# The Anomalous Shortfall in T Pulse Effectiveness

Thomas A.J. Fournier

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UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

## Abstract

Paired pulse stimulation is used to estimate the refractory period of the directly stimulated substrate of intracranial self stimulation. When the delay between the conditioning (C) pulse and the test (T) pulse is shorter than the refractory period, the T pulse elicits very little behaviour. The T pulse is ineffective because the substrate has not had time to recover from refractoriness. Conversely, when the C-T delay is longer than the refractory period, one would expect the T pulse to be fully effective. In practice, however, T pulses often fall 20% short of full effectiveness. This anomalous shortfall has received scant attention. The present study documented this anomaly, and tested a theory about its cause.

Experiment 1 tested at very long C-T delays and at closely spaced C-T delays. It was found that T pulses always became fully effective, but often only at C-T delays of 30 ms. Experiment 2 determined that the shortfall is not an undersampling or scaling artifact. T pulse effectiveness was calculated using standard 0.1 $\log_{10}$ unit gradations, and then again with 0.05 $\log_{10}$ unit gradations, the finest practical scaling. The fine scaling had no effect on the shortfall.

T pulse effectiveness is usually estimated with six replications per C-T delay. This limits statistical power, and estimates are necessarily approximate. Experiment 3 used an automated testing apparatus to generate very large

numbers of replications: as many as 120 per C-T delay. The resulting effectiveness curves are extremely precise. They show that effectiveness usually rises to 80% within 5 ms. Effectiveness stays near that level until 25 ms. Then it gradually rises to 100% at 35 ms. Effectiveness remains steady near 100% until at least 50 ms, the longest C-T interval that was tested.

Experiment 4 tested the theory that the shortfall is caused by a relative refractory period, a subnormal period, or a supernormal period by using T pulse currents that were 40% larger than the C pulse currents. Large T pulses eliminate relative refractory, subnormal, and supernormal effects, but they did not eliminate the shortfall. It follows that the shortfall is not caused by any of these factors.

Dédicace

La belle Carole.

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First and foremost, I would like to thank my wife, Carole. The road to a doctorate is long and tortuous. Progress is glacial, and setbacks are legion. Through it all Carole has been unwavering in her support, and unrestrained in her affection. I could not have done it without her. If the university senate had a grain of sense, it would give Carole the doctorate.

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Labmates, I am going to miss you. Janet, Lisa, Monika, and Tamara, I enjoyed the seven years we spent crowded together in a vermin infested office. Don't tell the professors, but you were the best part of grad school. Hey Claude, I didn't forget you and Trista. It was nice to have a man about the lab.

My family is extraordinary. No matter what I needed (groceries, laundry facilities, Sunday dinner, software, a place to hunt, a dog to pat, or just a few kind words), they always lined up to provide it. Enjoy the brain slices, gentle reader. My brother Marc CorelDrew them into submission.

If I learned anything in the course of my studies, it is that nothing important can be achieved without the help of family and friends. More to the point, they make achievement worthwhile.

Enough mush. Let's do some science!

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## General Introduction

Electrical brain stimulation is remarkable for its ability to elicit or motivate a wide variety of behaviours. One of the most notable is the self-administration of electrical brain stimulation, also known as intracranial self-stimulation (ICSS). This behaviour is an extremely powerful research tool because, coupled with psychophysical techniques, it allows us to examine the neuronal substrates of reward and motivation even when those substrates are commingled with others (Gallistel, Shizgal & Yeomans, 1981).

Intracranial self-stimulation has broad applications, some of which are listed below. The references indicate some recent, interesting articles that illustrate those applications. One of ICSS's principal uses is to help map the locations of reward relevant substrata (Forgie & Shizgal, 1995; Panagis, Miliaressis, Anagnostakis & Spyraki, 1995). It is also used to trace connections between reward-relevant structures (Malette & Miliaressis, 1995), and to reveal interactions between reward and aversion (Anderson, Diotte & Miliaressis, 1995). In combination with drugs, ICSS is used to probe receptor systems involved in reward and motivation (Hatcher, Boyland and Hagan, 1995; Moser, Moran, Frank & Kehne, 1995; Olds, 1995; Rompré, 1995;

Rompré, Injoyan & Hagan, 1995; Willick & Kokkinidis, 1995). In combination with lesions, it is used to explore the involvement of different structures in reward and motivation (Lepore & Franklin, 1995; Waraczynski & Shizgal, 1995). Intracranial self-stimulation is used to model drug kinetics (Lepore & Franklin, 1992), and to evaluate the abuse potential of drugs (Reid, 1987). Intracranial self-stimulation can also reveal how reward circuits integrate signals over time (Fouriezos, 1995; Walker & Fouriezos, 1995), the carrying capacity of those circuits (Miliaressis & Malette, 1987), and the relationship between signal strength and the subjective magnitude of reward (Mark & Gallistel, 1993).

One of ICSS's fundamental uses is to identify the basic electrophysiological characteristics of its substrate, particularly the refractory periods. There are other non-behavioural ways to measure refractory periods; see for example Kiss and Shizgal's (1989, 1990) recordings of compound action potentials. However, behavioural refractory tests have an important advantage over other techniques. By basing refractory period estimates on ICSS behaviour, researchers can ensure that the estimates reflect the properties of those neurons at the electrode tip that subserve ICSS. In effect, researchers can dissect away the neurons that are not related to ICSS (Gallistel, Shizgal & Yeomans, 1981).

When these refractory period estimates are made, there is often a curious anomaly; T pulse effectiveness (a measure of neuronal excitability)

does not rise as high as one would expect. The derivation of T pulse effectiveness predicts that it should always rise to a theoretical maximum of 1.00 (see "Appendix 1: Derivation of T Pulse Effectiveness"). In practice, however, T pulse effectiveness often falls short of its theoretical maximum. The present study documents this anomaly, and then attempts to uncover its cause. Before launching into the study proper, some background is in order. The following section, therefore, reviews the history of refractory period tests and the use of ICSS to estimate refractory periods.

## Previous Research

### History of Paired-Pulse Refractory Period Tests

In 1854 Helmholtz reported his experiments with the electrical stimulation of frog nerve-muscle preparations. He delivered pairs of electrical pulses to the nerve and recorded how much the muscle twitched in response. He found that when the delay between the two pulses was less than 1.6 ms, the pair of pulses was no more effective than a single pulse (described by Gallistel, 1973). The significance of Helmholtz's discovery was not fully appreciated until 1899 when Gotch and Burch (1899) measured the neuronal refractory period with a capillary electrometer. That same year Boycott (1899)

showed that the effectiveness of pairs of pulses in the nerve-muscle preparation corresponded to the electrophysiological measurements; when the delay between the pulses was less than 1.6 ms, the second pulse was ineffective because it arrived during the refractory period induced by the first pulse. In their article "On the summation of propagated disturbances in nerve and muscle", Adrian and Lucas (1912) documented a period of supernormal excitability following the refractory period. Five years later, Lucas (1917) published his work with crayfish nerve-claw-muscle preparations. He distinguished two populations of neurons with different refractory periods.

A century after Helmholtz's nerve-muscle experiments, Olds and Milner (1954) published their discovery of intracranial self stimulation (ICSS) in rats. They implanted enamelled silver electrodes in several structures including the septum, the cingulate cortex, and the mammillothalamic tract (adjacent to the lateral hypothalamus). They connected the electrodes to a 60-cycle power line through a step down transformer, and gave the rats access to a lever in a Skinner box. While the rats pressed the lever, the electrodes delivered between 0.5 and 4.8 volts r.m.s. of stimulation. While the rats were in the box, they spent between 36% and 92% of their time pressing the lever.

Ten years later, Deutsch (1964) made the connection between Helmholtz's double pulse experiment and Olds and Milner's ICSS experiment.

Deutsch delivered trains of pairs of conditioning (C) and test (T) pulses[1] through electrodes implanted either in the ventral tegmental area (VTA) or in the lateral hypothalamus (LH). The interval between the C and T pulses ranged from 0.2 to 2.1 ms. At intervals of 0.2 to 0.7 ms the rats pressed the lever very little if at all. Between 0.8 and 1.1 ms rates climbed to about 25 bar presses per minute. At intervals of 1.3 to 2.1 ms, rates remained steady near 25 presses per minute. This indicated to Deutsch that the refractory period of the neurons that subserve ICSS is between 0.8 and 1.1 ms.

Deutsch's 1964 article is a landmark, but it is not the last word. He used the rate of bar pressing as a measure of the rewarding quality of the stimulation, but Hodos and Valenstein (1962) had already shown that rate is a poor measure. When they gave rats a choice between pressing either a lever that delivered medium intensity septal stimulation or a lever that delivered low intensity LH stimulation, the rats preferred the septal lever. This was remarkable because when the rats had access to only one lever at a time, they pressed much faster for the LH stimulation than for the septal stimulation. When Hodos and Valenstein compared different intensities of LH stimulation, the rats preferred medium intensity over low intensity, and they preferred high intensity best of all. However, the rats pressed fastest for medium intensity and slowest for high intensity stimulation. The rate and preference tests

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[1]For the definitions of trains, delays, frequencies *et cetera*, see the section below titled "Definition of Terms."

contradicted each other, so Hodos and Valenstein concluded that rate is a poor measure. Valenstein and Meyers (1964) came to the same conclusion when they compared rates and place preference, and Valenstein (1964) reiterated his objections to rates in his review of the problems of measuring reward.

The essence of Valenstein's objections is that rate measurements such as Deutsch (1964) used do not provide an interval scale. Deutsch would have expected that a manipulation that doubles the effectiveness of the stimulation would increase the rate of bar pressing from 25 per minute to 50 per minute. The rat, however, might not be able to press that fast. Even if the rat could press 50 times per minute, the relationship between rate and the effort required to maintain that rate is not linear. In human terms, it is almost as easy to walk a mile in 20 minutes as it is to stand in one spot for 20 minutes. A ten minute mile requires moderate exertion; one would have to jog. For most people, a five minute mile is impossibly difficult. Clearly, therefore, it is unreasonable to suppose that if a person were willing to walk a mile in 20 minutes for one dollar, then they would jog a mile for two dollars, and they would sprint a mile in five minutes for four dollars.

Deutsch was no doubt aware of Valenstein's objections to rates, but in 1964 no one had worked out an interval scale for measuring the quality of rewarding stimulation; Deutsch therefore relied on rates. When he estimated refractory periods using a preference measure (T-maze) and bar pressing rates, he got two different results: 0.5 to 0.6 ms with the T-maze, and 0.8 to 1.1

with the rates. Instead of concluding that rate is a bad measure, Deutsch concluded that there are two different substrates: one for reinforcement (T-maze) and one for drive (bar press rate). Other authors have used the same logic to support similar conclusions. Hawkins and Chang (1974) claimed to have distinguished separate substrates for stimulation induced feeding and ICSS, and Carr and Bak (1987) believed that they had found different substrates for LH stimulation-induced analgesia and LH self stimulation.

The debate between the proponents of these hypotheses (bad measure versus different substrates) continued until Yeomans (1975) formulated his threshold shift paradigm. Yeomans inferred changes in reward from shifts in frequency thresholds. That is, he used the stimulation frequency that is required to sustain a behavioural criterion as an index of the rewarding value of the stimulation. For example, if a manipulation (such as increasing the interval between C and T pulses so that the T pulse comes after the refractory period) halves the frequency that is required for a rat to press a lever 50 times per minute, then it follows that the manipulation doubles the stimulation's effectiveness. Halving the frequency halves the number of pulses. If half as many pulses elicit the same behaviour, then each pulse must work twice as well. Similarly, a manipulation that doubles the frequency threshold cuts the

effectiveness of the stimulation in half[2]. Yeomans's method therefore provides an interval scale for the measurement of refractory period effects (Hawkins, Roll, Puerto & Yeomans, 1983).

Threshold shifts have another important advantage; they can be used with any behaviour that meets two conditions. First, it must be possible to define a threshold for the behaviour. For example, the threshold for stimulation induced feeding can be defined as the minimum frequency of stimulation at which a rat will feed for at least three seconds either during or immediately following the stimulation (Bielajew & Trzcińska, 1994). The threshold for vocalization can be defined as the minimum stimulation that elicits an audible squeak (Schenk & Robinson, 1988). Second, there must be a monotonic relationship between the stimulus and the behaviour (Gallistel, Shizgal & Yeomans, 1981). In other words, if everything is held constant except for the stimulation frequency, then there can be only one frequency that elicits the threshold behaviour. Otherwise, the threshold behaviour does not correspond to a single, fixed level of activity in the directly stimulated substrate. The derivation of Yeomans's effectiveness assumes that the threshold corresponds to a fixed level of activity, so Yeomans's technique can only be used if there is a monotonic relationship between the stimulus and the behaviour (see

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[2]Threshold shifts and effectiveness are explained in detail in the section below titled "Definition of Terms," and in "Appendix 1: Derivation of T Pulse Effectiveness."

"Appendix 1: Derivation of T Pulse Effectiveness). As long as these two conditions are satisfied, Yeomans's technique can be applied to behaviours as disparate as lever pressing, drinking, and startle. Threshold shifts therefore enable researchers to measure and compare the refractory periods of the substrates of otherwise incommensurable behaviours.

## Yeomans's Technique Applied

Researchers have applied Yeomans's refinement of Deutsch's refractory period estimates to a wide variety of behaviours. For example, Schenk and Robinson (1988) measured the refractory periods of the substrates of analgesia and of vocalization[3]. Stimulation of the periaqueductal grey (PAG) produces analgesia (as measured by the latency for a rat to flick its tail out of 54 °C water) as well as an audible high frequency vocalization. The refractory period for the analgesia is 2 to 5 ms, and the refractory period for the vocalization is 1.2 to 2 ms. Schenk and Robinson concluded that there are two different substrates with overlapping distributions.

In a stimulation escape paradigm, subjects work to stop stimulation. Dennis, Yeomans, and Deutsch (1976) implanted electrodes in the medial lemniscus. Then they divided their rats into two groups; those that adapted to

[3]The articles cited in the present section are listed in Table 1 near the end of the section.

a constant background of non-contingent aversive stimulation by becoming insensitive, and those that remained sensitive to aversive stimulation. Dennis, Deutsch, and Yeomans estimated that the refractory period for stimulus escape was 0.6 ms to 1.0 ms in the rats that adapted, and only 0.4 ms to 0.8 ms in non-adapters. They suggest that the different refractory periods represent different substrates for adaptive and non-adaptive stimulus escape.

In an On-Off paradigm, subjects work both to turn stimulation on and to turn it off. Presumably, subjects turn stimulation on because it is rewarding, and then turn it off because it becomes aversive. Using LH implants in rats, Skelton and Shizgal (1980) estimated that the refractory periods for both On- and Off-responding were 0.4 to 1.4 ms. This finding seems to suggest that On- and Off-responding are served by a common substrate. Period-intensity trade-off data and local potential summation results, on the other hand, suggest that the two behaviours are served by different substrates. Skelton and Shizgal conclude that On- and Off-responding are served by different populations of neurons with similar refractory periods.

Hawkins, Roll, Puerto, and Yeomans (1983) used LH electrodes to measure the refractory periods of the substrates of stimulation induced feeding and ICSS. They found that for both feeding and reward, the effectiveness of paired pulses rose sharply as the delay between the pulses increased from 0.4 to 1.2 ms, and then rose gradually as the delay increased from 1.2 to 2.0 ms. They concluded that the feeding and the reward substrates both had refractory

periods of 0.4 to 2.0 ms, and that both behaviours might therefore share a single substrate. Gratton and Wise (1988) obtained similar results with electrodes scattered along the medial forebrain bundle (MFB) from the anterior LH to the VTA.

Bielajew and Trzcińska (1994) measured the refractory period of the substrate of stimulation induced feeding in the sulcal prefrontal cortex (SPFC). They found that the recovery of excitability begins at 0.5 ms and ends at 3.0 ms, which overlaps with what was found in the MFB by Hawkins *et al.* (1983) and by Gratton and Wise (1988). This overlap between the refractory period estimates for stimulation induced feeding in the MFB and the SPFC suggests that a single substrate may underlie the behaviour at both sites. On the other hand, Bielajew and Trzcińska could find no evidence of self-stimulation in the SPFC. Their results suggest either that stimulation induced feeding has different substrates in the SPFC and the MFB, or that stimulation induced feeding and ICSS have different substrates in the MFB. Other authors, however, have elicited ICSS in the SPFC (Robertson, Laferrière & Milner, 1986; for a review see Robertson, 1989). D. Corbett (personal communication, July 30, 1996) therefore suggested that Bielajew and Trzcińska's failure to elicit ICSS may merely reflect deficiencies in their training and shaping procedures.

Miliaressis and Rompré (1980) studied the refractory periods of median raphe (MR) stimulation induced circling, and of LH ICSS. They found that the circling substrate began to recover from refractoriness 0.3 ms earlier than the

self-stimulation substrate. They concluded that two different substrates subserve circling and self-stimulation. In 1981 Miliaressis again measured the refractory period of MR stimulation induced circling. He found that some of the stimulated neurons began to recover within 0.36 ms, and that 90% of the substrate had recovered within 1.2 ms. Since the refractory period of serotonergic neurons is longer than 1.2 ms, Miliaressis concluded that serotonergic neurons contribute little or nothing to stimulation induced circling. Other studies at a variety of sites have also yielded estimates of about 0.3 to 1.7 ms (Miliaressis & Philippe, 1983, 1984; Tehovnik & Yeomans, 1986, 1987; Yeomans & Linney, 1985; Yeomans, Prior & Bateman, 1986). However, some studies have found evidence of longer refractory periods. Buckenham and Yeomans (1993) estimated that refractoriness lasted 0.45 ms to 3 ms in the superior colliculus, and 0.4 ms to 1.0 ms in the ventrolateral pons. Tehovnik and Yeomans (1988) estimated that refractoriness lasted 0.5 ms to 4.5 ms in the internal capsule and the substantia nigra. Using very small electrode tips (which increases current density) Yeomans, Mercouris, and Ellard (1985) also obtained slightly longer estimates. Recovery began at 0.3 ms, but it sometimes did not level off until 5 ms.

Yeomans and Buckenham (1992) studied turning rather than circling. They estimated that the refractory period in the anteromedial cortex and in the striatum was 0.6 to 4.0 ms. More recently, Chapman and Yeomans (1994) studied forelimb flexion. Their refractory period estimates ranged from 0.5 to

1.0 ms in pyramidal sites, from 0.6 to 1.5 ms in the internal capsule, and from 0.6 to 2.0 ms in surface cortical sites.

Startle-like responses are remarkable because they can be elicited by single pulses of as little as 200 $\mu$A. The response is measured with an accelerometer attached to a cage which is suspended between springs. When the stimulation is delivered, the rat jumps, the cage shakes, and the accelerometer produces a voltage. This voltage defines the threshold; the behavioural criterion is the production of 1 mV within 200 ms of the stimulation (Yeomans & Pollard, 1993). Yeomans, Rosen, Barbeau, and Davis (1989) measured the ability of T pulses to elicit startle-like responses in the cochlear nucleus, the ventral lateral lemniscus, and the caudal pontine reticular formation. They estimated that the refractory period was 0.3 to 0.5 ms at the pontine site, and 0.4 to 2.0 ms at the other sites. Yeomans and Pollard estimated the refractory period for startle-like responses in the midbrain, the medulla, and the ventral amygdalofugal pathway (VAF). In the midbrain and in the medulla recovery was complete within 0.2 to 0.5 ms. Recovery was slower in the VAF: 0.4 to 0.8 ms. Yeomans, Hempel, and Chapman (1993) estimated that the refractory period in pontine and medullary sites is 0.25 to 0.6 ms.

In 1975 Yeomans estimated the refractory period of the ICSS substrate in the LH. He found that recovery began at 0.4 ms and continued until 1.2 ms. Yeomans (1979) obtained similar results with electrodes in the posterior

hypothalamus and in the dorsal pons. Subsequently, many studies have used Yeomans's technique to estimate the refractory periods of self-stimulation substrates in different brain sites. Bielajew, Jordan, Ferme-Enright, and Shizgal (1981) compared ICSS in the periaqueductal grey (PAG) and the LH. Recovery began within 0.4 to 0.6 ms at both sites, but it took longer in the PAG. Bielajew, Lapointe, Kiss, and Shizgal (1982) measured refractory periods in the LH and in the ventral midbrain (the area dorsal to the VTA near the medial lemniscus, and the periventricular grey). They estimated that absolute refractory periods lasted between 0.4 ms and 1.5 ms. Schenk and Shizgal (1982) compared the LH and the medial pre-frontal cortex (MPFC). In the LH recovery began at 0.66 ms and ended at 1.5 ms. In the MPFC recovery began at 1.59 ms and finished at 3.5 ms. Trzcińska and Bielajew (1992) estimated the refractory periods of the substrates underlying ICSS in the caudate putamen (CP) and the MPFC. Recovery began at 0.65 and 0.95 ms in the CP and the MPFC respectively. Excitability rose to an asymptote at 6.0 ms in the CP and at 6.25 ms in the MPFC[4]. Fouriezos, Walker, Rick, and Bielajew (1987)

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[4]The apparent discrepancy between Schenk and Shizgal's results and Trzcińska and Bielajew's results is factitious. The discrepancy reflects differences in methods for estimating the beginning and end of the recovery from refractoriness rather than differences in effectiveness curves. Schenk and Shizgal used the points where effectiveness reached 20% and 80% of its maximum value to estimate the beginning and end of recovery. Trzcińska and Bielajew, on the other hand, used the point where the effectiveness curve started to climb and the point where the curve approached its upper asymptote (see Experiment 1). Visual inspection shows that both studies' curves are substantially similar.

found that the anterior basal forebrain recovers half as quickly as the LH. Bielajew, Thrasher, and Fouriezos (1987) performed estimates in the lateral preoptic area (LPO) and in the LH. They reported that 80% of the recovery occurred between 0.4 and 1.2 ms in the LH, and between 0.4 and 6.3 ms in the LPO. Testing in the mediodorsal thalamus (MDT), Bielajew and Fouriezos (1985) found that recovery began no earlier than 1 ms and ended as late as 10 ms. Vachon and Miliaressis (1994) estimated refractory periods in diencephalic sites. There were wide variations between sites, but in most cases recovery began between 0.5 and 1.0 ms, and ended within about 2.0 ms. At a few sites recovery continued until 5 or even 10 ms. MacMillan, Simantirakis, and Shizgal (1985) estimated refractory periods for self-stimulation in the ventrolateral tegmentum (VLT) and in the LH. The VLT recovery curve began to rise at 0.8 ms and finished rising at 2.0 ms, and the LH curve began at 0.4 ms and finished at 1.3 ms. Rompré and Miliaressis (1987) estimated the refractory periods of brainstem substrates of ICSS. They found that at pontine sites recovery started at 0.6 to 0.8 ms, and at midbrain sites recovery began 0.35 to 0.4 ms. Shizgal, Schindler and Rompré (1989) estimated refractory periods in the LH and in the VTA. In the LH recovery began at an average of 0.43 ± 0.03 ms (*SEM*) and levelled off at an average of 1.46 ± 0.08 ms. In the VTA recovery began at 0.48 ± 0.03 ms and ended at 1.55 ± 0.06 ms.

Gratton and Wise (1985) estimated refractory periods in the hypothalamus. They found that excitability climbed steadily between 0.4 and

0.6 ms. The recovery paused between 0.6 and 0.7 ms, and then excitability climbed again until about 1.2 ms. Atropine sulfate, a cholinergic blocker, eliminated growth of effectiveness before the pause. Gratton and Wise concluded that the LH ICSS substrate is composed of at least two different fibre populations. They estimated that 25% of the substrate is fast and cholinergic, and the other 75% is slower and non-cholinergic. The validity of this conclusion has been called into doubt because Gratton and Wise's results have not been replicated; Panagis, Spyraki, Anagnostakis, and Miliaressis (1995), Rompré and Miliaressis (1987), and Vachon and Miliaressis (1994) found similar pauses or steps, but they did not do a pharmacological analysis like Gratton and Wise. Moreover, it is curious that atropine sulfate did not raise baseline frequency thresholds; one would expect that thresholds would have to rise to compensate for the blockade of 25% of the substrate.

The ICSS papers cited above have shown that neurons with refractory periods of between 0.3 ms and 1.0 ms account for some 50% to 70% of the MFB stimulation's ability to elicit ICSS in the MFB. Yeomans, Mercouris, and Ellard (1985) shifted this balance by using electrodes with very small tips. This increased the current density in the centre of the stimulation field, and recruited fibres that continued to recover out to C-T intervals of 2.0 ms. This finding is consistent with evidence that neurons with long refractory periods also have high stimulation thresholds (Erlanger & Gasser, 1937; Swadlow & Waxman, 1978).

| Table 1: Summary of Behavioural Refractory Period Estimates | | | |
|---|---|---|---|
| Authors | Behaviour | Structure | Refractory Period |
| Schenk & Robinson, 1988 | Analgesia, vocalization | PAG | 2 to 5 ms, 1.2 to 2 ms |
| Dennis, Yeomans & Deutsch, 1976 | stimulation escape | midbrain lemniscus | 0.4 to 1.0 ms |
| Skelton & Shizgal, 1980 | On- and Off-responding | LH | 0.4 to 1.4 ms |
| Hawkins et al., 1983 | Feeding, ICSS | LH | 0.4 to 2.0 ms, |
| Gratton & Wise, 1988 | Feeding, ICSS | MFB from LH to VTA | 0.4 to 2.5 ms. Growth of effectiveness paused between 0.6 and 0.7 ms. |
| Bielajew & Trzcińska, 1994 | Feeding | SPFC | 0.5 to 3.0 ms |
| Miliaressis & Rompré, 1980 | Circling, ICSS | MR, LH | 0.3 to 1.2 ms, 0.6 to 2.4 ms |
| Miliaressis, 1981 | Circling | MR | 0.36 to 1.2 ms |
| Miliaressis & Philippe, 1983 | Circling | Mesencephalon | 0.24 to 2 ms |
| Miliaressis & Philippe, 1984 | Circling | Pons, tectopontine pathway | 0.28 to 1.2 ms |
| Tehovnik & Yeomans, 1986 | Circling | Rostromedial tegmentum, medial pons (MP), superior colliculus | 0.3 to 1.6 ms, 0.3 to 1.7 ms, 0.5 to 1.6 ms |
| Tehovnik & Yeomans, 1987 | Circling | anteromedial cortex, MP | 2.4 ms, 0.8 ms |
| Yeomans & Linney, 1985 | Circling | Tectospinal tract, medial longitudinal fasciculus | 0.3 to 1.6 ms |
| Yeomans, Prior & Bateman, 1986 | Circling | Mediocaudal midbrain | 0.3 to 1.4 ms |
| Buckenham & Yeomans, 1993 | Circling | Superior colliculus, ventrolateral pons | 0.45 to 3 ms, 0.4 to 1 ms |
| Tehovnik & Yeomans, 1988 | Circling | Internal capsule, substantia nigra | 0.5 to 4.5 ms |

| Table 1: Summary of Behavioural Refractory Period Estimates | | | |
|---|---|---|---|
| Authors | Behaviour | Structure | Refractory Period |
| Yeomans, Mercouris & Ellard, 1985 | Circling, ICSS | Medial brainstem, LH | 0.3 to 1.2 ms, 0.4 to 5 ms (both with small electrode tips) |
| Yeomans & Buckenham, 1992 | Turning | Anteromedial cortex, striatum | 0.6 to 4 ms |
| Chapman & Yeomans, 1994 | Forelimb flexion | Pyramidal tract, internal capsule, cortical surface | 0.5 to 2 ms |
| Yeomans et al., 1989 | Startle | cochlear nucleus, ventral lateral lemniscus, pontine reticular formation | 0.3 to 2 ms |
| Yeomans & Pollard, 1993 | Startle | Midbrain, medulla, VAF | 0.2 to 0.8 ms |
| Yeomans, Hempel & Chapman, 1993 | Startle | Pons, medulla | 0.25 to 0.6 ms |
| Yeomans, 1975 | ICSS | LH | 0.4 to 1.2 ms |
| Yeomans, 1979 | ICSS | Posterior hypothalamus, dorsal pons | 0.4 to 1.4 ms |
| Bielajew et al., 1981 | ICSS | PAG, LH | 0.4 to 2 ms, 0.4 to 1.2 ms |
| Bielajew et al., 1982 | ICSS | LH, ventral midbrain | absolute refractory period 0.4 to 1.5 ms, relative refractory period 1.0 to 3.5 ms |
| Schenk & Shizgal, 1982 | ICSS | LH, MPFC | 0.66 to 1.5 ms, 1.59 to 3.5 ms |
| Trzcińska & Bielajew, 1992 | ICSS | CP, MPFC | 0.65 to 6.25 ms |
| Fouriezos et al., 1987 | ICSS | Anterior basal forebrain, LH | 0.6 to 4 ms, 0.4 to 1.2 ms |
| Bielajew, Thrasher & Fouriezos, 1987 | ICSS | LPO, LH | 0.4 to 6.3 ms , 0.4 to 1.2 ms |

| Table 1: Summary of Behavioural Refractory Period Estimates | | | |
|---|---|---|---|
| Authors | Behaviour | Structure | Refractory Period |
| Bielajew & Fouriezos, 1985 | ICSS | MDT | 1 to 10 ms |
| Vachon & Miliaressis, 1994 | ICSS | Diencephalon | 0.5 to 2 ms, a few sites took as long as 10 ms |
| MacMillan, Simantirakis & Shizgal, 1985 | ICSS | VLT,<br>LH | 0.8 to 2 ms,<br>0.4 to 1.3 ms |
| Rompré & Miliaressis, 1987 | ICSS | Pons,<br>Midbrain | growth in effectiveness began at 0.6 ms, and at 0.35 ms |
| Shizgal, Schindler & Rompré, 1989 | ICSS | LH, VTA | 0.43 to 1.46 ms |
| Gratton & Wise, 1985 | ICSS | Hypothalamus | 0.4 to 1.2 ms |
| Panagis, Spyraki & Miliaressis, 1995 | ICSS | Ventral Pallidum | 0.5 to 1.6 ms |
| Yeomans, Mercouris & Ellard, 1985 | ICSS | LH | 0.4 to 2.0 ms with small electrode tips |

These estimates of refractory periods have given rise to three types of conclusions. First, if the estimated refractory period matches the known refractory period of a particular class of neuron, then the substrate may be made up of that class of neuron. For example, most of the recovery of effectiveness in MFB ICSS substrates occurs between 0.4 and 1.2 ms. This corresponds to non-dopaminergic, myelinated axons between 0.5 and 2 $\mu$m in diameter. It follows that the directly stimulated MFB ICSS substrate is probably made up mostly of these small myelinated axons (Yeomans, 1989).

The second type of conclusion addresses the equivalence of substrates. If the refractory period estimates in different sites are similar, then either both sites share the same substrate, or the two substrates are alike. Similarly, when the estimates are different, then one can infer that the two sites may have different substrates. For example, refractory period estimates for ICSS in the LH and in the VTA are very similar (Shizgal, Schindler & Rompré, 1989), so it follows that the same fibres may subserve ICSS at both sites. Conversely, estimates in the MPFC are very different from estimates in the MFB (Schenk & Shizgal, 1982; Trzcińska & Bielajew, 1992). It follows, therefore, that different substrates may be responsible for ICSS in cortical sites and in the MFB. This same logic applies to comparisons between behaviours. Skelton and Shizgal (1980), for example, concluded that On- and Off-responding have similar substrates because they found that the two behaviours yielded very similar refractory period estimates.

The third type of conclusion is that if a single site yields different estimated refractory periods, then there may be multiple substrates. In other words, if the recovery curve has a distinct step, then ICSS may have two substrates: one of neurons that recover quickly, and a second of neurons with distinctly longer refractory periods. When Gratton and Wise (1985) found a step, they repeated the tests using a variety of blockers. They found that atropine sulfate, a cholinergic receptor blocker, eliminated growth of effectiveness before the step. Gratton and Wise concluded that 25% of the

ICSS substrate comprises quick cholinergic fibres, and 75% comprises slower non-cholinergic fibres.

Taken together, these refractory period studies have painted the following portrait of ICSS's substrates. The substrates in hypothalamic sites, the VTA, and the PAG are very similar (Bielajew, Jordan, Ferme-Enright & Shizgal, 1981). These sites may therefore share the same substrate. With moderate currents and electrode tip exposures most of the growth in effectiveness occurs between 0.4 and 1.2 ms. This corresponds to small, myelinated, non-dopaminergic fibres (Gallistel, Shizgal & Yeomans, 1981). The substrate is a heterogenous mix of different kinds of fibres. The quickest fibres (those that recover within 0.6 ms) may be cholinergic, and the slower fibres (0.7 to 1.2 ms refractory periods) are non-cholinergic (Gratton & Wise, 1985). High currents and small electrode tips add slow, high threshold fibres that may be dopaminergic (Yeomans, Mercouris & Ellard, 1985; Yeomans, 1989).

The substrates of ICSS in other brain sites are very different. Effectiveness recovers from refractoriness between 0.8 ms and 2 ms in the VLT (MacMillan, Simantirakis & Shizgal, 1985), between 0.4 ms and 6.3 ms in the LPO (Bielajew, Thrasher & Fouriezos, 1987), between 0.5 ms and 10 ms in diencephalic sites (Vachon & Miliaressis, 1994), and between 1 ms and 10 ms in the MDT (Bielajew & Fouriezos, 1985). The discrepancies between the refractory periods in these sites and those in hypothalamus, VTA, and PAG indicate that ICSS's substrates vary from site to site.

Substrates also vary with behaviour. The substrates for analgesia and vocalization in the PAG are slower than the ICSS's substrate at the same site (Schenk & Robinson, 1988). The substrates for circling and startle include elements that begin to recover within 0.2 ms to 0.3 ms (Miliaressis & Philippe, 1984; Yeomans & Pollard, 1993). This is quicker than the fastest elements in ICSS's substrate (Panagis, Spyraki & Miliaressis, 1995; Yeomans, 1975). The range for stimulation escape in the medial lemniscus overlaps with ICSS's, but the medial lemniscus does not support ICSS (Dennis, Yeomans & Deutsch, 1976). It seems unlikely, therefore, that stimulation escape and ICSS share the same substrate. The same logic may also apply to stimulation induced feeding and ICSS. The range of refractory period estimates for stimulation induced feeding overlaps ICSS's range (Gratton & Wise, 1988; Hawkins et al., 1983), but feeding can be elicited in the SPFC, a structure that sometimes does not support ICSS (Trzcińska & Bielajew, 1994). The range for On- and Off-responding also overlaps with the range for ICSS (Skelton & Shizgal, 1980). These overlaps suggest similarities between the substrates of stimulation escape, On- and Off-responding, and ICSS.

Clearly, Yeomans's (1975) refinement of Deutsch's (1964) technique is very powerful. It identifies the electrophysiological characteristics of behaviourally relevant neurons, and this allows researchers to focus their efforts. For example, if the substrate in one site is made up of fast myelinated fibres, and the substrate in another site is made up of slow unmyelinated fibres,

then there is probably no direct axonal link between the two sites. Researchers might therefore expect to find transsynaptic collision between the two sites, but they should not expect to find axonal collision. Behavioural refractory period estimates are therefore interesting both for their own sake, and because they provide basic, useful knowledge for researchers who are interested in other phenomena.

## Refractory Estimate Anomalies

Paired pulse measurements of refractoriness in MFB reward fibres have shown that the effectiveness of the T-pulse is minimal when the C-T interval is between 0.25 and 0.4 ms. The T-pulse's ability to elicit behaviour climbs steadily as the C-T interval increases from 0.4 to 1.2 ms. This agrees with the refractory period one would expect given the diameter and conduction velocity of reward relevant fibres (Yeomans, 1989). Frequently, however, the T-pulse does not become fully effective within five or ten milliseconds. This shortfall is surprising because, theoretically at least, T pulse effectiveness should always rise to 1.00 (see "Appendix 1: Derivation of T Pulse Effectiveness").

It is not always possible to tell if researchers found shortfalls; sometimes they only reported standardized effectiveness values (see for example Yeomans, Mercouris, and Ellard, 1985). In other cases they only reported very short delays (see for example Gratton and Wise, 1988). Even so, many studies

(including the one that introduced Yeomans's threshold technique) have reported shortfalls[5]. Yeomans (1975) implanted six posterior hypothalamic electrodes in five rats (one had a bilateral implant). The highest estimate of effectiveness was 86% at a C-T delay of 2.0 ms (rat E). Yeomans (1979) tested three rats. Two had electrodes in the posterior hypothalamus, and one had an electrode in the dorsal pons. They all had 80% effectiveness at 5 ms, the longest reported C-T interval. While testing in the LH, Skelton and Shizgal (1980) found that only one out of six rats attained 100% T-pulse effectiveness within 5 ms; the others only reached 60% to 90%. Bielajew, Jordan, Ferme-Enright, and Shizgal (1981) found that LH T-pulse effectiveness rose very slowly. One out of five rats reached 100% in 2 ms. One reached 90% in 8 ms, another reached 70 % in 25 ms, and two others reached 100% in 25 ms. Bielajew and Shizgal (1986) had mixed results. They implanted six LH electrodes. One reached 120% T-pulse effectiveness at 1 ms, two reached 100% at 2 ms, and a fourth at 4 ms. The two others did not attain 100% within 25 ms; one reached 90% and the other 50%. Similar results were obtained with six ventral tegmental area (VTA) electrodes. Two reached 100% T-pulse effectiveness at 1 ms, one at 1.5 ms, one at 3 ms, and another at 25 ms. The sixth only attained 80% at 25 ms. Bielajew, Lapointe, Kiss, and Shizgal (1982) measured T-pulse effectiveness at a C-T interval of 10 ms.

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[5]The articles cited in the present section are listed in Table 2 at the end of the section.

Their two LH implants produced effectivenesses of 100% and 95%. One of their four VTA implants yielded 70%, another 85%, and two others 90%. Bielajew and Fouriezos (1985) implanted in the mediodorsal thalamus and tested T-pulse effectiveness at 10 ms; two rats attained 100% and the third only reached 70%. Bielajew and Shizgal (1982) implanted four LH and four VTA electrodes. One of the VTA implants took 25 ms to reach 100%. The other seven LH and VTA implants all reached 100% within 5 ms. Trzcińska and Bielajew (1992) reported their average asymptotic effectiveness values achieved at a C-T delay of 12 ms: 0.73 in the CP and 0.69 in the MPFC. In their review of ICSS, Gallistel, Shizgal and Yeomans, (1981) noted that there is a 10% to 25% increase in effectiveness at C-T delays of 1.2 to 15 ms, but they did not cite any data in particular.

