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The Effects of Anterior Medial Forebrain Bundle Lesions on Self-Stimulation of the Lateral Hypothalamus and Ventral Tegmental Area

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A Thesis

in

The Department

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ABSTRACT

The Effects of Anterior Medial Forebrain Bundle Lesions on Self-Stimulation of the Lateral Hypothalamus and Ventral Tegmental Area

Beverley Murray

Psychophysical data suggest that descending reward fibers directly link the lateral hypothalamus (LH) and ventral tegmental area (VTA) (Bielajew & Shizgal, 1982, 1986). As a step toward identifying the nuclei from which these fibers arise, electrolytic lesions were made in the anterior medial forebrain bundle (MFB) and the effect on self-stimulation of both the LH and VTA was examined. Changes in the rewarding effect were inferred from lateral displacements of the function relating the rate of responding to the number of pulses per train of stimulation. Since changes in the rewarding effect can occur following damage at any point along the reward circuit, a behavioural version of the collision technique was used to render the anatomical interpretation of the lesion data less ambiguous. It was reasoned that if a lesion destroyed some of the reward neurons undergoing collision, then the collision effect would decrease in size. Seven rats with self-stimulation electrodes in the LH and/or VTA and lesioning electrodes in the anterior LH served as subjects. Lesions in 5 of the 7 rats displaced the rate-number functions for the LH and/or VTA sites toward higher pulse numbers (26-58% above baseline) consistent with a reduction in the rewarding

impact of the stimulation. Both of the ineffective lesions were restricted to compartment 'c' of the anterior MFB while the five effective lesions invaded the more lateral compartments 'a', 'd', and 'e' (Nieuwenhuys et al., 1982). Lesions reduced the size of the collision effect by 27% to 35% in 2 of the 4 collision subjects. The simplest interpretation of these data is that the lesions in the antero-lateral MFB damaged reward neurons linking the LH and VTA.

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The other members of the Shizgal lab have provided a stimulating and friendly environment in which to work. Stewart Francis and Ivan Kiss were always willing to discuss the issues and offer much needed advice. David Morton, Dwayne Schindler and Stewart Francis wrote the programs for the testing facilities and data analysis. Dwayne has been very helpful and patient during my own attempts at programming. I'd like to thank my office-mates (Beth, Lois, Margaret, Renée and Sandra) for providing an atmosphere of camaraderie and for their stress-management program. Drs. John Yeomans and Ora Kofman introduced me to the field of brain stimulation reward and gave me the confidence to continue.

My deepest gratitude goes to Tuan for his love and for providing an endless supply of encouraging words.

Dedication

This thesis is dedicated to my parents, Berney and Elsie Murray, for their love and support throughout my education.

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Animals implanted with chronic intracranial electrodes can be trained to self-administer trains of electrical pulses to many brain areas. The vigorous manner in which animals engage in this behaviour, even at the expense of satisfying physiological needs (Deutsch, Adams & Metzner, 1964; Routtenberg & Lindy, 1965), has attracted an enduring interest in brain stimulation reward (BSR) as a model for studying motivational behaviour. The neural circuits activated by these pulses are believed to be involved in the rewarding experience of naturally occurring reinforcers such as food (Hoebel & Thompson, 1969; Rolls, Burton & Mora, 1980) and the rewarding impact of drugs of abuse (Wise, 1980).

After over thirty years of research on the phenomenon of brain stimulation reward (BSR), the directly activated neurons that carry the reward signal have eluded discovery. The complexity of the brain areas that support the most robust self-stimulation behaviour and the use of inadequate measures to assess the rewarding value of the stimulation have been some of the impediments to progress.

The psychophysical approach to the study of BSR has addressed both of these problems by providing techniques for characterizing the reward-relevant neurons and by developing scaling procedures that are not affected by the arbitrary choice of stimulus parameters. In the last decade an impressive body of research involving the psychophysical characterization of the directly activated neurons in medial forebrain bundle (MFB) self-stimulation has accumulated. Although the neurons activated at the electrode tip have not yet been identified, the number of candidate pathways has been considerably

reduced (Gallistel, Shizgal & Yeomans, 1981). These more recent advances have provided hope that the neural pathways subserving BSR will soon be identified and that psychologists may one day have a model for studying the neural basis of motivation and learning in vertebrates.

The Psychophysical Approach

Given that there are approximately fifty fiber pathways coursing through the MFB (Nieuwenhuys, Geeraedts & Veening, 1982), it is very likely that an electrode placed within the bundle will activate many neurons besides the ones that actually contribute to the rewarding effect. Although recording the activity of cells activated by self-stimulation electrodes can provide us with their physiological characteristics, this information alone does not provide a basis for deciding whether a particular neuron actually carries the reward signal. If this information is coupled with data on the physiological and anatomical characteristics of reward neurons, then it becomes possible to identify individual neurons that are likely to carry the reward signal. For example, if we know that the reward-relevant neurons have conduction velocites ranging from 1 to 8 m/sec then a neuron with a conduction velocity of 5 m/sec would be a likely candidate whereas a neuron with a conduction velocity of 0.3 m/sec would be an unlikely candidate.

The psychophysical approach is able to characterize those neurons responsible for the rewarding effect of the stimulation through the use of trade-off functions, functions that determine the combinations of two stimulus parameters that produce the same level of behaviour. These trade-off functions are explained in terms of the physiological and anatomical

properties of the directly activated reward-relevant neurons. Inferring physiological characteristics of the directly activated cells from the behaviour of the animal requires that each level of the system being measured behaves in a monotonic fashion. If for every stimulus input to the first stage there is one and only one output from the final stage, then a system is considered monotonic and all of its constituent stages must also be monotonic (Gallistel et al., 1981). If the final output of a monotonic system is held constant, then the initial output of the first stage will also be held constant. Trade-off functions will therefore determine the combinations of two stimulus parameters that produce the same level of excitation in the first stage. In this way, information about the properties of the first stage can be inferred from manipulating the pattern of the stimulation and holding constant the final output. The BSR system has been shown to possess this property of monotonicity over a wide range of stimulation parameters (Edmonds, Stellar & Gallistel, 1974; Gallistel, 1978).

