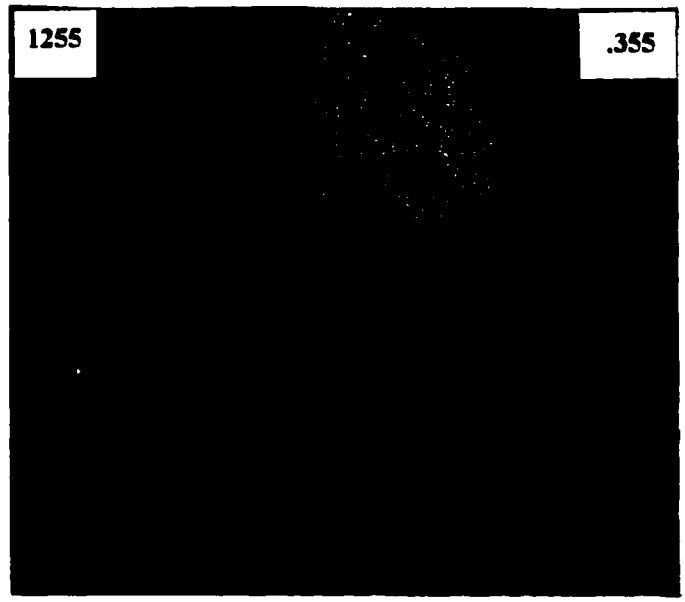


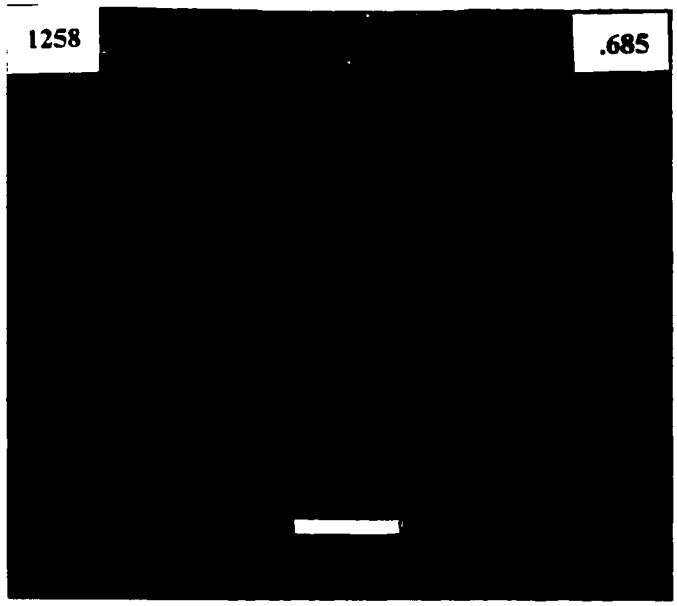




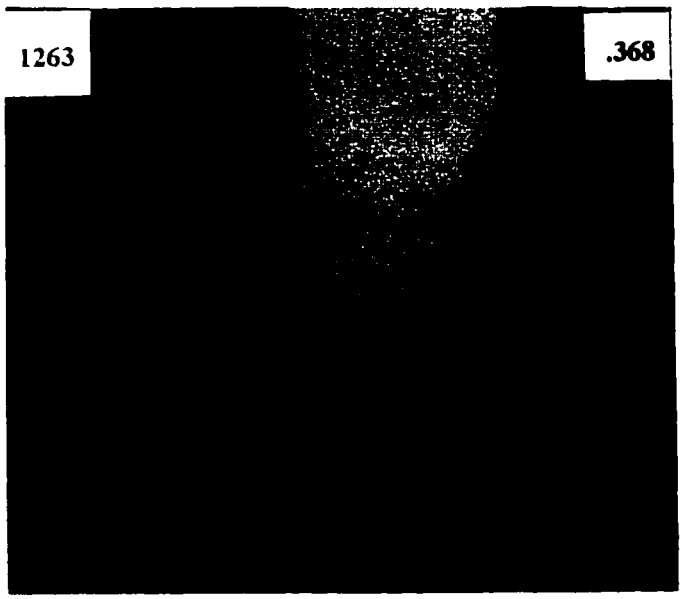
Large Functional Lesion

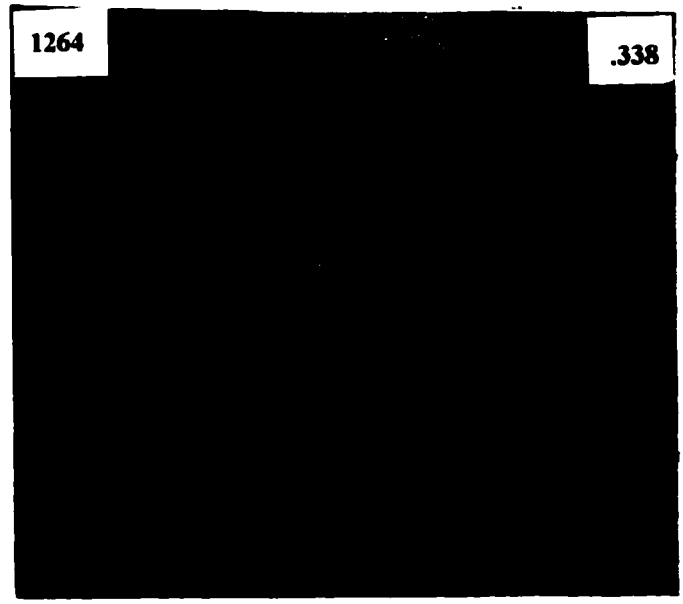


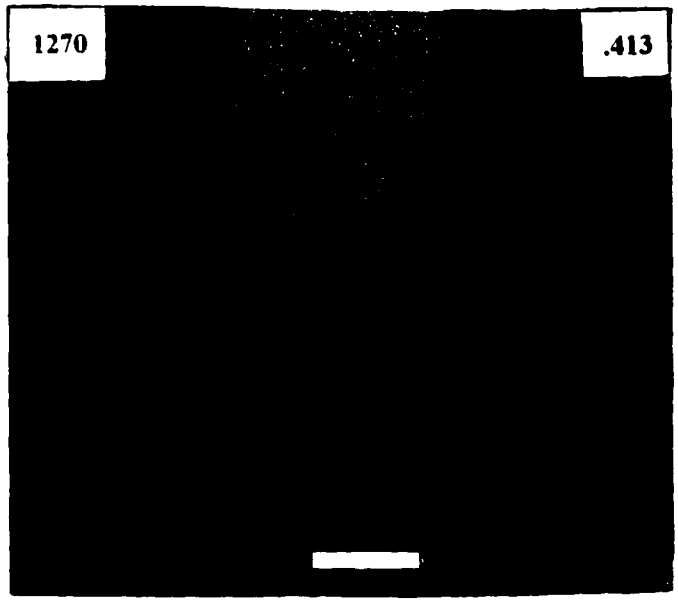




Large Functional Lesion



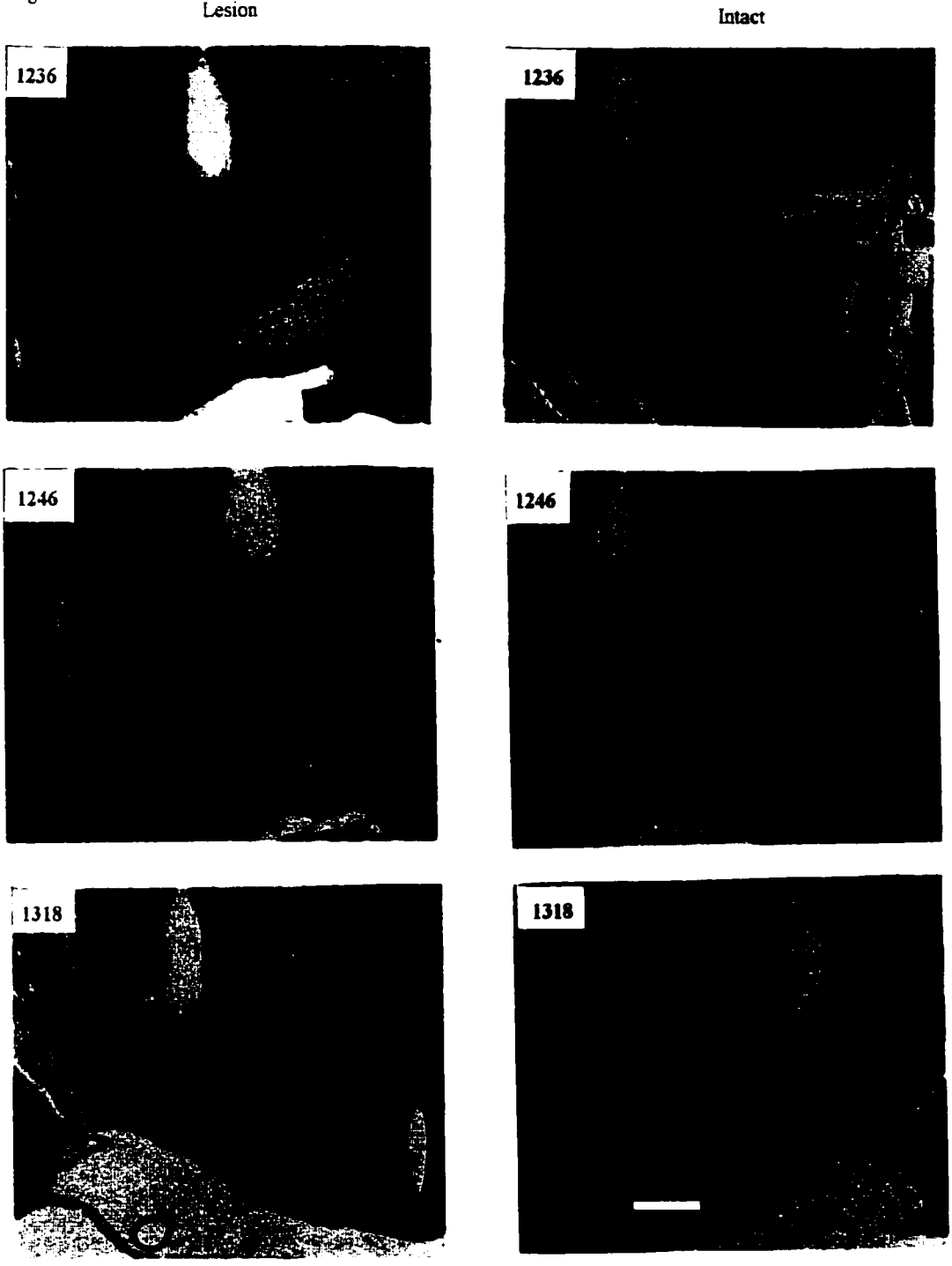




Experiment 2

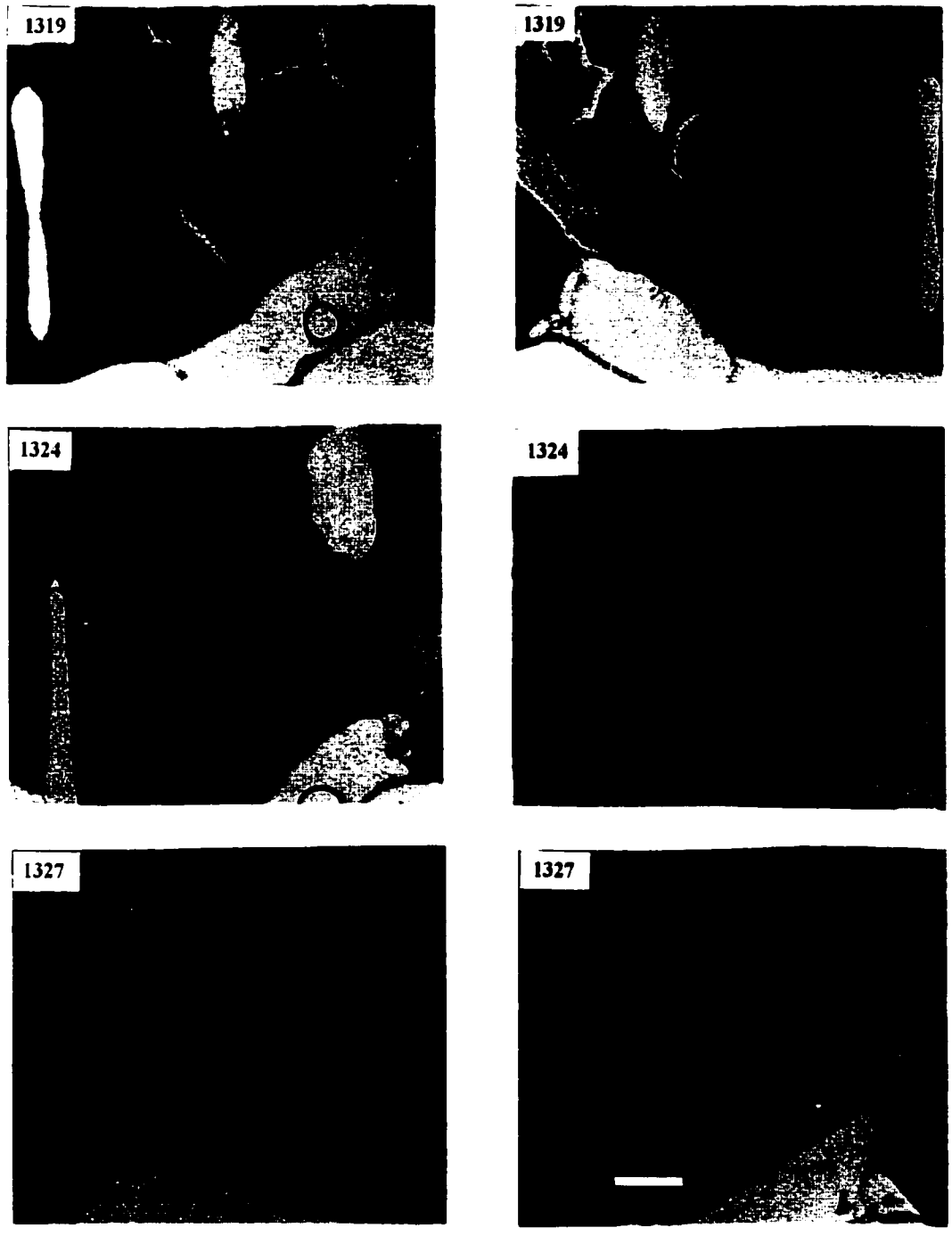
Figure B-9. Photomicrographs of coronal sections showing bilateral LH lesion/stimulating electrode tip locations in 6 rats. Numbers in the top left corners identify the rats. Pictures in the left column show locations of lesions, while pictures in the right column show intact electrode locations. Bar = 0.5 mm.

Figure B-9



Lesion

Intact



Experiments 3 and 4

Figure B-10. Photomicrographs of coronal sections showing LH lesion/stimulating electrode and probe electrode tip locations for 6 rats. Numbers in the top left corners identify the rats. Bar = 0.5 mm.

Figure B-11. LH lesion/stimulating electrode tip locations for 4 rats. Numbers in the top left corners identify the rats. Bar = 0.5 mm.