Table 2: Anomalous Refractory Period Estimates

| Authors | Structure | Number of Subjects/ Electrodes | Maximum E | Longest C-T Delay |
|---|---|---|---|---|
| Yeomans, 1975 | Posterior hypothalamus | 5/6 | 86% | 5 ms |
| Yeomans, 1979 | Posterior hypothalamus, dorsal pons | 3/3 | 80% | 5 ms |
| Skelton & Shizgal, 1980 | LH | 6/6 | 60% to 100% | 5 ms |
| Bielajew et al., 1981 | LH | 5/5 | 80% to 100% | 25 ms |

| Table 2: Anomalous Refractory Period Estimates | | | | |
|---|---|---|---|---|
| Authors | Structure | Number of Subjects/ Electrodes | Maximum E | Longest C-T Delay |
| Bielajew & Shizgal, 1986 | LH, VTA | 6/12 | 60% to 120% | 25 ms |
| Bielajew et al., 1982 | LH, VTA | 6/6 | 70% to 100% | 10 ms |
| Bielajew & Fouriezos, 1985 | Mediodorsal thalamus | 3/3 | 70% to 100% | 10 ms |
| Bielajew & Shizgal, 1982 | LH, VTA | 4/8 | 100% | 25 ms |
| Trzcińska & Bielajew, 1992 | CP, MPFC | 3/5 | Average CP = 73% MPFC = 69% | 12 ms |

Note two things about the shortfalls in the literature. First, they are frequently reported by several different laboratories. This shows that the shortfalls are not merely a symptom of methodological or technical problems in one particular laboratory. Second, the shortfalls are not counterbalanced by an equal number of overshoots. This suggests that the shortfalls are not part of a normally distributed cloud of experimental errors which is centred on E = 1.00.

## Relevance to Current Research

The anomalous 10% to 25% shortfall in T-pulse effectiveness is about ten times longer than a refractory period is supposed to last, but for the most part the shortfall has been ignored. Moreover, most of the refractory studies cited above were published in the 1980's. One must therefore question the relevance of the shortfall to current research. The present section will explore the reasons the shortfall has been ignored. Then it will show how the shortfall is related to tests that are foci of current research.

There are three reasons why the shortfall has attracted little attention. First, although it occurs often, it has been difficult to produce at will. Very similar placements produce very different recovery curves (see for example rats GB5 and Hype in Experiment 1). As Experiment 3 will demonstrate, this reason is no longer valid. If measurements are done with care and precision, shortfalls are the rule rather than the exception.

The second reason why the shortfall has been ignored is that it is a small effect; effectiveness rises only 10% to 25% recovery between 1.2 and 15 ms. Because most studies only use six replications per C-T delay, they do not have the power to resolve such small effects. Note that this impuissance does not invalidate those studies. During the shortfall effectiveness rises to within 10% or 25% of its predicted maximum, so 75% to 90% of the recovery is explained even when the shortfall is ignored (Gallistel, Shizgal & Yeomans, 1981).

This is not to say that small effects are trivial. Gratton and Wise (1985), for example, found a brief lull on the rising portion of the LH ICSS effectiveness curve between 0.6 and 0.7 ms. When they administered atropine sulfate (a cholinergic blocker) and repeated the refractory period tests, Gratton and Wise found that they had eliminated any growth of effectiveness before 0.7 ms. These results led them to the conclusion that the hypothalamic reward substrate has a cholinergic component with short refractory periods. Small effects, therefore, can open the door to big conclusions; we can not afford to ignore phenomena just because they are small.

The third reason why the shortfall has been ignored is that most authors consider that phenomena that occur at delays of more than five or ten milliseconds are irrelevant to the study of refractoriness. Some even scale out the shortfall by dividing all effectiveness values by the value obtained at 5 ms (Yeomans, Mercouris, & Ellard, 1985; see also Bielajew, Jordan, Ferme-Enright, and Shizgal, 1981).

I do not question the wisdom of disregarding changes in effectiveness at C-T delays greater than 10 ms. Given the diameters of the fibres found in (for example) the MFB, one would not expect the refractory periods to last more than 5 ms (Swadlow & Waxman, 1978; Szabo, Lenard & Kosaras, 1974). It is reasonable, therefore, to assume that whatever happens at 10 ms or more is unrelated to refractoriness. Because it is impossible to address intelligently all of the phenomena one observes while doing an experiment, researchers have

to choose which to examine and which to ignore. Logic dictates that we should ignore apparently irrelevant phenomena, so it is only reasonable for researchers interested in refractory periods to ignore the shortfall.

The foregoing does not suggest that the shortfall should be ignored: it just means that if one accepts that refractoriness cannot extend beyond 5 ms or 10 ms, then within the strictly defined bounds of a refractory period study it may be legitimate to disregard the shortfall. However, there are many excellent reasons for focusing on the shortfall. First, if one is open to the possibility of long refractory periods, then it is clearly necessary to study the rise of effectiveness at long delays. Second, if the fact that refractory period tests produce anomalous results causes one to question the reliability of refractory period estimates, then it is vital to investigate the shortfall. Refractory periods are a fundamental electrophysiological characteristic, so casting doubt on our knowledge of refractory periods also casts doubt on much of the rest of what we know.

Third, it may also be essential to study the shortfall if our interests extend beyond refractoriness. For example, super- and subnormal periods (see Experiment 4) become apparent at long C-T intervals. If one is interested in super- and subnormal periods, therefore, then the shortfall is of concern because it distorts the effectiveness curve at long C-T intervals.

Fourth, the shortfall also demands investigation if one is interested in psychophysics. The behavioural collision test is a psychophysical technique

that is very closely related to refractory period estimation. In collision tests, the C pulse is delivered through one electrode, and the T pulse is delivered through a second electrode. If the two electrodes are aligned on the same axon bundle, then the action potentials generated by the C pulse course past the second electrode. Neurons are refractory during action potentials, so the shared substrate can not respond to the T pulses while the C pulse action potentials are traversing the second electrode's stimulation field. T pulse effectiveness is therefore reduced while C pulse action potentials course past the second electrode. If the T pulse is delivered after the C pulse action potentials have gone by, then T pulse effectiveness is not suppressed. Consequently, when T pulse effectiveness is plotted against C-T delay, collisions show up as a temporary drop in effectiveness. If there is a functional anatomical link between the two electrodes, therefore, then C pulse action potentials and T pulses collide, and T pulse effectiveness drops temporarily. If there is no functional link, then there is no collision, and T pulse effectiveness remains constant at all C-T delays.

Since Shizgal, Bielajew, Corbett, Skelton, and Yeomans (1980) developed the behavioural collision test in 1980, the technique has been used to infer functional anatomical links between many different sites. The results of these studies are summarized in Table 3.

| Table 3: Collision Studies | | |
|---|---|---|
| Authors | Behaviour | Structures |
| Malette & Miliaressis, 1995 | ICSS | Left and right LH, left and right ventral tegmentum |
| Frankland & Yeomans, 1995 | Startle | Caudal ventral amygdalofugal pathway (VAF) |
| Yeomans, 1995 | Turning, startle | Various (review) |
| Murray & Shizgal, 1994 | ICSS | LH, VTA |
| Buckenham & Yeomans, 1993 | Circling | Superior colliculus, ventrolateral pons |
| Bushnik Harris, 1993 | ICSS | LPO, VTA |
| Hempel et al., 1993 | Startle | Caudal pons, medullary reticular formation |
| Yeomans & Cochrane, 1993 | Startle | Rostrolateral pons, caudomedial medulla |
| Yeomans & Pollard, 1993 | Startle | VAF, medial medulla |
| Yeomans et al., 1993 | Startle | Caudal pons, medulla |
| Yeomans & Buckenham, 1992 | Turning | Anteromedial cortex, striatum |
| Shizgal, 1989 | ICSS | Various (review) |
| Shizgal & Murray, 1989 | ICSS | Various (review) |
| Kofman & Yeomans, 1988 | ICSS | LH, dorsal tegmentum |
| Tehovnik & Yeomans, 1988 | Circling | Internal capsule, substantia nigra |
| Gratton & Wise, 1988 | ICSS, feeding | LH, VTA |
| Bielajew, Thrasher & Fouriezos, 1987. | ICSS | LH, LPO |

| Table 3: Collision Studies | | |
|---|---|---|
| Authors | Behaviour | Structures |
| Durivage & Miliaressis, 1987 | ICSS, exploration | LH, VTA |
| Bielajew & Shizgal, 1986 | ICSS | LH, VTA |
| Tehovnik & Yeomans, 1986 | Circling | Rostromedial tegmentum, medial pons |
| Yeomans & Linney, 1985 | Circling | Pons, mesencephalon |
| Miliaressis & Philippe, 1983 | Circling | Pons, mesencephalon |
| Bielajew & Shizgal, 1982 | ICSS | MFB |
| Schenk & Shizgal, 1982 | ICSS | LH, MPFC |
| Bielajew et al., 1981 | ICSS | LH, PAG |
| Bielajew & Shizgal, 1980 | ICSS | LH, VTA |
| Shizgal et al., 1980 | ICSS | LH, VTA |

These collision studies have drawn a detailed portrait of the pathways that underlie ICSS and circling. The ICSS studies have shown that there is a chain of connected reward relevant fibres that stretch from the LPO back to the VTA (Shizgal 1989). One of the recent studies (Malette & Miliaressis, 1995) has shown that both LHs are apparently on opposite branches of the same forked fibre bundle. Another recent study (Murray & Shizgal, 1994) has shown that this chain includes fibres with a wide range of conduction velocities or

refractory periods. Some of the slower fibres may be dopaminergic (Yeomans, 1989; Yeomans, Maidment, & Bunney,1988) . The circling and turning studies have shown that these behaviours are subserved by crossed and uncrossed pathways that extend from the pons to the rostromedial tegmentum and the superior colliculus, from the substantia nigra to the internal capsule, and from the striatum to the anteromedial cortex (Yeomans, 1995).

Refractory period estimates and collision tests are very similar. Although the former use single electrodes and the latter use pairs of electrodes, they both use DP trains, and they both generate curves with T pulse effectiveness on the ordinate and C-T delay on the abscissa. Collision and refractory tests even use the same formula to calculate effectiveness; the frequency thresholds at the two electrodes are substituted for the frequency thresholds at the two currents in formula #17 in "Appendix 1: The Derivation of T Pulse Effectiveness." In fact, just as the equal pulse refractory test can be thought of as a special case of the unequal pulse test, single electrode refractory period tests can be thought of as collision tests with the two electrodes superimposed.

Because refractory period estimates and collision tests are so closely related, whatever causes the shortfall in refractory period tests may also distort the results of collision tests. This is not just a theoretical concern; effectiveness sometimes rises with anomalous sluggishness in collision tests just as in refractory period tests. For example, in nine of the 26 collision

curves reported by Murray and Shizgal (1994), visual inspection shows that effectiveness appears still to be rising at the longest C-T delay that was tested (as much as 17.3 ms). It is difficult to account for this protracted growth in terms of conduction time and refractoriness. In most of the subjects the distance between electrodes was 1.5 or 1.6 mm, so even if the conduction velocity was only 0.8 m/s it would only take about 2 ms for the action potentials from the C electrode to reach the T electrode. If one allows another 5 ms for refractoriness, then that leaves at least 10 ms of growth unexplained. It appears, therefore, that something like the shortfall sometimes occurs in collision studies.

This interpretation of Murray and Shizgal's results is somewhat at odds with their own. They reported that the rising portion of the curve was much shorter (2.2 to 7.7 ms), but they used a conservative criterion. They began by evening out the effectiveness curves using a LOWESS smoothing procedure. Then they interpolated the points where the curves reached 20% and 80% of their maximum height. They used these points as conservative estimates of the beginnings and ends of the rising portions. This conservatism was appropriate because Murray and Shizgal were challenging other studies that had found that the rise was complete within 5 ms. However, if the shape of the empirical effectiveness curve reflects the actual development of effectiveness better than the 80% criterion does, then the shortfall occurs both in collision and in refractory period tests.

To recapitulate, this brief review of collision has revealed three points that demonstrate the relevance of the shortfall to current research. First, collision studies are a current area of research; note that 11 of the 25 original papers (44%) in Table 3 were published since 1992. Second, the techniques, equipment, and parameters used in collision tests are very similar to those in used in the refractory period tests that show the shortfall. Third, there is evidence that something like the shortfall is sometimes found in collision tests. It follows from these three points that the shortfall is relevant to current research.

Similar arguments can be made to show that the shortfall is of concern to those who use other tests. For example, Willick and Kokkinidis (1995) have found evidence that GABAB receptors modulate ICSS in the VTA, and that GABAA receptors do not. Microinjections of baclofen (a GABAB agonist) into the VTA raised ICSS current thresholds, but microinjections of muscimol (a GABAA agonist) did not. Willick and Kokkinidis measured thresholds by varying current while holding the C-C delay constant at 10 ms. That is a sensible strategy if GABA's action at 10 ms is representative of its action at other periods. However, if GABA receptors are responsible for the shortfall, then it follows that their effects on ICSS vary with C-C delay over a span of dozens of milliseconds. This raises the possibility that some GABA effects may only become apparent at delays of 20 ms or 30 ms. The shortfall might

therefore explain Willick and Kokkinidis's failure to find evidence of GABAA involvement in MFB ICSS.

Arguments like the one above can be extended to establish the shortfall's relevance to many different tests. We do not know what causes the shortfall in T pulse effectiveness when we estimate refractory periods, so it is difficult to predict what other tests might also be affected by the shortfall. If shortfalls affect all tests that use interpulse intervals shorter than about 20 ms, then shortfalls may distort or obscure the results of most of the tests cited at the beginning of the General Introduction above.

In summary, there are two reasons why the shortfall has such wide implications. First, refractory periods are a fundamental electrophysiological property, so anomalous results in refractory period tests challenge our understanding of neurons' most basic functions. Second, we do not know what causes the shortfall when we estimate refractory periods, so shortfalls or other similar phenomena may affect the results of other related tests.

Given the shortfall's broad implications, it is important to document it rigorously and to uncover its cause. These are the tasks undertaken in the experiments that follow. Before launching into the experiments proper, it is necessary to provide a gloss of their rationale, and then to define the terms used to describe the experiments.

# The Present Study

## Rationale

Clearly, several issues needed to be addressed in the present study. First, it is essential to document the shortfall. Other authors have observed the shortfall, but it has always been an incidental finding. Because no one has ever studied the shortfall systematically, there were no precise measurements of its depth or duration. Some authors only tested out to C-T delays of 5 ms, so it was not even known whether the shortfall is permanent or if effectiveness does eventually rise to 1.00. The present study therefore began with refractory period tests at very long C-T delays (Experiment 1). This revealed the shortfall's duration. Once it was known how long the shortfall lasts, appropriate C-T delays could be chosen for the experiments that followed.

The second task was to eliminate the possibility that the shortfall is an artifact. Refractory period tests were repeated, but the sweeps were scaled differently (Experiment 2). If this rescaling had eliminated the shortfall, then the shortfall would have been an artifact, and the third task would have been to develop a method to measure accurately the recovery from refractoriness. However, the shortfall survived rescaling, so the third task was to measure its depth and duration precisely (Experiment 3). Precise measurement provided an authoritative description of the shortfall, something that had been lacking.

Precise measurement of the shortfall was also important for the fourth task: uncovering the cause of the shortfall. One way to uncover the cause is to try different parametric manipulations that might change the depth or duration of the shortfall. Experiment 4 used T pulse currents that were 40% larger than the C pulse currents to test the hypothesis that the shortfall is caused by relative refractory, supernormal, or subnormal periods. Another experiment tested the hypothesis that the shortfall is caused by the incidental stimulation of inhibitory afferents. This last experiment's results are more promising than conclusive; its design was cumbersome, and only one out of nineteen subjects satisfied all the protocol's requirements. Consequently, the incidental inhibition experiment was relegated to "Appendix 3: Is the Shortfall Caused by Inhibitory Afferents?"

In the experiments below, extensive use is made of Yeoman's refractory period test. The terminology used to describe these tests is defined in the section that follows. For a formal derivation of Yeomans's T pulse effectiveness, see "Appendix 1: Derivation of T Pulse Effectiveness."

Definition of Terms

In the present study, the stimuli were 100 $\mu$s cathodal, constant current pulses. Current was held constant because the impedance of brain tissue is affected by the activity of glial cells, and by electrical stimulation. It is

therefore impossible to keep both the voltage and the current of the stimulation constant. Since it is current rather than voltage that determines the size of the transmembrane voltage changes that excite neural membranes, constant current pulses are more useful than constant voltage pulses (Yeomans, 1990a).

Four terms are required to describe the administration of rewarding pulses: train, trial, sweep, and session. A train is a series of consecutive pulses. The length of time between the onset of the first pulse in the train and the onset of the last pulse is the train duration. Two second, one second, and 0.5 second trains were used in the present study. The time between the onsets of consecutive pulses in the train is the period of the stimulation. The reciprocal of the stimulation period is the stimulation frequency.

During the initial stages of training, reinforcing trains were available *ad libitum*, but during testing bar pressing was rewarded with stimulation only during discrete periods called trials. The number of responses the animal makes during a trial divided by the duration of the trial is the response rate. The present study used 10, 15, and 60 second trials. Other studies have used a wide variety of reinforcement schedules, but in the present experiments one rewarding train was administered every time the rat pressed the lever (unless the rat pressed while a train was already underway).

A sweep is a series of trials. Each trial in a sweep is run with a lower pulse frequency trains than its predecessor. Eventually the frequency drops too low to support the behaviour, the subject stops responding, and the

experimenter terminates the sweep. A series of sweeps is a session. In the present study, sessions usually lasted two or three hours. Session length was limited to keep fatigue from contaminating the results.

For each sweep, a threshold between responding and non-responding can be calculated. A behavioural criterion is selected: usually either a fixed response rate or one half of the maximum response rate obtained earlier in that sweep. The frequency that corresponds to the criterion is interpolated between

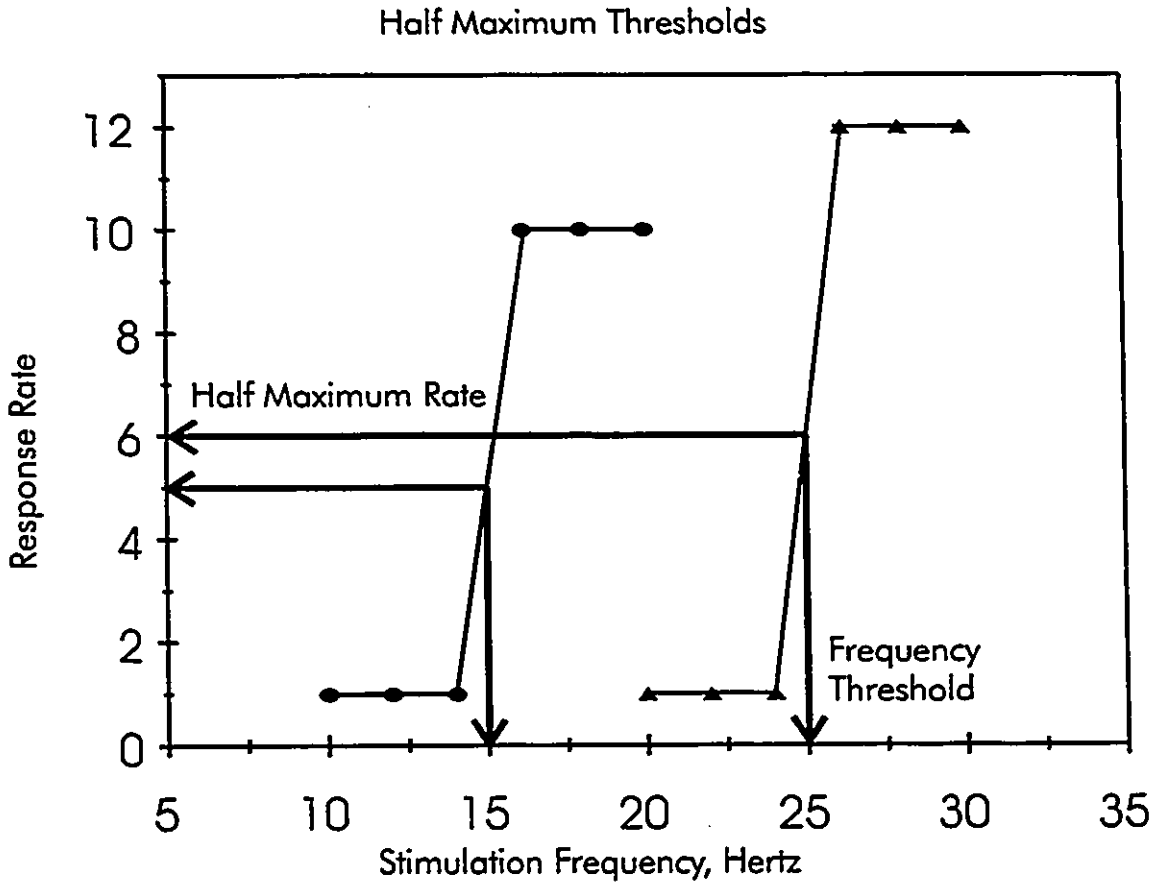



**Figure 1:** An illustration of how to determine a threshold. Each point represents the data from one trial, and each Z-shaped line represents the data from one sweep. The significance of the horizontal and vertical arrows is explained in the text.

the two consecutive trials that yielded rates above and below the criterion rate. Perhaps this may be expressed more clearly in a graph (Figure 1 above). If stimulation frequency is plotted on the abscissa and response rate on the ordinate, a sigmoidal curve results. A line can then be drawn through the rate frequency curve at the level of the criterion rate (the horizontal arrows). If the threshold criterion is well chosen, this intersection will fall on the rising portion of the rate frequency curve. A second line drawn through this intersection (the vertical arrows) will cut the abscissa at the frequency threshold.

Changes in thresholds provide evidence of changes in the rewarding value of stimulation. If each pulse in a train is very rewarding, then the subject will keep pressing until the stimulation frequency is very low (the sweep with circular markers in Figure 1). If the rewarding value of each pulse is reduced (for example, by lowering the current), then more pulses will be required to motivate the subject to press. The subject will therefore stop pressing when the frequency is still high (the sweep with triangular markers in Figure 1). Lowering current, then, reduces reward and raises frequency thresholds. Similarly, manipulations that increase the rewarding value of each pulse lower frequency thresholds.

Refractory period tests engender their own jargon. The refractory period is the period after an action potential when a cell's excitability is reduced. In these tests, the rewarding value of test (T) pulses is manipulated by preceding them with conditioning (C) pulses that induce refractoriness. Trains that are

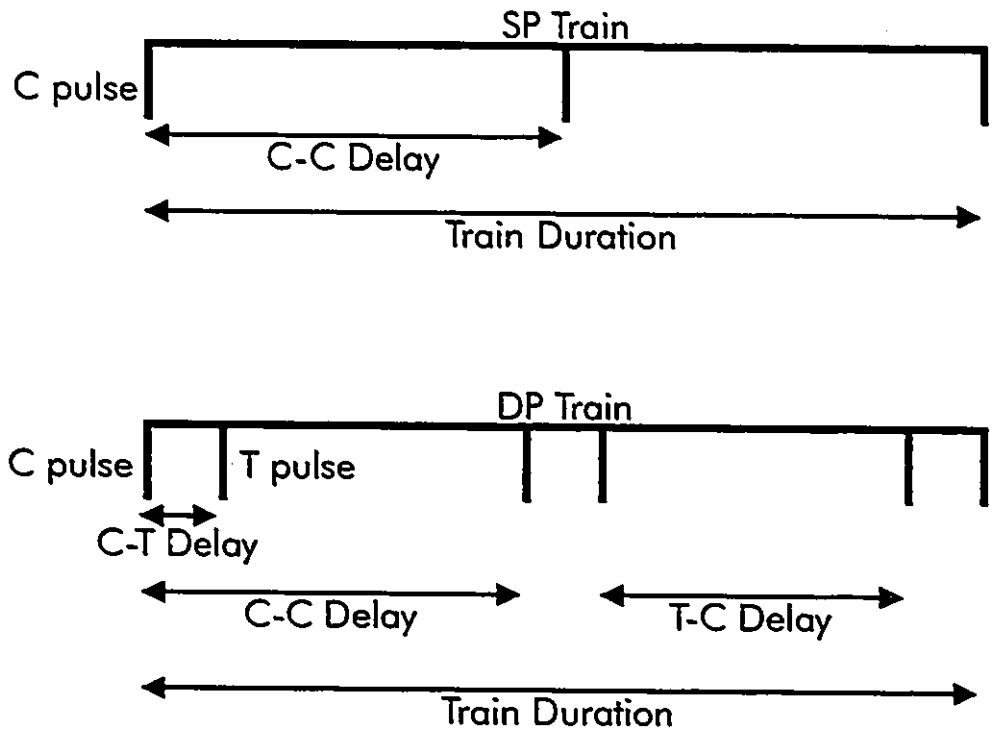



**Figure 2:** An illustration of C-C, C-T, and T-C delays. The vertical bars represent pulses, and the horizontal bars represent trains of stimulation. The upper bar represents a single pulse train, and the lower bar represents a double pulse train. All of the delays are measured from the leading edges of the pulses.

made up of pairs of C and T pulses are called double pulse (DP) trains. Trains made up of unpaired, equally spaced pulses are called single pulse (SP) trains. All of the pulses in an SP train are considered to be C pulses. The interval between the onsets of consecutive C pulses is the C-C delay. The interval between the onset of a C pulse and the T pulse that follows is the C-T delay. The interval between the onset of a T pulse and the C pulse in the next pair is the T-C delay. In the case of DP trains, pulse frequency refers to the frequency of pairs of C and T pulses rather than to the frequency of individual pulses.

## Experiment 1: Effectiveness Does Rise to 100%

### Introduction

In some refractory period studies, T pulses took dozens of milliseconds to become fully effective (e.g. Bielajew, Jordan, Ferme-Enright, and Shizgal, 1981; see also "Refractory Estimate Anomalies" above). In other studies, full T pulse effectiveness was never observed (e.g. Yeomans, 1975). Because previous studies produced such different results, and because they focused on refractoriness rather than on the slow rise of effectiveness, it is not known if or when effectiveness eventually rises to 100%. In the present experiment, effectiveness was measured at very long C-T intervals to determine whether or not effectiveness recovers fully. Effectiveness was also measured at closely spaced long intervals to determine when such a recovery might occur.

### Method

#### Subjects

The subjects were six male Long Evans rats supplied by Charles River Canada: Bit, GB2, GB5, Ka, Hype, and #950. They weighed between 314 and 430 grams at the time of surgery. The rats were individually housed in 47 cm

X 25 cm X 20 cm Plexiglas cages in a climate controlled animal care facility. The temperature was kept at 22°C, and the relative humidity at 45%. Automatic timers provided a 12 hour/12 hour light/dark cycle with the lights coming on at 7 am. Purina® Rat Chow and water were available *ad libitum*.

Surgery

The rats were anaesthetized with a standard dose (65 mg/kg i.p.) of sodium pentobarbitol (Somnotol, MTC Pharma®). This dose was insufficient; the rats still reacted to noise and to tail pinch. The standard dose was therefore supplemented to a total of 100 mg/kg i.p. of sodium pentobarbitol. Xylazine (1.0 mg i.m., Rompun, Bayvet®) was also occasionally used to supplement anaesthesia. Atropine sulfate (0.025 mg s.c.) was given to control bronchial mucus secretions. Rat #950 was given 0.2 mg/kg s.c. of atropine sulfate. A petroleum jelly based ophthalmic ointment was smeared on the eyes to keep them from drying out. A topical anaesthetic (2% lidocaine hydrochloride, Xylocaine, Astra Pharmaceuticals®) was applied to the ear canals and the mouth to mitigate the discomfort of the ear and incisor bars. The rats were then mounted in a stereotaxic apparatus. The incisor bar was adjusted to maintain a flat skull position. If the rats started to wake up before the end of the operation, then a plastic cone with an ether or halothane (Fluothane,

Wyeth-Ayerst Canada®) soaked swab was placed over their noses until they returned to a deeper plane of anaesthesia.

A longitudinal cut was made in the scalp to expose lambda and bregma. The fascia was scraped away and adrenalin chloride (1 mg/ml) was applied topically to control bleeding. A hole for the electrode was drilled with a dental burr. Four 0·80 1/8 inch stainless steel screws were toed in to holes drilled through the skull to anchor the crown. A 7 cm stainless steel wire was wrapped around the four screws. A 2·56 3/8 inch stainless steel screw soldered to the end of this wire served as a ground return and as strain relief for the wire leads used in testing.

The electrodes were made of 0.25 mm stainless steel wire soldered to Amphenol® brand Reli-a-tac® gold plated plugs. Phosphoric acid was used as flux. The wire was insulated with epoxy resin and then sharpened to expose a shallow conical point. The electrode was plumbed and then sunk to flat skull coordinates from Paxinos and Watson's (1986). In the case of the Rats GB-2 and Bit, the electrode was aimed at the lateral hypothalamus. For the others (GB-5, Ka, Hype, and #950), the electrode was aimed at the ventral tegmental area. These sites were chosen because they are often used in ICSS experiments (see Table 1), and because they produce shortfalls (see Table 2). The electrode was fixed in place with a mushroom shaped crown built out of acrylic dental cement. The crown's overhang protected the edges of the incision. Normal saline (2 to 3 ml i.p.) was administered at the end of the

operation. After surgery, the rats were allowed to convalesce for at least a week before they were trained to lever press.

## Histology

When testing was completed, the rats were euthanised with overdoses of sodium pentobarbitol. They were then transcardially perfused with saline solution and formalin. The brains were fixed in formalin for at least a week. They were then frozen and sliced into 40 $\mu$m coronal sections. These sections were mounted on glass slides and stained with cresyl violet. The locations of the electrode tips were determined by comparing these sections with the illustrations in Paxinos and Watson's (1986) atlas of the rat's brain. This comparison confirmed that GB-2 and Bit's electrodes were implanted in the LH, and that GB-5, Ka Hype, and #950's electrodes were implanted in the VTA. Electrode placements are illustrated in Figures 3 and 4 below.

## Apparatus

The rats were tested in a room lit by fluorescent ceiling fixtures. During the summer, a window mounted air conditioner kept the temperature below 25 °C. A radio was left on in the room to maintain a constant level of ambient noise.

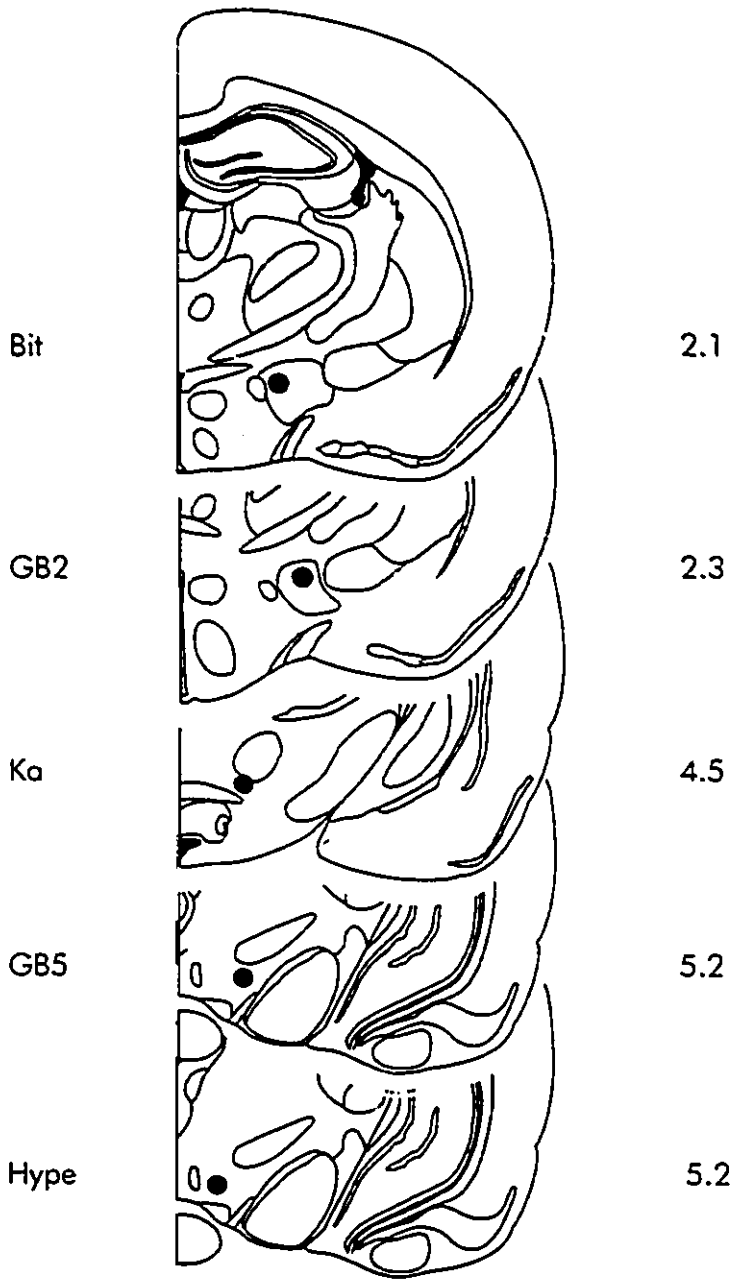



**Figure 3:** Electrode placements for the Rats Bit, GB2, GB5, Ka, and Hype. The large black dots show where the electrode tips were. The rats' names are on the left, and the distance behind bregma (in millimetres) is on the right.

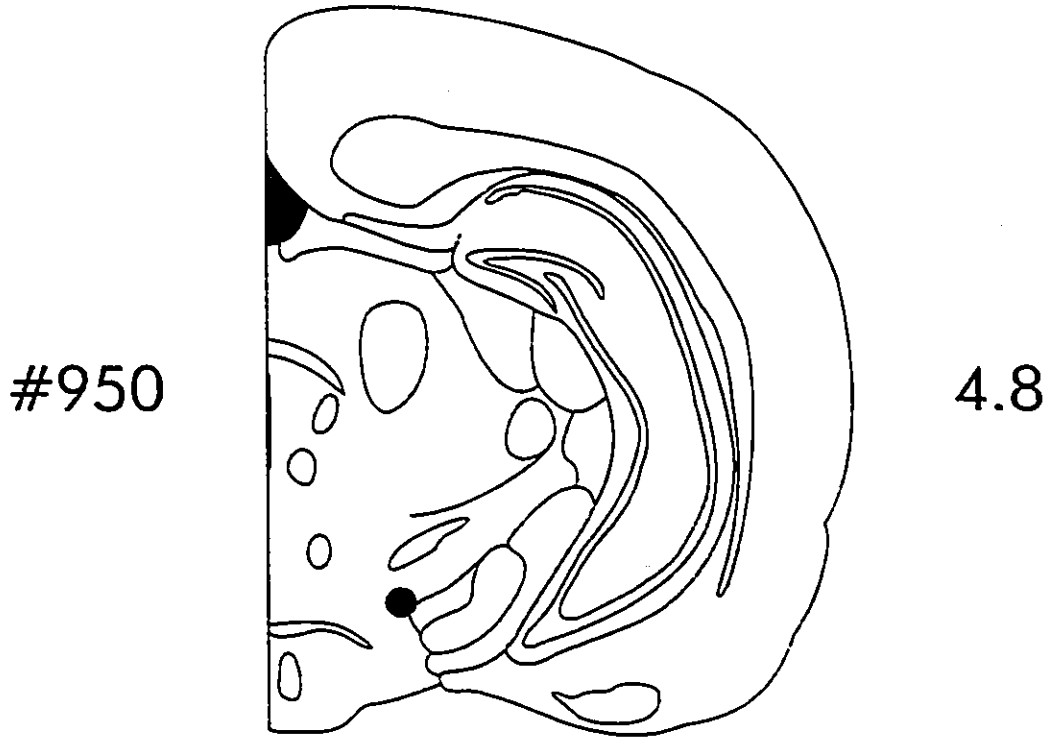



**Figure 4**: Electrode placement for Rat #950. The large black dot shows where the electrode tip was. The rat's name is on the left, and the distance behind bregma (in millimetres) is on the right.

The test cages were 38 cm wide, 28 cm deep, and 50 cm tall. The side and back walls were varnished plywood, and the front wall was Plexiglas. The floor was a plastic tray covered with sawdust. A Gerbrands® test lever was mounted 3 cm from the cage floor in the middle of one of the side walls. The leads were connected to a commutator mounted on a counterbalanced boom above the cage.

The laboratory built stimulators used digital pulse generators to control constant current amplifiers which were based on a design published by Mundl (1980). The stimulators generated square, cathodal, 100 $\mu$s pulses. The

electrode was grounded between pulses to prevent polarization of the electrode tip. The shape and current of the pulses were monitored with an oscilloscope by measuring the voltage across a 1 kohm, 1% tolerance resistor that was placed in series between the rat and the ground.

Training

After a week of postoperative recovery, the rats were trained to lever press to self administer trains of electrical brain stimulation. All training and testing was done between 9 am and 5 pm (the middle of the rats' light cycle). The time required for training varied widely between rats; some learned in less than one hour while others took several two hour sessions. The rats were hooked up, placed in the cage, and then given trains of successively higher currents until the stimulation elicited sniffing and exploration. The experimenter attracted the rat to the lever by scratching the wall of the cage and by rattling the lever. Then the experimenter shaped the rat's behaviour by using the stimulation to reward successively closer approximations of lever pressing. The experimenter triggered the stimulator by pressing the lever from the outside of the cage. If the rat did not catch on, the stimulation parameters were readjusted. Once they had learned to lever press, the rats were given 15 minutes to an hour of *ad libitum* access to lever pressing contingent stimulation.

Testing Procedure

During the testing, lever pressing was rewarded with stimulation only during one minute trials. The beginning of each trial was signalled by three non-contingent trains of stimulation spaced a half second apart. The 60 second duration of the trial was measured from the moment of the first lever press. If the rat did not start pressing within 12 seconds of the third priming train, then the trial started automatically. Once the rat started reliably when primed, sweeps could be run. The frequency of the priming and reward stimulation administered during each trial within a sweep was either 0.10 $\log_{10}$ units (21%) lower than in the previous trial (in the cases of Bit, GB2, Ka, GB5, and Hype), or 0.05 $\log_{10}$ units lower (in the case of #950). Sweeps were terminated when the rate of responding (the number of presses per one minute trial) dropped to less than half of the maximum rate obtained previously in that sweep. Practice sweeps were run until thresholds were stable; that is, until thresholds did not vary more than 10% on successive trials.