The Collision Test

The most powerful of the psychophysical trade-off functions is obtained using the collision technique which establishes the existence of a direct axonal link between two self-stimulation sites (Shizgal, Bielajew, Corbett, Skelton & Yeomans, 1980; Bielajew & Shizgal, 1982). The collision technique has provided an important means for mapping out the circuitry of the directly activated reward neurons. Trains of conditioning (C) and test (T) pulses are delivered to two self-stimulation electrodes, each electrode receiving one of the pulses in each pair. Each pulse will trigger two volleys

of action potentials at each electrode; one travelling towards the terminal region (orthodromic) and one travelling towards the somata (antidromic). If the two electrodes activate the same reward fibers at different sites along their trajectory then, at short C-T intervals, the orthodromic volley from the electrode closest to the somata will collide with the antidromic volley from the electrode closest to the terminals. When this collision occurs, only the orthodromic volley from the electrode closest to the terminals will reach the terminals and therefore transmit the reward signal to the next stage of the system. When the C-T interval is increased so that the first volley and its trailing refractory period zone has had time to pass by the second electrode before the T-pulse arrives, then the orthodromic volley from both electrodes will propagate successfully to the terminal region.

Evidence of collision between two self-stimulation sites is inferred from a decrease in the rewarding efficacy of the stimulation when the C-T interval is decreased. This decrease in the rewarding impact of the stimulation is presumably due to the loss of action potentials due to collision. In order to keep constant the number of action potentials reaching the terminals, and thus the behaviour of the animal, the firings lost due to collision are replaced by increasing the frequency (i.e. the number of pulse pairs in a train of fixed duration). Thus, a decrease in the rewarding efficacy of the stimulation is inferred from a lateral shift in the rate-frequency (rate-number) function towards higher frequencies (number of pulse pairs). If all of the fibers stimulated at one site are also stimulated at the other then, at short C-T intervals, half of the action potentials generated by the two pulses will be removed due to collision. In this case, a train of pulse pairs will be

no more effective than a train of equally spaced single pulses, and pulse-pair effectiveness values (E-values) of zero will be assigned to those intervals. If none of the reward fibers are stimulated at both sites then collision cannot occur and both volleys of action potential will arrive at the terminals. In this case, a train of pulse pairs will be twice as effective as a train of equally spaced single pulses, and E-values of 1.0 will be assigned. If only half of the fibers are common to the two sites, then only 1/4 of the action potentials will be removed due to collision. In this case, a train of pulse pairs will be 1.5 times more effective than a train of equally spaced single pulses, and E-values of .5 will be assigned at short C-T intervals. In all the above cases, stimulation at long C-T intervals will be twice as effective as a train of single pulses (assuming perfect summation of the rewarding effects produced by the two electrodes) and will therefore be expressed as E-values of 1.0. The difference between the pulse-pair effectiveness values obtained at short and long C-T intervals (assuming perfect summation at long C-T intervals) therefore gives the proportion of fibers common to the two sites of stimulation.

The C-T interval at which the increase in stimulation effectiveness is observed is called the collision interval and can be broken down into the time it takes for the volley of action potentials to travel between the two electrodes and the refractory period of the neurons stimulated by the T-pulse. If behavioural estimates of the refractory period are obtained and the distance between the two electrodes measured, then the collision interval can provide an estimate of the conduction velocity of the fibers undergoing collision. Since there exists a strong correlation between fiber diameter and

conduction velocity (Hursch, 1939; Waxman & Bennett, 1972), the caliber of the directly activated neurons can be estimated from the collision test.

Combining Lesions with the Psychophysical Approach

Earlier efforts to map the circuitry for BSR made extensive use of the lesion technique. Various brain regions were damaged by a host of lesioning methods and the effects on self-stimulation behaviour examined. The rationale for the lesion technique is that if any region is a critical component of the circuitry for BSR, then removing that region should alter the rewarding effect of the stimulation. Unfortunately these early lesion studies examined the effect of lesions on the rate of operant responding (e.g. Boyd & Gardner, 1967; Lorens, 1966; Olds & Olds, 1969), a methodology that confounds changes in reward with changes in performance. Hodos and Valenstein (1962) were the first to show that rate measures were not good indices of the rewarding value of the stimulation. Rats given a choice between two levers that deliver different levels of stimulation learn to alternate their behaviour between the two levers. Hodos and Valenstein found that rats always preferred the stronger stimulus regardless of their rate of responding for each stimulus when presented alone. They concluded that rate measures were not a good indication of the rewarding value of the stimulation.

In contrast to rate measures, the curve-shift paradigm allows an experimenter to dissociate changes in the rewarding efficacy of the stimulation from changes in the subject's performance for the stimulation.

The rate of lever pressing is measured for a range of stimulation frequencies such that the behaviour ranges from zero to maximum responding. These curves are generally very steep so that a change in frequency of only 0.1 $\log_{10}$ units (26%) can be enough to change the subject's response rate from zero to asymptote. Changes in the position of the rising portion of the rate-frequency function along the frequency axis are thought to reflect changes in the rewarding impact of the stimulation. If the rising portion of the curve is shifted towards higher frequencies, then the subject requires more stimulation before responding begins and the stimulation is therefore assumed to be less rewarding. Changes in asymptotic rate are thought to indicate alterations in the subject's capacity or motivation to perform the operant response. Validation studies have shown that the position of the rate-frequency functions along the frequency axis is relatively insensitive to performance manipulations. Making the animal run up a gradient, injection of paralytic agents, and adding weights to the lever have all been shown to produce a marked reduction in the asymptotic rate while producing little or no shift in the position of the rising portion of the curve (Edmonds & Gallistel, 1974; Miliaressis, Rompré, Laviolette, Philippe & Coulombe, 1986). These studies suggest that a reduction in the rate of responding for a single level of stimulation following a lesion may be due to either a reduction in the rewarding impact of the stimulation or a performance deficit. Similarly, if a level of stimulation is chosen that produces an asymptotic rate of response, the rate of responding may not change following a lesion even though the rising portion of the rate-frequency function has been shifted laterally. Thus, the entire rate-frequency function must be obtained in order to fully characterize the changes that have occurred after the lesion.