Figure B-10

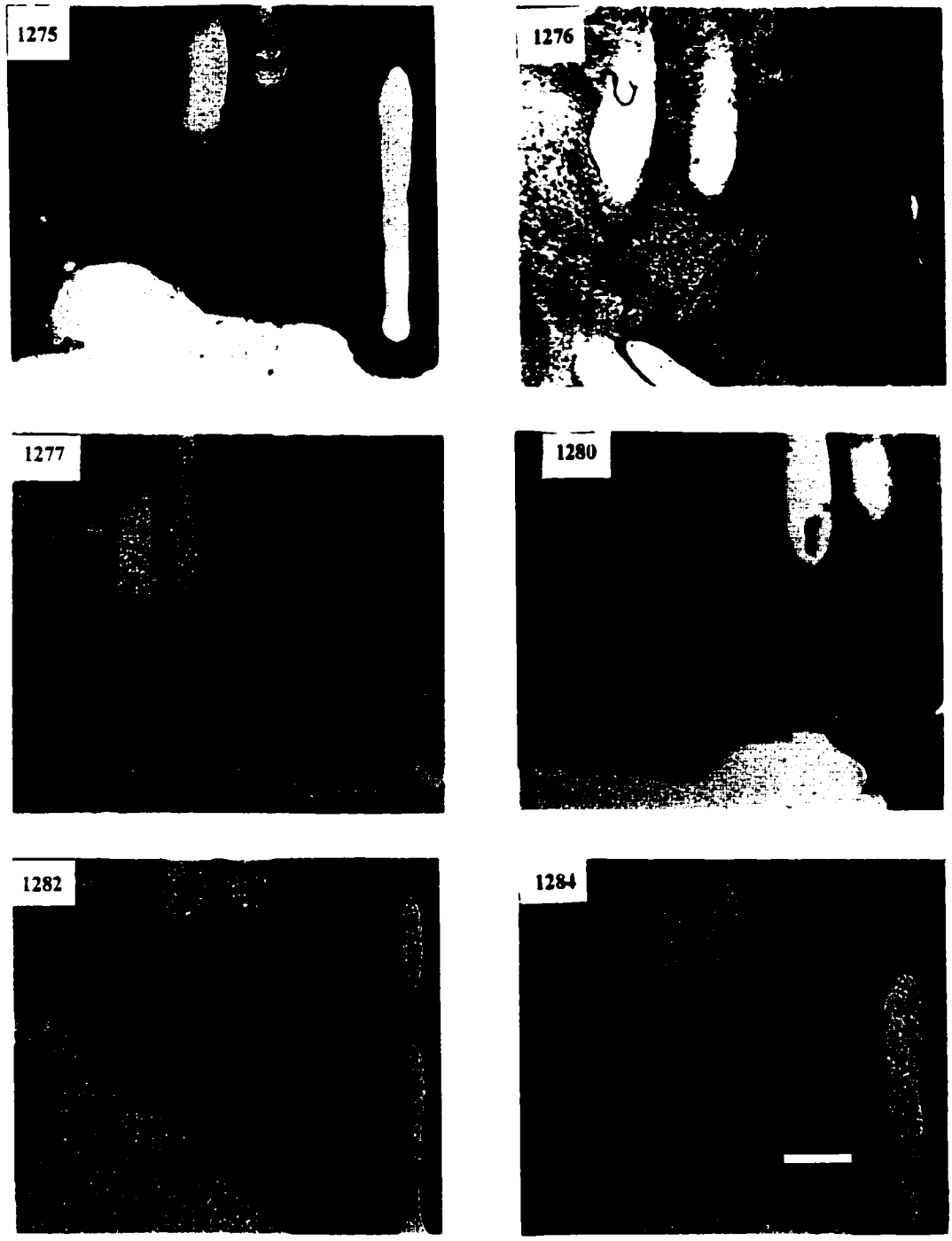