Each two to three hour testing session began with a warm-up of three sweeps. This was necessary because at the beginning of the session the rats would press vigorously for low frequency stimulation that they would normally ignore. After the warm up, single pulse baseline sweeps were interdigitated with blocks of double pulse test sweeps. This was necessary because fatigue tends to raise frequency thresholds over the course of a session. Using an SP

baseline sweep from the beginning of a session to calculate the effectiveness in a DP test sweep at the end of a session would therefore underestimate the true value. Interdigitation eliminates this artifact by closely juxtaposing test and baseline sweeps. Calculations of effectiveness were based on the average of the two SP baseline thresholds that most closely preceded and followed the test sweep. There were never more than five DP test sweeps in a block.

Effectiveness was measured at C-T delays of 0.25, 0.4, 0.63, 1, 1.6, 2.5, 4, 6.3, 10, 16, 25 and 40 ms (test parameters are summarized in Table 4 below). The Rat GB-2 was also tested at 63 ms. Every C-T delay was accorded the same number of test sweeps in any given session. The order of the test sweeps was randomized. There were six replications of every delay in every subject.

The Rats GB-2, GB-5, Ka, and Hype were tested with 2 second long trains of 1000 $\mu$A pulses, and the Rat Bit was tested with 0.5 second long trains of 1600 $\mu$A pulses. The nature of the experiment demanded high stimulation currents. It was necessary to use long C-C delays so that the 40 ms and 63 ms C-T delays would always be less than the T-C delays. Otherwise the T pulses would be closer to the following C pulses than to the preceding C pulses. The C pulses would, in effect, become T pulses and effectiveness would be measured at the T-C delay rather than at the C-T delay. Increasing the C-C delay (the period) reduced the frequency, and hence the number of pulses per train. The individual pulses, therefore, had to be very

strong in order to support lever pressing. High currents, however, caused motor artifacts and seizures. In an effort to reduce these problems, the lowest currents that would support the necessary C-C delays were used. When a subject had a seizure, the experimenter interrupted the session until the rat had recovered. The criterion for recovery was that the rat returned to the lever without priming or prompting; five minutes was usually sufficient.

Rat #950 was tested the same way as the other subjects (100 $\mu$s pulses, 0.5 second sweeps, one minute trials), but two refinements were implemented to more precisely characterise the recovery of effectiveness. First, more long C-T delays were tested. The other subjects were tested at C-T delays of 0.25, 0.4, 0.63, 1.0, 1.6, 2.5, 4, 6.3, 10, 16, 25, and 40 ms. Rat #950 was also tested at 30, 32, 34, 35, 36, 38, and 45 ms. The test points were concentrated like this because, in pilot work, Rat #950's T pulse effectiveness rose sharply between 30 and 35 ms. The shorter delays (0.25 to 25 ms) were tested six times with a current of 360 $\mu$A, and the longer delays (10 to 45 ms) were tested six times with a current of 700 $\mu$A. Another six sets of measurements were then taken at 700 $\mu$A at C-T delays of 16, 25, 30, 32, 34, 36, 38, and 40 ms. There was therefore a total of six replications with 360 $\mu$A at delays of 0.25 to 25 ms, six replications with 700 $\mu$A at 10, 32, 34, 35, 36, 38, and 45 ms, and twelve replications with 700 $\mu$A at 16, 25, 30, and 40 ms. The overlap between the two currents at 10, 16, and 25 ms bridged the long delays collected at 700 $\mu$A with the short delays collected at 360 $\mu$A. Two

different currents were used because, although the higher current was necessary to maintain responding at the longer delays, 700 $\mu$A induced gnashing, facial twitches, and motor artifacts. The higher current was only used, therefore, when necessary.

The second refinement in #950's testing was the adoption of finely scaled sweeps. Between the trials in a sweep, the stimulation pulse frequency was reduced by 0.05 $\log_{10}$ units (as in Experiment 2) rather than by 0.10 $\log_{10}$ units (as with the other subjects in Experiment 1). This was done because although 0.05 and 0.10 $\log_{10}$ steps produce similar results, the smaller steps in stimulation pulse frequency may allow finer estimates of frequency thresholds (see Experiment 2).

Table 4: Summary of Test Parameters in Experiment 1

| Rat | Train Length (s) | Intertrial Decrement ($\log_{10}$ units) | Current ($\mu$A) | C-T Delays (ms) |
|---|---|---|---|---|
| Bit | 0.5 | 0.10 | 1600 | 0.25, 0.4, 0.63, 1, 1.6, 2.5, 4, 6.3, 10, 16, 25, and 40. |
| GB2 | 2 | 0.10 | 1000 | Same as Bit plus 63. |
| GB5 | 2 | 0.10 | 1000 | Same as Bit. |
| Hype | 2 | 0.10 | 1000 | Same as Bit. |
| Ka | 2 | 0.10 | 1000 | Same as Bit. |
| #950 | 0.5 | 0.05 | 360 | 0.25, 0.4, 0.63, 1, 1.6, 2.5, 4, 6.3, 10, 16, and 25. |

| Table 4: Summary of Test Parameters in Experiment 1 | | | | |
|---|---|---|---|---|
| Rat | Train Length (s) | Intertrial Decrement ($log_{10}$ units) | Current ($\mu$A) | C-T Delays (ms) |
| #950 | 0.5 | 0.05 | 700 | 10, 16, 25, 30, 32, 34, 35, 36, 38, 40, and 45. |

## Results

Bit had motor artifacts and seizures. High current stimulation (700 $\mu$A) induced gnashing, facial twitches, and motor artifacts in #950, but low current stimulation (360 $\mu$A) produced no such effects. Records of motor artifacts and seizures were not kept for the other subjects. However, high response rates indicate that performance was not seriously compromised. Over the course of the testing the maximum response rate for each of the rats was 98 presses per minute for Bit, 89 for GB-2, 115 for Hype, 135 for #950, 37 for KA, and 32 for GB-5. Ka and GB-5's response rates were lower than the other rats' rates, but Ka and GB-5 were tested with two second long trains, and long trains typically elicit low rates.

The recovery of T pulse effectiveness is illustrated in Figures 5 through 8. In each of these graphs, the horizontal axis is the C-T delay in milliseconds, and the vertical axis is T pulse effectiveness calculated according to the

procedure outlined in "Appendix 1: Derivation of T Pulse Effectiveness". The error bars represent standard errors of the means (*SEM*).

The different shapes of the six effectiveness curves below were quantified by calculating the points at which they approached their upper asymptotes (Bielajew, Jordan, Ferme-Enright & Shizgal, 1981; Bielajew & Trzcińska, 1994). The *SEM* of the longest C-T delay was compared with the *SEM* of the preceding C-T delay (in the case of the rat GB2, 63 and 40 ms). If the two *SEM's* overlapped, then all of the effectiveness values that contributed to either *SEM* were pooled. A new omnibus mean and *SEM* were calculated, and this combined *SEM* was compared to that of the preceding C-T delay (in the case of GB2, 25 ms). This procedure was repeated until there was no more overlap. The beginning of the upper asymptote was defined as the shortest C-T delay that contributed to the combined *SEM*. The results of this analysis are summarized in Table 5 below.

Table 5: Asymptotic Effectiveness Values

| Rat | Beginning of Asymptote (ms) | Average Asymptotic Effectiveness |
|---|---|---|
| GB2 | 1.6 | 0.97 |
| GB5 | 25 | 0.95 |
| Bit | 25 | 1.10 |
| Hype | 4 | 0.91 |
| Ka | 40 | 0.98 |
| #950 | 32 | 0.93 |
| Overall | | 0.97 |

In every subject, effectiveness dropped slightly between 0.25 and 0.63 ms, and then rose steadily. In the case of the Rat GB-2, full effectiveness (defined here as the point where mean effectiveness rose to within one *SEM* of 1.00) was achieved at 2.5 ms. Except for a dip at 25 ms, effectiveness remained near 1.00 at all longer delays. Effectiveness also recovered quickly in the case of the Rat Hype. Full effectiveness was reached at 6.3 ms. Effectiveness remained steady out to 40 ms (Figure 5).

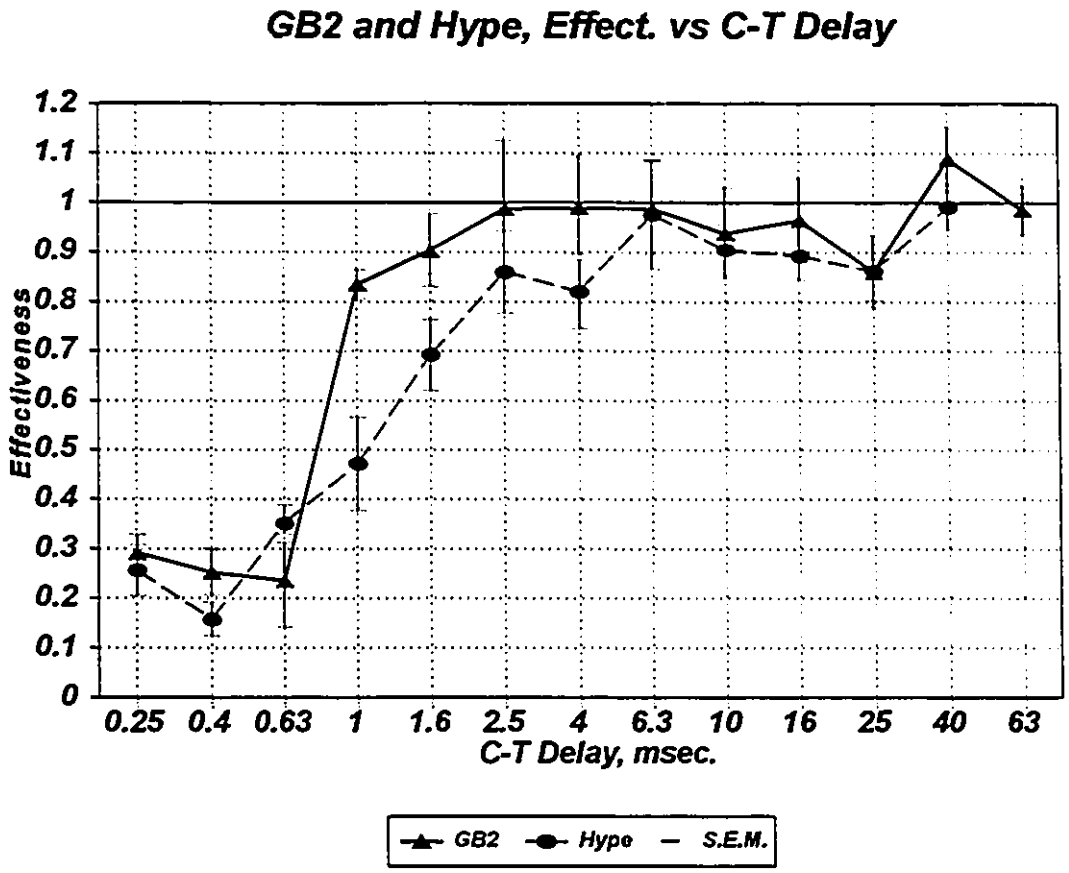



**Figure 5:** Effectiveness curves for Rats GB-2 and Hype. The triangles and circles show the mean effectivenesses achieved, respectively, by GB-2 and Hype. The error bars show standard errors of the means (*S.E.M.*). In both of these rats effectiveness rose quickly.

The Rats GB-5, Ka, and Bit showed very different effectiveness profiles. In the case of GB-5 (Figure 6), effectiveness rose slowly, and finally approached 1.00 at 25 ms. In the cases of Ka and Bit (Figure 7), effectiveness

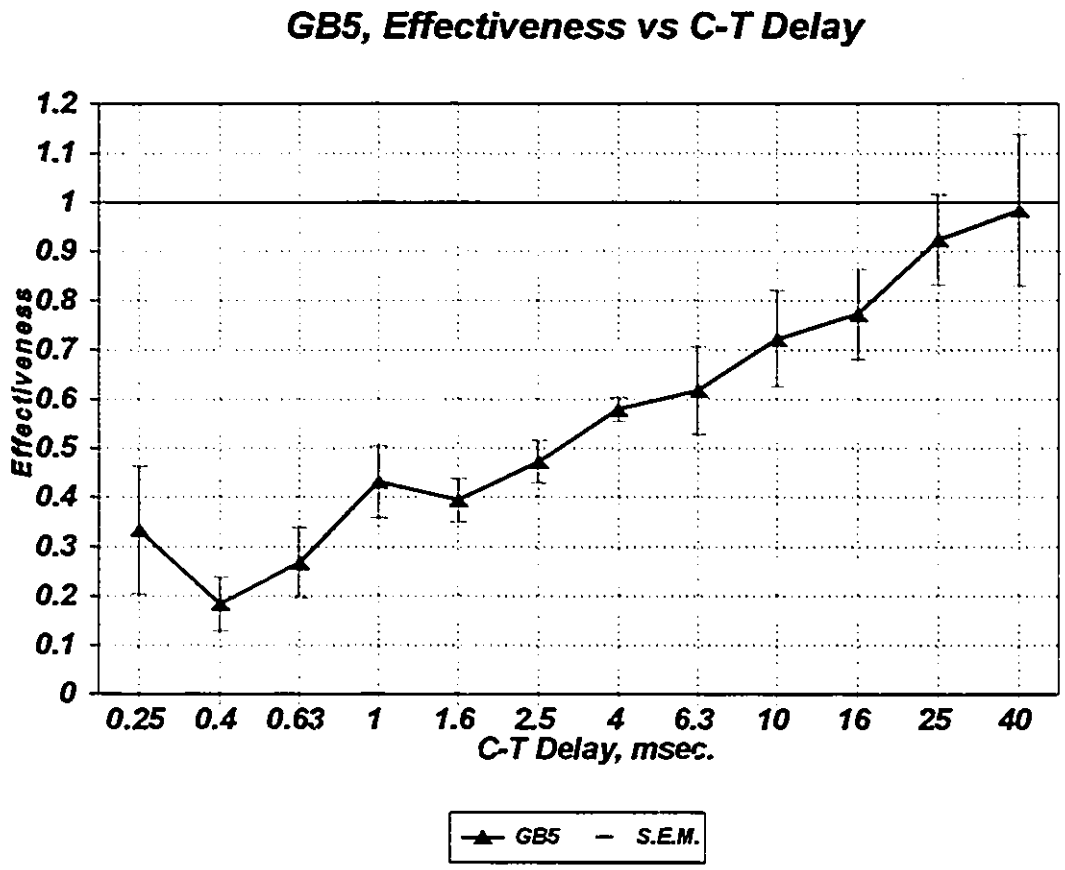



**Figure 6**: Effectiveness curve for the Rat GB5. The triangular markers represent mean effectivenesses, and the error bars represent standard errors of the means (*S.E.M.*). The X axis is log scaled, so although effectiveness seems to rise steadily, the slope is much steeper at shorter delays..

stalled at a plateau before rising to 1.00. Ka's effectiveness remained near 0.6 from 2.5 to 25 ms, and then rose to near 1.00 at 40 ms. Bit's effectiveness stalled near 0.8 between 1.6 and 10 ms. Effectiveness then rose to near 1.00 at 16 ms, and remained there at 25 and 40 ms .

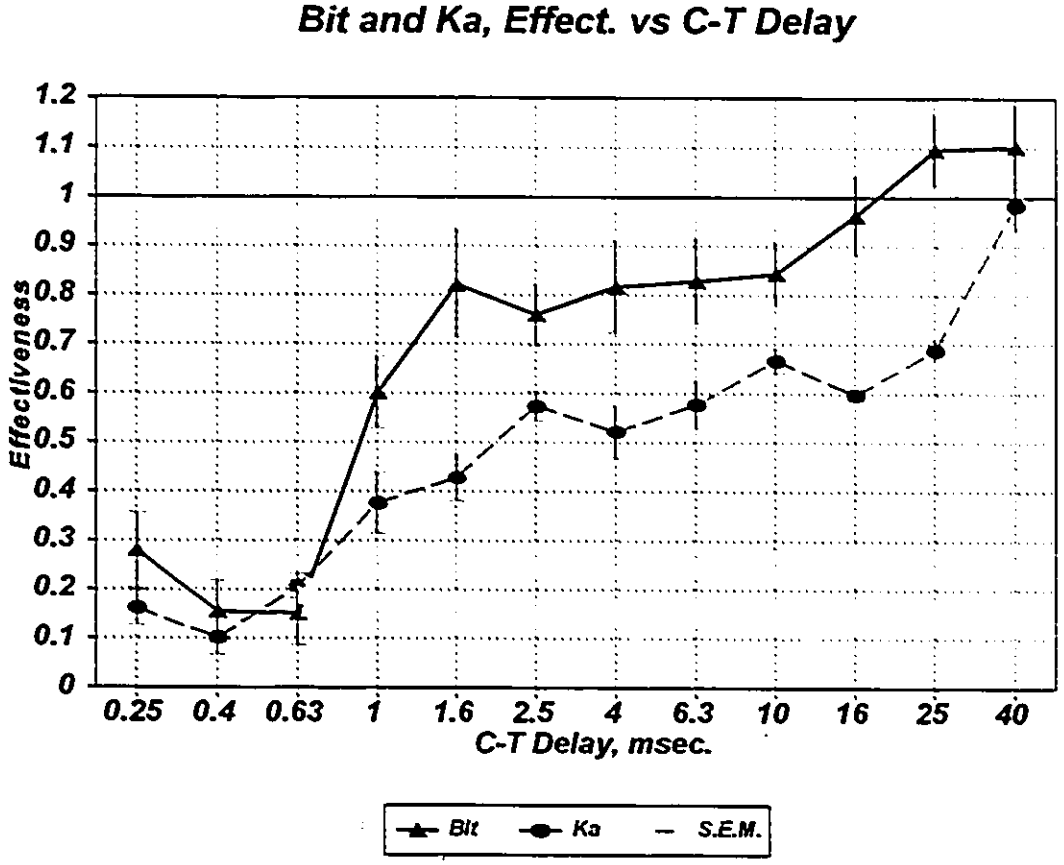



**Figure 7**: Effectiveness curves for the Rats Bit (triangle markers) and Ka (circle markers). The filled markers represent mean effectivenesses, and the error bars represent standard errors of the means. In both of these rats effectiveness stalled at a plateau between 2.5 and 10 ms.

T pulse effectiveness grew in much the same manner with #950 as with Bit. With 360 $\mu$A, effectiveness began low (0.13) at a C-T delay of 0.25 ms, dropped at 0.4 ms, and then rose steadily between 0.4 and 1.0 ms. Between 1.0 and 25 ms effectiveness rose very gradually from about 0.60 to 0.75. With 700 $\mu$A effectiveness was only 0.51 at 10 ms. At 16 ms it rose to 0.74 and remained near that level at 25 and 30 ms. Between 30 and 32 ms effectiveness rose abruptly to 0.89. At C-T delays of 32 to 45 ms,

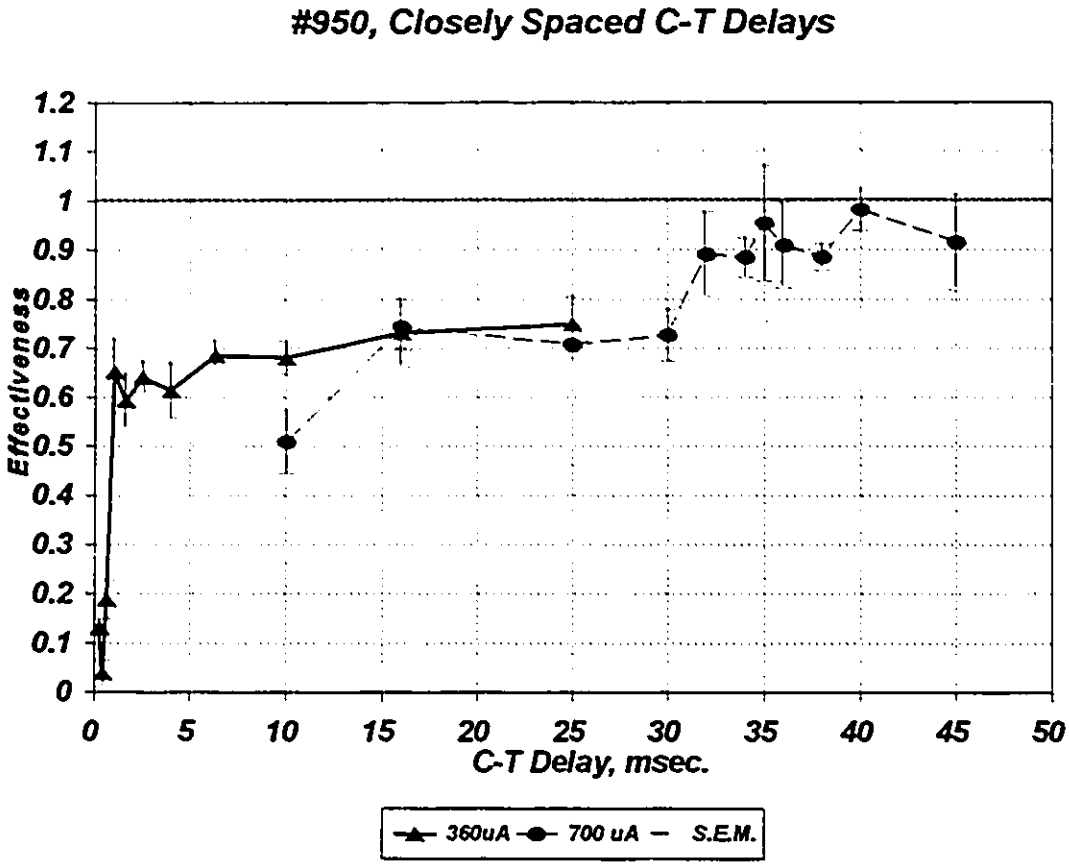



**Figure 8**: Closely spaced C-T delays. The triangles indicate the means of estimates made with 360 $\mu$A pulses, and the circles indicate the means of estimates made with 700 $\mu$A pulses. The error bars indicate standard errors of the means (*S.E.M.*).

effectiveness levelled off between 0.88 and 0.98. The average effectiveness during this final plateau was 0.93.

Rat #950's 360 $\mu$A and 700 $\mu$A effectiveness curves were nearly superimposed where at two of the three delays where they overlapped. There was a marked difference only when the C-T delay was 10 ms (0.68 at 360 $\mu$A versus 0.51 at 700 $\mu$A).

# Discussion

The development of T pulse effectiveness can be divided into three phases: a local potential summation phase, a refractory phase, and a post-refractory phase. These phases are defined jointly by the shape of the effectiveness curve, and by the phenomena that are presumed to mould the curve. In all three phases, the results of the present experiment are consonant with other reported refractory period tests, and with current explanations for the effectiveness curve's shape (Yeomans, 1990a).

The 0.25 and 0.4 ms delays were part of the first phase. When the C-T delay is less than half a millisecond, effectiveness is greater than zero even though the fibres fired by the C pulse are fully refractory. T pulses are slightly effective during the first phase because there is a ring of fibres at the outer edge of the C pulse's stimulation field that did not quite reach the depolarization threshold required for an action potential. It takes these fibres about half a millisecond to repolarise. When the C-T delay is very short, therefore, the T pulse summates with the residual potential left over from the C pulse. This local potential summation causes the fibres in that outer ring to fire (Yeomans, Matthews, Hawkins, Bellman & Doppelt, 1979). As the C-T period increases to more than 0.4 ms the residual potential from the C pulse fades, and local potential summation no longer contributes to effectiveness. This signals the end of the first phase.

The second phase begins as the C-T delay increases to more than half a millisecond. At this point, most of the fibres in the core of the stimulation field are in the middle of their absolute refractory period, so the T pulse cannot make them fire. T pulse effectiveness is therefore at low ebb. As the C-T delay increases from 0.63 ms to 1.0 ms, the fibres that were fired by the C pulse begin to recover. T pulse effectiveness therefore starts to rise. The absolute refractory period is essentially finished by 1.2 ms, but the relative refractory lasts a little longer. Effectiveness therefore continues to rise until the C-T delay reaches 2.5 ms (Yeomans, 1975; Yeomans & Davis, 1975).

The third phase extends from 5 ms to 63 ms. During this post refractory phase one would expect T pulse effectiveness to be near 1.00 because the refractory period is finished, and because the T pulse has the same current and duration as the C pulse. Just as in previous studies, however, T pulse effectiveness sometimes failed to reach 1.00 within five milliseconds (the maximum span of time that is usually allotted for the refractory period; Bielajew, Jordan, Ferme Enright & Shizgal, 1981; Yeomans, Mercouris & Ellard, 1985). In four out of the six cases reported in the present experiment, T pulse effectiveness did not reach 1.00 when the C-T delay was 10 ms or less. This is roughly the same proportion of slow rises as reported in the literature; in 32 out of the 54 cases reported above in "Refractory Estimate Anomalies", T pulse effectiveness failed to approach 1.00 when the C-T delay was 10 ms or less. The slow rise to full effectiveness found in the present experiment, therefore,

does not represent an unusual problem with the present study's equipment or procedures. On the contrary, the frequency of effectiveness shortfalls in the present experiment is the same as what is reported in the literature. This suggests that the present study's subjects, electrode placements, and methodology are comparable with those used in other studies.

There were also some novel results. In the present experiment, it was found that effectiveness always rose to near 1.0, even when it took longer than 10 ms to reach full effectiveness. In the review in the introduction, there were 32 cases reported that failed to achieve full effectiveness within 10 ms. In 26 out of those 32 cases effectiveness never reached 1.00. Unlike those previous studies, the present experiment tested out to delays of 40 ms and 63 ms rather than just 5 ms, 10 ms, 12 ms or 25 ms. In those 26 cases, therefore, effectiveness may never have reached 1.00 simply because the C-T delays were too short. One would expect T pulses to become fully effective at long delays because C and T pulses are identical except for timing. They have the same current and duration, so when the C-T delay gets too long for the C pulse to interfere with the T pulse, C and T pulses should become equally effective.

It was also found that whether effectiveness rose early or late had no bearing on the maximum level that was achieved. GB-2 and Hype reached full effectiveness in 2.5 and 6.3 ms respectively. Bit, GB-5, #950, and Ka took much longer: 16 ms, 25 ms, 31 ms, and 40 ms respectively. The starting

points of upper asymptotes ranged from 1.6 ms to 40 ms. In all six cases, however, once effectiveness reached 1.00 it remained near that level even at C-T delays of 40 ms, 45 ms, and 63 ms; asymptotic effectiveness ranged from 0.91 to 1.10, and the average across all subjects was 0.97. These results suggest that even though effectiveness curves often take between 16 ms and 40 ms to approach 1.00, they usually do rise to their theoretical maximums.

These results do not suggest that the refractory periods of reward fibres last 16 ms to 40 ms. The sharp rise in effectiveness between 0.5 ms and 2.5 ms, estimates of conduction velocity (Bielajew & Shizgal, 1982; Shizgal, Bielajew, Corbett, Skelton & Yeomans, 1980), microscopic measurements of reward fibre diameters (Szabo, Lenard & Kosaras, 1974; Yeomans, 1989), and measurements of digitally isolated action potentials (Kiss & Shizgal, 1989) all point to refractory periods of 0.4 ms to 5 ms. These are such reliable and coherent indicators of refractory period duration that it makes more sense to ascribe the slow rise in effectiveness to some other, unknown factor.

Rat #950 was tested with closely spaced long delays to locate precisely the final rise to full effectiveness. The sharp increase from the plateau between 1 and 30 ms to the plateau between 32 and 45 ms indicates that the final rise occurred at a C-T delay of 31 ms. Thirty-one milliseconds is almost an order of magnitude longer than the longest CNS refractory period reported by Swadlow and Waxman (1978). It is unlikely, then, that the final rise to full effectiveness represents a recovery from refractoriness. It follows that there

was some other, longer lasting inhibitory process at work. Subnormal periods are one alternative process because they can last tens or hundreds of milliseconds (Yeomans, 1990b). The experiment below titled "Unequal Pulses" tests the hypothesis that the shortfall is caused by a subnormal period.

There was a difference between the effectiveness values obtained with #950 at a C-T delay of 10 ms with 360 $\mu$A and 700 $\mu$A (0.68 at 360 $\mu$A versus 0.51 at 700 $\mu$A). There are two reasons why this difference does not throw the results into question. First, the goal of testing with #950 was to locate the final rise in effectiveness between 25 and 40 ms, so what occurred at 10 ms had no direct bearing on the conclusions. The measurements at short C-T delays served primarily to show that the development of effectiveness in #950 was similar to that found in the other subjects. Second, there were as few as six replications with each of the 22 combinations of current and C-T delay, so outliers were to be expected.

The small number of replications may also account for the fact that T pulse effectiveness never quite rose to 1.0; #950's average effectiveness during the final plateau was 0.93. This is slightly lower than 0.97, the average effectiveness in the final plateaus of the other five subjects. Given the limitations of the methodology (11% steps between sweeps, and *N*'s as low as six), a 7% shortfall is minimal. It does, however, point to the need for higher numbers of replications. Furthermore, a large number of subjects should be tested because there were considerable individual variations in the timing of the

final rise to full effectiveness. Bit rose between 10 and 16 ms, GB 5 rose between 16 and 25 ms, Ka rose between 25 and 40 ms, and #950 rose at 31 ms. Larger numbers of subjects and replications, and smaller gaps between C-T delays would show if the final rise usually happens at about 15, 25, or 30 ms.

## Experiment 2: The Shortfall Is Not a Scaling Artifact

### Introduction

The shortfall in effectiveness that occurred with the Rats GB-5, Ka, Bit, and #950 is about the same size as the intertrial decrement in stimulation pulse frequency (15-40% vs 21%). This raises the possibility that the shortfall is an artifact of the scaling. The question arises because, near the end of a sweep, response rates often drop from nearly maximum in one trial to zero in the next. When the response rate falls suddenly like this, the half maximum rate and the threshold frequency are interpolated precisely halfway between the two test points. As a result, there is a disproportionately high number of these halfway frequency threshold measurements. When measurements clump like this, average differences between threshold measurements can be inflated to the size of the intertrial decrement in stimulation pulse frequency. In other words, a very small shortfall in effectiveness could be inflated up to 21%.

Murray and Shizgal (1994) explained this inflation in terms of undersampling. When we collect the response rate/stimulation frequency data that we use to calculate thresholds and effectiveness, we sample points along an underlying, continuous rate/frequency *function*. Using this data, we generate a rate/frequency *curve* that is a digitized approximation of the continuous function. Then we estimate the shape of the rate/frequency

function and the positions of thresholds by interpolating between adjacent points on the rate/frequency curve. When the gaps between the points on the curve are too large (i.e., when we undersample the function), our threshold estimates are prone to error.

Murray and Shizgal showed that undersampling errors can be significant. They performed an LH-VTA collision experiment using 0.05 log unit intertrial decrements. Then they calculated effectiveness three times: first using all of the trials, second using every other trial (i.e. data points 0.1 log units apart), and third using only the data points that had been excluded from the second calculation (again, data points 0.1 log units apart). They found that the 0.05 log unit effectiveness curve rose more gradually than its 0.1 log unit counterparts. The slower rise of the 0.05 log unit curve indicates a broader range of conduction velocities than does the quicker rise of the 0.1 log unit curve. The 0.05 log unit curve therefore suggests a dopaminergic reward component that the 0.1 log unit curve appears to rule out. Undersampling errors can be large enough to lead to false conclusions.

If 0.1 log unit intertrial decrements are too big, then what size is appropriate? It is clear that the smaller the decrement, the better the match between the rate/frequency curve and function. Ideally, then, the decrement should be as small as possible. In practice, however, we must limit the lengths of sweeps by restricting the number of trials per sweep. Murray and Shizgal suggest that sampling is adequate when at least two data points fall within the

span of the dynamic interval (the rising segment of the rate/frequency function). The intertrial decrement should therefore be one half or a third the width of the dynamic interval.

In the present experiment it was found that the dynamic interval was often only 0.05 log units wide. This would suggest that Gratton and Wise's (1988) 0.02 log unit (5%) decrements are appropriate. However, long C-T intervals (40 ms) require C-C intervals that are at least twice as long (about 100 ms). Consequently, even with 1 second long trains, there are only 11 pulse pairs per train. Because numbers of pulse pairs are integers, the smallest decrement is 0.05 log units (11%). The 0.05 log unit decrements suggested by Murray and Shizgal were therefore adopted.

The goal of the present experiment is to test the hypothesis that the effectiveness shortfall is an undersampling artifact. To this end, effectiveness was calculated using 0.10 $\log_{10}$ unit (21%) intertrial frequency decrements (as in Experiment 1), and again using 0.05 $\log_{10}$ unit (11%) decrements. If the shortfall is an artifact of the intertrial steps, then reducing the size of those steps should reduce or eliminate the shortfall.

## Method

Bit and #950, two of the rats from Experiment 1, were used again for Experiment 2. Bit had one electrode implanted in the LH (see Figure 3), and

#950 had one electrode in the VTA (see Figures 4 and 9). Four other rats were also used: #957, #958, #1007, and #1009 (Figure 9). They all had electrodes implanted in the VTA, a structure that supports ICSS (see Table 1) and that produces shortfalls (see Table 2 and Experiment 1). The subjects weighed between 370 and 430 grams at the time of surgery. Bit was given 0.025 mg s.c. of atropine sulfate to control mucus secretions. Rat #950 was given 0.2 mg s.c. of atropine sulfate, and the other rats were all given 0.125 mg s.c. of atropine sulfate. The rats were anaesthetized with 65 mg/kg i.p. injections of sodium pentobarbitol (Somnotol, MTC Pharma®). This standard dose was inadequate (the rats still reacted to noise or to tail pinch), so it was supplemented up to a maximum total of 100 mg/kg i.p.. Rats #950, #957, #958, and #1007 also required xylazine (1.0 mg i.m., Rompun, Bayvet®) to supplement anaesthesia. Rats #957 and #958 were also treated with ether, and Rat #1007 was treated with halothane (Fluothane, Wyeth-Ayerst Canada®) because they began to wake up before the end of the operation. Otherwise, the surgery, housing conditions, and histology were the same as described in Experiment 1. The same apparatus, and the same training methods were also used.

There were three principal differences between Experiments 1 and 2: two in how the testing was conducted, and one in the treatment of the data. The first procedural difference was that the sweeps were run using intertrial decrements of stimulation pulse frequency of 0.05 $\log_{10}$ units rather than 0.10

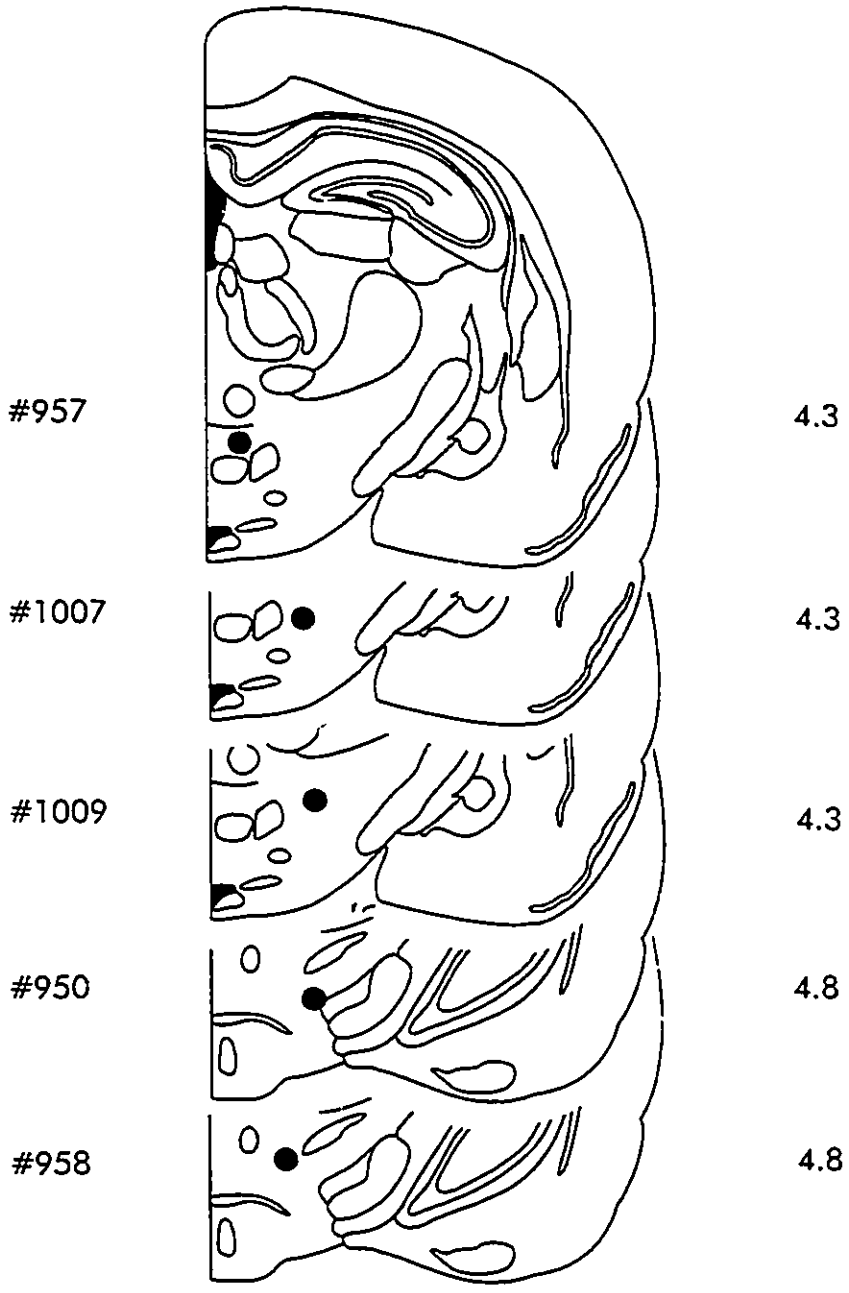



**Figure 9:** Electrode placements for the Rats #950, #957, #958, #1007, and #1009. The large black dots show where the electrode tips were. The rats' names are on the left, and the distance behind bregma (in millimetres) is on the right. Bit's histology is illustrated in Figure 3.

$\log_{10}$ units. In Experiment 1, the stimulation pulse frequency was reduced by 0.10 $\log_{10}$ units (21%) between the trials that made up a sweep. For example, if the stimulation pulse frequency in the first trial in a sweep was 50.0 Hz, then the stimulation pulse frequency in the second trial was 39.8 Hz. In the present experiment, the stimulation pulse frequency was reduced by 0.05 $\log_{10}$ units (11%) between trials. Thus, if the stimulation pulse frequency in the first trial was 50.0 Hz, then the frequency in the second trial was 44.6 Hz.