In contrast to the precise anatomical interpretation of the decrease in stimulation efficacy seen in the collision test, decreases in stimulation efficacy following lesions cannot be linked to any one stage of the reward system. The lesion may be damaging reward-relevant fibers at any number of places along the reward circuitry. For example, a lesion efferent to the stimulation electrode will disconnect the directly activated fibers from their output whereas a lesion damaging the directly activated fibers afferent to the stimulation electrode will sever their connection to the soma and cause them to atrophy and die. Alternatively, a lesion may not act directly on the pathway carrying the reward signal but on some gating mechanism that modulates transmission in this pathway (see Biejalew & Shizgal, 1986).

Given the ambiguity of the lesion technique it would seem preferable to delineate the reward circuitry by exclusive use of the psychophysical tests currently available. However, lesions play an important corroborative role when used in conjunction with the psychophysical approach. Even if electrophysiological recording studies find a cell that possesses all the characteristics of the reward-relevant fibers as determined by the psychophysical approach, the possibility still remains that the cell is only an "imposter"; a cell that resembles a reward-relevant cell but in fact is not. If a lesion placed in the region of the cell body was found to decrease the rewarding impact of the stimulation, the plausability of it being an imposter would be considerably reduced. Lesion studies could also serve as a guide for the time-consuming recording studies, pointing them towards likely candidate sites for the reward nuclei. If a lesioned area failed to alter the

rate-frequency function then the electrophysiologist might be advised to search elsewhere.

A more powerful use of the lesion technique, suggested by the present experiment, is in combination with the collision technique. The collision test establishes that reward-relevant fibers link two self-stimulation sites. If a given nucleus is believed to give rise to the fibers that link the two sites, then lesioning all or some of the cell bodies should eliminate or reduce the size of the collision effect. The collision effects reported to date have always been less than 100%; indicating that some proportion of the fibers stimulated at one site are not stimulated at the second site. This implies that self-stimulation would still be possible even if a lesion were to damage all of the collision fibers due to the activation of fibers by only one of the two electrodes.

A lesion that reduced the size of the collision effect would be logically tied to the directly activated neurons linking the two sites. The simplest interpretation of such an effect would be that the lesion had damaged the fibers linking the two sites. It is also possible that the lesion damaged the efferents of the collision fibers, an interpretation which requires that the efferents of the collision and non-collision fibers be spatially segregated. Such a possibility becomes more implausible when the data from lesion and recording studies are considered together. It would be unlikely that a collision-reducing lesion had damaged efferents to the directly activated neurons if the lesioned nucleus also contained cells with anatomical and physiological characteristics identical to those of the directly activated reward neurons.

Since the collision fibers represent only a proportion of the reward fibers stimulated, other outcomes besides a reduction in the size of the collision effect are possible with a reward-degrading lesion. The total number of reward fibers (N_t) stimulated at the two sites can be broken down into collision (N_c) and non-collision (N_{nc}) fibers. The proportion of fibers undergoing collision can be expressed as the ratio of collision to total fibers or N_c / (N_c + N_{nc}). If a lesion only damaged the non-common fibers, then the denominator (N_c + N_{nc}) would decrease and the proportion of fibers undergoing collision would increase. If an equal number of collision and non-collision fibers were damaged by the lesion, then the proportion of fibers undergoing collision would remain the same.

Rationale For The Present Experiment

Previous psychophysical studies have shown that common reward-relevant fibers link the LH and VTA (Shizgal et al., 1980; Bielajew & Shizgal, 1982) and that at least a subset of these fibers descend from the LH to the VTA (Bielajew & Shizgal, 1986). Refractory period estimates for these fibers range from 0.5 to 1.2 msec (Yeomans, 1975, 1979) and conduction velocity estimates (1-8 m/s) suggest that the fibers are thin and myelinated (Bielajew & Shizgal, 1982). It is still not known where these fibers arise or terminate.

Several forebrain nuclei give rise to fibers that pass through the MFB and are therefore candidate sites for the origin of the descending MFB reward neurons. However, several recent lesion studies employing the curve-shift paradigm have called into question the importance of fibers arising anterior to the LH.

Stellar and Neeley (1982) implanted electrodes at an anterior and posterior MFB site and assessed the effect of an electrolytic lesion at one site on self-stimulation of the other. They concluded that the large anterior lesions did not degrade the reward at posterior stimulation sites and had mixed effects on performance. However, one of their subjects with an anterior lesion showed a large shift in the rate-frequency function of approximately 0.4 $\log_{10}$ units (150%) that recovered to baseline values by the eighth day post-lesion. It is not clear why this particular lesion produced such a large effect while similar lesions were ineffective.

Janas and Stellar (1987) found more consistent effects with large knifecuts that transected the anterior MFB at the level of the caudal lateral preoptic area (LPO). Stable shifts in the rate-frequency function that ranged from 0.16 to 0.50 $\log_{10}$ units were seen for up to ten days of post-knifecut testing in three of the four rats. A fourth subject also showed a shift in the rate-frequency function of 0.28 $\log_{10}$ units but this shift was only seen on the first day of testing. When the knifecut was moved to a more anterior level of the MFB below the anterior commissure, the lesions were much less effective in degrading reward in the three animals tested. Shifts in the rate-frequency function of 0.17-0.19 $\log_{10}$ units were seen in two subjects but only on the first day of testing.

Waraczynski (1988) made knifecuts in various forebrain regions that have been considered potential sites for the nuclei of the descending MFB reward fibers. She found that knifecuts in the region of the diagonal band of Broca were either ineffective or produced small transient shifts in the rate-frequency function obtained at the LH. Knifecuts in the LPO resulted in a

variety of effects on the rate-frequency function. One group of 6 rats had appreciable shifts in the rate-frequency function towards higher frequencies (approximately 0.1-0.3 $\log_{10}$ unit shift) that often lasted for more than a week of post-knifecut testing. A second group of subjects with similarly placed knifecuts showed either a transient or erratic shift in the rate-frequency function. A third group of animals, with knifecuts more medially situated, showed a shift in the rate-frequency function towards lower frequencies, indicating an increase in the rewarding impact of the stimulation following the lesion. When knifecuts were situated just anterior to the LH stimulating electrode, a similar pattern of effects was seen. Only one of the anterior MFB knifecuts produced a substantial shift in threshold lasting for the 12 days of testing. Other knifecuts in this region had no effect on the rate-frequency function, produced only small transient shifts or shifted the rate-frequency function toward lower frequencies.