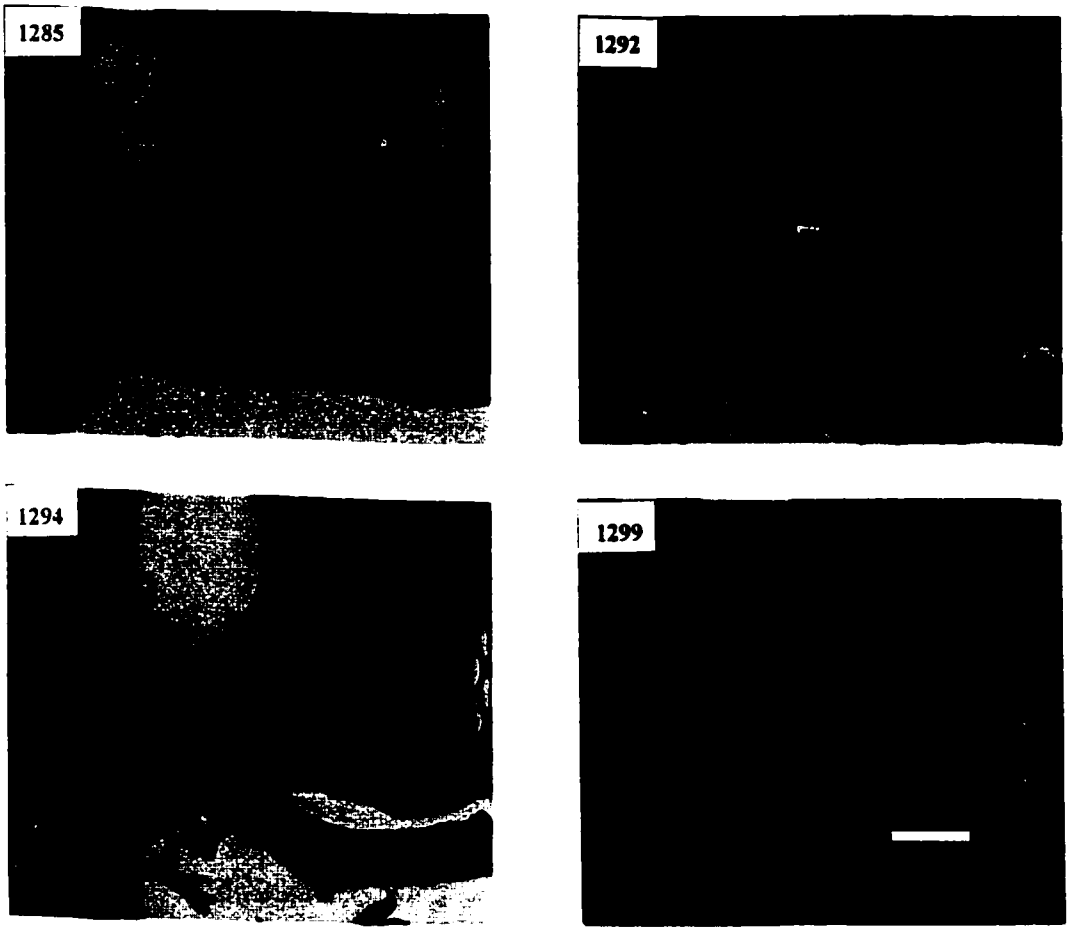