The second procedural difference was that for Bit, the stimulation current was reduced from 1600 $\mu$A to 500 $\mu$A (test parameters are summarized in Table 5 below). This was done in order to reduce the motor artifacts and seizures that had been induced by the larger current. The weaker current only supported responding down to stimulation frequencies of 22.4 Hz or 20.0 Hz in the double pulse condition. This reduced the maximum C-C delay to 50 ms, and the maximum C-T delay to 25 ms. It was not possible, therefore, to test at C-T delays of 25 ms, 40 ms, or 63 ms. However, the purpose of the present experiment was to test the effect of scaling changes rather than to examine effectiveness at very long C-T delays. It was sufficient, then, to include only the early part of the post refractory phase described in Experiment 1. Bit was tested, therefore, at C-T delays of 0.25, 0.4, 0.63, 1.0, 1.6, 2.5, 4, 6.3, 10, and 16 ms. The other rats did not have seizures, so they were tested at longer C-T delays. Rat #950 was tested with 0.5 sec trains of 700 $\mu$A pulses at 10, 16, 25, 30, 35, 40, and 45 ms. Rats #957 and #958 were tested with one

second trains of, respectively, 900 μA and 350 μA pulses at delays of 0.25, 0.4, 0.63, 1, 1.6, 2.5, 4, 6.3, 10, 16, 25, and 40 ms. Rats #1007 and #1009 were tested with one second trains of 700 μA pulses at 0.5, 1, 2.5, 5, 10, 20, 30, and 40 ms. Each of the subjects was tested six times at each C-T delay.

Table 6: Summary of Test Parameters in Experiment 2

| Rat | Train Length (s) | Current (μA) | C-T Delays (ms) |
|---|---|---|---|
| Bit | 0.5 | 500 | 0.25, 0.4, 0.63, 1, 1.6, 2.5, 4, 6.3, 10, and 16. |
| #950 | 0.5 | 700 | 10, 16, 25, 30, 35, 40, and 45. |
| #957 | 1 | 900 | 0.25, 0.4, 0.63, 1, 1.6, 2.5, 4, 6.3, 10, 16, 25, and 40. |
| #958 | 1 | 350 | 0.25, 0.4, 0.63, 1, 1.6, 2.5, 4, 6.3, 10, 16, 25, and 40. |
| #1007 | 1 | 700 | 0.5, 1, 2.5, 5, 10, 20, 30, and 40. |
| #1009 | 1 | 700 | 0.5, 1, 2.5, 5, 10, 20, 30, and 40. |

The third difference between the present experiment and Experiment 1 was that thresholds and effectiveness were calculated twice with the same data. First, frequency thresholds were interpolated between the test points that were 0.05 $\log_{10}$ units apart. Then another set of thresholds were interpolated using data only from the trials that were run at frequencies that had been used in Experiment 1, i.e., trials that were 0.10 $\log_{10}$ units apart. In other words, the data from every second trial was excluded. Effectiveness,

then, was calculated twice: once with the small step thresholds, and once with the large step thresholds.

## Results

Rat #950 had a motor artifact; when it was stimulated it would twist its head and shoulders to the left. It also gnashed its teeth and had facial twitches. None of the other subjects had motor artifacts or seizure signs.

In the introduction to the present experiment, it was predicted that if the shortfall were an undersampling artifact, then finely scaled sweeps should reduce or eliminate the shortfall. To verify this prediction, the 0.10 and the 0.05 $\log_{10}$ curves were compared at each C-T delay. Even when no correction was made for familywise error, *t* tests ($p = .05$) could detect no significant differences in any of the rats. Note that the tests' power was limited by the small number of replications at each C-T delay. Because of this limitation, the error bars in the graphs below represent standard errors of means (*SEM*) rather than confidence intervals.

Bit's 0.10 and the 0.05 $\log_{10}$ unit step effectiveness curves were similar to those obtained in Experiment 1 (see Figure 7 above and Figure 10 below). There was an initial dip from 0.25 ms to 0.4 ms. This was followed by a rising portion between 0.63 ms and 4.0 ms. Effectiveness then levelled off between 0.89 and 1.03 at the longer delays. Just as in Experiment 1, effectiveness

never rose significantly higher than 1.00. It is notable that full effectiveness was first achieved at a C-T delay of 4 ms rather than at 16 ms as in Experiment 1. There was a slight shortfall at 6.3, and at 10 ms. In the 0.05 $\log_{10}$ step condition, there was also a shortfall at 16 ms.

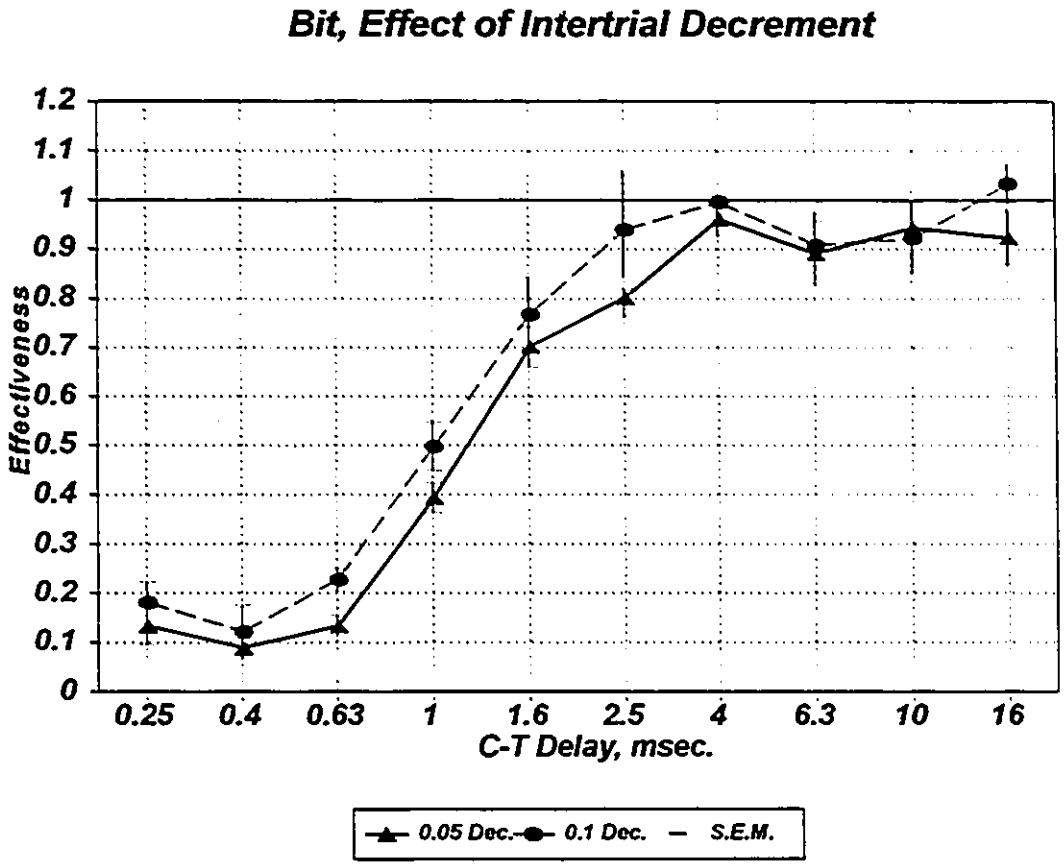



**Figure 10:** Effect of intertrial decrement on the Rat Bit. The triangles and circles represent, respectively, the means obtained with 0.05 and 0.1 $\log_{10}$ unit frequency decrements. The error bars represent standard errors of the means (*SEM*).

Rat #950's 0.10 and the 0.05 $\log_{10}$ unit curves both rose from approximately 0.55 at 10 ms to 0.70 at 16 ms (see Figure 11 below). Effectiveness remained close to that level at 20, 25, and 30 ms and then rose

to about 0.97 at 35 ms. It stayed near that level at 40 and 45 ms, except that in the 0.05 condition it dipped to 0.78 at 40 ms.

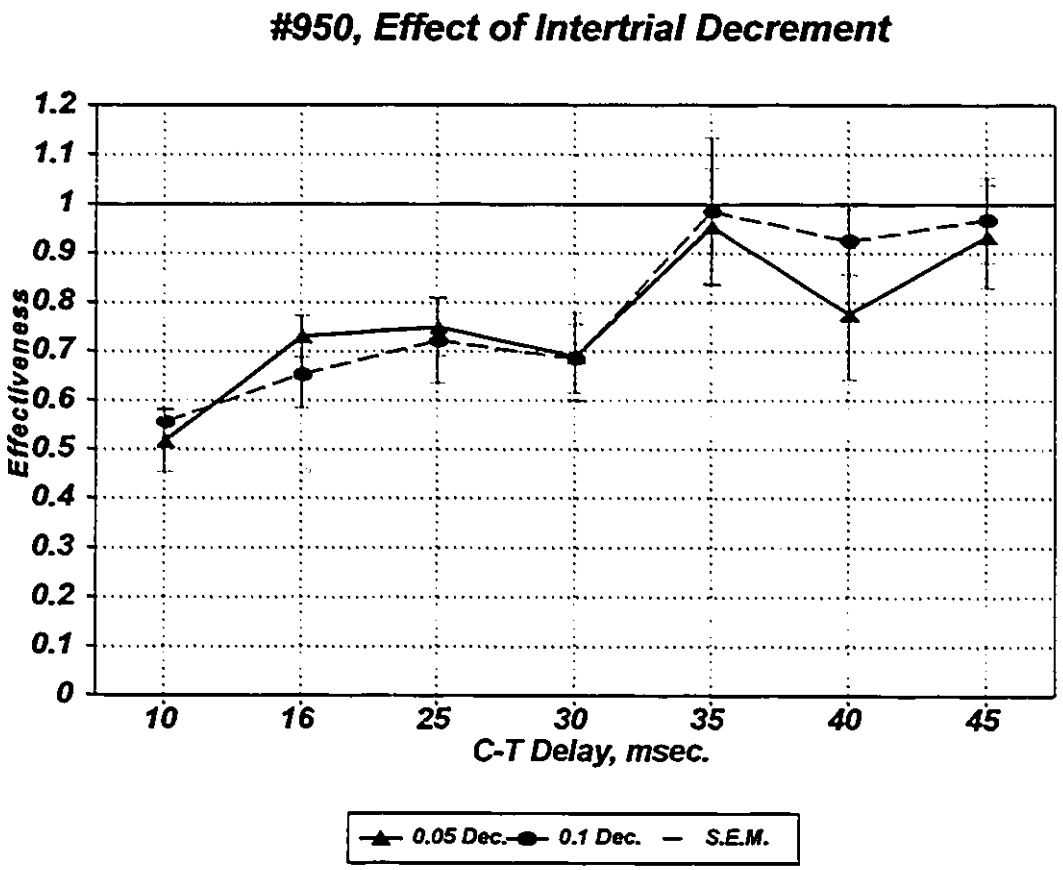



**Figure 11**: Effect of intertrial decrement Rat #950. The triangles and circles represent, respectively, the means obtained with 0.05 and 0.1 $\log_{10}$ unit frequency decrements. The error bars represent standard errors of the means (*SEM*).

In the case of Rat #957 effectiveness started at approximately 0.20 at 0.25 ms and rose to 0.85 at 4 ms (Figure 12). It remained near that level until 25 ms when it reached 1.00. Effectiveness reached its maximum (1.06 and 1.13 in, respectively, the 0.05 and 0.1 $\log_{10}$ unit conditions) at a C-T delay of 40 ms. Rat #958's curves were similar (see Figure 13 below). Effectiveness

rose to 0.85 within 1.6 ms and remained near that level until the C-T delay exceeded 10 ms. At 16 ms effectiveness was close to 1.04. It then declined to about 0.94 at 40 ms.

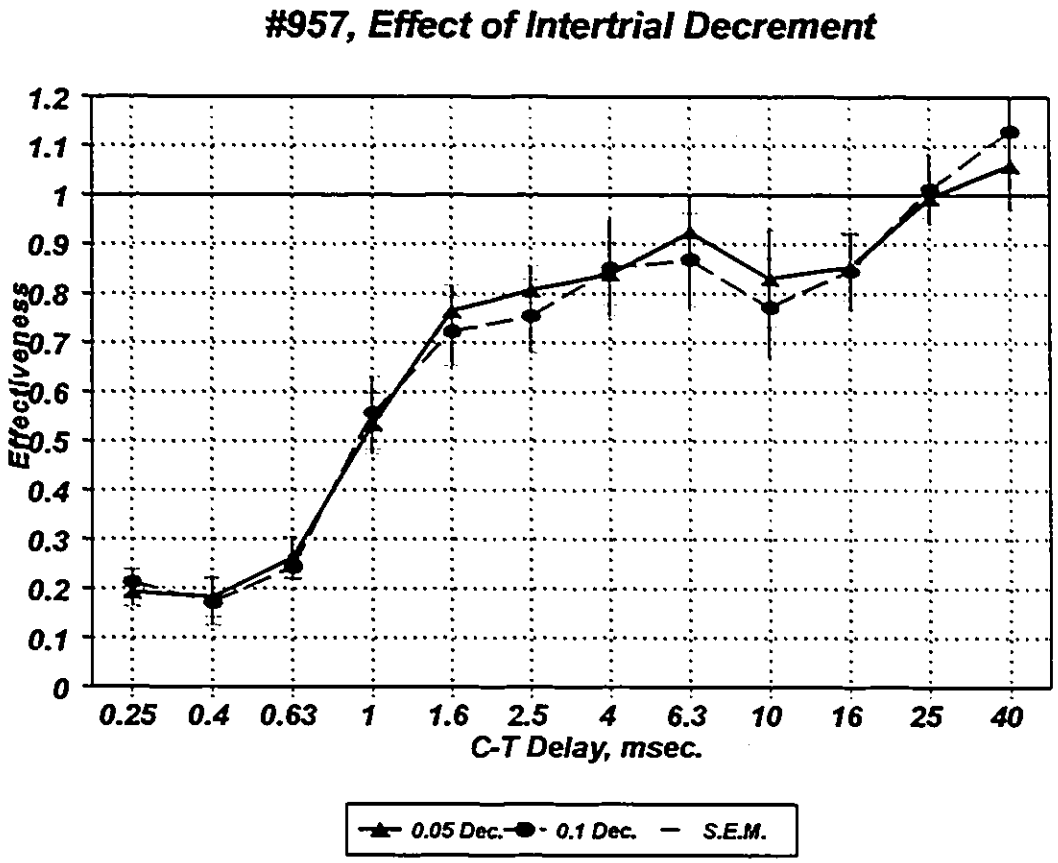



**Figure 12:** Effect of intertrial decrement on Rat #957. The triangles and circles represent, respectively, the means obtained with 0.05 and 0.1 $\log_{10}$ unit frequency decrements. The error bars represent standard errors of the means (*SEM*).

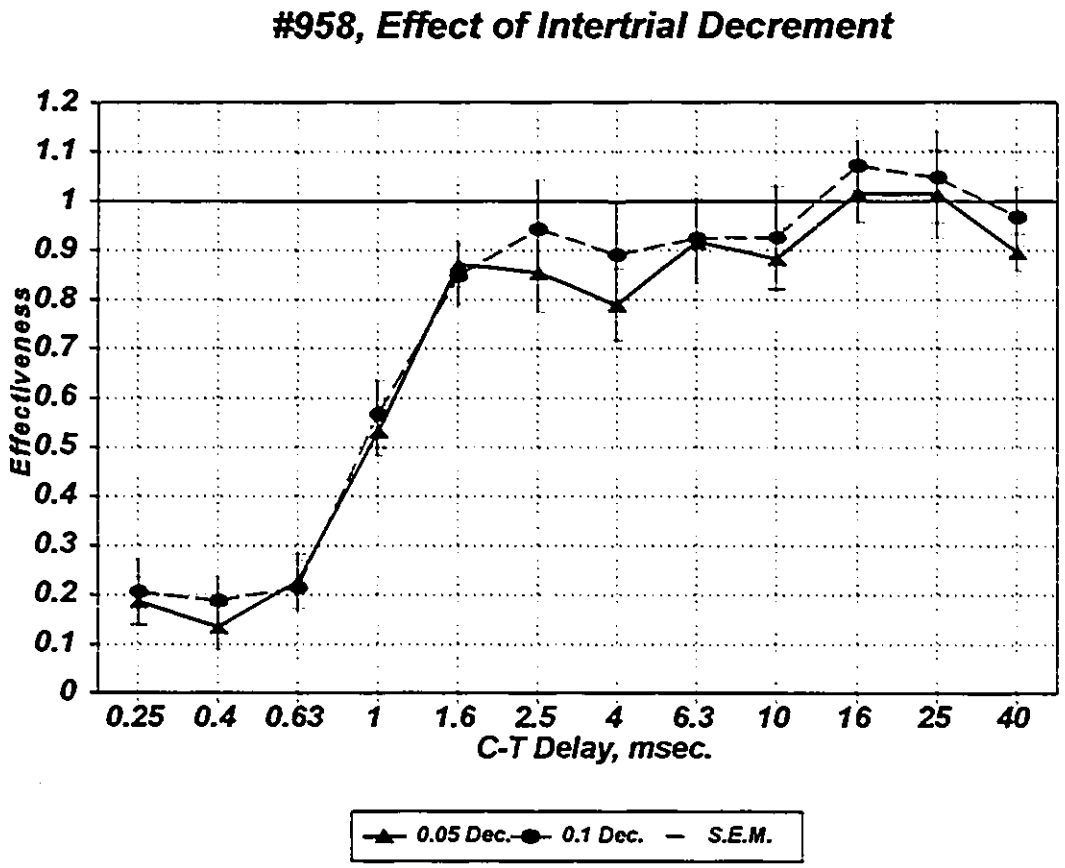



**Figure 13**: Effect of intertrial decrement on Rat #958. The triangles and circles represent, respectively, the means obtained with 0.05 and 0.1 $\log_{10}$ unit frequency decrements. The error bars represent standard errors of the means (*SEM*).

In the cases of Rats #1007 and #1009 full effectiveness was reached within 5 and 2.5 ms respectively (Figures 14 and 15). Effectiveness stayed near 1.00 at all longer delays, except for a dip to 0.90 at 20 and 40 ms in the case of #1009.

In every subject the curves obtained with 0.10 and the 0.05 $\log_{10}$ unit steps were very closely superimposed. The 0.05 $\log_{10}$ unit curves did not consistently either overshoot or undershoot the 0.1 $\log_{10}$ unit curves.

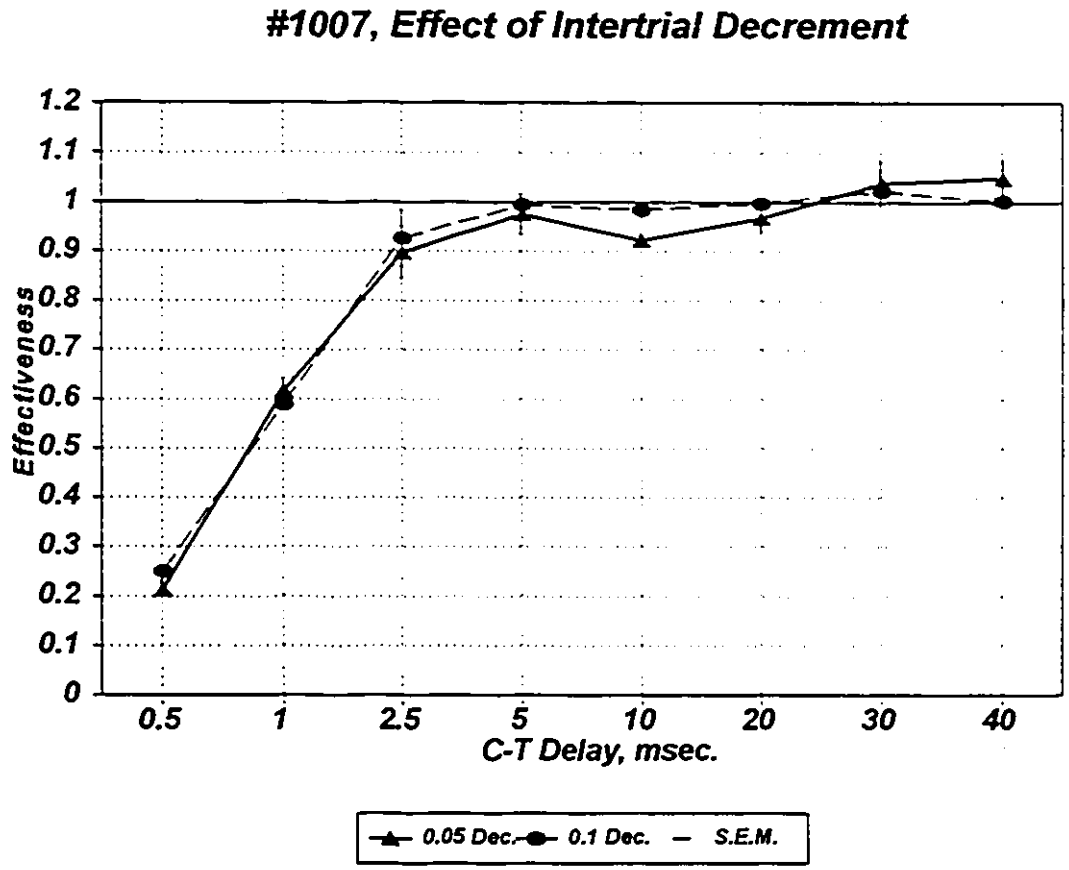



**Figure 14:** Effect of intertrial decrement on Rat #1007. The triangles and circles represent, respectively, the means obtained with 0.05 and 0.1 $\log_{10}$ unit frequency decrements. The error bars represent standard errors of the means (*SEM*).

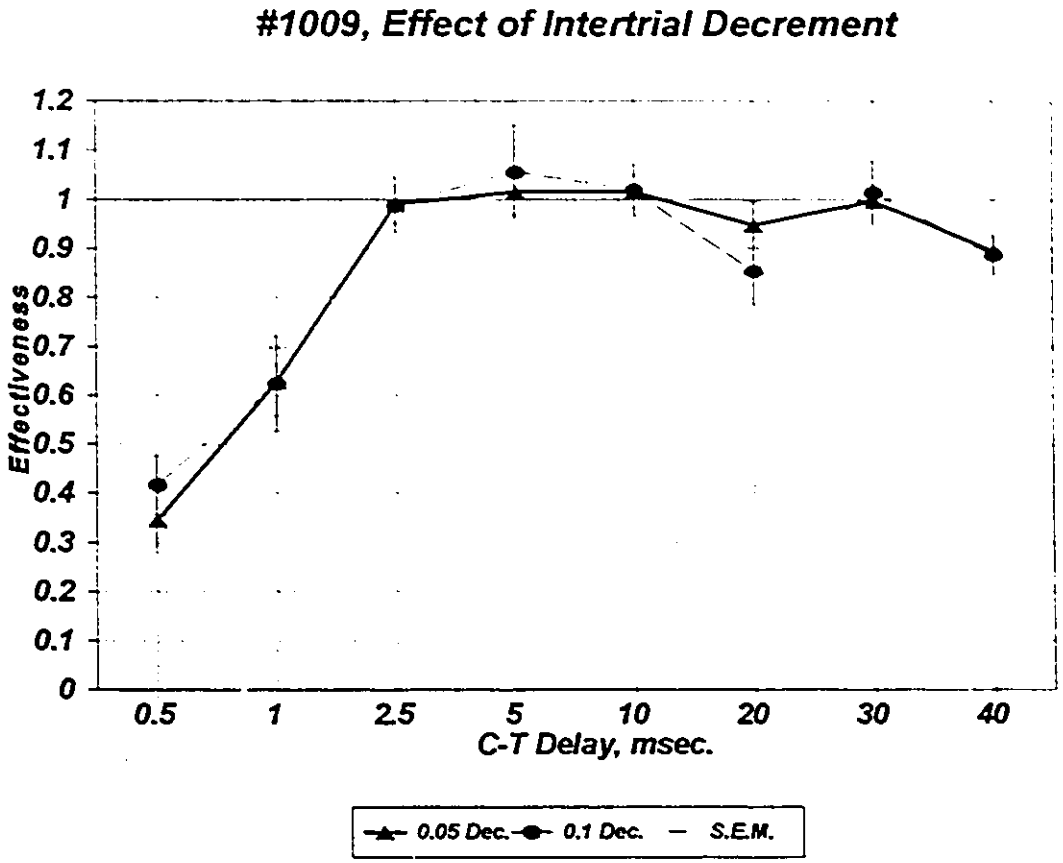



**Figure 15:** Effect of intertrial decrement on Rat #1009. The triangles and circles represent, respectively, the means obtained with 0.05 and 0.1 $\log_{10}$ unit frequency decrements. The error bars represent standard errors of the means (*SEM*).

## Discussion

It was predicted that if the effectiveness shortfall was an undersampling artifact, then reducing the size of the intertrial steps would reduce or eliminate the shortfall. It was found that the reducing the size of the intertrial decrement had very little effect; the 0.05 $\log_{10}$ step curves did not consistently overshoot

or undershoot the 0.1 $\log_{10}$ unit curves. There was no evidence, therefore, that the shortfall was just an undersampling or scaling artifact.

The similarity of the results obtained with both 0.10 and 0.05 $\log_{10}$ unit intervals shows that the present study's methodology is robust; the basic findings are independent of the scaling that was used. It is therefore reasonable to compare the results of experiments that use the two different sizes of intertrial decrement. The smaller decrements do not change the basic findings, but they may improve precision. With the larger decrements, thresholds are estimated by interpolating between test points that are 0.10 $\log_{10}$ units apart. With 0.05 $\log_{10}$ unit intervals the interpolation spans an interval that is half as wide. The threshold is still estimated, but there is less room for error. Since the smaller decrements may help and do not hinder, it makes sense to adopt the small intertrial decrements endorsed by Murray and Shizgal (1994).

Just as in Experiment 1, effectiveness rose to near 1.00 but never greatly exceeded it. Also, there were similar variations in the magnitude of the effectiveness shortfall and in the timing of final rise to full effectiveness. The present experiment therefore reinforces the basic findings of Experiment 1.

Since Bit was used both in the present experiment and in Experiment 1, the results of Experiments 1 and 2 can be compared directly. First, one thing did not change. Neither changing from 0.10 to 0.05 $\log_{10}$ steps, nor lowering the current from 1600 $\mu$A to 500 $\mu$A raised the maximum effectiveness above

1.00. This reinforces what was found with the other subjects; T pulses do not become more than fully effective. Second, the final rise to full effectiveness came earlier with the lower current used in the present experiment. Full effectiveness was first achieved at a C-T delay of 4.0 ms rather than 16 ms (as in Experiment 1).

The earlier rise to full T pulse effectiveness obtained with the lower current may be due to a reduction in maximum current density. Current density is similar at the fringes of both the 500 $\mu$A and the 1600 $\mu$A stimulation fields. At the centre of the 1600 $\mu$A field, however, the current density is greater than at the centre of the 500 $\mu$A field. Yeomans, Mercouris, and Ellard (1985) found evidence that high current densities recruit axons that are resistant to low current densities, and that these resistant fibres recover slowly. If the higher current density produced by Bit's 1600 $\mu$A pulses did recruit a population fibres that were resistant to the lower density produced by the 500 $\mu$A pulses, and if the resistant fibres did recover slowly, then that could account for the earlier rise of the 500 $\mu$A curve.

## Experiment 3: Large *N* Confirmation

### Introduction

In Experiments 1 and 2 there were never more than 12 replications at a particular combination of current and C-T delay. Consequently, standard errors were large, and estimates of effectiveness were necessarily approximate. It could not be determined, therefore, if the shortfalls at long C-T intervals were significant. The goal of the present experiment, therefore, was to generate a very precise picture of the effectiveness curve. This was accomplished by implementing a rapid, automated technique for determining thresholds. The automated technique allowed for large numbers of replications, and hence for more precise estimates of effectiveness.

### Method

The subjects were seven male Long Evans rats from Charles River Canada: #1080, #1082, #1085, #1086, #1106, #1111, and #1115. The rats were housed and fed as described in Experiment 1. With the exception of #1111 (which weighed 504 gm), they all weighed between 335 gm and 383 gm at the time of surgery.

Surgery was performed as described in Experiment 1. The rats were anaesthetized with 100 mg/kg i.p. injections of sodium pentobarbitol (Somnotol, MTC Pharma©). This is more than the recommended dose (65 mg/kg), but in previous experiments it was found that the standard dose was inadequate. Moreover, when 0.125 mg s.c. of atropine sulfate was given to control mucus secretions, the larger dose of anaesthetic was well tolerated. Rats #1085, #1086, and #1111 required xylazine (1.0 mg i.m., Rompun, Bayvet©) to supplement anaesthesia.

Each of the subjects had three electrodes: one aimed at the left lateral hypothalamus (LH), a second aimed at the right medial hypothalamus (MH), and a third aimed at the right LH. These multi-electrode arrays were required for the MH-LH inhibition tests in "Appendix 3: Is the Shortfall Caused by Inhibitory Afferents?" Multiple implants were performed in much the same way as the single implants in the previous experiments. The only important difference was that the electrodes were fixed in a jig before surgery. It is difficult to implant closely spaced electrodes because the dental cement anchoring the first electrode tends to cover up the holes that were bored for the other electrodes. It is more practical to cement the electrodes together, and then to implant them simultaneously. Once the electrodes are cemented together, however, it is impossible to adjust their spacing. It is therefore necessary to hold them parallel to each other with their points properly spaced while they are cemented. Several techniques were tested. Attempts to cement the

electrodes after implanting them in plastic foam or plasticine proved unsuccessful. If the foam or plasticine was tough enough to hold the electrodes securely, then it was also tough enough to bend the electrodes. The best method was to stick a piece of millimetre grid graph paper to a pencil eraser with transparent cellophane tape, and then to tape the electrodes to the graph paper. Transparent tape was used so that the electrodes could be aligned against the graph paper backdrop. The cellophane tape held the electrodes securely, but it was slightly loose so that the electrodes could be nudged into position. The final spacing of the electrode tips was measured with a microscopic scale etched on a glass slide, so the jigs were precise to within 0.1 mm. Once the spacing was correct, dental acrylic cement was dripped on to the bases of the electrode plugs. This cement was gradually built up until there was a solid bridge connecting the bases of all the electrode plugs. Rats #1080, #1085, and #1086 were tested only with their right LH electrodes (the ones that were ipsilateral to the MH electrodes). Rat #1082 was tested only with its left (contralateral) LH electrode. Rats #1106 to #1115 were tested with both their left and right LH electrodes. Electrode placements were confirmed histologically as described in Experiment 1 (see Figures 16 and 17 below).

In order to generate a large number of replications within a reasonable amount of time, a rapid threshold technique was adopted. Previously, a sweep consisted of a series of one minute trials. An efficient experimenter could run a

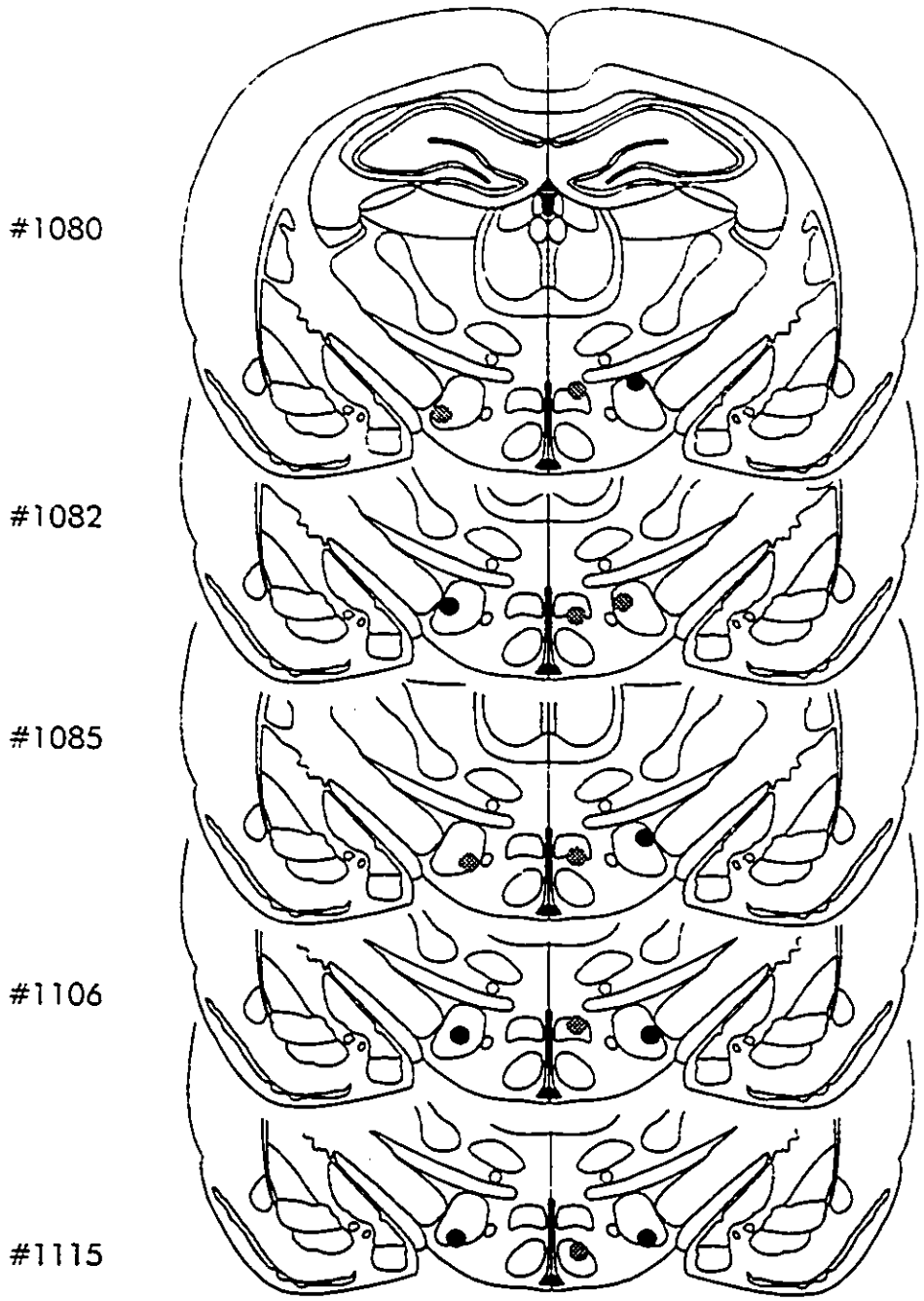
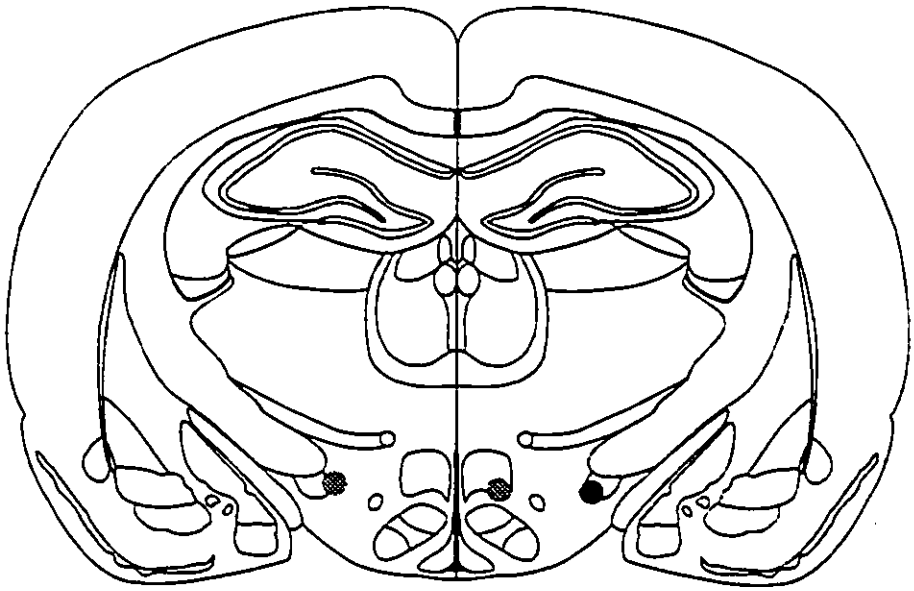



**Figure 16:** Electrode placements for the Rats #1080, #1082, #1085, #1106, and #1115. The large black dots show the locations of the electrode tips that were used in the present experiment. The grey dots show the locations of the electrode tips that were not used in the present experiment. The rats' names are on the left. All of the electrodes in this figure were 2.8 mm behind bregma.

#1086



#1111

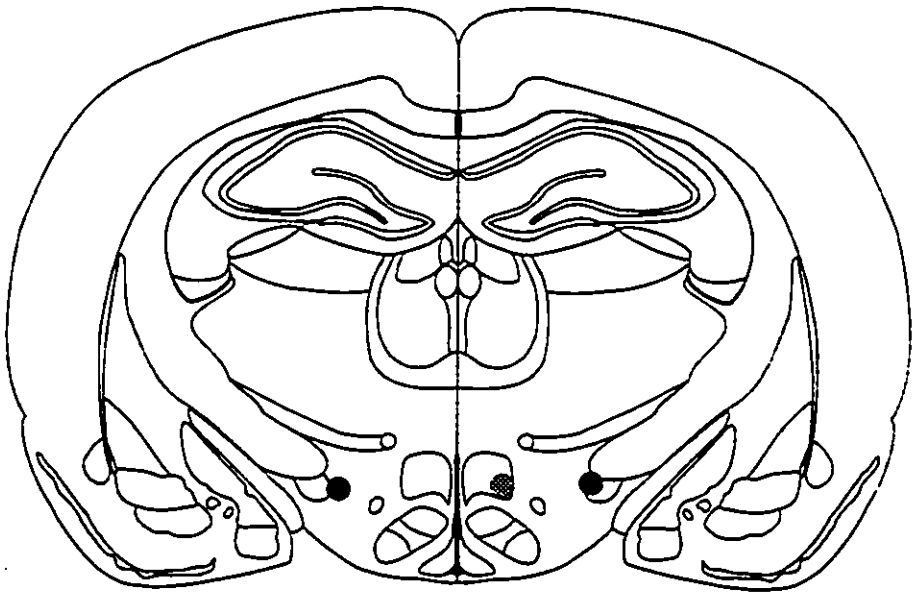


**Figure 17**: Electrode placements for Rats #1086 and #1111. The large black dots show the locations of the electrode tips that were used in the present experiment. The grey dots show the locations of the electrode tips that were not used in the present experiment. The rats' names are to the left of each section. All of the electrodes in this diagram were 3.1 mm behind bregma.