In all of these studies, some lesions to the anterior MFB were able to produce small decreases in the rewarding effect of stimulating more caudal portions of the MFB. These data indicate that the anterior MFB does play some role in MFB reward. The inconsistencies in the lesion data presented above need not cast doubt on this conclusion, but instead suggest that subtle differences in the location of the lesion and/or stimulation site may determine whether or not the lesion alters the rewarding effect. If so, then small, well-defined lesions may be more useful than large lesions or knifecuts in assessing the role of the anterior MFB in MFB reward.

In order to further investigate the role of the anterior MFB on MFB reward, electrolytic lesions were made in the anterior LH and the effect on

collision between the middle LH and VTA was examined. It was reasoned that if a lesion damaged neurons that were undergoing collision, then a decrease in the size of the collision effect would be observed after the lesion. Since rate-number curves obtained at the LH and VTA are used to scale the pulse-pair data, the effect of lesions on the required number of single pulses at the LH and VTA was also assessed.

Method

Subjects

Seven male, old colony rats of the Long-Evans strain (Charles River Breeding Farms) served as subjects. The animals were individually caged with unlimited access to food and water and maintained on a reverse 12 hour light/12 hour dark cycle. Weight at the time of surgery varied from 350 - 440 grams.

Surgery

Subjects were food deprived for approximately 12 hours before surgery. Atropine sulphate was administered 20 minutes prior to anaesthesia in order to reduce mucous secretions. Surgery was performed under sodium pentobarbitol anaesthesia (Somnatol, 60 mg/kg i.p.) with supplements administered as required.

Fixed electrodes were constructed from 0.25 mm stainless steel rods insulated with Formvar except for their rounded tips. Male Amphenol pins were attached to a flexible wire soldered to the electrode. Moveable electrodes were of the type designed by Miliaressis (1981). The upper portion of the moveable electrode fit snugly within a nylon tube and was secured by a set screw; the tube was attached to the skull and anchoring screws by dental acrylic. In order to lower the electrode, a calibrated driver was attached, the set screw was loosened and the threaded feed shaft

of the driver rotated in quarter turn increments. Each quarter turn caused the electrode to move approximately 80 um.

Fixed stimulating electrodes were aimed at the LH using the following flat-skull coordinates: -2.8 mm from bregma, 1.7 mm lateral to the mid-sagittal suture, and 7.8 mm below the dura mater. The coordinates for the fixed lesioning electrodes aimed at the anterior LH were: -1.3 mm from bregma, 2.2 mm lateral, and 7.8 mm below dura. Moveable electrodes were aimed at the anterior VTA using the coordinates: -4.8 mm from bregma, 1.0 mm lateral, and 7.2 mm below dura. Petroleum jelly applied to the top of the moveable electrode shaft prevented dental acrylic from binding to the electrode. A stainless steel wire wrapped around four jeweller's screws imbedded in the skull served as the anode.

After the electrodes were implanted and secured to the skull with dental acrylic, the male Amphenol pins attached to the fixed electrodes and the stainless steel wire were inserted into a 9-pin, externally threaded connector and cemented onto the head of the rat with dental acrylic. By means of an internally-threaded ring, this connector was mated firmly during testing with a matching connector mounted at the end of the stimulation cable.

Several days were allowed for recovery before testing began.

Stabilization

Apparatus

Subjects were initially screened for self-stimulation and their performance subsequently stabilized in wooden boxes measuring 25 cm (w) x 25 cm (d) x 70 cm (h), with Plexiglas front panels and wire mesh floors. A Lehigh Valley rodent lever was located in the center of the left wall approximately 5 cm from the floor. Located 5 cm above the lever was a yellow 'jewel' light measuring 1.5 cm in diameter. The stimulation cable attached to the 9-pin connector on the subject's head was connected to the stimulator by a 7-channel, slip ring commutator fixed in the center of the ceiling of the testing cage.

Depression of the lever resulted in a 0.5 sec train of 0.1 msec, cathodal, rectangular pulses. Since the train duration was held constant for all testing, the number of pulses (or pulse pairs) per train of stimulation co-varied with the frequency. The temporal parameters of the stimulation were controlled by hand-set integrated circuit pulse generators. The stimulation pulses were produced by dual constant-current amplifiers (Mundl, 1980) and the amplitude of the pulses was set by a potentiometer. Current was monitored by measuring the voltage drop across a 1 kohm resistor in series with the rat. Accumulation of charge at the electrode-brain interface was minimized by a circuit that shorted the stimulator outputs through a 1 kohm resistor when no pulse was present.

Procedure

Following recovery from surgery, the impedance of the electrode-brain interface for each electrode was measured in each subject by noting the voltage drop across the electrode for a current of 200 uA. Electrodes with very low resistance (less than 5 kohms) were not used in any of the experiments since it was assumed that there was a leak in the insulation covering the electrode shaft.

Subjects were initially screened for self-stimulation using a current of 200 uA and a frequency of approximately 40 Hz. If sniffing and exploration were observed then the current was gradually increased and conventional shaping procedures were used in an effort to train subjects to self-stimulate. Screening was terminated if the stimulation appeared aversive (e.g., the subject vocalized or withdrew from the lever). Animals that could be trained to self-stimulate were allowed unlimited access to the stimulation for approximately 30 minutes on the first day. On subsequent training sessions, the animals were again shaped to self-stimulate and then access to the stimulation was withdrawn until lever pressing had extinguished. This process was repeated until, following an extinction trial, animals would reliably return to the lever after 5 trains of priming pulses. At this point in the training, animals were given access to the stimulation for 30 second trials and the number of pulses per train was decreased after each trial until the animals would no longer respond for the stimulation. This process was repeated several times until the animal would reliably stop responding at approximately the same number of pulses per train. Animals were tested with a descending series of pulses for various current intensities in order to

determine the range of frequencies for which the subject would reliably self-stimulate. These parameters were used as a guide for later testing in the computer-operated equipment.