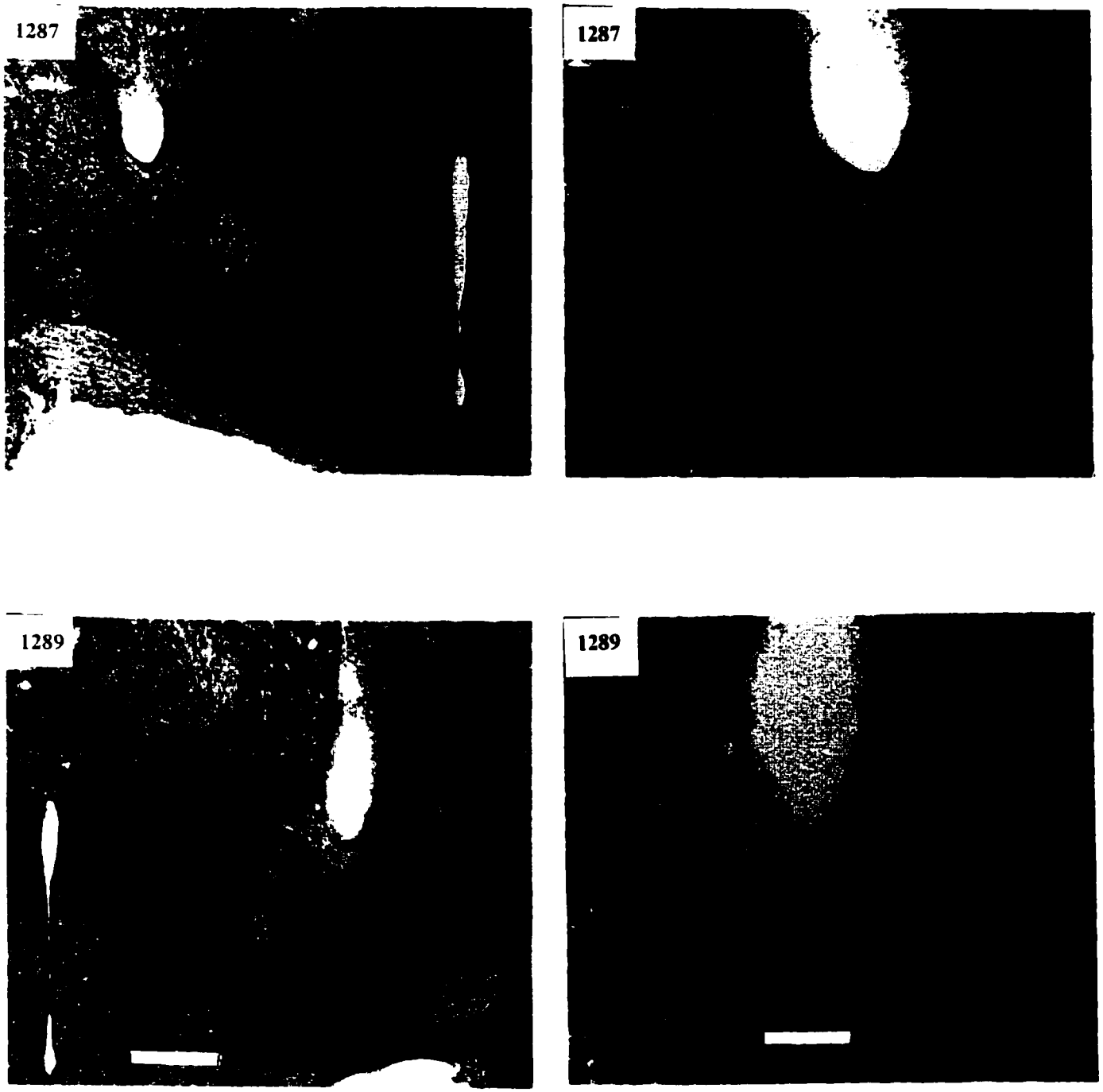
Figure B-11



Experiment 5

Figure B-12. Photomicrographs of coronal sections in the left column show LH lesion/stimulating electrode tip locations for 4 rats (bar = 0.5 mm). Numbers in the top left corners identify the rats. A magnification of each electrode track