60 second trial, record the data, and reset the stimulator in 90 seconds. A five trial sweep therefore took a minimum of seven minutes. A rapid threshold sweep took no more than two minutes because it consisted of an uninterrupted series of ten second trials. The single exception was Rat #1085; it was tested with 15 second trials because its response rates were unusually low. Increasing trial length raised the number of responses per trial, and with #1085 this produced more reliable thresholds. After three priming trains of stimulation, a ten second trial began (15 seconds in the case of #1085). During trials, lever pressing was rewarded with one second trains of stimulation. Immediately following this first trial, the stimulation pulse frequency was reduced by 0.05 $\log_{10}$ units (11%), and a second trial began. Thereafter, a new trial (at a 0.05 $\log_{10}$ unit lower stimulation pulse frequency) began every ten (or fifteen) seconds for the duration of the sweep. The sweep lasted for ten trials, or until the rat stopped pressing (whichever came first). The rat was judged to have stopped pressing when the total number of presses in two consecutive trials had dropped to less than four (nine in the case of #1085).

The rapid threshold technique required a computer controlled stimulator because it was not feasible for a human experimenter to change frequencies every ten seconds. Current was set by hand before testing began, but the timing and the frequency of the stimulation were computer controlled. This provided several benefits. First, the computer automatically transcribed the

data and calculated the thresholds, so transcription and calculation errors were greatly reduced. Second, sweeps started and stopped exactly on time, and frequencies always changed precisely when they were supposed to. Third, there was no opportunity for the experimenter to influence the results unconsciously by subtly egging the rats on. Digital speed, precision, and objectivity made it possible to generate a large body of accurate, reliable data.

The computer controlled stimulators used the same Mundl constant current generators as the manual apparatus, so the electrodes were grounded between pulses. The same test cages, levers, and testing room were also used. The training routine was also the same as in Experiment 1. The experimenter attracted the subject to the lever by scratching the wall of the cage and by rattling the lever from the outside. The experimenter rewarded approaches to the lever by pressing the lever from the outside of the cage and delivering trains of stimulation.

Testing sessions lasted no more than three hours. They began with four single pulse warm up sweeps. Single pulse baseline sweeps were then interdigitated with blocks of four double pulse test sweeps. The rats were tested with C-T delays of 0.5, 1, 2, 5, 10, 15, 20, 25, 30, and 35 ms. Rats #1106, #1111, and #1115 were also tested at C-T delays of 40, 45, and 50 ms.

| Table 7: Summary of Test Parameters in Experiment 3 | | | |
|---|---|---|---|
| Rat | Electrode | Current (μA) | C-T Delays (ms) |
| #1080 | Right LH | 400 | 0.5, 1, 2, 5, 10, 15, 20, 25, 30, and 35. |
| #1082 | Left LH | 400 | Same as #1080. |
| #1085 | Right LH | 900 | Same as #1080. |
| #1086 | Right LH | 560 | Same as #1080. |
| #1106 | Right LH | 400 | 0.5, 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50. |
| | Left LH | 400 | Same as #1106 Right LH. |
| #1111 | Right LH | 600 | Same as #1106 Right LH. |
| | Left LH | 600 | Same as #1106 Right LH. |
| #1115 | Right LH | 700 | Same as #1106 Right LH. |
| | Left LH | 800 | Same as #1106 Right LH. |

As before, the interpolated stopping frequency was the threshold. However, by trial and error it was determined that it was more reliable to cut the rate frequency curve at three responses per trial (four per trial for #1085) rather than at one half the maximum rate. An AmigaBASIC program used a model proposed by Campbell, Evans, and Gallistel (1985) to do the interpolation. See also Coulombe and Miliaressis (1987) for a comparison of different models. The program fitted three straight line segments to the data. One horizontal segment spanned the high response rates at the beginning of the sweep, a second diagonal segment spanned the intermediate rates in the

middle of the sweep, and a third horizontal segment spanned the low rates at the end of the sweep. The program fitted all possible combinations of beginning, middle, and end segments, and chose the combination that explained the most variance. The three-response threshold was then interpolated along the middle segment. This method for determining the threshold had three advantages. First, it greatly reduced the need for calculation by hand. Second, it used all of the data so one unusual trial could not distort the threshold. This was important because a momentary distraction could halve the number of responses in a ten second trial. The third advantage was that the program calculated the percentage of variance explained by the three segment model. This provided an objective criterion for accepting or rejecting thresholds. Unless the three line segment model explained at least 75% of the variance in a sweep, the data from that sweep was disregarded. Effectiveness was calculated by hand as in Experiment 1.

The results of the present experiment are the pooled controls from Experiment 4, Appendix 3, and pilot drug experiments. These experiments used an A B A C A D A design where A is a control condition and the other letters are experimental conditions. That is, control sessions were run the first, third, fifth, and seventh weeks, and experimental sessions were run during the second, fourth, and sixth weeks. This design was complicated by three factors. First, it took at least three weeks to run all of the experimental sweeps in Appendix 3, so the control weeks that preceded and followed the

experimental condition were separated by as much as a month. Second, #1106, #1111, and #1115 were tested with both the right and left LH electrodes, so with them it took at least two weeks to run all of the sweeps required for a control or experimental condition. Third, there was an interruption at Christmas time, so there were weeks when no measurements were taken. The chronology of the testing is detailed in Appendix 1.

Rat #1085 had stimulation induced seizures. Sodium valproate (150 mg/kg i.p., Sigma®), an anticonvulsant (Altrup, Gerlach, Reith, Said & Speckman, 1992; Bradley, 1989; Kupferberg, 1980), provided no relief. Two and a half weeks after the valproate session, #1085 was tested again with brotizolam, a long acting anticonvulsant benzodiazepine (7.5 mg/kg i.p., Boehringer®, one hour before the test session; Bechtel, 1983; Boke-Kuhn, Danneberg, Kuhn & Lehr, 1986). This treatment eliminated all seizure signs. Rat #1085 then had three more informal sessions with brotizolam so that its thresholds could stabilize. Thereafter, #1085 was treated with brotizolam one hour before every session. Data collected before the brotizolam treatments (Control 1 and the unequal pulse tests in Experiment 4) were disregarded.

Because several months sometimes separated the first week of control sessions from the final week, SP thresholds were measured. This provided an index of stability. If the thresholds in any given week differed from the average by 0.1 $\log_{10}$ units or more, then the results from that week and from all subsequent weeks were excluded.

# Results

Two rats, #1115 and #1106, had to be withdrawn from the study. Rat #1115 developed stimulation induced seizures and was withdrawn from the study when it had only produced 10 to 14 replications per delay. Until the seizures interfered, it worked for stimulation through either of its LH electrodes. During its final week of control sessions, Rat #1106's SP frequency threshold increased by 0.14 $\log_{10}$ units (38%). This exceeded the 0.1 $\log_{10}$ unit criterion laid out in the method section above, so the data from the final week was excluded; the large shift raised concerns that the tissues at the electrode tips were deteriorating. No baseline in any other rat strayed more than 0.1 $\log_{10}$ units (26%) from the average (see Figures 18 and 19 below).

The goal of the present experiment was to generate a very precise picture of the effectiveness curve rather than to test a hypothesis. Analysis was therefore limited to the calculation of 95% confidence intervals. These confidence intervals are represented by the error bars in the graphs below.

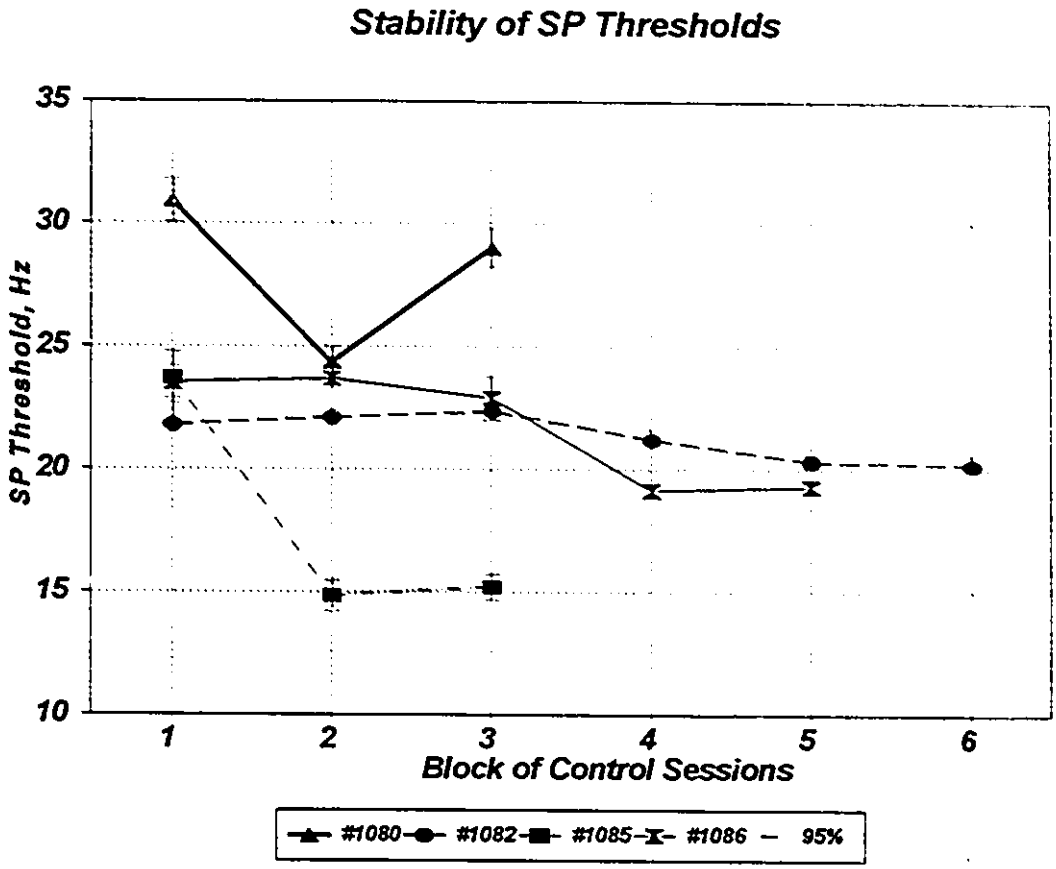



Figure 18: Stability of SP frequency thresholds for Rats #1080, #1082, #1085, and #1086. The solid markers represent means, and the error bars represent 95% confidence intervals. Rat #1085 developed seizures between its first and second block of sessions. The seizures were controlled by medication during the second and third blocks. The results of the first block were discarded. Aside from #1085's first block, none of the means for individual blocks strays more than 0.1 $\log_{10}$ units from total mean for each rat.

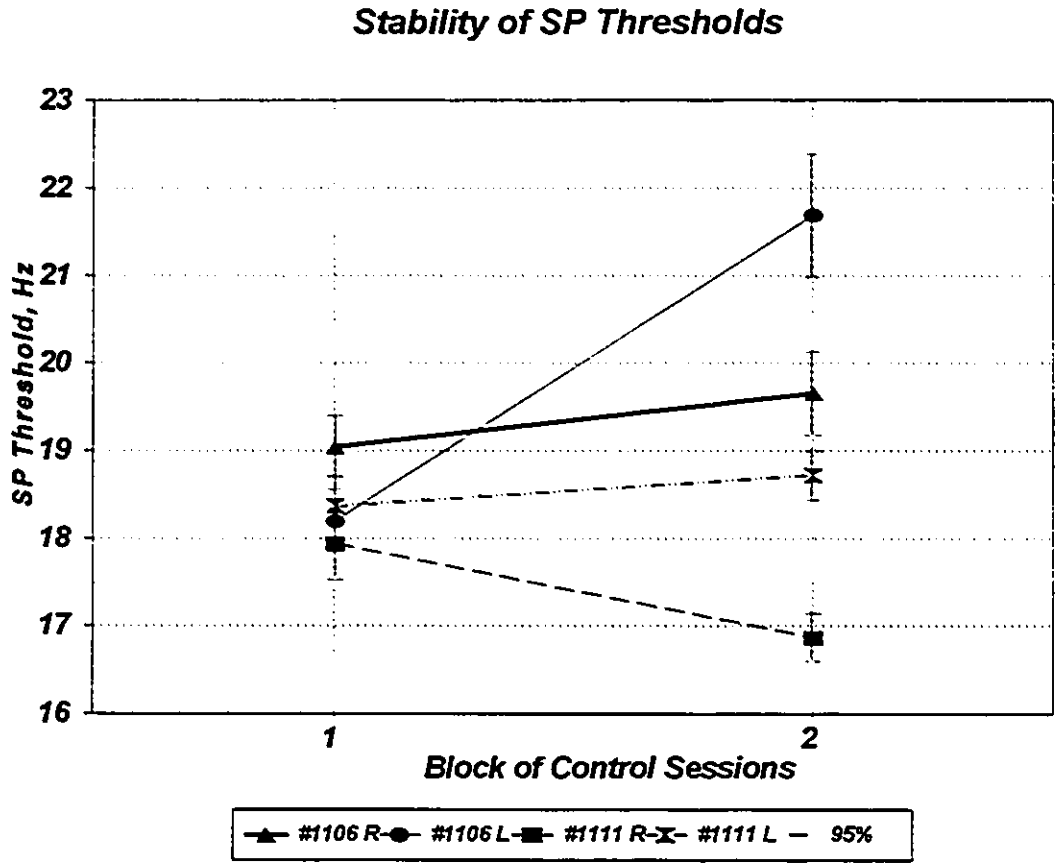



**Figure 19:** Stability of SP frequency thresholds at the right (R) and left (L) electrodes of Rats #1106 and #1111. The solid markers represent means, and the error bars represent 95% confidence intervals. Rat #1106's third control block is not plotted because it was discarded. Rat #1115's SP thresholds are not plotted because it only had one control block.

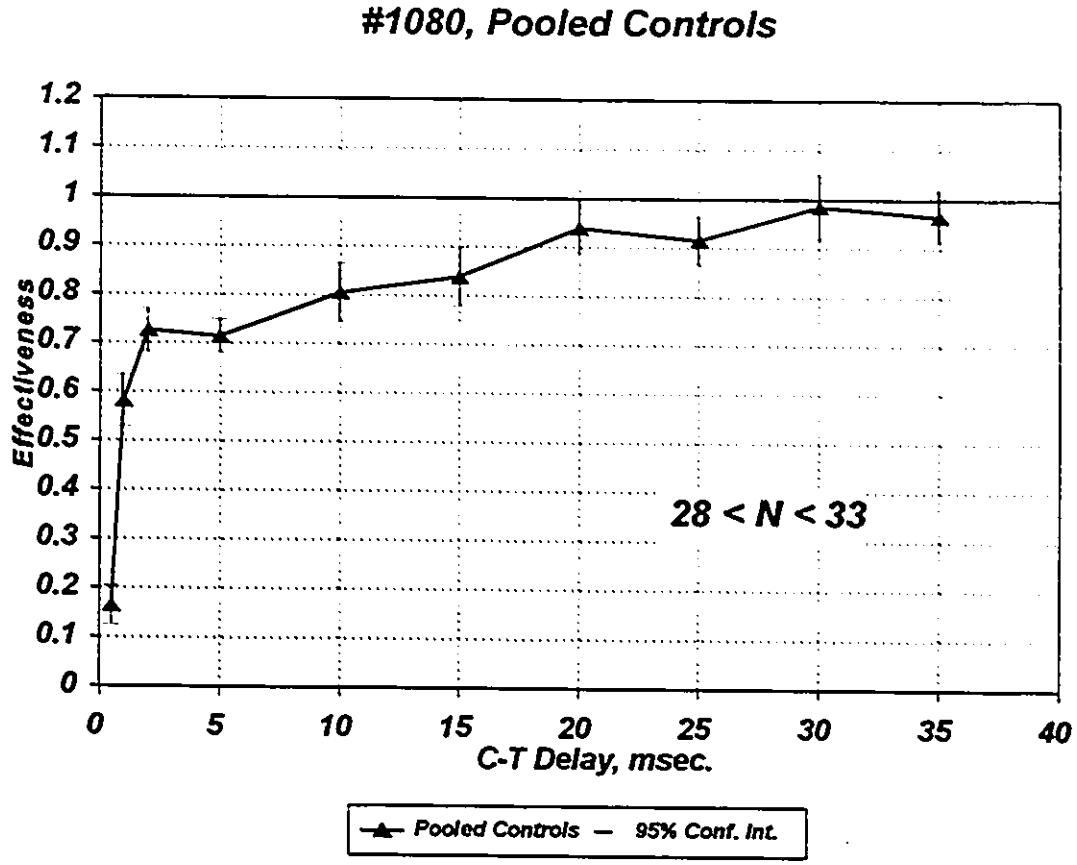



**Figure 20:** Large *N* measurement of effectiveness in Rat #1080. The triangles represent means obtained by pooling together blocks of control sessions. The error bars represent 95% confidence intervals. The "*N*" refers to the number of replications per C-T delay.

Rat #1080 had 29 to 32 replications per C-T delay (see Figure 20 above). At a delay of 0.5 ms effectiveness was 0.17. It rose to 0.58 at 1.0 ms and levelled off near 0.72 at 2.0 and 5.0 ms. Effectiveness rose steadily at 10 and 15 ms, and then levelled off between 0.92 and 0.99 at 20, 25, 30, and 35 ms.

Rats #1082, #1085, and #1086 had between 26 and 120 replications per delay (see Figures 21, 22, and 23 below). They presented very similar profiles at delays between 0.5 and 15 ms. Effectiveness started near 0.15,

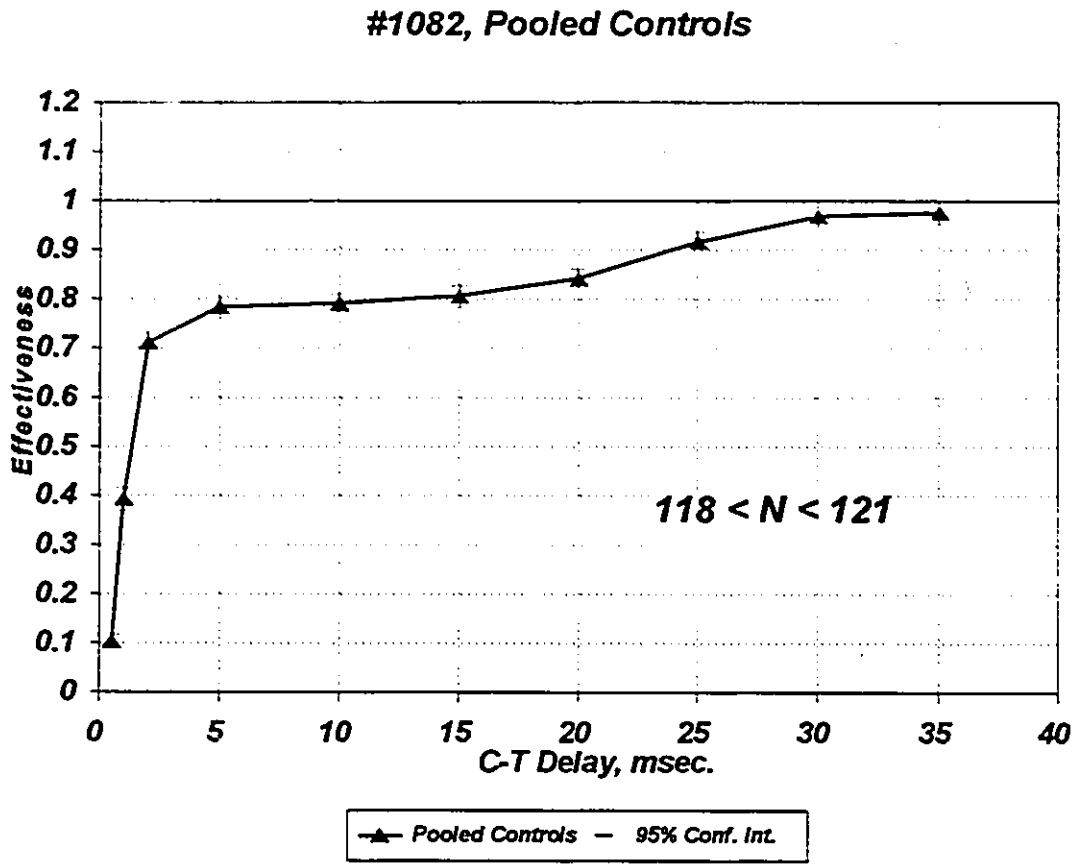



**Figure 21**: Large *N* measurement of effectiveness in Rat #1082. The triangles and error bars represent, respectively, means and 95% confidence intervals obtained by pooling together blocks of control sessions. The "*N*" refers to the number of replications per C-T delay.

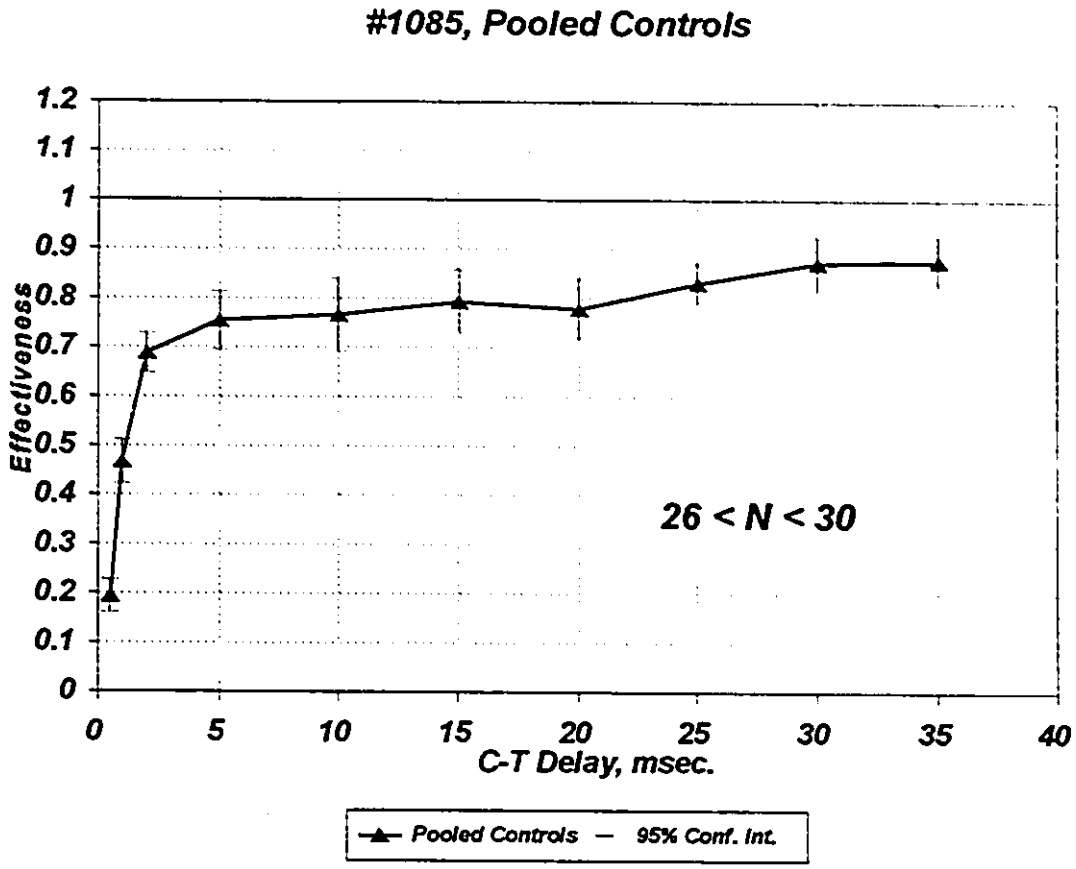



**Figure 22:** Large *N* measurement of effectiveness in Rat #1085. The triangles and error bars represent, respectively, means and 95% confidence intervals obtained by pooling together blocks of control sessions. The "*N*" refers to the number of replications per C-T delay.

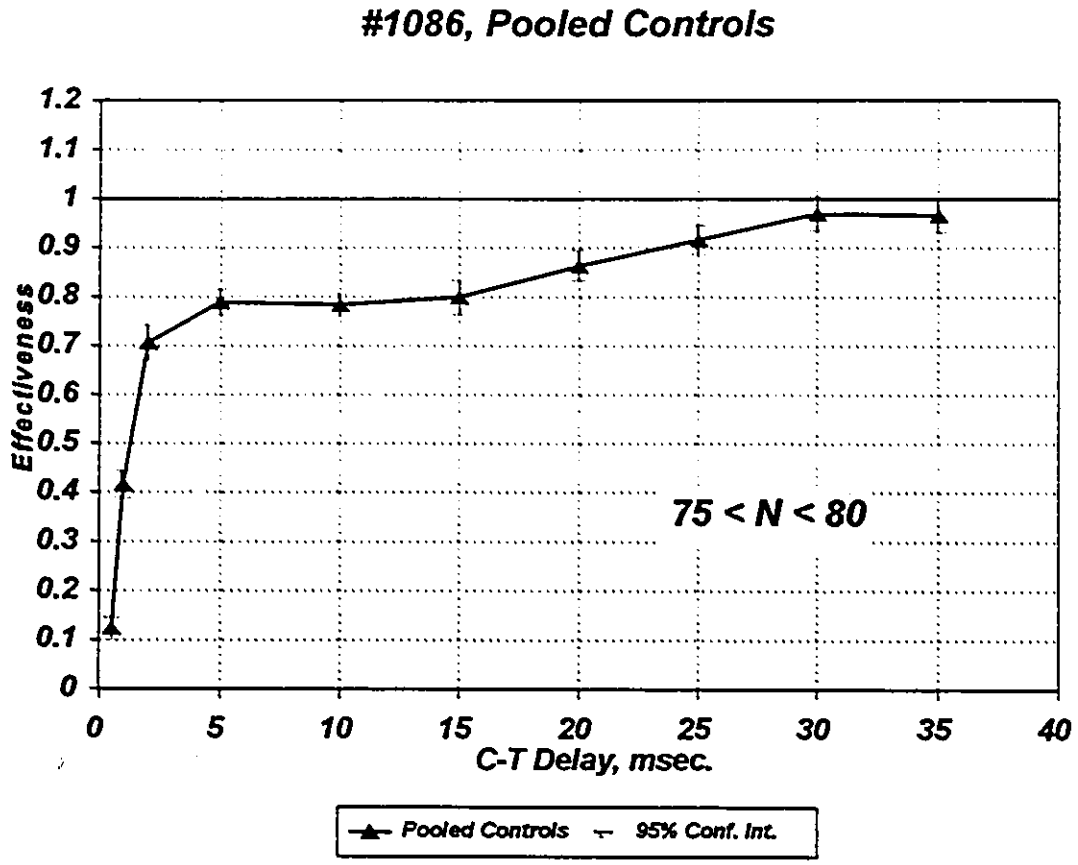



**Figure 23:** Large *N* measurement of effectiveness in Rat #1086. The triangles and error bars represent, respectively, means and 95% confidence intervals obtained by pooling together blocks of control sessions. The *"N"* refers to the number of replications per C-T delay.

rose to about 0.80 at 5 ms, and levelled off at 10 and 15 ms. Effectiveness began to rise at 20 ms in the case of #1082, and at 25 ms in the cases of #1085 and #1086. Effectiveness then rose gradually until it levelled off at 30 and 35 ms. The final effectivenesses achieved by #1082, #1085, and #1086 were, respectively, 0.98, 0.88, and 0.97.

Rats #1106 and #1111 both had between 18 and 54 replications at C-T delays up to 50 ms, and they both lever pressed for stimulation of either LH (see Figures 24 and 25 below). Both of them also presented very similar

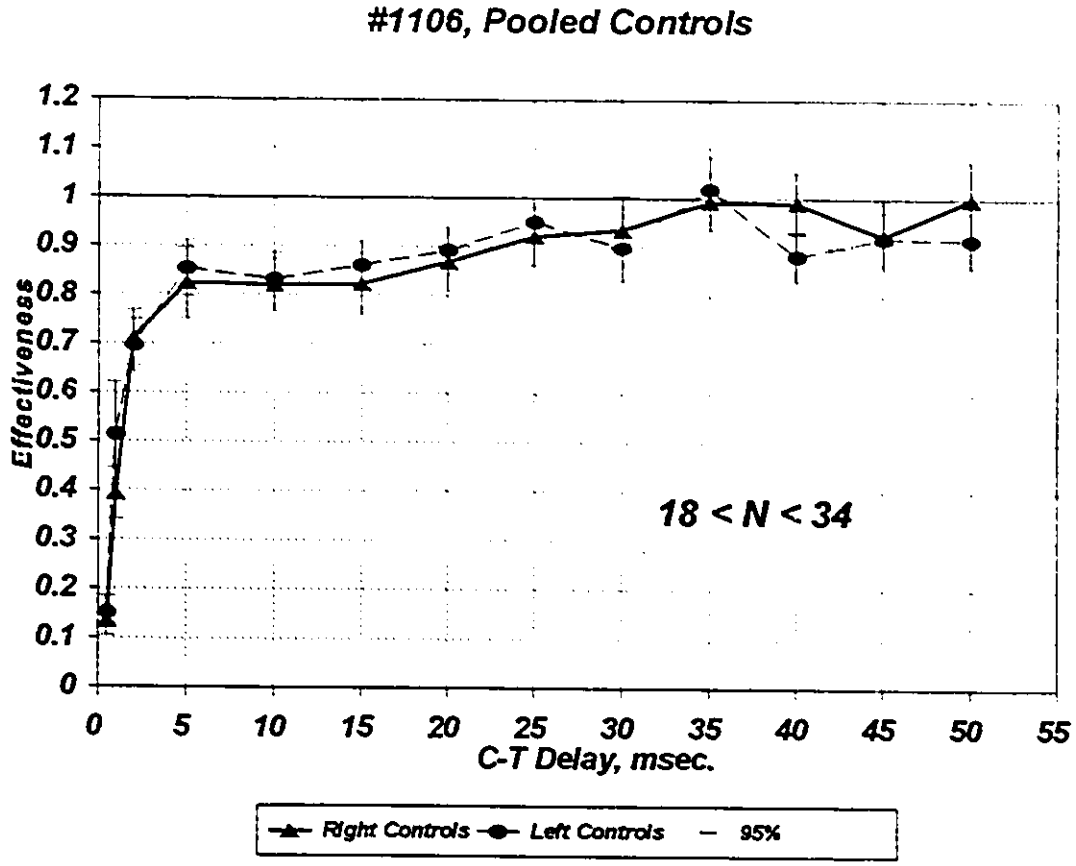



**Figure 24:** Large *N* measurement of effectiveness in Rat #1106. The triangles and circles represent means from, respectively, the right and left LH electrodes. The error bars represent 95% confidence intervals. "N" refers to the number of replications per C-T delay at each electrode.

effectiveness profiles at both LH electrodes. In the case of #1106, effectiveness was approximately 0.15 at a C-T delay of 0.5 ms. Effectiveness rose to about 0.85 at 5 ms, and remained steady at 10 and 15 ms. At 20 ms effectiveness began to rise until it approached 0.95. From 30 to 50 ms it held steady between 0.88 and 1.00. In the case of #1111 effectiveness rose from approximately 0.16 at a delay of 0.5 ms to about 0.82 at 2 ms. Effectiveness remained steady near that level at delays from 2 to 20 ms. Then it rose to about 0.95 at 30 ms, and stayed at that level at delays of 35 to 50 ms.

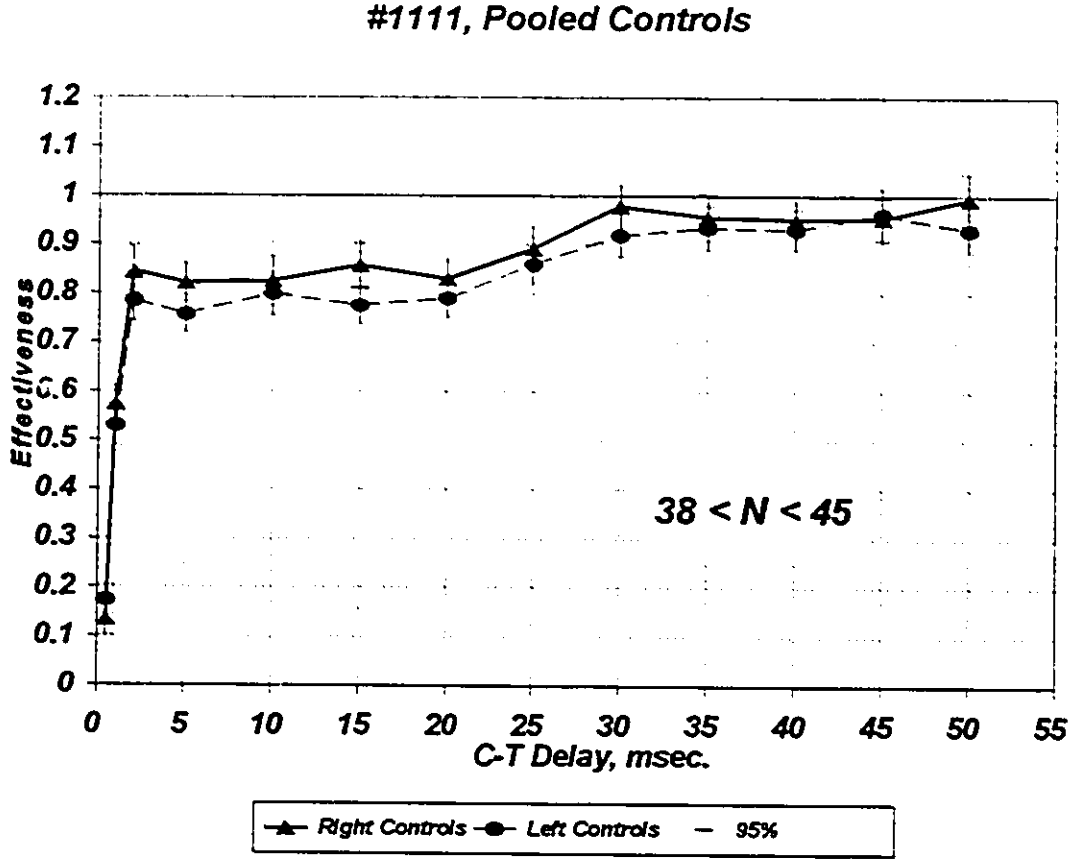



**Figure 25:** Large N measurement of effectiveness in Rat #1111. The triangles and circles represent means from, respectively, the right and left LH electrodes. The error bars represent 95% confidence intervals. "N" refers to the number of replications per C-T delay at each electrode.

The development of T pulse effectiveness was very similar at both of Rat #1115's LH electrodes (see Figure 26 below). Effectiveness was near 0.14 at 0.5 ms, rose to near 0.75 at 2 ms, and hovered between 0.81 and 0.71 until 15 ms. Then effectiveness rose gradually to near 0.92 at 30 ms. Thereafter, it hovered between 0.88 and 0.96.

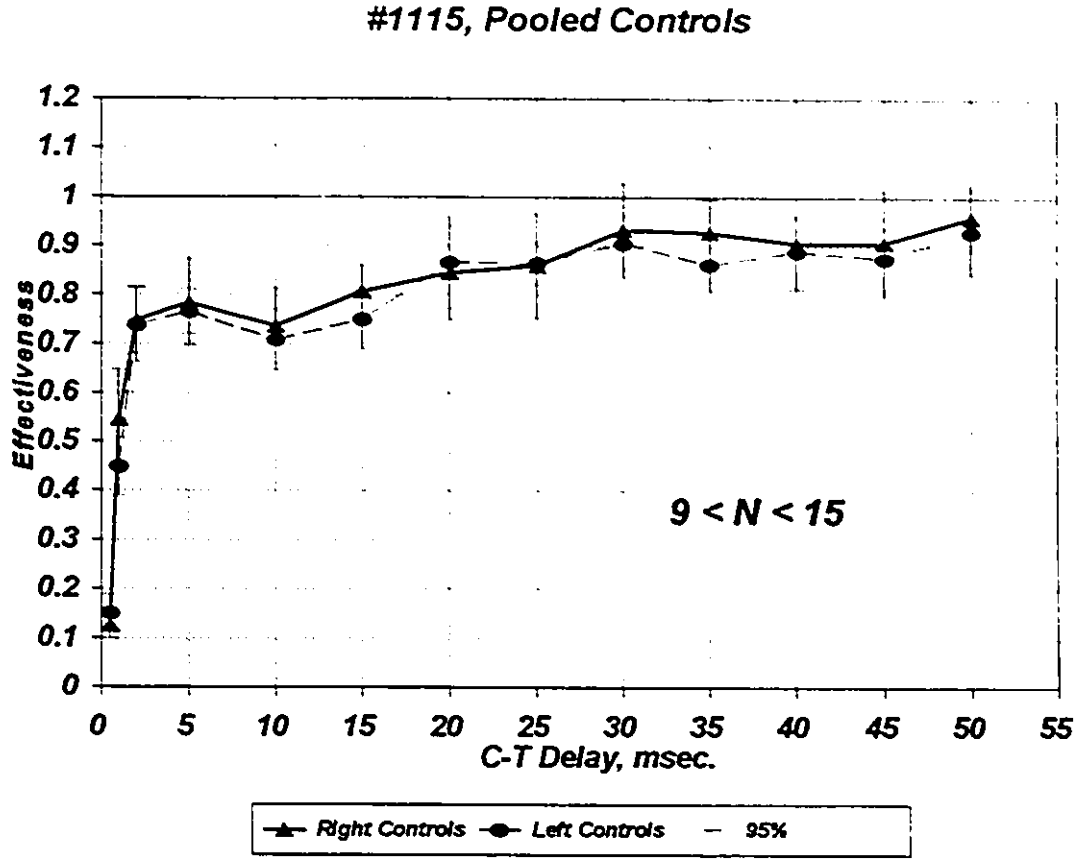



**Figure 26:** Large *N* measurement of effectiveness in Rat #1115. The triangles and circles represent means from, respectively, the right and left LH electrodes. The error bars represent 95% confidence intervals. "N" refers to the number of replications per C-T deiay at each electrode.

## Discussion

The present experiment used automated techniques to generate unusually large *N*'s, but otherwise the equipment and techniques were typical of those that are used in other refractory period studies (see the sections above titled "Yeomans's Technique Applied" and "Refractory Estimate Anomalies"). Furthermore, at C-T delays of 0.5 to 10 ms the shape of the effectiveness

curves agrees very well with other reports of refractory period tests. This suggests that the results of the present experiment are applicable to MFB refractory tests in general.

One of the main results of the present experiment was that every electrode in every subject had a shortfall of about 20% at delays of 5 to 20 ms. There was not a single instance of 100% T pulse effectiveness falling within the 95% confidence intervals when the C-T delay was less than 25 ms. It follows that given sufficiently precise measurements and sufficiently large numbers of replications, effectiveness shortfalls should be considered a normal part of refractory period tests.