If a subject could not be shaped to self-stimulate for stimulation delivered to the moveable electrode, the subject was briefly anaesthetized with a short-acting inhalant (Metafane) and the electrode lowered by 320 um. Screening was then repeated as above.

Collision Test

Apparatus

The computer-operated setup used for the collision test was similar to the hand-operated equipment used for screening and training. Only those aspects of the computer-operated setup that differ from the hand-operated setup will be described below.

Test chambers for the computer-operated setup consisted of Plexiglas boxes measuring 25 cm x 25 cm x 75 cm with hinged doors on the upper half of the front face and removeable floors. Lehigh Valley rodent levers were located on opposite walls of each test box 5 cm from the floor and 5 cm from the nearest corner. A yellow jewel light measuring 1.5 cm in diameter was located 3 cm above one lever and a red jewel light was similarly placed above the other lever. The test chambers were mounted in 50 cm x 50 cm x 90 cm plywood boxes insulated with 2.5 cm of Styrofoam. Removeable front panels containing Plexiglas inserts allowed viewing of the

subject via remote control video camera from an adjoining room. A single 40 watt bulb illuminated the test chamber.

Temporal parameters of the stimulation for each test cage were controlled by a dedicated microprocessor with a custom-built interface. A bank of relays controlled by the parallel port of the dedicated microprocessor determined which electrode would deliver the stimulation. Pulse amplitude was determined by a digital to analog converter attached to a voltage-controlled constant current amplifier (Mundl, 1980).

Procedure

In order to test for collision between the LH and VTA electrodes, trains of pulse pairs (conditioning (C) and test (T) pulses) were delivered, each electrode receiving one of the pulses in each pair. The time between the delivery of the C-pulse and the delivery of the T-pulse (C-T interval) was varied from 0.2 to 10.0 msec. In order to determine the number of pulse pairs required to support half-maximal responding (the "required number") at each C-T interval, the number of bar presses in a 30 second trial was recorded for a range of frequencies. Each trial began with the overhead light turning off and on followed by 5 priming trains of stimulation. Each train of priming stimulation was identical to the stimulation that would be available to the animal during the rest of the trial. Following the priming stimulation, the light located above the lever came on to signal that the subject could press the lever in order to self-administer the stimulation.

A starting frequency was chosen so that the the subject would respond near his maximum rate on the first trial. If the animal did in fact respond

during the first 30 second trial then the maximum response rate was estimated by increasing the number of pulse pairs delivered in subsequent trials by 0.1 $\log_{10}$ unit steps until the number of responses did not exceed the number recorded on the the previous trial by more than 10%. If the rat did not respond on the first trial, then the number of pulse pairs was increased by 0.3 $\log_{10}$ units on the next trial and the search for the maximum response rate was continued as above. A ceiling value was placed on the maximum number of pulse pairs that could be delivered so that the algorithm would never deliver an excessive stimulus. After the maximum response rate had been estimated, the number of pulse pairs was then lowered to 0.1 $\log_{10}$ units below the starting number of pulses and decreased in 0.1 $\log_{10}$ unit steps until the number of lever presses for two consecutive trials was less than 10% of the maximum response rate recorded for that C-T interval. In this manner, the function relating rate of lever pressing to the number of pulse pairs per train was obtained. The number of pulse pairs required to support half-maximal responding was then interpolated from this function for each C-T interval.

The C-T intervals tested were presented in two counter-balanced quasi-random orders, each order presented in alternate sessions. In order to obtain a pulse-pair effectiveness value (E-value) for each C-T interval, the required number of pulse-pairs was compared to the required number of single pulses. These single pulse determinations were obtained in an analogous manner to the paired pulse determinations except that the trains of stimulation consisted of evenly spaced single pulses instead of pulse pairs. The single pulse determinations were interspersed throughout the session (every four to

five paired pulse determinations) in order to check for shifts due to fatigue. The session began with four single pulse determinations (two for each electrode) which were used as a warm-up and therefore not included in the data analysis. Each session lasted about 1.5 hours. Sessions with the C-pulse applied to the anterior electrode (A-P condition) were alternated with sessions with the C-pulse applied to the posterior electrode (P-A). If after approximately two sessions of each condition (A-P and P-A) a rise in effectiveness of the stimulation was not apparent with increasing C-T interval, the moveable electrode was lowered by 160 um. This process was continued until evidence of collision (rise in stimulation effectiveness) was apparent or the subject would no longer self-stimulate. If a rise in effectiveness was evident at a given electrode site, six replications for each condition (A-P and P-A) were run and then a lesion was made through the lesioning electrode which was aimed at the anterior LH. The first post-lesion session was run approximately 1 hour after the lesion. At least six replications of the A-P and P-A condition were obtained post-lesion. Following the collection of the post-lesion sessions, further lesions were made through the same electrode in some subjects.

In those cases where collision was not found or self-stimulation was never obtained at the VTA, single-pulse determinations were run at the LH. Four to six rate-number curves were collected per session and at least 5 sessions were run before lesioning. Serial lesions were made through the same electrode following the collection of post-lesion data.

Lesion Parameters

Direct current was passed using the lesioning electrode as the anode and the skull screws as the cathode. Initial lesion parameters varied, depending on the subject, from 0.2 mA - 1.0 mA for 10 seconds. In some subjects second and third lesions were made, following post-lesion data collection, through the same electrode but at a higher current. Later lesion parameters varied, depending on the subject, from 1.0 mA - 2.0 mA for 10 seconds.