is shown in the right column. Bar = 0.2 mm.

Figure B-12

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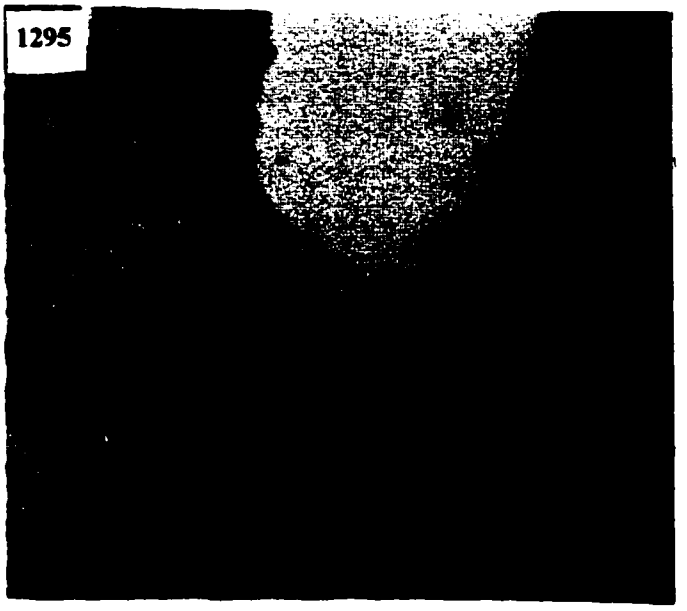
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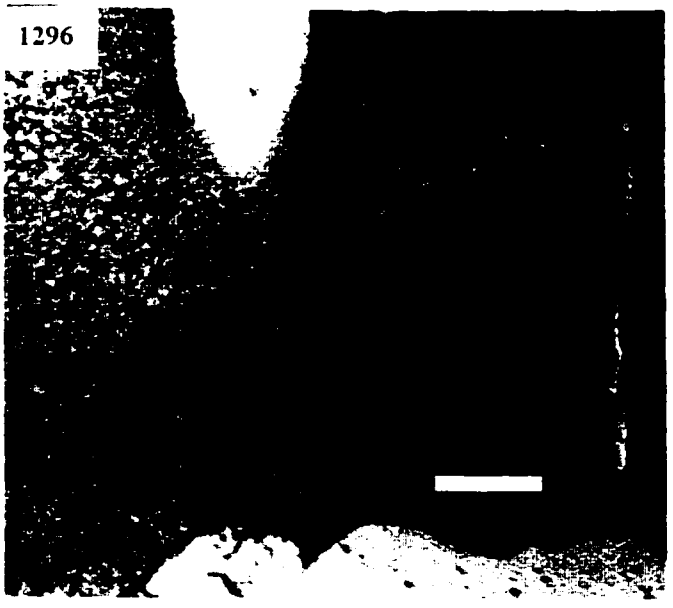
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1296