In the present experiment there were no overshoots. The highest average effectiveness was 1.02, and the highest 95% confidence interval limit was 1.08 (both in Rat #1080 at 30 ms). Even when testing was extended out to C-T delays of 50 ms (as in the cases of Rats #1106 and #1111), effectiveness remained steady after the maximum value of about 1.00 was achieved at 30 or 35 ms. This agrees with the findings of Experiments 1 and 2; effectiveness always rose to near 1.00 and remained there. In the cases of Rats #1007 and #1009 (Experiment 2) effectiveness rose to near 1.00 quite early, 5 and 2.5 ms respectively, but then it did not rise any further at 30 ms. Full effectiveness, therefore, really is the final level irrespective of the timing of the final rise. In other words, the T pulses did not become more than fully effective, they only became as effective as the C pulses. Since the C and T

pulses have the same current and duration, they are distinguished only by their order in the train. It makes sense, therefore, that they would become equally effective. The final plateau near 1.00 is a reliable and sensible finding, so it follows that it is a real phenomenon, not an artifact. By extension, so are the other parts of the effectiveness curve including the shortfall in the 5 to 25 ms range.

The absence of overshoots is particularly noteworthy because the results of Yeomans (1990b) suggested that there might be large overshoots. In that study, effectiveness peaked as high as 2.1 at C-T delays of 5 or 10 ms, and then dropped to about half that value at delays of 30 to 100 ms. If that pattern were transposed onto the present study, then at 5 ms there would have been an overshoot to 1.9 instead of a shortfall to 0.8. Yeomans's results, however, were unusual. Indeed, they were the highest effectiveness values ever reported. In order to elicit these overshoots he used very long, slow trains of high current pulses: trains up to 2 seconds long, currents as high as 1700 $\mu$A, and SP thresholds of less than 10 Hz. Few subjects will bar-press for such low frequencies. Yeomans implanted LH electrodes bilaterally in 30 rats, but only reported data from 7 of those 60 electrodes; the other 53 did not meet the criterion rate of bar-pressing (60 presses per minute for 0.5 s, 10 Hz trains of stimulation) and were excluded. His subjects, therefore, were a small minority that were especially selected for their ability to display these unusual overshoots. In contrast, the present study used less extreme stimulation

parameters, so its results are more representative of what might normally be expected in a refractory period study.

In Experiment 2, Rat #950 achieved full effectiveness suddenly between 30 and 32 ms. In the present study the final rises occurred gradually over a span of ten or fifteen milliseconds. This could reflect a genuine difference between Rat #950 and the subjects in the present experiment. However, given the relatively small number of replications in Experiment 2 (*N*'s of six to twelve) no firm conclusion is possible. Since the *N*'s in the present experiment are very large, and since the final rise was similarly gradual for every electrode in every subject, it would seem that a gradual rise is the norm rather than the exception. This is an important finding because it suggests that the shortfall is caused by a phenomenon that wears off gradually.

In six of the seven subjects effectiveness rose to about 0.95 or 1.00 at C-T delays of 35 ms or more. In Rat #1085, however, effectiveness only rose to 0.88 at the longest tested delays, 30 and 35 ms. Because #1085 could not be tested without brotizolam, it was impossible to determine if the drug lowered the maximum T pulse effectiveness. This subject's SP period threshold was 66 ms, so it could have been tested out to C-T delays of 50 or even 60 ms. Unfortunately it was not, and there is no way to tell if #1085's effectiveness would have eventually risen to 1.00 had longer delays been tested. When delays out to 50 ms were tested in other rats, there was no indication of increased effectiveness after 35 ms. Although #1085's

effectiveness curve did not climb all the way to 1.00, it had the same shape as the other subjects' curves. That is, if every value in #1085's effectiveness curve had been divided by 0.88 (effectiveness at 35 ms), then it would have been indistinguishable from the other subjects' curves.

A similar scaling strategy (dividing by the value of effectiveness at a particular delay) was used by Yeomans, Mercouris, and Ellard (1985) (see also Bielajew, Jordan, Ferme-Enright, and Shizgal, 1981). Yeomans, Mercouris, and Ellard used different currents to collect effectiveness curves at C-T delays of 0.1 to 5 ms in order to study the effects of current density on refractoriness. It was difficult, however, to compare the curves collected at different currents because the SP period thresholds varied from 15 to 95 ms. When currents are high and delays are long, subnormal excitability can raise SP frequency thresholds (Yeomans, 1990b). This, in turn, can inflate T pulse effectiveness. In order to examine the effects of refractoriness apart from the effects of the C-C interval, they divided each value of effectiveness collected at C-T delays of 0.1 to 5 ms by the value of effectiveness at 5 ms. This transformation standardized the heights of the effectiveness curves so that their shapes could be compared; the transformed effectiveness value at 5 ms was always equal to 1.00, but the transformed values at other delays were free to vary. In this way they isolated the shape of the early part of the curve (which is affected by refractoriness) from non-refractory variations that take place at longer delays.

The present study is concerned with shape of the effectiveness curve at C-T delays of 0.5 to 50 ms, especially the shortfall between 5 and 20 ms and the final rise between 20 and 35 ms. These phenomena could be isolated by dividing every value by the value of effectiveness at 35 ms. This would erase #1085's 12% shortfall at 30 and 35 ms. However, the present experiment's effectiveness curves all rise so close to 1.00 that the shortfall and final rise are clearly visible. It is unnecessary, therefore, to standardize the raw effectiveness values.

Experiments 1 through 3 clearly show that if T pulse effectiveness is measured with moderate currents and SP baseline frequencies, then there is often a 20% shortfall at C-T delays of 5 to 20 ms. This shortfall is followed by a gradual rise to full effectiveness at about 30 ms. Effectiveness then remains steady near 100% at all delays from 35 to 50 ms. In other words, the post refractory phase described in Experiment 1 can be divided into two previously undescribed phases: the shortfall (including the final rise to full effectiveness) which extends from 5 to about 30 ms, and the final plateau which extends from about 35 ms to at least 50 ms, the longest delays tested in the present experiment.

What produces this pattern? The fact that the final rise is both gradual and late suggests that it is due to an inhibitory process that lasts about 20 ms and then dissipates slowly. Relative refractory periods, supernormal periods, and subnormal periods fit this description. They wear off gradually, and

subnormal periods can last for tens or hundreds of milliseconds (Yeomans 1990b). Experiment 4 used unequal C and T pulse currents to test the hypothesis that the shortfall is due to relative refractory, supernormal, or subnormal periods.

The shortfall in T pulse effectiveness may also be caused by the incidental stimulation of inhibitory neurons by the C pulses. This hypothesis was tested in a fifth experiment, but the results were inconclusive. That experiment suffered from an unwieldy design; the protocol was so demanding that only one out nineteen subjects qualified for the final phase of testing. The fifth experiment has therefore been relegated to "Appendix 3: Is the Shortfall Caused by Inhibitory Afferents?" Other alternative explanations are discussed in "Suggestions for Further Research" in the Overview below.

## Experiment 4: Unequal Pulses

### Introduction

T pulse effectiveness is usually used to measure refractory periods, but refractoriness is just one of the factors affecting the excitability of neurons; the refractory period is sometimes followed by a period of supernormal excitability, and then by a period of subnormal excitability (Adrian & Lucas, 1912; Erlanger & Gasser, 1937; Graham 1934, 1935). The processes underlying super- and subnormal periods are still being worked out. It appears, however, that repeated firing can cause a build up of extracellular $K^+$ and of intracellular $Na^+$. An increase in extracellular $K^+$ would depress the potassium equilibrium potential and lower the threshold for excitation; hence supernormal excitability. Conversely, an increase in intracellular $Na^+$ would reduce the sodium equilibrium potential and raise the threshold for excitation; hence subnormal excitability (Eng & Kocsis, 1985). Because ICSS trains repeatedly fire reward relevant neurons, these trains may induce sub- and supernormal periods. A second explanation for supernormality has been proposed, and this second reason also suggests that reward fibres may be prone to supernormal periods. Some small axons have a slow depolarizing afterpotential caused by the capacitance of their myelin sheaths. These axons therefore remain close to threshold after their $Na^+$ channels have recovered (Barrett & Barrett, 1982;

Bowe, Kocsis & Waxman, 1985). While these axons are close to threshold, they are easily excited. Small myelinated fibres are implicated in reward (Bielajew & Shizgal, 1982; Gallistel, Shizgal & Yeomans, 1981), so this second explanation also suggests that reward fibres are prone to supernormal effects.

Super- and subnormal effects have in fact been observed at ICSS sites. Yeomans (1979) found a supernormal period between 2 and 5 ms in the posterior hypothalamus and in the dorsal pons. Bielajew and Fouriezos (1985) found a supernormal period between 3.5 and 10 ms in the mediodorsal thalamus, and Miliaressis and Rompré (1980) found a supernormal period in the LH at 2.4 and 5.0 ms. Yeomans (1990b) delivered very high current, low frequency trains through LH electrodes. He found a subnormal period that extended from 10 to 200 ms. There are, then, precedents for ICSS trains producing super- and subnormal periods; they are not just a theoretical concern.

If ICSS trains induce subnormal periods, and if T pulses fall within the subnormal period induced by the C pulses, then that may account for the shortfall in T pulse effectiveness. This theory can be tested by using a T pulse current that is 40% larger than the C pulse current. The large T pulse's field of excitation is made up of a halo that is stimulated only by the T pulse, and of a core that is also stimulated by the C pulse. The unequal pulse effectiveness formula (derived in "Appendix 1: Derivation of T Pulse Effectiveness") isolates the core by cancelling out the halo's contribution. In the core, the large T

pulses fire the subnormally excitable neurons because the T pulse current is so large that it overcomes the neuron's reluctance to fire. The size of the subnormal effect can be measured, therefore, by comparing the equal pulse effectiveness curve with the large T pulse effectiveness curve. If large T pulses eliminate the shortfall obtained with equal pulses, then the shortfall is caused by a subnormal period.

Supernormal periods could also cause an apparent shortfall. If the C-C interval in the single pulse baseline condition places each C pulse in the supernormal period induced by the previous pulse, then that would inflate the effectiveness of the baseline C pulses. This, in turn, would reduce the effectiveness of T pulses relative to those inflated C pulses. If supernormal excitability causes the shortfall, then the supernormal period must begin at about 35 ms because that is when T pulses are fully effective, i.e., when the T pulses are as effective as the (presumably) supernormally effective C pulses.

Usually, one would only expect to see a supernormal period when the C pulse is larger than the T pulse. A large C pulse increases the odds that a T pulse will fire the fibres at the outer edge of its field of excitation because those fringe fibres fall within the C pulse's larger field. The C pulse, therefore, already fired those fringe fibres and induced supernormal excitability. Hypothetically, supernormality may also play a part when the C and T pulses are equal. There is a layer of fibres near the outer edge of the field of excitation that have only a moderate chance of firing in response to the first

pulse in a train. If the first pulse induces a supernormal period, and if the second pulse is delivered during that supernormal period, then the fibres that fired the first time would be more likely to fire the second time. A portion of the fibres that did not fire in response to the first pulse would fire in response to the second because those unfired fibres would still have the original probability of firing. Because the second pulse would recruit supernormally excitable fibres as well as previously unfired fibres, more fibres would fire the second time than the first time. Similarly, the third pulse would fire more fibres than either the first or second pulses. In theory, this process of cumulative recruitment could inflate the effectiveness of baseline trains of equal current C pulses.

Because cumulative recruitment would only work in a thin rind near the edge of the field of excitation, the supernormal period's hypothetical contribution to the effectiveness of baseline single pulse trains would be small. However, the shortfall in T pulse effectiveness is only 20%, so supernormality would only have to make a small contribution in order to produce the shortfall. It would be prudent, therefore, to test for supernormal periods.

There are two ways to test for supernormal excitability. The first is to use large C pulses. This would magnify the supernormal effect by enlarging the pool of neurons fired by the C pulses. Large C pulses would therefore increase the difference in effectiveness between the T pulses that come before the supernormal period and those that fall within the supernormal period. In

other words, large C pulses would increase the effectiveness gap between the shortfall (5 to 25 ms) and the final plateau (35 to 50 ms). The second test is to use large T pulses. Large T pulses erase supernormal effects in two ways. First, large T pulses give all of the neurons in the core of the T pulse field of excitation the same chance to fire; subnormally excitable fibres fire because the large T pulse overcomes their subnormality, and supernormally excitable fibres fire because they are easily excited. Second, the unequal pulse effectiveness formula cancels out the halo of the T pulse field of excitation Unequal pulse effectiveness, therefore, can only reflect the equalized excitability of the neurons in the C pulse field (see "Appendix 1: Derivation of T pulse Effectiveness). It follows that if the shortfall is caused by supernormality, then large T pulses should lower the effectiveness of T pulses delivered during the supernormal period to 0.8, the level of the shortfall. Previously it was argued that if the shortfall is caused by supernormality, then the supernormal period must start at about 35 ms because at that point the T pulses are as effective as the presumably supernormal baseline C pulses. Large T pulses should therefore lower effectiveness between 35 and 50 ms to 0.8.

Large T pulses not only test for supernormal periods and subnormal periods, they also test for relative refractory periods. Immediately following the absolute refractory period, neurons undergo a relative refractory period; they are reluctant to fire because some of their $Na^+$ channels are still open. Large T pulses erase relative refractory effects by overwhelming this reluctance.

One manipulation therefore tests for three phenomena at once. First, if large T pulses raise effectiveness at short delays (0.5 to 5 ms) then effectiveness in the equal pulse condition is suppressed by relative refractoriness. Second, if large T pulses raise effectiveness at delays of 5 to 25 ms, then the shortfall is caused by a subnormal period. Third, if large T pulses lower effectiveness at delays of 35 to 50 ms down to the shortfall level, then the shortfall is caused by a supernormal period.

## Method

The subjects were two rats that were used in Experiments 1 and 2 (Bit and #950), and three rats that were used in Experiment 3 (#1082, #1086, and #1106). Bit had an abbreviated shortfall in Experiment 1, and a shallow shortfall in Experiment 2. The other four rats all had distinct shortfalls and final plateaus. Housing conditions, surgery, and histology were as described in Experiments 1 and 3. Bit had one LH electrode, and #950 had one VTA electrode. Rats #1082 and #1086 were each tested with one LH electrode, and Rat #1106 was tested with two: one in the left LH and another in the right LH. Bit's electrode placement is illustrated in Figure 3, #950's in Figure 4, #1082's and #1106's in Figure 16, and #1086's in Figure 17.

Bit and #950 were tested with the manual stimulator described in Experiment 1, and #1082, #1086, and #1106 were tested with the computer

controlled stimulators used in Experiment 3. However, the stimulators were not hooked up the same way they were before. The stimulators had two channels, but previously only one was used. Each channel's current could be adjusted independently of the other's. Channels A and B were connected with jumper cables so that both channels fed into the same electrode; #1106 had two electrodes, but they were tested one at a time. Channel A generated the C pulses, and channel B generated the higher current T pulses. While a pulse was being delivered through one channel, the other channel was thrown into high impedance so none of the stimulation current was bled off. To keep the electrode from becoming polarized, both channels were grounded between pulses.

The rats were trained as described in Experiment 1. Bit and #950 were tested with one minute trials and 0.05 $\log_{10}$ unit intertrial decrements. Rats #1082, #1086, and #1106 were tested with two minute sweeps and 0.05 $\log_{10}$ unit intertrial decrements. Bit and #950 were tested at C-T delays of 0.25, 0.4, 0.63, 1, 1.6, 2.5, 4, 6.3, 10, 16, and 25 ms (test parameters are summarized in Table 7). Each delay was tested one time per session. Rats #1082 and #1086 were tested at C-T delays of 0.5, 1, 2, 5, 10, 15, 20, 25, 30, and 35 ms. Rat #1106 was tested at those ten delays plus 40, 45, and 50 ms. Each delay was tested four times per session. The order of the delays was randomized. Testing sessions were run as before, except that the blocks of double pulse test sweeps were interdigitated with pairs of high and low

current single pulse baseline sweeps. Bit and #950's sessions lasted an hour and a half. Bit had six sessions and #950 had five. Rats #1082 and #1086's sessions lasted two and a half hours. They each had five sessions. Rat #1106 was tested at more delays, so its sessions lasted three hours. It sometimes disconnected its electrodes, so it had six sessions of left LH tests and seven sessions of right LH tests.

Bit was tested with 1000 $\mu$A and 1400 $\mu$A pulses, and #950 with 360 $\mu$A and 500 $\mu$A pulses. Rat #1082 was tested with 400 $\mu$A and 560 $\mu$A pulses, and Rat #1086 with 560 $\mu$A and 800 $\mu$A pulses. Rat #1106 was tested with 400 $\mu$A and 560 $\mu$A pulses at both its left and right LH electrodes. The T pulses were 40% larger than the C pulses because that ratio is large enough to suppress super- and subnormal effects, and because it minimizes noise by restricting the size of the T pulse halo (Yeomans, 1979, 1990a; see also "Appendix 1: Derivation of T Pulse Effectiveness"). Other investigators have compared T pulses that were either 41% or 71% larger than the C pulses, and they found that the two ratios produced substantially similar results (Bielajew, Lapointe, Kiss & Shizgal, 1982). The C pulse currents were the same as the currents used in the equal pulse control sessions that were run in the weeks before and after the unequal pulse sessions. This ensured that the C pulse field of excitation (the core of the T pulse field) was comparable to the control field of excitation.

| Table 8: Summary of Test Parameters in Experiment 4 | | | |
|---|---|---|---|
| Rat | C Pulse Current ($\mu$A) | T Pulse Current ($\mu$A) | C-T Delays (ms) |
| Bit | 1000 | 1400 | 0.25, 0.4, 0.63, 1, 1.6, 2.5, 4, 6.3, 10, 16, and 25. |
| #950 | 360 | 500 | 0.25, 0.4, 0.63, 1, 1.6, 2.5, 4, 6.3, 10, 16, and 25. |
| #1082 | 400 | 560 | 0.5, 1, 2, 5, 10, 15, 20, 25, 30, and 35. |
| #1086 | 560 | 800 | 0.5, 1, 2, 5, 10, 15, 20, 25, 30, and 35. |
| #1106 | 400 | 560 | 0.5, 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50. |

Analysis consisted of *t* tests of the difference between the mean effectiveness at each delay in the equal pulse curve with the mean at the same delay in the unequal pulse curve. This was appropriate because it was predicted that the equal and unequal pulse curves would differ at particular C-T delays rather than in overall shape. Because the comparisons of the means at each C-T delay were planned, it was not necessary to correct for familywise error (Keppel, 1982).

## Results

There were three predictions. First, if the shortfall in T pulse effectiveness were caused by a subnormal period that deflated the

effectiveness of T pulses, then high current T pulses would raise effectiveness at C-T delays of 5 to 25 ms. Second, if the shortfall were caused by a supernormal period that inflated the effectiveness of C pulses, then high current T pulses would lower effectiveness at delays of 30 to 50 ms. Either way, large T pulses would flatten the effectiveness curve; it would remain at the same level from 5 through to 50 ms. Third, it was predicted that if there were a relative refractory period, then large T pulses would raise effectiveness

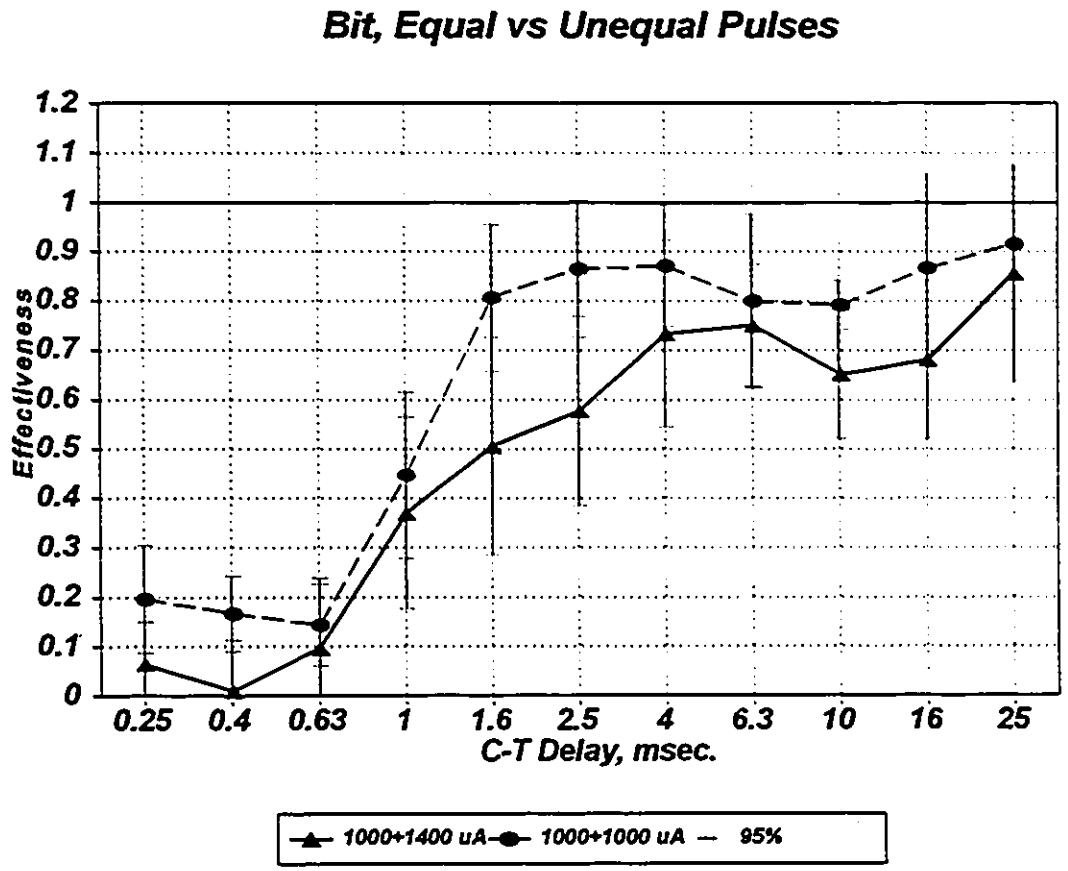



**Figure 27:** Effect of large T pulses on the Rat Bit. The triangles represent means obtained with 1000 $\mu$A C pulses and 1400 $\mu$A T pulses. The circles represent means obtained with 1000 $\mu$A C and T pulse. The error bars represent 95% confidence intervals.

at delays of 0.5, 1, and 2 ms.

These predictions were not borne out. Instead, large T pulses tended to deepen the shortfall. Bit's large T pulse curve was lower than its equal pulse curve at 0.25, 0.4, 1.6, 2.5, and 10 ms (only significant differences are reported; $t$ tests, $p = 0.05$). Rat #950's large T pulse curve was higher than its equal pulse curve at 0.4 ms. Otherwise there were no significant differences.

In the case of #1082, large T pulses lowered T pulse effectiveness at all

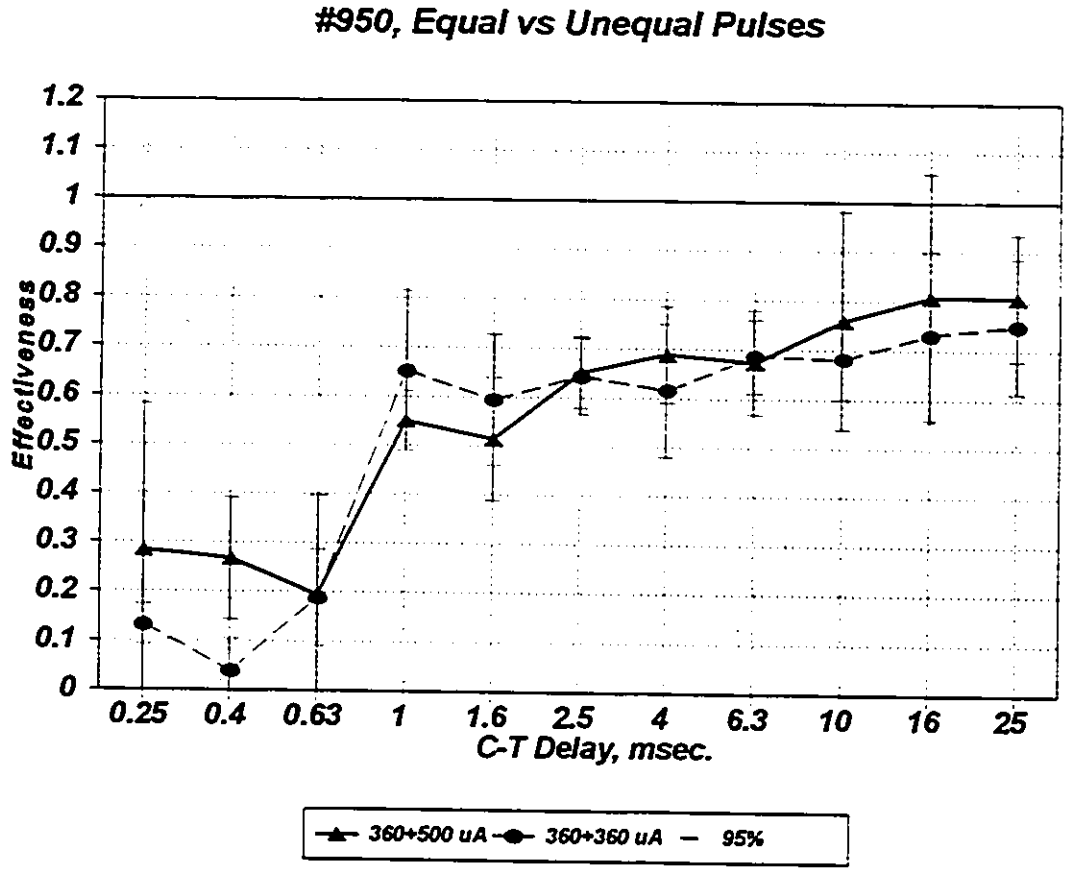



**Figure 28:** Effect of large T pulses on Rat #950. The circles and triangles represent mean effectiveness values obtained with, respectively, equal and unequal C and T pulse currents. The error bars represent 95% confidence intervals.

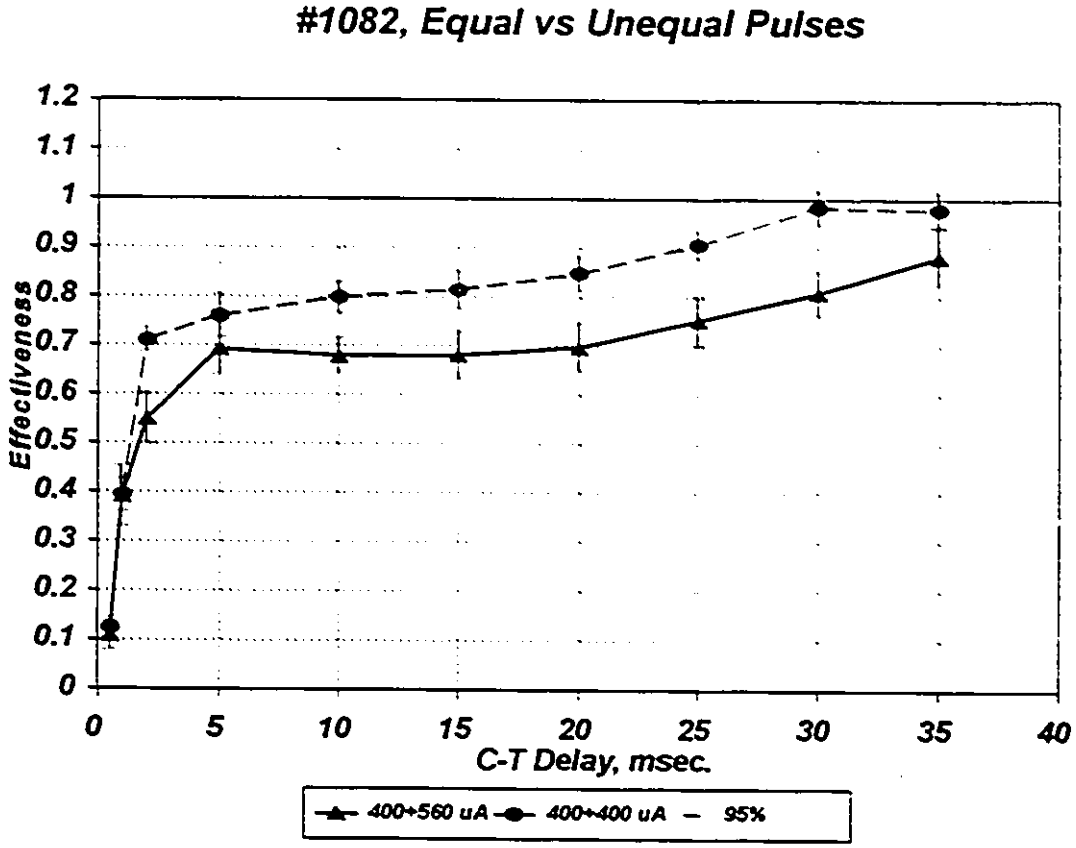



**Figure 29:** Effect of large T pulses on Rat #1082. The circles and triangles represent mean effectiveness values obtained with, respectively, equal and unequal C and T pulse currents. The error bars represent 95% confidence intervals.

delays from 2 to 35 ms. In the case of #1086, there was little difference between the equal pulse and unequal pulse curves. When there was a difference, the unequal pulse curve was lower.

At #1106's right LH electrode, the unequal curve was slightly lower from 10 through to 25 ms. The equal and unequal pulse curves converged at 30 ms. The unequal curve fell at 35 and 40 ms, and then rose to the equal curve level again at 45 and 50 ms. At #1106's left LH electrode, the unequal curve was lower from 5 through to 25 ms. The two curves converged at 30 ms.

The unequal pulse curve fell at 35 ms, and then rose to meet the equal pulse curve at 40, 45, and 50 ms.

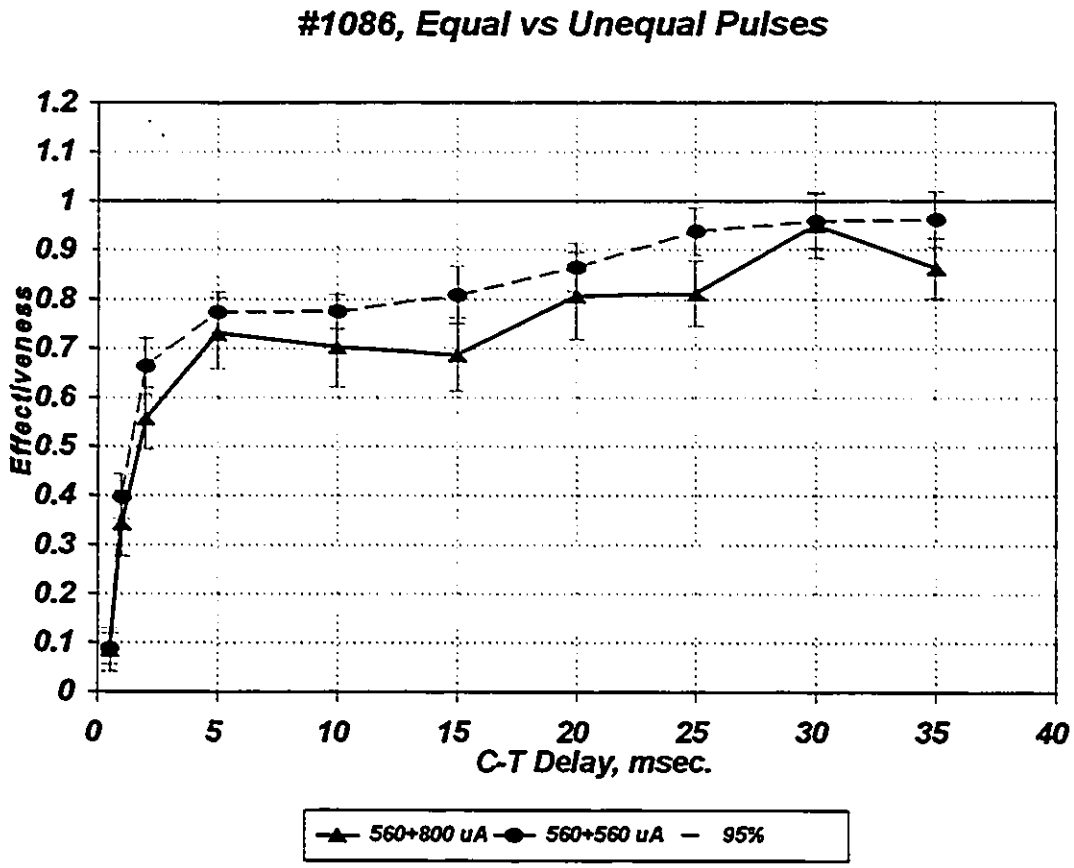



**Figure 30:** Effect of large T pulses on Rat #1086. The circles and triangles represent mean effectiveness values obtained with, respectively, equal and unequal C and T pulse currents. The error bars represent 95% confidence intervals.

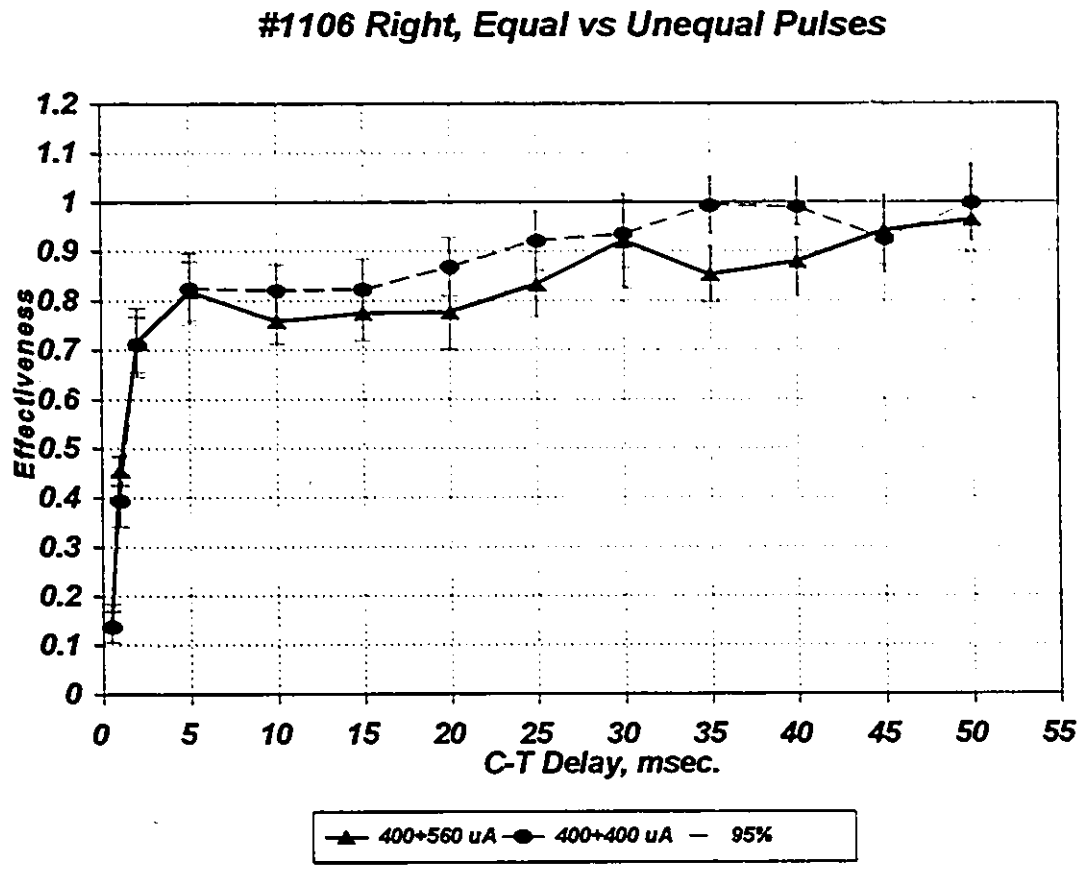



**Figure 31:** Effect of large T pulses on Rat #1106 at its right LH electrode. The circles and triangles represent mean effectiveness values obtained, respectively, with equal and unequal C and T pulse currents. The error bars represent 95% confidence intervals.

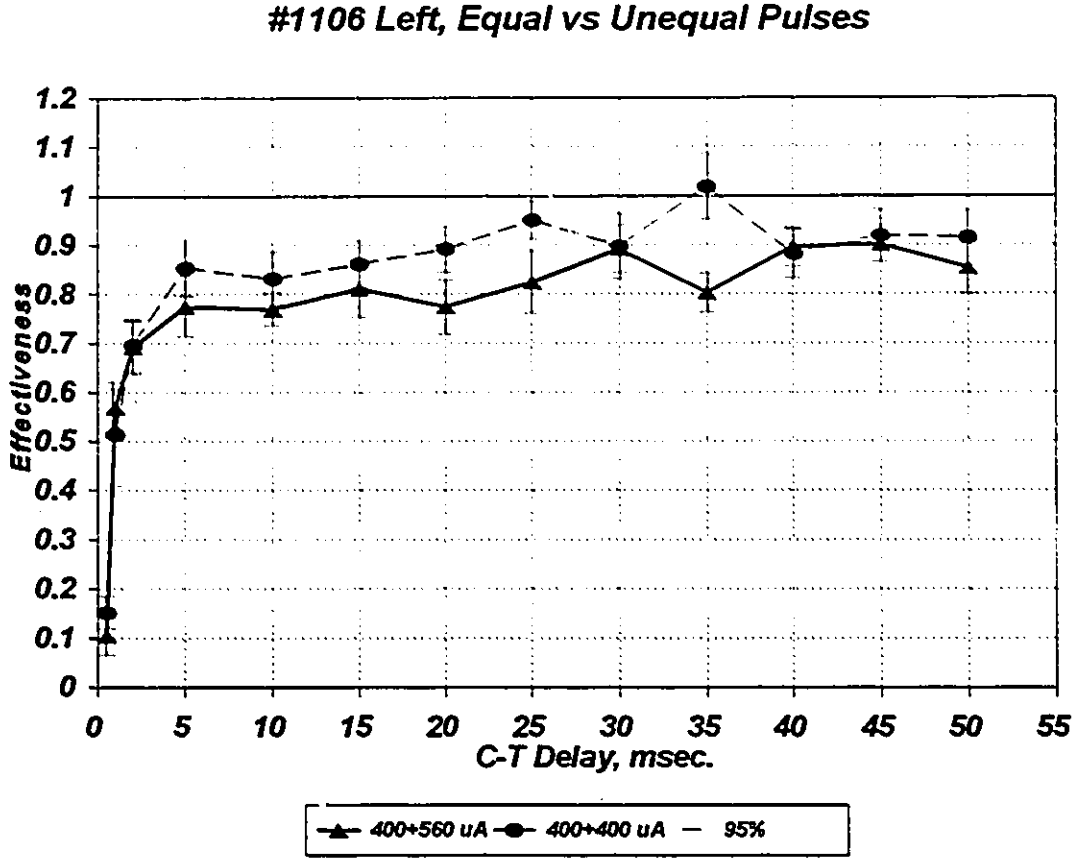



**Figure 32:** Effect of large T pulses on Rat #1106 at its left LH electrode. The circles and triangles represent mean effectiveness values obtained, respectively, with equal and unequal C and T pulse currents. The error bars represent 95% confidence intervals.