Data Analysis

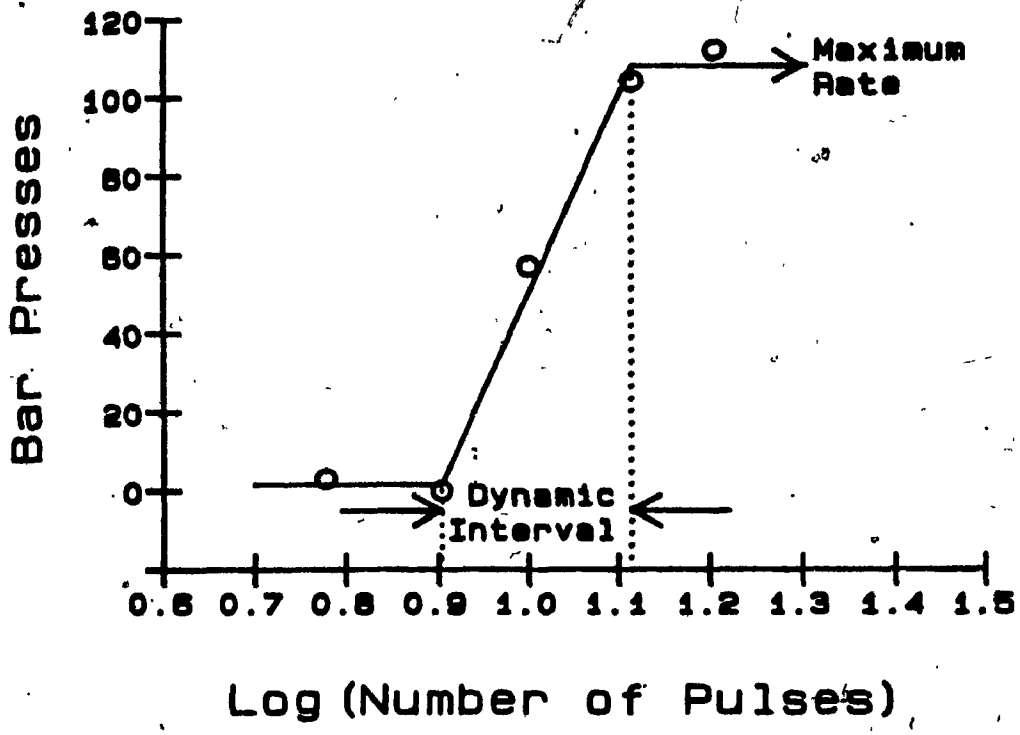
Single Pulse Data

The number of single pulses required to support a half-maximal rate of responding was interpolated from each function relating bar pressing rate to the logarithm of the number of pulses per train. The average of the required number of single pulses was determined for each session pre- and post-lesion. The logarithim of the required number of single pulses was plotted for each session as a function of time after lesioning.

A "broken-line" function, as used by Gallistel and Freyd (1987), was fit to each rate-number curve. An example of such a function is shown in Figure 1. The broken-line function is composed of a straight line joining an upper and lower asymptote. The four parameters of the broken-line function were chosen so that the residual sum of squares was minimized. The maximum rate for each required number determination was estimated from the upper asymptote of the broken-line function. The range over which the

Figure 1. Example of a broken-line function fit to actual rate-number data (open circles) from subject B1. The maximum rate for each determination was estimated from the upper asymptote and the dynamic interval from the difference between the upper and lower break-points.

BROKEN-LINE FIT



functions rose (the dynamic interval) was obtained from the difference between the upper and lower break-points.

Collision Data

The number of pulse pairs required to support a half maximal rate of responding was interpolated for each C-T interval from the functions relating bar pressing rate to the logarithm of the number of pulse pairs per train. The required number of single pulses was then used to scale the required number of pulse pairs using a modified version of Yeomans' effectiveness formula:

$$E = \frac{RN_l}{RN_{ct}} - 1 \times \frac{RN_h}{RN_l}$$

where E = effectiveness of paired-pulse stimulation

RN_l = lower of the required number values for the SP condition

RN_{ct} = number of pulse pairs required to meet the behavioural criterion (CT condition)

RN_h = higher of the required number values for the SP condition

E-values for the A-P and P-A conditions were averaged across those sessions where the range of the logarithm of the required number of single pulses did not exceed 0.15. E-values for the pre-lesion and post-lesion sessions were averaged separately and plotted as a function of C-T interval.

A broken-line function was fit to the data relating E-values to the C-T interval in order to estimate the beginning and end of recovery as well as the percent change in E-values before and after the rise.

Histology

At the completion of the experiment, animals were given an overdose of Somnotol (sodium pentobarbitol) and perfused intracardially with physiological saline followed by 10% Formalin. The brains were removed and soaked in 10% Formalin for at least one week. Brains were sliced in 20 um thick sections and mounted onto glass slides coated with gelatine. The slides were then stained with formol thionine. Lesions were reconstructed by locating the section with the first sign of the lesion, the largest cross-section of the lesion and the last sign of the lesion. Landmarks located near the lesion and the electrode tips were used to identify their coordinates according to the Paxinos and Watson (1986) stereotaxic atlas.

Results

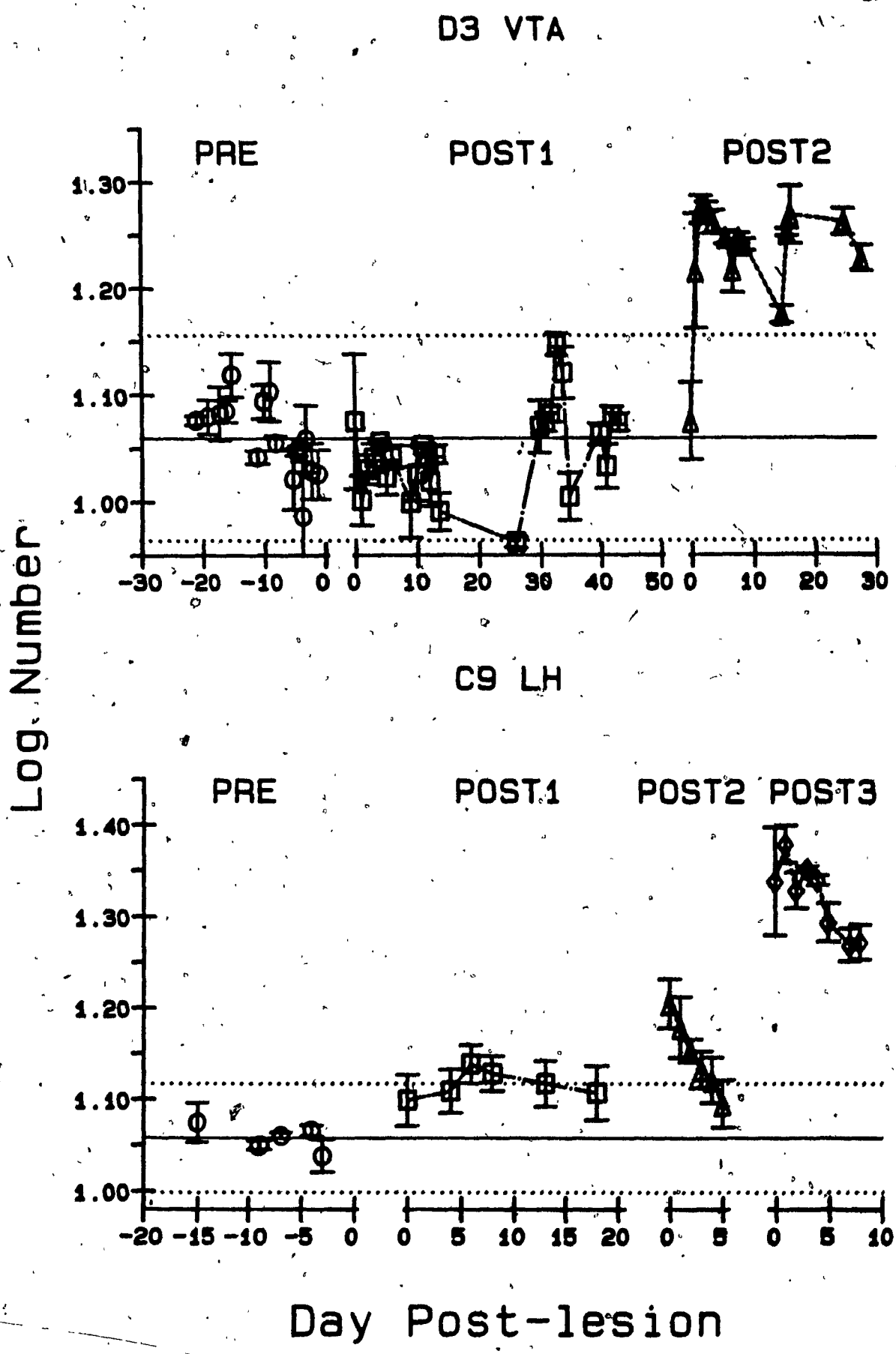
Effects on Rate-Number Functions

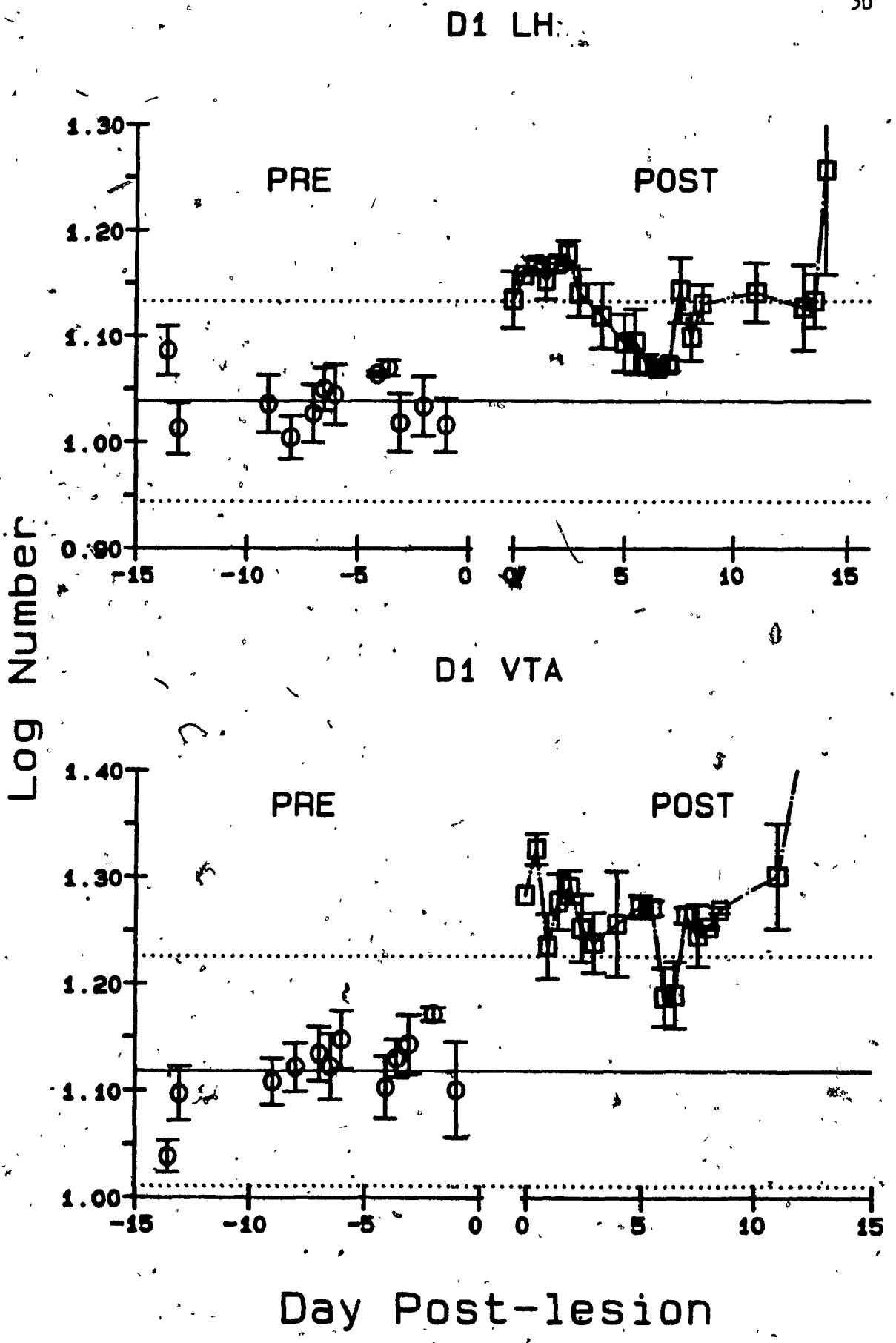
Effective Lesions

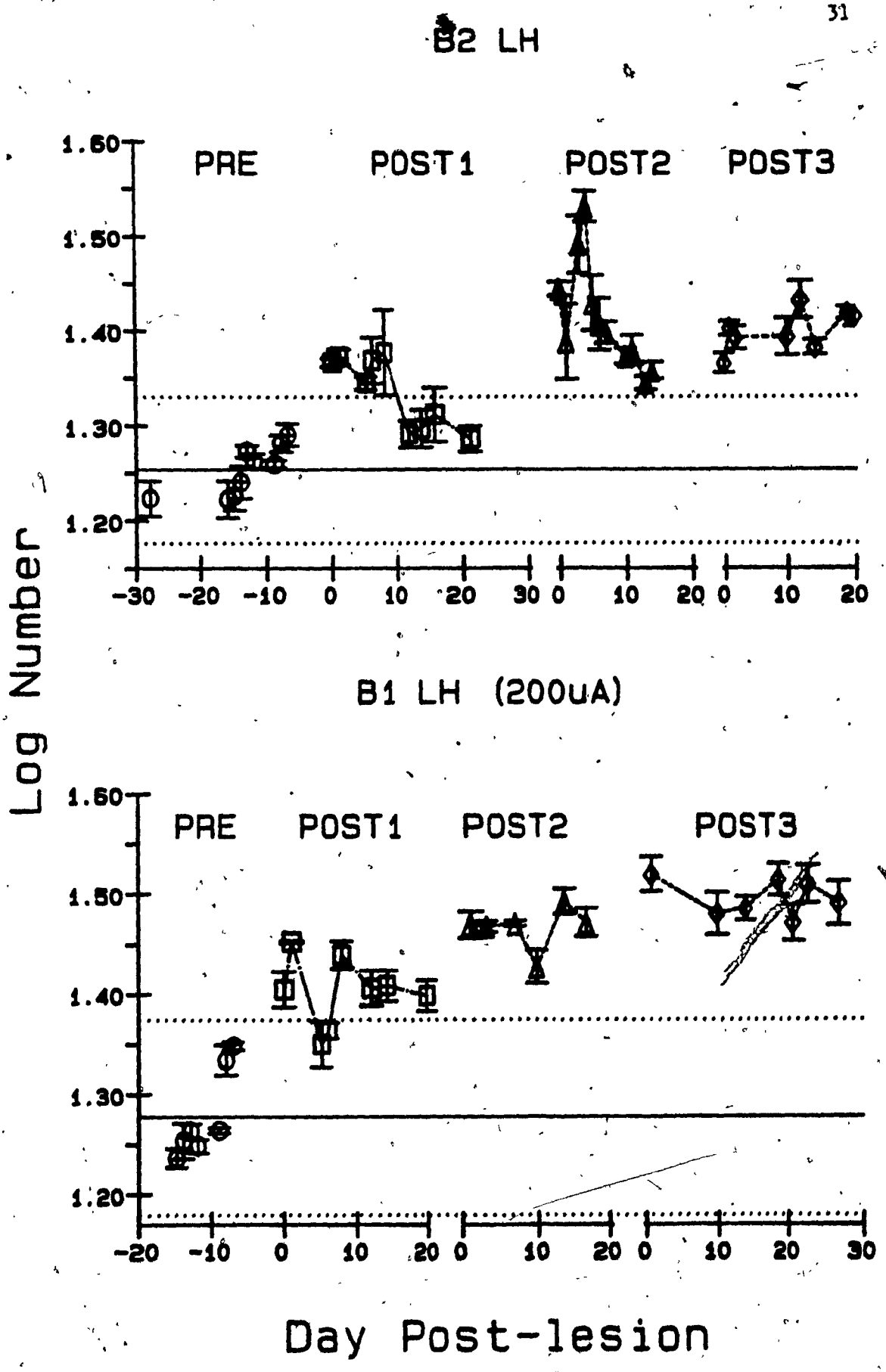
Following lesions to the anterior LH, increases in the required number were seen for 2 VTA electrodes and 4 LH electrodes (Figures 2 to 4). The solid horizontal line extending across each graph indicates the mean of the required number for the baseline data. The dotted lines indicate 2 standard deviations from the mean. In the case of subjects D3 (Figure 2, top panel), B2 (Figure 4, top panel), and B1 (Figure 4, lower panel) the baseline data are not randomly distributed around the mean but instead show definite positive or negative trends. In these cases, post-lesion differences from the mean do not provide a reasonable estimate of the size or significance of the shift in the required number. A better estimate can be obtained from the value of the last few baseline points before the lesion.