## Discussion

There was no evidence of relative refractory, supernormal, or subnormal effects. At none of the six electrodes was there a consistent, significant shift in one of the predicted directions. We must therefore reject the hypotheses that relative refractory, supernormal, or subnormal periods are responsible for the shortfall in T pulse effectiveness.

These results do not contradict previous findings of subnormal effects by Yeomans (1990b). Yeomans found a subnormal period, but he used very high currents (a mean of 1257 $\mu$A) and very low frequencies (10 Hz or less). He used these parameters to maximize the chances of generating a subnormal effect. Even so, he only used seven out of the sixty electrodes he implanted. One would not expect, therefore, to see results like Yeomans's very often, especially with the relatively moderate parameters that generate the shortfall in T pulse effectiveness. Because one would not expect the present study's stimulation parameters to replicate Yeomans's results, the two studies do not contradict each other.

Nor do the present results contradict studies that found supernormal effects. For example, Bielajew and Fouriezos (1985) found a supernormal period, but they were looking for supernormal effects that were invisible to equal pulse trains. Bielajew and Fouriezos therefore compared large C pulse trains (which are sensitive to supernormal effects) to equal pulse trains (which are insensitive to supernormal effects). The present experiment, on the other hand, sought to discover a hypothetical supernormal effect produced by equal pulse trains. It therefore compared equal pulse trains (which are insensitive to supernormal effects) with large T pulse trains (which are even more insensitive to supernormal effects). Even though the logic of the two experiments is similar, it would be surprising if they produced the same results. It is therefore

impossible for the present study to contradict other investigations of supernormal effects.

If the shortfall is not produced by super- or subnormal periods, then it must be produced by some other process. Alternative causes are considered in the omnibus discussion that follows.

## Overview

### The Present Study's Contributions

Previous studies suggested that T pulses require ten milliseconds or more to achieve full effectiveness, but those studies were primarily concerned with the recovery from refractoriness, which is generally considered a five or ten millisecond phenomenon (Bielajew, Jordan, Ferme-Enright, & Shizgal, 1981; Yeomans, Mercouris, & Ellard, 1985). Testing, therefore, was not conducted at long C-T intervals, so it could not be determined if or when full effectiveness is achieved. Experiments 1, 2, and 3 combined long, closely spaced C-T intervals, small intertrial decrements, and large numbers of replications to produce a very precise picture of the development of T pulse effectiveness.

The present study revealed four phases in the development of T pulse effectiveness: a local potential summation phase, a refractory phase, a shortfall phase, and a final plateau. The first two phases are well known (Erlanger & Gasser, 1937), but the latter two have not been described previously. The first phase begins at the shortest delays (0.25 ms in Experiments 1 and 2), and lasts for about half a millisecond (0.4 ms in Experiments 1 and 2, and 0.5 ms in Experiment 2 and 3). During this phase, effectiveness is ascribed to local potential summation. When the T pulse follows the C pulse this closely, the residual potential from the C pulse does not have time to dissipate. This

residual potential can summate with the T pulse to fire previously unfired cells at the outer margin of the field of excitation (Yeomans, Matthews, Hawkins, Bellman & Doppelt, 1979). Because of this local potential summation, during the first phase T pulses are about 25% effective even though the previously fired cells are absolutely refractory.

The second or refractory phase begins at about half a millisecond and extends to about 5 milliseconds. The growth of effectiveness during this phase is ascribed to recovery from refractoriness (Yeomans, 1975, 1979). In Experiment 3 it was found that during the refractory phase effectiveness climbs to about 80%.

Experiments 1, 2, and 3 show that the post refractory phase described in Experiment 1 can be subdivided into two more phases which had not been described previously. The first of these (the third overall) is the shortfall phase which extends from 5 to about 30 ms. During this phase, effectiveness hovers near 80% and then gradually rises to near 100%. The shortfall is followed by a fourth phase that extends from about 30 ms to at least 50 ms (the longest delay that was tested in Experiment 3). During this final plateau effectiveness remains steady near 100%.

Regardless of the exact timing of the final rise, or of the precise height of the final plateau, the basic shape of the effectiveness curve was invariant. There were always four phases as described above. For example, in Rat #1080 in Experiment 3 the rise was complete at 20 ms, but in Rats #1082,

#1085, #1086, #1106, #1111, and #1115 the rise began at 15 or 20 ms and ended at 30 ms. Nonetheless, in every one of these cases, each of the four phases is clearly distinguishable.

Experiments 1, 2, and 3 show that the full effectiveness achieved in the final plateau really is the final level. When Bit's current was lowered from 1600 $\mu$A in Experiment 1 to 500 $\mu$A in Experiment 2, T pulse effectiveness still rose to 100% and stayed there. In Experiment 3 effectiveness was measured at delays of up to 50 ms, but effectiveness did not creep upwards as the C-T delay was increased; effectiveness always remained steady near the level that was achieved at 30 or 35 ms. The final level is truly final.

The achievement of full effectiveness might be reasonably described as full recovery, but that term must be carefully defined. When T pulses are 100% effective at 35 ms, they elicit as much behaviour as the C pulses do in the single pulse baseline condition. In other words, the T pulses have completely recovered the full measure of effectiveness enjoyed by the baseline C pulses. For all practical purposes, therefore, the T pulses have fully recovered. A researcher who uses moderate stimulation parameters to map reward circuits will probably never use T pulses that are more effective than the 100% documented in the present study. That does not mean, however, that within 35 ms the substrate has returned to the state it was in before the train of stimulation started. Using extremely high currents and extremely low frequencies, Yeomans (1990b) found that some reward fibres have a

subnormal period at interpulse delays of several hundred milliseconds. Excitability, therefore, is not static between 35 ms and 1000 ms, so it would be a mistake to interpret 100% effectiveness at 35 ms as a sign that the substrate has returned to an unstimulated state. Full effectiveness at 35 ms represents full recovery relative to a practical baseline, not relative to an absolute, theoretical benchmark.

Experiment 2 shows that the shortfall is not merely a symptom of inadequate sampling density. A comparison of the effectiveness curves produced using either 0.1 or 0.05 $\log_{10}$ unit intertrial decrements (respectively, low and high sampling densities) showed that the size of the decrements had no effect on either the shape or the height of the effectiveness curve. The shortfall is not, therefore, merely what Murray and Shizgal (1994) would call an undersampling artifact.

The shortfall is a reliably documented phenomenon, and it is not an undersampling artifact. What, then, is its cause? Experiment 4 tried to answer this question (see also "Appendix 3: MH-LH Inhibition"). It was proposed that a relative refractory period, a subnormal period, or a supernormal period might be responsible for the shortfall. On the basis of this hypothesis, it was predicted that large T pulses would erase either the shortfall or the final rise to full effectiveness. Large T pulses did not erase either the shortfall or the final rise. We must therefore conclude that relative refractory periods, subnormal periods, and supernormal periods are not responsible for the shortfall.

## Suggestions for Further Research

The present study has established that relative refractory, subnormal, and supernormal periods definitely are not responsible for the shortfall, but it has not identified the shortfall's cause. Other alternatives must therefore be considered. When Gallistel, Shizgal, and Yeomans (1981) noted a gradual 10% to 25% rise in T pulse effectiveness over C-T intervals of 1.2 to 15 ms, they suggested that there may be a population of axons with extremely long absolute refractory periods. They did not suggest, however, what kind of fibres these extremely slow axons might be; a 15 ms absolute refractory period is much longer than one would expect given the diameters of the smallest fibres in areas like the MFB (Swadlow & Waxman, 1978; Szabo, Lenard & Kosaras, 1974). None of the experiments in the present study can rule out absolute refractory periods, but until an extremely slow substrate is identified the absolute refractory hypothesis can not be given much weight. Once a candidate substrate is identified, then its involvement in the shortfall can be tested after the manner of Gratton and Wise (1985). If an appropriate antagonist flattens out the effectiveness curve between 5 and 50 ms, then that will show that the substrate susceptible to that antagonist is responsible for the shortfall.

Gallistel, Shizgal, and Yeomans's (1981) second suggestion was that the slow rise of effectiveness might reflect some peculiarity of electrical

stimulation. Perhaps the fact that electrical stimulation stimulates several nodes at once somehow retards recovery. This theory cannot be tested because it is impossible to stimulate single nodes. The problem is that large volumes of tissue are excited by the high current pulses that support long C-T delays; even in the present study's most sensitive rats the field of excitation was about 400 μm across (Fouriezos & Wise, 1984). This is considerably larger than the internodal distance. Myelinated fibres in the LH range from 0.5 to 3.5 μm (Szabo, Lenard & Kosaras, 1974), and the internodal distance is 100 times the fibre diameter (Nicholls, Martin & Wallace, 1992), so the internodal distance is between 50 and 350 μm. Each pulse therefore excites a minimum of two to nine nodes per fibre. Even with sensitive rats, therefore, it is impossible to compare single node stimulation with multi-node stimulation.

Gallistel, Shizgal, and Yeomans suggested a third reason for the slow rise of effectiveness; maybe the high currents necessary to support ICSS somehow distort the excitability cycle. This theory is difficult to test because high current pulses are required to support long C-T delays. It is therefore impossible to compare the effects of high and low currents at long C-T delays. It might be possible compare high and extremely high currents, and then to correlate the height of the shortfall with the current. The range of useful currents is limited, however, and this truncation would make correlation problematic. Furthermore, raising the current increases the size of the field of excitation, so even if a correlation were found its significance would not be

clear. A correlation might capture the effect of raising the current on the substrate within the high current field of excitation, or a correlation might merely reflect the recruitment of new substrates in the extremely high current halo surrounding the high current field. Instead of increasing the current, it would be better to increase current density by reducing the exposed surface at the electrode tip (Yeomans, Mercouris & Ellard, 1985). Unfortunately, it is not feasible to significantly reduce current density because the present study used shallow conical points which exposed a large surface; again, range truncation would be a problem. Also, reducing the exposed surface would drastically reduce the power of any test. Because it is impossible to change the exposed surface after implantation, all comparisons would have to be between electrodes or between subjects. It would not be sufficient, therefore, simply to accumulate a 50 or 100 replications at each electrode. It would also be necessary to accumulate a large number of subjects.

Gallistel, Shizgal, and Yeomans's (1981) fourth and final suggestion was that the slow rise in effectiveness may reflect synaptic phenomena that come into play at long interpulse delays. Again, they did not suggest what these phenomena might be. More recently, some candidates have appeared.

Yeomans (1990a) discussed a cortical model where stimulation elicits both direct and indirect firings. In this model, the effectiveness curve is affected by three different factors: the refractory periods of pyramidal cells, the refractory periods of interneurons that elicit indirect firings, and collisions

between direct and indirect action potentials. Yeomans suggested that these three factors can extend the rising portion of the effectiveness curve for another four milliseconds after the end of refractoriness. This is much less than the 30 or so milliseconds that are required to account for the shortfall, but the model could be modified to lengthen the rising portion of the effectiveness curve.

One way to extend the effects of Yeomans's (1990a) model is to add GABA neurons to the mix. There are two reasons why this is a reasonable addition. First, GABA inhibition lasts a long time; the average duration for GABAA $Cl^-$ channels ($\tau$) is $20 \pm 6.6$ ms (mean $\pm$ *SD*)(Barker & McBurney, 1979). Second, GABA has been implicated in the modulation of reward. Willick and Kokkinidis (1995) have shown that micro-infusions of baclofen (a GABAB agonist) into the VTA raised thresholds for ICSS. This shows that GABAB receptors in the MFB modulate reward circuitry. Also, Klitenick, DeWitte and Kalivas (1992) have demonstrated interactions between GABAA and GABAB receptors and dopamine, a transmitter that is associated with ICSS (Murray & Shizgal, 1994; Yeomans, 1989; Yeomans, Maidment & Bunney 1988; Yeomans, Mercouris & Ellard,1985) . Klitenick, DeWitte and Kalivas proposed a model to explain these interactions. In this model, GABA afferents and interneurons both inhibit and disinhibit the release of dopamine in the MFB. GABA's effects on ICSS and dopamine show that GABA and reward are intertwined. Because GABA and reward are intertwined, and because GABA

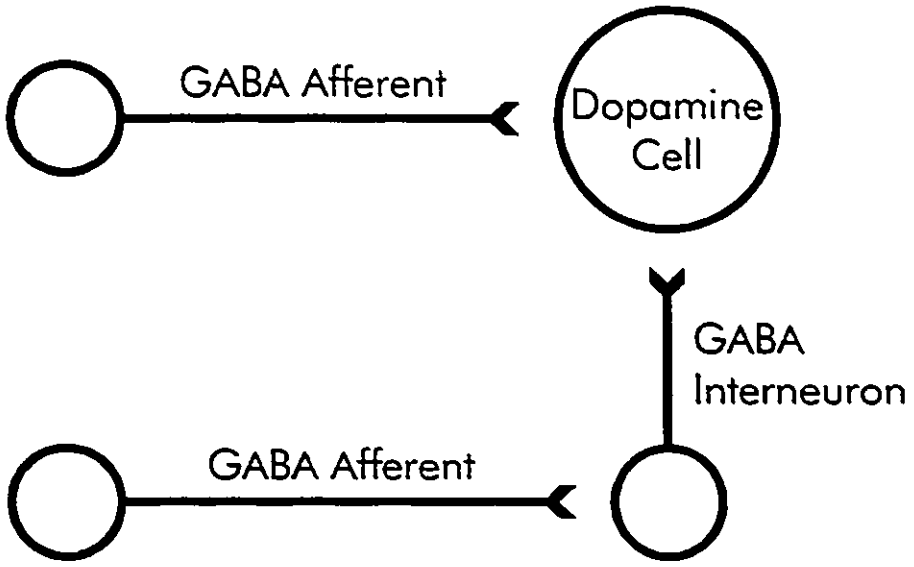



**Figure 33:** A simplified version of Klitenick, DeWitte, and Kalivas's (1992) model. GABA afferents inhibit dopamine cells as well as GABA interneurons. Because GABA has two opposite effects, the net effects of GABAergic agents are unpredictable.

inhibition is long-lived, it is reasonable to suspect that GABA is involved in the shortfall.

If GABA is added to Yeomans's cortical model, and if that model is transposed to the MFB, then that might account for the shortfall. GABA neurons and reward fibres are commingled in the MFB just as pyramidal cells and interneurons are commingled in the cortex. C pulses delivered to the MFB might therefore trigger GABA neurons as well as reward fibres. If C pulses fired both GABA afferents and reward fibres, then C pulses would cause GABA inhibition as well as refractory periods. If C pulses had these two effects, then both GABA inhibition and refractoriness would depress T pulse effectiveness.

Because GABA inhibition lasts 20 ms or more, this model could account for the shortfall. The hypothesis that the shortfall is caused by the incidental stimulation of inhibitory afferents is tested in "Appendix 3: Is the Shortfall Caused by Inhibitory Afferents?"

The shortfall may also be caused by the incidental stimulation of inhibitory efferents . The action of efferents could be distinguished from the actions of afferents and local interneurons by adapting the collision test (see "Relevance to Current Research" above). If shortfalls were found when the C and T pulses were delivered through different electrodes, then it would follow that the shortfall's cause is not confined to the vicinity of the C pulse electrode; the substrate at the T pulse electrode and other downstream sites would be beyond the reach of afferent terminals and local interneurons near the C pulse electrode. The only alternative would be something like efferents that can act at some distance from the C pulse electrode.

A recent study supports the idea that C pulses can induce inhibition at a distance. Vachon and Miliaressis (1994) proposed a model to explain two cases where T pulse effectiveness fell from a maximum of about 0.80 at 0.2 ms to a minimum of 0.00 to -0.20 between 1.0 and 5.0 ms. In their model, C pulses stimulate two populations of neurons: one that is fast and excitatory, and a second that is slower and inhibitory. They suggest that the slower, inhibitory signal induced by the C pulse would reach the synapse just after the faster excitatory signal. If that were the case, then the excitatory signal from

the following T pulse might arrive at the synapse while it is still unresponsive. A synaptic blockade like this could reduce T pulse effectiveness at the same time as the substrate at the electrode tip is recovering from refractoriness. In Vachon and Miliaressis's model C pulse inhibition lasted only a few milliseconds, but if the inhibitory signal stimulated the release of GABA, then the inhibition could last much longer; the average duration for GABA-activated $Cl^-$ channels ($\bar{\tau}$) is $20 \pm 6.6$ ms (mean $\pm$ *SD*)(Barker & McBurney, 1979). If GABA inhibition replaced the short lived inhibition in Vachon and Miliaressis's model, then the result could be something like the shortfall.

## Conclusion

This thesis has made three important contributions. First and foremost, it documented the development of T pulse effectiveness at long C-T delays. The thousands of finely resolved T pulse effectiveness estimates (nearly 4000 in Experiment 3 alone) leave no doubt; the third and fourth phases (the shortfall and the final plateau) are consistent and reliable parts of the excitability cycle. Second, it showed that the shortfall is not just a scaling artifact. Third, it showed that the shortfall definitely is not caused by a relative refractory, supernormal, or subnormal period.

Now we know that there is a shortfall, and we know how deep it is and how long it lasts. We also know that it is not caused by relative refractoriness,

subnormality, or supernormality, but we still do not know what does cause the shortfall. As always, every answer spawns another question.

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## Appendix 1: Derivation of T Pulse Effectiveness

When the C-T delay is very short, T pulses have very little rewarding value because the neurons in their field of excitation are refractory[6]. Because the T pulses are ineffective, the researcher must add extra pairs of C and T pulses to elicit a threshold level response. The frequency threshold is correspondingly high. When the C-T delay is long enough, the neural substrate has time to recover and the T pulses' rewarding value is high. The frequency threshold is therefore low. In other words, shifts in DP thresholds provide an indication of the T pulses' ability to elicit behaviour; the higher the DP frequency threshold, the lower the T pulses' contribution. Yeomans (1975) quantified this principle with his formulation of T pulse effectiveness (E).

The derivation of E requires two assumptions. The first is that equal numbers of action potentials in the relevant neural pathways produce equivalent behaviour. Given stimuli that produce moderate response rates, this assumption holds true. There is a range of very weak stimuli that elicit no response, and a range of very strong stimuli that all produce maximal response intensities. Between these behavioural floors and ceilings, however, there is a monotonic relationship between stimulus intensity (hence the number of relevant action potentials) and response intensity. That is to say that when

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[6] C and T pulses, delays, and thresholds are explained in the section entitled "Definition of Terms" in the "General Introduction".

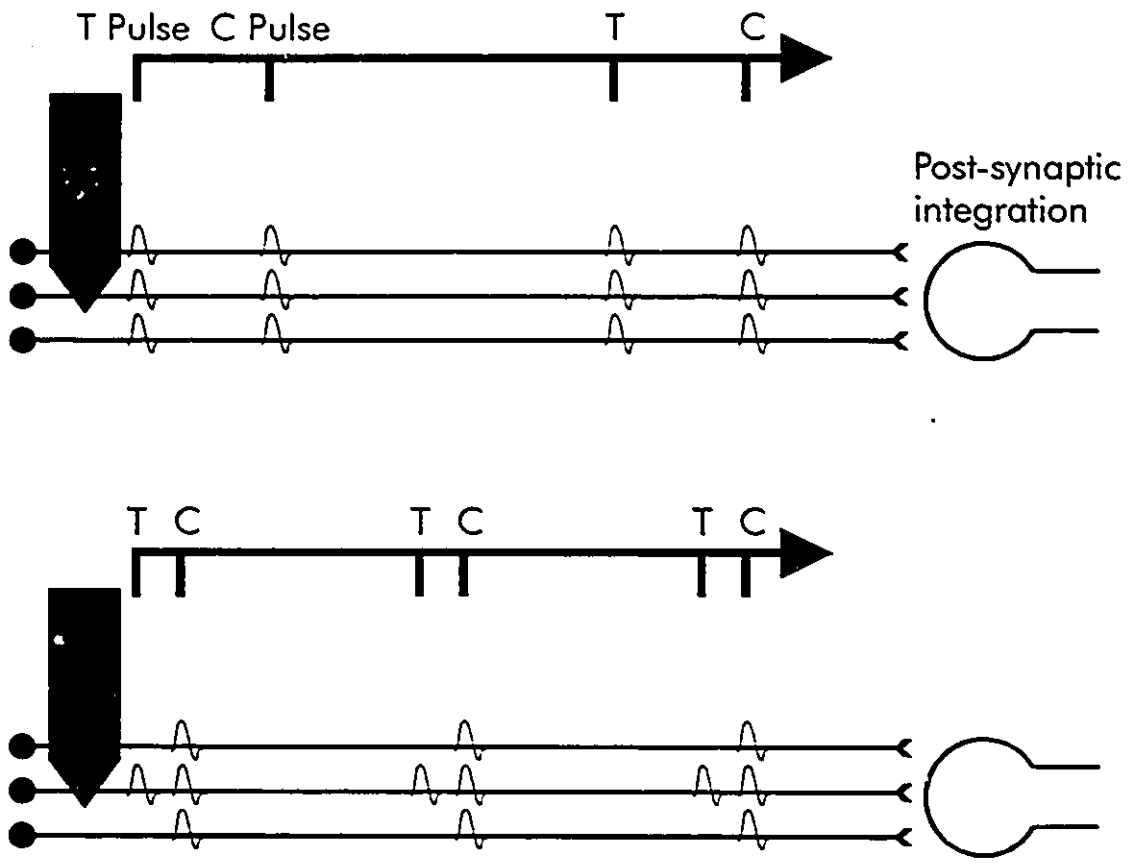


**Figure 34**: Equal numbers of action potentials produce equivalent behaviour. Both the upper and the lower trains of stimulation elicit the same behaviour because they both cause 12 action potentials in the relevant fibres. The difference is that the T pulses in the lower train are delivered during the relative refractory period induced by the C pulses. The frequency of the lower train was therefore increased to compensate for the ineffectiveness of the T pulses. Adapted from Gallistel (1988).

response intensity is plotted against stimulus intensity, the resultant curve is sigmoidal. In the central rising part of this curve (sometimes called the dynamic interval), there is a one to one relationship between response intensity and stimulus intensity. A threshold is a unique point along this dynamic interval, so it corresponds to a fixed number of action potentials. If two

different sets of stimulation parameters produce the same threshold behaviour, it follows that they produce the same number of action potentials.

The second assumption is that for any given current, all of the C pulses in a train are equally capable. If the delay between the C pulses and the preceding T pulses is long enough, then this is reasonable.

Yeomans (1975) used these two assumptions to develop his index of the T pulse's ability to elicit behaviour. Given that equivalent behaviour implies equal numbers of action potentials, it follows that threshold SP and DP trains generate the same number of action potentials. Since the number of pulses in a train is proportional to the pulse frequency, this can be expressed algebraically.

$$N_C F_{DP} + N_T F_{DP} = N_C F_{SP} \qquad (1)$$

The symbols $N_C$ and $N_T$ represent, respectively, the number of action potentials generated by each C and T pulse, and $F_{SP}$ and $F_{DP}$ represent the SP and DP frequency thresholds.

The C and T pulses are almost identical; they have the same current, and they are delivered through the same electrode. The only difference is that the T pulses suffer some interference from the C pulses with which they are paired. This interference is expressed as effectiveness (E), the ratio of the number of action potentials generated by T and C pulses.

$$E = \frac{N_T}{N_C} \qquad (2)$$

Using E, $N_T$ can be expressed in terms of $N_C$.

$$N_T = E\,N_C \qquad (3)$$

The right side of Equation 3 can be substituted for $N_T$ in Equation 1.

$$N_C\,F_{DP} + E\,N_C\,F_{DP} = N_C\,F_{SP} \qquad (4)$$

Subtracting the DP C pulse action potentials from both sides isolates the T pulse action potentials.

$$E\,N_C\,F_{DP} = N_C\,F_{SP} - N_C\,F_{DP} \qquad (5)$$

Dividing both sides by $N_C$ and $F_{DP}$ isolates the effectiveness of the T pulses.

$$E = \frac{F_{SP}}{F_{DP}} - \frac{F_{DP}}{F_{DP}} = \frac{F_{SP} - F_{DP}}{F_{DP}} \qquad (6)$$

This is an efficient computational formula, but factoring out $F_{DP}$ produces the equivalent of Yeomans's (1975) formulation.

$$E = \frac{F_{SP}}{F_{DP}} - 1 \qquad (7)$$

When effectiveness is equal to zero, the T pulses elicit no action potentials at all; the C pulses elicit all of the action potentials (and all of the

behaviour). When effectiveness is equal to one, the T pulses are as capable of firing neurons as the baseline conditioning pulses. If the C and T pulses are the same in all respects except for their order within the train, then T pulse effectiveness must rise to 1.00 as C pulse interference subsides. This is why the shortfalls reviewed above are so puzzling (see the section titled "Refractory Estimate Anomalies"). If there is a shortfall, then there must be interference, but the nature of that interference is unknown.

Note that there is no mention of rate anywhere in the derivation. The only numbers are the frequencies that elicit threshold behaviour in the SP and DP conditions. E is therefore what Valenstein (1964) would call a rate-free measure, and it provides a true interval scale for the measurement of the rewarding value of stimulation (Hawkins, Roll, Puerto & Yeomans, 1983). Also, E can be used with any behaviour for which one can define a behavioural threshold on a monotonic dynamic interval: lever pressing, vocalization, feeding, drinking, etc.

In Experiment 4, T pulse currents are 40% larger than C pulse currents. When the T pulses are larger than the C pulses, the DP frequency threshold can be less than half of the SP C pulse baseline even when the T pulse does suffer some interference from the C pulse. An E of one, therefore, does not indicate zero interference as it would when C and T pulse currents are equal. A new E is required to make sense of unequal pulse experiments.

The derivation of the effectiveness of unequal T pulses ($E_{T>C}$) is very similar to the derivation of E, but allowances must be made for the different sizes of the C and T pulses' fields of excitation. When the T pulse is larger than the C pulse, the field stimulated by the T pulse is made up of a core of fibres that are also stimulated by the C pulse, and of a halo of fibres that are stimulated only by the T pulse. The number of action potentials generated in this halo can be estimated by subtracting the number of action potentials generated by the C pulse ($N_C$) from the number of action potentials generated by the T pulse when there is no interference from a preceding C pulse ($N_{TSP}$, the number of action potentials generated by the 40% larger T pulses in a single pulse condition).

$$\textit{Halo Action Potentials} = N_{TSP} - N_C \qquad (8)$$

Given that equal numbers of action potentials generate equivalent behaviour, the ratio of the baseline SP frequency thresholds obtained with C pulse and T pulse currents ($F_{CSP}$ and $F_{TSP}$ respectively) provides a good estimate of $N_{TSP}$.

$$N_{TSP} = N_C \frac{F_{CSP}}{F_{TSP}} \qquad (9)$$

Having described what a large T pulse does in the single pulse condition, it is now possible to describe what it does in the double pulse condition. As in the case of equal C and T pulses, an effectiveness factor ($E_{T>C}$) is used to quantify the interference caused by the C pulse. The purpose of unequal pulse

experiments is to study the central core, so $E_{T>C}$ is applied to the number of action potentials generated by the T pulse in the central core.

$$N_T=(E_{T>C}*\textit{Core Action Potentials})+(\textit{Halo Action Potentials}) \qquad (10)$$

Just as in Equation 1, $N_T$ is the number of action potentials generated by the T pulse in the double pulse condition.

$$N_T = (E_{T>C}\ N_C) + (N_{TSP} - N_C) \qquad (11)$$

By substituting the right side of Equation 9 for $N_{TSP}$ one obtains

$$N_T = (E_{T>C}\ N_C) + \left(N_C \frac{F_{CSP}}{F_{TSP}} - N_C\right) \qquad (12)$$

The derivation of $E_{T>C}$ can now begin exactly the same way as the derivation of E, except that Equation 12 rather than Equation 3 is substituted for $N_T$ in Equation 1.

$$N_C\ F_{DP} + \left((E_{T>C}\ N_C) + \left(N_C \frac{F_{CSP}}{F_{TSP}} - N_C\right)\right) F_{DP} = N_C\ F_{CSP} \qquad (13)$$

Again, subtracting the DP C pulses from both sides isolates the T pulse action potentials.

$$\left((E_{T>C}\ N_C) + \left(N_C \frac{F_{CSP}}{F_{TSP}} - N_C\right)\right) F_{DP} = N_C\ F_{CSP} - N_C\ F_{DP} \qquad (14)$$

Subtracting the halo action potentials ($(N_C(F_{CSP}/F_{TSP}) - N_C)\ F_{DP}$) from both sides isolates the T pulse core action potentials.

$$E_{T>C}\, N_C\, F_{DP} = N_C\, F_{CSP} - N_C\, F_{DP} - (N_C\, \frac{F_{CSP}}{F_{TSP}} - N_C)\, F_{DP} \qquad (15)$$

Dividing both sides by $N_C$ and $F_{DP}$ isolates $E_{T>C}$.

$$E_{T>C} = \frac{F_{CSP}}{F_{DP}} - 1 - (\frac{F_{CSP}}{F_{TSP}} - 1) = \frac{F_{CSP}}{F_{DP}} - \frac{F_{CSP}}{F_{TSP}} \qquad (16)$$

This is an efficient computational formula, but factoring out $F_{CSP}/F_{TSP}$ produces the equivalent of Yeomans's (1979) formulation (Bielajew, Lapointe, Kiss & Shizgal, 1982).

$$E_{T>C} = (\frac{F_{TSP}}{F_{DP}} - 1)\, \frac{F_{CSP}}{F_{TSP}} \qquad (17)$$

Note that when the T and C pulse currents are equal, $F_{CSP}$ and $F_{TSP}$ are equal, so $F_{CSP}/F_{TSP}$ is equal to one, and the formula for $E_{T>C}$ is the same as the formula for E. The equal pulse condition can therefore be thought of as a special case of the unequal condition.

The beauty of Yeomans's approach is that each animal is its own control. Every experimental DP sweep is compared to a SP baseline that is run during the same session. That way individual differences, motor artifacts, sedation, and other performance deficits are controlled for. There is only one potential hazard. If a rat has trouble pressing, then the researcher must be careful to select a threshold criterion that reliably identifies a point in the dynamic interval; thresholds that fall on the upper or lower asymptotes do not identify unique points on the rate/frequency function. As long as the

researcher chooses an appropriate criterion, however, E provides a reliable measure of the quality of the rewarding stimulation. The shortfalls in T pulse effectiveness found in Experiments 1 through 4 and in Appendix 3, therefore, cannot be dismissed as performance problems (see Edmonds and Gallistel, 1974).

## Appendix 2: Order of Experimental Manipulations in Experiment 3

This table lists the experimental manipulations that were interdigitated with the blocks of control sessions that were pooled in Experiment 3. Brotizolam, picrotoxin, and strychnine refer to pilot drug tests. MH-LH refers to the inhibition tests in Appendix 3. Unequal refers to the unequal pulse current tests in Experiment 4. The electrode that was used in each block of tests is listed in the comment column.

| RAT | MANIPULATION | DATE | COMMENT |
|---|---|---|---|
| #1080 | Surgery | 21/5/93 | |
| | Training | 26/5/93--8/6/93 | |
| | Control 1 | 9/6/93--13/6/93 | Tested with right LH. |
| | Picrotoxin (pilot test) | 15/6/93--19/6/93 | Tested with right LH. |
| | Control 2 | 21/6/93--24/6/93 | Tested with right LH. |
| #1082 | Surgery | 25/5/93 | |
| | Training | 31/5/93--8/6/93 | |
| | Control 1 | 9/6/93--13/6/93 | Tested with left LH. |
| | Picrotoxin | 15/6/93--19/6/93 | Tested with left LH. |
| | Control 2 | 21/6/93--24/6/93 | Tested with left LH. |
| | Unequal (Expt. 4) | 28/6/93--2/7/93 | Tested with left LH. |
| | Control 3 | 5/7/93--9/7/93 | Tested with left LH. |
| | Brotizolam (pilot study) | 11/7/93--14/7/93 | Tested with left LH. |

| RAT | MANIPULATION | DATE | COMMENT |
|---|---|---|---|
| #1082 (cont.) | Control 4 | 19/7/93--22/7/93 | Tested with left LH. |
| | MH-LH (Appendix 3) | 26/7/93--10/8/93 | Tested with left LH. |
| | Control 5 | 13/8/93--19/8/93 | Tested with left LH. |
| | Strychnine (pilot study) | 24/8/93--28/8/93 | Tested with left LH. |
| | Control 6 | 31/8/93--3/9/93 | Tested with left LH. |
| | | | |
| #1085 | Surgery | 28/5/93 | |
| | Training | 9/6/93--11/7/93 | |
| | Control 1 | 12/7/93--22/7/93 | Tested with left LH. Results discarded. |
| | Unequal | 11/8/93--25/8/93 | Tested with left LH. Developed seizures. Results discarded. |
| | Control 2 | 26/8/93--28/8/93 | Tested with right LH and brotizolam. |
| | Strychnine | 31/8/93--3/9/93 | Tested with right LH and brotizolam. |
| | Control 3 | 7/9/93--10/9/93 | Tested with right LH and brotizolam. |
| | | | |
| #1086 | Surgery | 28/5/93 | |
| | Training | 10/6/93--15/6/93 | |
| | Control 1 | 17/6/93--24/6/93 | Tested with right LH. |
| | Unequal | 28/6/93--2/7/93 | Tested with right LH. |
| | Control 2 | 5/7/93--9/7/93 | Tested with right LH. |
| | Brotizolam | 12/7/93--14/7/93 | Tested with right LH. |
| | Control 3 | 19/7/93--22/7/93 | Tested with right LH. |

| RAT | MANIPULATION | DATE | COMMENT |
|---|---|---|---|
| #1086 (cont.) | MH-LH | 29/7/93--19/8/93 | All three electrodes used. |
| | Control 4 | 26/8/93--28/8/93 | Tested with right LH. |
| | Strychnine | 31/8/93--3/9/93 | Tested with right LH. |
| | Control 5 | 7/9/93--10/9/93 | Tested with right LH. |
| #1106 | Surgery | 18/10/93 | |
| | Training | 27/10/93--3/11/93 | |
| | Control 1 | 5/11/93--19/11/93 | Tested with right and with left LH electrodes. |
| | Unequal | 22/11/93--15/12/93 | Tested with right and with left LH electrodes. |
| | Control 2 | 16/12/93--21/12/93 | Tested with right and with left LH electrodes. |
| | Control 3 | 14/1/94--2/2/94 | Current boosted from 400 $\mu$A to 550 $\mu$A. Results discarded. |
| | MH-LH | 3/2/94--4/3/94 | Results discarded. |
| #1111 | Surgery | 25/11/93 | |
| | Training | 1/12/93--2/12/93 | |
| | Control 1 | 3/12/93--21/12/93 | Tested with both left and right LH electrodes. |
| | Control 2 | 14/1/94--2/2/94 | Tested with both left and right LH electrodes. |
| | MH-LH | 3/2/94--3/3/94 | Right MH and both LHs. Results reported in Appendix 3. |
| #1115 | Surgery | 21/12/93 | |
| | Training | 19/1/94--7/2/94 | |

| RAT | MANIPULATION | DATE | COMMENT |
|---|---|---|---|
| #1115 (cont.) | Control 1 | 8/2/94--21/2/94 | Tested with both left and right LH electrodes. Developed seizures, so the end of the block was discarded. |

## Appendix 3: Is the Shortfall Caused by Inhibitory Afferents?

### Introduction

There is reason to suspect that the shortfall in T pulse effectiveness that was documented in Experiments 1, 2, and 3 may be caused by the incidental stimulation of inhibitory afferents. Horseradish peroxidase studies have shown that the LH receives input from the ipsilateral MH, particularly from the dorsomedial nucleus and the lateral margin of the ventromedial nucleus (Kita & Oomura, 1981). It has also been shown that spontaneous and electrically stimulated activity in the MH inhibits activity in the LH (Oomura, Kimura, Ooyama, Maeno, Iki & Kuniyoshi, 1964; Oomura, Ooyama, Yamamoto, & Naka, 1967; van Atta & Sutin, 1971; Porrino, Coons & MacGregor, 1983). There are, therefore, inhibitory afferents as well as reward fibres in the LH. If rewarding C pulses stimulate both, then C pulses would induce both inhibition and refractoriness. If it could be shown that afferent inhibition of the LH by the MH has the same time course as the shortfall in T pulse effectiveness, then that would indicate that the incidental stimulation of these inhibitory afferents may be responsible for the shortfall. This is the incidental inhibition hypothesis.

Porrino, Coons, and MacGregor (1983) studied MH inhibition of the ipsi- and contralateral LHs by administering pairs of C and T pulses. They delivered the C pulses to the MH, and the T pulses to either the ipsi- or contralateral LH.

The rats did not press as quickly for these pairs of C and T pulses as they did for trains of single pulses delivered to one of the LHs. Porrino et al. used this reduction in rate as an index of inhibition. When they plotted the degree of contralateral inhibition against the C-T delay, they found that inhibition was invariant. Ipsilateral inhibition, however, was greatest at delays of 1, 5, and 15 ms. Inhibition fell at 20 ms, and bottomed out at 25 ms. These ipsilateral results closely parallel the time course of the shortfall in T pulse effectiveness. Porrino et al.'s results therefore suggest that afferents projecting from the MH to the ipsilateral LH may cause the shortfall in T pulse effectiveness.

There is an important shortcoming in Porrino et al.'s methodology. Instead of using a rise in frequency threshold as an index of inhibition, they used the reduction of the response rate. They could not, therefore, quantify the degree of inhibition. The problem with rates is that they do not measure the efficacy of stimulation. If a rat is given the opportunity to press for intensely rewarding stimulation, then it will typically press at a maximal rate. If the frequency of the stimulation decreases gradually, the rat will not gradually slacken its pace to match that decay. Instead, the rat will press at the same maximal pace for intensely, strongly, or moderately rewarding stimulation. Eventually the frequency will drop below a threshold, and the rat will suddenly stop pressing. Frequency thresholds, on the other hand, do measure efficacy of stimulation; when the rewarding value of stimulation is cut in half, the frequency threshold doubles (Hawkins, Roll, Puerto & Yeomans, 1983). If

Porrino, Coons, and MacGregor's tests were repeated using threshold shifts instead of rate changes, then the similarity between MH-LH inhibition and the effectiveness shortfall could be quantified.

The purpose of the present experiment is to quantify direct MH-LH inhibition, and to compare its time course with that of the shortfall in T pulse effectiveness. Stimulation of the MH, however, does not just inhibit LH reward via direct inhibitory connections. MH stimulation is generally aversive, and rats will work to escape it (Olds & Olds, 1963; Schmitt, Sandner & Karli, 1976). It is necessary, therefore, to distinguish between inhibition due to direct connections on the one hand, and general inhibition on the other hand.

Direct inhibition can be distinguished from general inhibition by comparing ipsi- and contralateral inhibition. The LH has extensive afferent connections with the ipsilateral MH but not with the contralateral MH (Kita & Oomura, 1982; Luiten & Room, 1980; Ter Horst & Luiten, 1986, 1987). When MH stimulation attenuates the rewarding effects of stimulation of the contralateral LH, therefore, it can only do so indirectly. Ipsilateral reward, on the other hand, is subject to both direct and indirect attenuation. All else being equal, therefore, the difference between ipsi- and contralateral inhibition is a measure of direct inhibition.

In other words, if a rat has matched electrodes in the left and right LHs, and if C pulses delivered to the MH inhibit T pulse reward more in the ipsilateral LH than in the contralateral LH, then that difference may be ascribed to the

direct connections between the MH and the ipsilateral LH. Plotting ipsi- and contralateral inhibition against C-T delay will therefore reveal the time course of the direct inhibition. When ipsilateral inhibition is greater than contralateral inhibition, direct inhibition is active. When ipsilateral inhibition decreases and approaches the contralateral level, direct inhibition comes to an end.

It was proposed above that the shortfall in T pulse effectiveness may be caused by the incidental stimulation of inhibitory afferents. If this hypothesis is correct, then it follows that the time course of direct inhibition should match the duration of the shortfall; it should remain steady for about 25 ms, and disappear within 35 ms. That is, ipsilateral inhibition should be greater than contralateral inhibition for about 25 ms. Ipsilateral inhibition should then gradually decrease and approach the contralateral level at about 35 ms.

The incidental inhibition hypothesis also predicts that contralateral Remaining Effectiveness should remain steady at all C-T delays. In the absence of direct MH-LH projections, total contralateral inhibition should remain steady at a level that is representative of general, indirect inhibition.

## Method

Nineteen rats were used in this experiment, including the seven that were also used in Experiment 3. The lightest of the nineteen weighed 330 g at the time of surgery, and the heaviest weighed 608 g. Atropine sulfate (0.125

mg s.c.) was given to control mucus secretions. The rats were anaesthetized with between 65 and 100 mg/kg i.p. of sodium pentobarbitol (Somnotol, MTC Pharma©); the standard dose of 65 mg/kg was often insufficient. When required, xylazine (1.0 mg i.m., Rompun, Bayvet©) or halothane (Fluothane, Wyeth-Ayerst Canada©) was used to supplement anaesthesia. Surgery was performed as described in Experiment 1. After at least a week of convalescence, the rats were trained to press for one second trains of stimulation as described in Experiment 3. The same session and trial lengths, and the same intertrial decrements were also used. When testing was complete, the rats were euthanised with overdoses of sodium pentobarbitol. The electrode placements were then histologically verified as described in Experiment 1. Seven of the rats self stimulated reliably; #1080, #1082, #1085, #1086, #1106, #1111, and #1115. The locations of their electrode tips are illustrated above in Experiment 3.

Each of the rats was implanted with at least three electrodes: one in each LH, and one in the MH. Three rats had four electrodes: one in each LH and one in each MH. Multiple implants were performed in much the same way as the single implants in the previous experiments. The only important difference was that the electrodes were fixed in a jig before surgery. Jig construction is described in detail in the method section of Experiment 3.

The computer controlled stimulator used in Experiment 3 was used again, but it was hooked up differently. The stimulators had two channels, but

in Experiment 3 only one had been used. In the present experiment, channel A generated the MH pulses, and channel B generated the LH pulses. Each channel's current could be adjusted independently of the other's. While a pulse was being delivered through one channel, the other channel was thrown into high impedance. This ensured that the current was grounded through the skull screws rather than through one of the other electrodes. To prevent the build up of polarization, both channels were grounded between pulses.

Using the procedures described in Experiments 1 and 3, the rats were trained to lever press for trains of LH stimulation.

Direct MH inhibition of the ipsilateral LH was inferred by comparing ipsilateral and contralateral MH inhibition of LH reward. If the LH electrodes were closely matched, then the difference in inhibition could be ascribed to ipsilateral MH-LH connections. If, on the other hand, the LH electrodes were not closely matched, then the difference in inhibition could be ascribed either to the direct ipsilateral connections, or to other differences such as placements, tip exposures, clotting, or scarring. During the first phase of testing, the LH electrodes were matched by adjusting the stimulation currents so that the rats had similar single pulse frequency thresholds on both sides. The electrodes were considered to be matched if, at a given single pulse frequency threshold, there was no more than a 50% difference in current at the two LH electrodes. If a rat required more than a 50% difference in current, or if it did not work

reliably for stimulation through one or both LH electrodes, then that rat was retired from the experiment.

Once the currents and frequency thresholds at the two LH electrodes were matched with single pulse trains, the rats were tested with double pulse trains. During this second phase of testing, C pulses were delivered to the MH, and T pulses were delivered to one or the other of the LH electrodes. The C-T delay was fixed at 15 ms. A dozen double pulse replications and single pulse LH baselines were collected on each side. Then the MH current was increased, the tests were repeated, and the MH current was increased again. This cycle was repeated until responding became erratic. The MH currents ranged between 200 $\mu$A and 800 $\mu$A. At the higher MH currents it was necessary to increase the LH currents. Otherwise the double pulse frequency thresholds rose too high, and it was impossible to test at long C-T delays.

The thresholds from this testing were used to calculate Remaining Effectiveness at each LH electrode. Remaining Effectiveness is the frequency threshold obtained with single pulse trains of LH pulses divided by the frequency threshold obtained with double pulse trains of MH and LH pulses. Essentially, therefore, Remaining Effectiveness = 1 - Inhibition. Remaining Effectiveness was used as an index because when it is plotted against C-T delay, it resembles T pulse effectiveness. If the shortfall in T pulse effectiveness is caused by MH-LH inhibition, therefore, it should be possible to overlay the effectiveness and Remaining Effectiveness curves.

The MH current that gave the biggest difference between the Remaining Effectivenesses on the ipsilateral and contralateral sides was tested again. This was done to ensure that the results of the initial testing were not spurious. If the difference survived another twenty replications, then that combination of MH and LH currents was used in the tests that followed. Otherwise the combination that gave the second biggest difference was tested.

If a rat had steady SP frequency thresholds at similar currents at both LH electrodes, and if it had significantly higher Remaining Effectivenesses (lower inhibition) at the contralateral LH electrode than at the ipsilateral LH electrode, then the rat went on to the third and final phase of testing. Remaining Effectiveness was measured as before, but the C-T delays were 1, 5, 15, 25, and 35 ms rather than just 15 ms. It was predicted that if the shortfall is caused by the direct MH-LH circuit, then at the contralateral LH electrode Remaining Effectiveness would remain constant at all C-T delays. At the ipsilateral LH electrode, Remaining Effectiveness would be lowest at delays that span the shortfall (1, 5, 15, and 25 ms), and Remaining Effectiveness would approach the contralateral level at the delay that falls outside of the shortfall (35 ms).

# Results

Eighteen of the nineteen rats were disqualified for one or more reasons: they did not self stimulate reliably, they had intractable seizures, the LH electrodes were poorly matched, the MH electrodes did not inhibit reliably, or Remaining Effectiveness was not lower (inhibition was not higher) at the ipsilateral LH electrode than at the contralateral LH electrode.

Rats #1067, #1069, #1071, #1072, and #1073 were pilot subjects with triple electrode arrays. Their placements were inaccurate, and they did not self stimulate reliably. Rat #1083 would not work for 400 $\mu$A stimulation, and it squealed when it was given 500 $\mu$A. Stimulation had an aversive effect on Rat #1104. It would jump when it was stimulated through its LH electrodes. Stimulation through the LH electrodes had very little effect on Rat #1107; currents up to 600 $\mu$A failed to elicit sniffing or exploration. Rat #1108 responded very well to LH stimulation. In the experimenter's notebook it is described as a "self shaper". Its response rates were low, however, and its single pulse frequency thresholds were unsteady. Rats #1123, #1124, and #1125 had quadruple implants: one in each MH and one in each LH. Rat #1123 died in surgery. Rats #1124 and #1125 had seizures that were poorly controlled with brotizolam (7.5 mg/kg i.p., Boehringer®, one hour before each test session). They both had unsteady frequency thresholds. Rat #1125 also had crooked upper incisors that needed weekly trimming.

Rats #1080, #1082, #1085, #1106, #1111, and #1115 all self stimulated well. However, #1080 was retired before the inhibition tests could begin because it developed a motor artifact when it was stimulated through its right (ipsilateral) LH electrode. It would not lever press for stimulation through its contralateral LH electrode. Rat #1082 was withdrawn because stimulation of its right (ipsilateral) LH began to induce seizures; it would only work for stimulation through its contralateral LH electrode. Rat #1085 could only be tested with brotizolam, and MH stimulation made it run frantically between sweeps. Rat #1106 had to be withdrawn from the study because its single pulse frequency thresholds rose from 18 hertz to 22 hertz over a span of two weeks. This was taken as an indication that there was clotting, scarring, or infection at the electrode tips. Rat #1115 was retired from the study because it developed intractable seizures.

With Rat #1111, stimulation currents of 600 $\mu$A at the right MH electrode and at both LH electrodes produced the greatest difference between ipsilateral and contralateral Remaining Effectivenesses (#1111's electrode placements are illustrated in Experiment 3; see Figure 17). However, there was more Remaining Effectiveness (less inhibition) at the right (ipsilateral) electrode than at the left (contralateral) electrode. According to the protocol described in the method section above, #1111 was ineligible for the full battery of third phase tests. Only one subject qualified for the final phase though, so #1111 was subjected to an abbreviated third phase even though it had not formally

qualified. It was tested at MH-LH delays of -5 ms (the LH pulse preceded the MH pulse), 15 ms, and 25 ms. An analysis of variance showed that Remaining Effectiveness at the ipsilateral LH electrode (0.88) was significantly higher than at the contralateral electrode (0.77): $F(1, 78) = 125.6$, $p < 10^{-18}$. The interaction of C-T delay and laterality (ipsilateral vs. contralateral LH electrodes) was not significant, $F(2, 78) = 1.08$, $p = .34$, but the effect of C-T delay was, $F(2, 78) = 5.48$, $p = .006$. The difference in Remaining

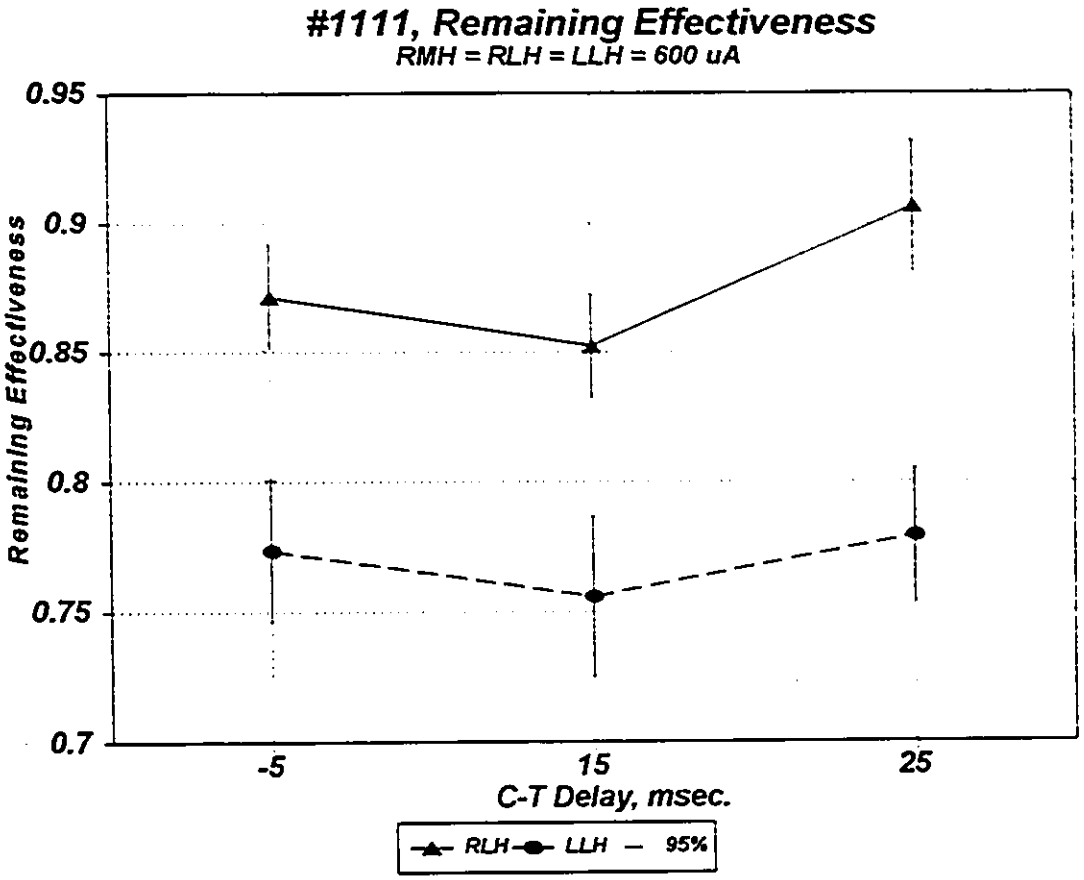


**Figure 35:** The results of the abbreviated third phase testing in rat #1111. The C-T delay of -5 ms denotes the condition where the LH pulse preceded the MH pulse by 5 ms. The upper and lower lines represent means obtained with the LH electrodes that were, respectively, ipsilateral and contralateral to the MH electrode. The error bars represent 95% confidence intervals.

Effectiveness at different C-T delays was evaluated with Tukey's least significant difference (LSD = 0.049, one tail, $p = .05$). On the contralateral side, there were no significant differences, but on the ipsilateral side Remaining Effectiveness was significantly higher at 25 ms (0.91) than at 15 ms ( 0.85 ).

Of the 19 rats that were tested, only #1086 had steady single pulse thresholds at comparable currents at both LH electrodes, as well as significantly higher Remaining Effectiveness at its contralateral LH electrode than at its ipsilateral LH electrode. The contralateral LH current was 800 $\mu$A, the ipsilateral LH current was 1000 $\mu$A, and the MH current was 450 $\mu$A. Rat #1086's electrode placements are illustrated in Experiment 3 (see figure 17).

Testing had to be stopped when there were between 21 and 25 double pulse replications at each C-T delay at each LH electrode. The male Reli-a-tac© plug connected to #1086's MH electrode broke off flush with the acrylic crown.

Rat #1086's results were subjected to an analysis of variance to test the effects of C-T delay and laterality (ipsilateral vs. contralateral LH electrodes). Remaining Effectiveness at the ipsilateral LH electrode (0.69) was significantly lower than at the contralateral electrode (0.81): $F(1, 214) = 176.3$, $p < 10^{-25}$. The effect of C-T delay was also significant, $F(4, 214) = 3.19$, $p = .01$, and the interaction was not significant, $F(4, 214) = 2.19$, $p = .07$. The difference in Remaining Effectiveness at different C-T delays was evaluated with Dunnet's honestly significant difference (HSD = 0.047, one tail, $p = .05$). On the

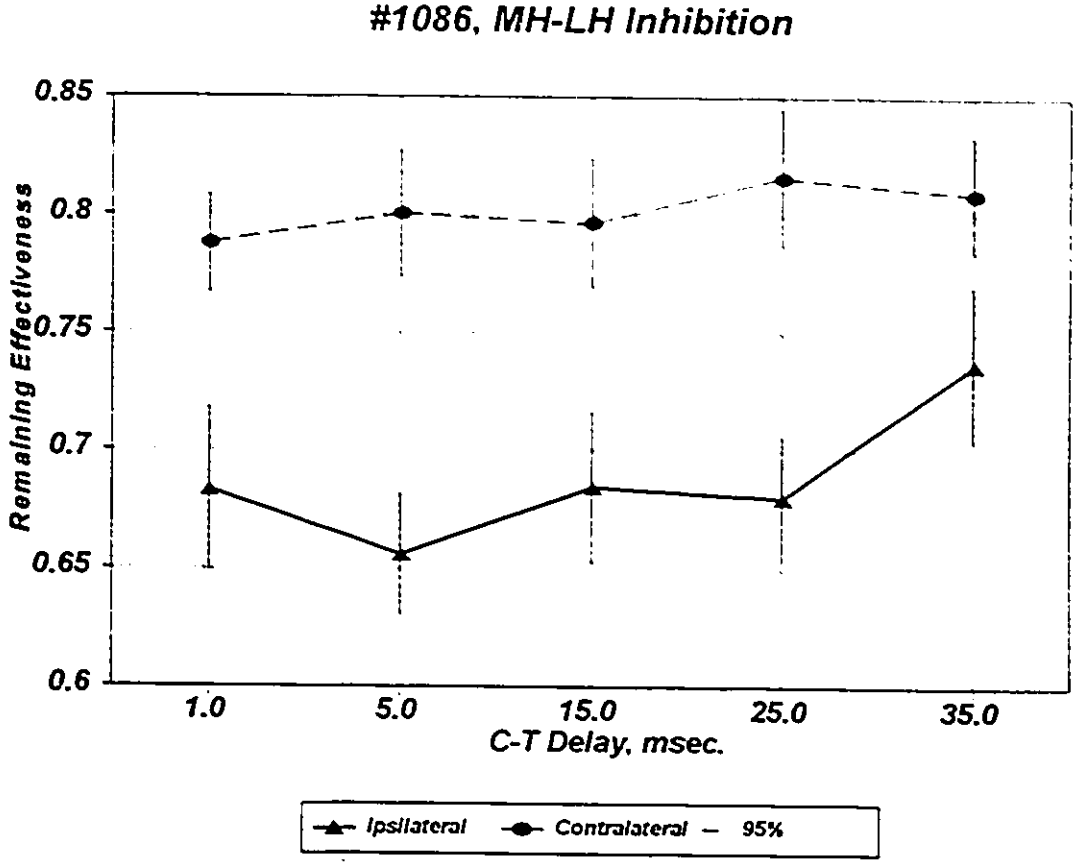


**Figure 36:** Time course of MH-LH inhibition of reward in Rat #1086. The upper and lower lines represent means obtained at the LH electrodes that were, respectively, contralateral and ipsilateral to the MH electrode. The error bars represent 95% confidence intervals. Analysis of variance and Dunnet's honestly significant difference show that the contralateral curve is flat, and that the ipsilateral curve turns up at 35 ms.

contralateral side, there were no significant differences, but on the ipsilateral side Remaining Effectiveness was significantly higher at 35 ms (0.74) than at 1, 5, 15, and 25 ms (mean = 0.68). At 35 ms, the ipsilateral level approached but did not reach the contralateral level (0.74 versus 0.81).

Rat #1186's Remaining Effectiveness curve approximates the shape of its effectiveness curve. In Figure 37, the Remaining Effectiveness axis was rescaled to underline the similarity in the two curves shapes.

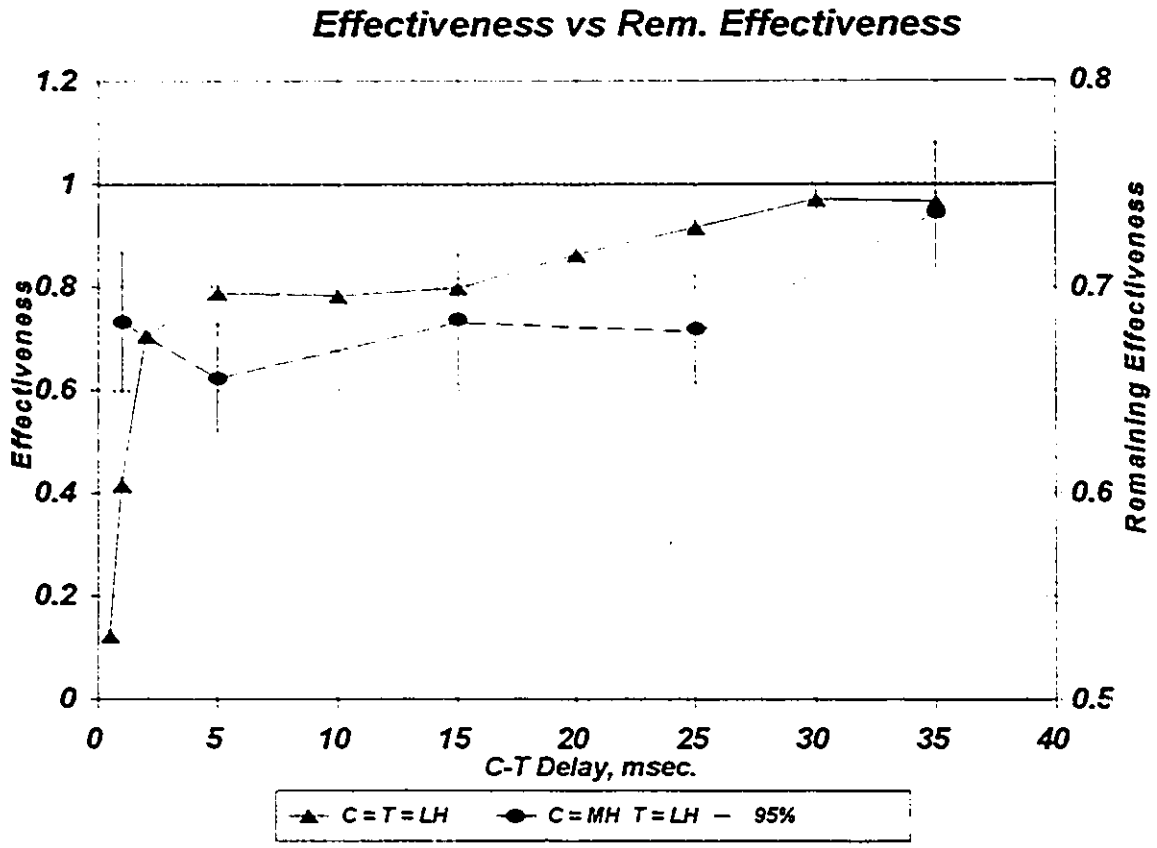


**Figure 37:** A comparison of the effectiveness curve (triangles) for rat #1086 from Experiment 3 with the Remaining Effectiveness curve (circles) from the present experiment. The solid markers represent means, and the error bars represent 95% confidence intervals. Note that the Remaining Effectiveness curve approximates the shape of the effectiveness curve.

# Discussion

Rat #1086's results have implications for the MH LH inhibition hypothesis, and #1111's have implications for the design of the present experiment. Rat #1086's results are discussed first, and #1111's are discussed second.

## Rat #1086

The predictions were borne out in #1086 by the Dunnet's *HSD* test. First, at the contralateral LH electrode Remaining Effectiveness did not vary with C-T delay. Second, at the ipsilateral LH electrode Remaining Effectiveness was lower at the delays that spanned the shortfall than at the delay that fell outside the shortfall. Third, at the post-shortfall delay, ipsilateral Remaining Effectiveness approached the contralateral level. In short, the shape of the ipsilateral Remaining Effectiveness curve mimicked the shape of the effectiveness curve.

The most notable difference between the effectiveness and the Remaining Effectiveness curves is that the latter does not rise sharply between 1 and 5 ms. This difference is to be expected because the effectiveness tests used one electrode, and the inhibition tests used two electrodes. In both cases the C pulses induced refractoriness, but in the inhibition tests the refractoriness

was induced in the MH, about 1.5 mm away from the T pulse electrode in the LH. At 1 ms, therefore, refractoriness reduced effectiveness but not Remaining Effectiveness. Refractoriness was therefore a factor for only for the effectiveness curve.

The predicted similarity between the effectiveness and Remaining Effectiveness curves shows that direct MH-LH inhibition lasts about as long as the shortfall in T pulse effectiveness that was documented in Experiments 1, 2, and 3. This supports the theory that the shortfall in T pulse effectiveness in the LH is caused by incidental stimulation of inhibitory afferents from the MH.

The flatness of the contralateral Remaining Effectiveness curve also supports the incidental inhibition hypothesis. If the shortfall is caused by ipsilateral MH-LH projections, and if MH stimulation stimulates those same projections at their origin, then MH stimulation should impose a shortfall-like shape on ipsilateral Remaining Effectiveness curves. In contrast, MH stimulation should not distort contralateral Remaining Effectiveness curves because there are no contralateral MH-LH projections. The incidental inhibition hypothesis therefore predicts that the contralateral curve should be flat. The flatness of the contralateral curve accords with this prediction, so the results support the incidental inhibition hypothesis.

Support for the incidental inhibition hypothesis is tempered by the fact that only one out of nineteen rats completed all three phases of testing (matching LH electrodes, selecting LH and MH currents, and measuring

Remaining Effectiveness at different C-T delays). When so few rats complete an experiment, either the experiment is very difficult, or the results are idiosyncratic. In the present case, the technical difficulties are formidable.

The first technical problem is that the effects are small. Currents were selected to maximize the difference between the Remaining Effectivenesses at the ipsilateral and contralateral LH electrodes, but in #1086 that difference was still only 0.11 (0.05 $\log_{10}$ units). Large numbers of replications are therefore required not only for the final testing, but also when selecting the three currents (MH, ipsilateral LH, and contralateral LH). A month or more of daily tests was therefore required to select an appropriate combination of MH, and ipsilateral and contralateral LH currents. Moreover, there was no guarantee that weeks of testing would yield an appropriate set of currents; if the MH and ipsilateral LH electodes were slightly misaligned, then no set of currents could produce the requisite gap between ipsi- and contralateral inhibition. Consequently, even though the individual screening and testing sessions did not present any particular difficulties, so many sessions were required that their sheer volume was a problem.

The second technical problem is that it is rare to get three properly aligned electrodes (two mirror image LH placements, and the MH electrode site projecting to the ipsilateral LH site). Collision studies (e.g. Bielajew & Shizgal, 1980) need only two good placements, but the correct juxtaposition is so critical that these studies are usually only attempted with moveable electrodes

(Miliaressis & Philippe, 1983). Since each additional electrode multiplies the chances for misalignement, the need for moveable electrodes is greater in the present, triple electrode experiment than in double electrode collision studies. In the present study, however, moveables are impractical because triple and quadruple arrays are too crowded to accommodate their pedestals. Even if moveable electrodes could be used, it would take several months of testing to try all the different combinations of placements and currents. That means that a rat might very well lose its crown before the three electrodes were aligned.

Necessarily, therefore, triple electrode MH-LH inhibition tests have a very high rate of attrition. Since practical considerations imposed a ceiling on the time and resources that could be invested in the present experiment, attrition limited the number of successful subjects. Because very few rats could be expected to complete all three testing phases, the fact that #1086 was the only one does not indicate that it was peculiarly idiosyncratic.

Indeed, Rat #1086 was an almost completely unremarkable subject. Its effectiveness curve closely resembled those of the other subjects (see the results sections of Experiments 1, 2, and 3). It had no seizures or motor artifacts. Its response rates were similar to those of the other rats; unlike #1085, #1086 did not require unusually long trials to produce reliable thresholds (see the Method section of Experiment 3). Rat #1086's electrode placements were also very similar to those of several other rats: #1080, #1082, #1085, #1106, and especially #1111 (see Figures 16 and 17). Rat

#1086's LH placements were almost perfect mirror images, and (perhaps as a consequence) it cleared all of the methodological hurdles necessary to qualify for the third phase of testing. Otherwise, however, #1086 did not stand out from the other subjects. There is every indication, therefore, that the results generated by #1086 in the present experiment are representative of what would be generated by other rats that successfully completed all three testing phases.

Clearly, the results would be more convincing if more rats had completed all three testing phases. On the other hand, the rat that did complete the experiment was representative, the probability of type I error was low ($.05 > p > 10^{-25}$), and there were no contradictory findings. Moreover, the present study has no precedent in the literature; it is the first to combine rate-free measurements, triple electrode arrays, and MH-LH inhibition tests. The most similar previous study was Porrino, Coons, and MacGregor (1983), and its results accord well with those of the present experiment. The results of the present experiment therefore support the hypothesis that the shortfall in T pulse effectiveness is caused by the incidental stimulation of inhibitory afferents by the C pulse.

Another recent study supports the incidental inhibition hypothesis. Vachon and Miliaressis (1994) found two cases where T pulse effectiveness fell from a maximum of about 0.80 at 0.2 ms to a minimum of 0.00 to -0.20 between 1.0 ms and 5.0 ms. They suggest that this might indicate that the C

pulse stimulates two populations of neurons: one that is fast and excitatory, and a second that is slower and inhibitory. If that were the case, then the inhibitory C pulse signal would reach the synapse just after the excitatory signal. The T pulse influx would then find the synapse in a hyperpolarized state. In Vachon and Miliaressis's model the inhibition only lasted a few milliseconds, but processes such as GABA inhibition are much longer lived; the average duration for GABA-activated $Cl^-$ channels ($\tau$) is $20 \pm 6.6$ ms (mean $\pm$ *SD*)(Barker & McBurney, 1979). If GABA inhibition were substituted for the short lived process in Vachon and Miliaressis's model, then the result would be a 20 to 35 ms shortfall.

Rat # 1111: Problems with the present experiment's design

The present experiment is noteworthy in part because it is unique. Porrino, Coons, and MaGregor's (1983) study is similar in that it compared ipsi- and contralateral MH inhibition of LH reward, but the present study is the first to use rigorous, threshold shifting techniques to quantify that comparison. As is the case with other first efforts, as much was learned about the experimental design as about the substrate under investigation.

The first problem with the present experiment's design is that it is very expensive, both in terms of rats and of time. As was explained above, only a very small proportion of the subjects can be expected to fulfill all the screening

criteria. Consequently, something in the order of a hundred subjects would have to be implanted and screened to find a half dozen succesful third phase candidates. If each candidate could be screened in one month, and if the experimenter could run four rats at a time, then it would take more than two years of uninterrupted testing just to do the screening.

Why was such an expensive design adopted? Because it provides a powerful, within subject control; the effect of MH stimulation on ipsilateral LH reward is compared with the effect of that same MH stimulation on contralateral reward. All else being equal, therefore, the difference between ipsi- and contralateral effects can be ascribed to direct ipsilateral connections. That brings us to the second problem; all else is very seldom equal.

Rat #1111's left and right LH currents were well matched. This suggested that that the two LH electrode placements were well matched, i.e., that all else was equal. It was expected, therefore, that indirect MH inhibition would affect both ipsi- and contralateral LH reward equally. Because previous studies (Kita & Oomura, 1981; Oomura, Kimura, Ooyama, Maeno, Iki & Kuniyoshi, 1964; Oomura, Ooyama, Yamamoto, & Naka, 1967; Porrino, Coons & MacGregor, 1983; van Atta & Sutin, 1971) indicated that ipsilateral reward is subject to both direct and indirect MH inhibition, it was also expected that ipsilateral inhibition would be greater than contralateral inhibition. In fact, the opposite was true; contralateral inhibition was greater than ipsilateral inhibition. Clearly, one or more assumptions were false.

As #1111's results demonstrated, it is unduly sanguine to assume that if SP current thresholds are equal at both LH electrodes, then indirect MH inhibition of LH reward will also be equal. Even though #1111's currents were well matched, its ipsilateral inhibition was significantly lower than its contralateral inhibition. It follows that ipsilateral inhibition could not be equal to the sum of indirect contralateral inhibition and direct ipsilateral inhibition. By themselves, therefore, matched currents do not warrant the arithmetic decomposition of inhibition.

If matched currents are not enough to justify an assumption of equal indirect inhibition, then what is? Certainly, the combination of matched currents and virtually identical LH placements (as in the case of #1086) carries considerable weight. If everything that we can see is equal (aside from laterality), then it is tempting to assume that everything actually is equal. For #1086 everything probably was in fact equal, and it probably was reasonable to dissect inhibition into its direct and indirect components. However, it is impossible to measure direct and indirect inhibition independently; they are inferred by comparing total inhibition at the ipsi- and contralateral LH electrodes. Consequently, it is impossible to validate independently either the assumption of equal indirect inhibitions, or the partition of inhibition into its direct and indirect components. In the end, there must always be some doubt that results that appear to support the incidental inhibition hypothesis may, in truth, merely fit our expectations.

A better design would rely on fewer assumptions. Originally it was hoped that if the LH electrodes were well matched, then ipsilateral Remaining Effectiveness would rise up to the contralateral level at 35 ms. Rat #1111 showed that it is impractical to rely on matched electrodes. Instead, it would be better to compare the shapes of contra- and ipsilateral Remaining Effectiveness curves (within or accross subjects) without regard to the height of the curves. This curve shape comparison could produce three outcomes. First, if the contra- and ipsilateral curves were all flat, then that would show that the shortfall and MH-LH inhibition were unrelated. Second, if the contra- and ipsilateral Remaining Effectiveness curves were both shaped like the standard effectiveness curve, then that would suggest that direct MH-LH projections were not responsible for the shortfall, but that MH-LH inhibition and the shortfall were nonetheless somehow related. Third, if the ipsilateral curves mimicked the shapes of the standard effectiveness curves, and if the contralateral curves were flat, then that would suggest that the direct ipsilateral connections that modulate MH-LH inhibition were also responsible for the shortfall.

Curve shape comparison can be used with Rat #1086. The ipsilateral curve is shaped like the standard effectiveness curve because Remaining Effectiveness at the post-shortfall delay (35 ms) is significantly higher than at the shortfall delays (1, 5, 15, and 25 ms). The contralateral is flat because

there are no significant differences in Remaining Effectiveness at the different C-T delays.

Another approach would be to compare positive and negative C-T delays. That is, the condition where the MH pulse precedes the LH pulse could be compared to the condition in the same subject where the LH pulse precedes the MH pulse. This is an interesting comparison because the MH-LH projections cannot directly inhibit an LH reward that has already been delivered. This is also a very powerful comparison because the only thing that would change is the order of the pulses; the subject, electrodes, placements, and currents would all be the identical in the positive and negative conditions. Comparisons between positive and negative delays, therefore, would not require the assumption of equal indirect inhibition at both LH electrodes.

Negative C-T delay comparisons were implemented in a modest way with Rat #1111. There was no difference in Remaining Effectiveness at -5, 15 and 25 ms with the contralateral MH-LH pair of electrodes. With the ipsilateral pair, Remaining Effectiveness was higher 25 ms at than at 15 ms, and there was no significant difference between -5 and 25 ms. These results support the incidental inhibition hypothesis. As predicted, Remaining Efectiveness was higher at -5 ms than at 15 ms, but the difference was not significant. There were only 14 replications per delay, so the statistical insignificance of this effect may be due to the small sample size.

Negative C-T delays and curve shape comparison have two important advantages. First, they do not have the stringent entry requirements of the within subject comparisons that were attempted in the present experiment. These alternative approaches are therefore economical and practicable. Second, they do not rely on as many assumptions as ipsi-contralateral Remaining Effectiveness comparisons. Negative C-T delays and curve shape comparison are therefore both practically and theoretically superior.

## Conclusion

In retrospect, it is clear that the present experiment should have used negative C-T delays or curve shape comparison instead of within subject comparisons between ipsi- and contralateral Remaining Effectiveness. This would have expanded the pool of suitable subjects while reducing the overhead of methodological assumptions. Even so, the results would probably have been quite similar. In the case of #1086, contralateral Remaining Effectiveness was flat across all C-T delays, while ipsilateral Remaining Effectiveness rose in step with effectiveness. In effect, curve shape comparison was used with #1086. With #1111, there were no differences in contralateral Remaining Effectiveness at different C-T delays. Ipsilaterally, Remaining Effectiveness was greater at 35 ms than at 15 ms, and there was no difference between the 35 and -5 ms conditions. Essentially, therefore, negative C-T delays were used with #1111.

Even when these alternative analyses are used with #1086 and #1111, the results support the incidental MH-LH inhibition hypothesis enunciated in the introduction to the present experiment.

Together with the anatomical findings discussed in the introduction to the present experiment, #1086's and #1111's results are highly suggestive. All the statistically significant differences that were found support the hypothesis that the shortfall in LH T pulse effectiveness is caused by the incidental stimulation inhibitory MH afferents. However, these results certainly do not prove that the shortfall is caused by inhibitory afferents. Nor do they prove that other inhibitory factors are not involved. Nonetheless, they do show that the incidental inhibition hypothesis merits serious consideration.

## Appendix 4: Acronyms

C: Conditioning (pulse)

C-C: Conditioning pulse to Conditioning pulse

C-T: Conditioning pulse to Test pulse

CP: Caudate Putamen

DP: Double Pulse

E: Effectiveness of equal current T pulses

$E_{T>C}$: Effectiveness of higher current T pulses

$F_{CSP}$: SP Frequency threshold with the C pulse current

$F_{DP}$: Frequency threshold in the Double Pulse condition

$F_{SP}$: Frequency threshold in the Single Pulse condition

$F_{TSP}$: SP Frequency threshold with the T pulse current

GABA: Gamma Aminobutyric Acid

*HSD*: Dunnet's Honestly Significant Difference

ICSS: Intracranial Self Stimulation

i.m.: intramuscular

i.p.: intraperitoneal

LH: Lateral Hypothalamus

LPO: Lateral Preoptic area

MFB: Medial Forebrain Bundle

MH: Medial Hypothalamus

MP: Medial Pons

MPFC: Medial Pre-Frontal Cortex

MR: Median Raphe

*N*: Number of replications per C-T delay

$N_C$: Number of action potentials generated by a C pulse

$N_T$: Number of action potentials generated by a T pulse

$N_{TSP}$: Number of action potentials generated by the large T pulse current in a Single Pulse train

*p*: Probability of type 1 error

PAG: Periaqueductal Grey

r.m.s.: root mean square

s.c.: subcutaneous

*SD*: Standard Deviation

*SEM*: Standard Error of the Mean

SP: Single Pulse

SPFC: Sulcal Prefrontal Cortex

T: Test (pulse)

T-C: Test pulse to Conditioning pulse

VAF: Ventral Amygdalofugal pathway

VLT: Ventrolateral Tegmentum

VTA: Ventral Tegmental Area