The size of the required number shifts ranged from approximately 0.1 log units (26%) to 0.2 log units (58%) although a transient shift of around 0.25 log units (78%) was seen in the case of subject C9 (Figure 2, lower panel, third lesion). Recovery is evident in some of the graphs (C9, second and third lesion; B2, second lesion) although the required number seldom returned to baseline values. In most cases, long-lasting elevations in the required number were seen for up to 27 days of post-lesion testing. The abrupt increase in the required number at the LH and VTA for subject D1, that

Figures 2-4. Lesion-induced changes in the required number of pulses for the effective lesion group. The horizontal, solid lines extending across each of the graphs indicate the mean for the pre-lesion data and the dotted lines indicate 2 standard deviations from the mean. Vertical lines represent the standard error of the mean (s.e.m.) for that session.


D3 VTA
PRE
POST1
POST2
1.30
1.20
1.10
1.00
-30 -20 -10 0 0 10 20 30 40 50 0 10 20 30
C9 LH
PRE
POST1
POST2
POST3
1.40
1.30
1.20
1.10
1.00
-20 -15 -10 -5 0 0 5 10 15 20 0 5 0 5 10
Log. Number
Day Post-lesion


D1 LH
PRE
POST
1.30
1.20
1.10
1.00
0.90
-15
-10
-5
0
0
5
10
15
D1 VTA
PRE
POST
1.40
1.30
1.20
1.10
1.00
-15
-10
-5
0
0
5
10
15
Log Number
Day Post-lesion

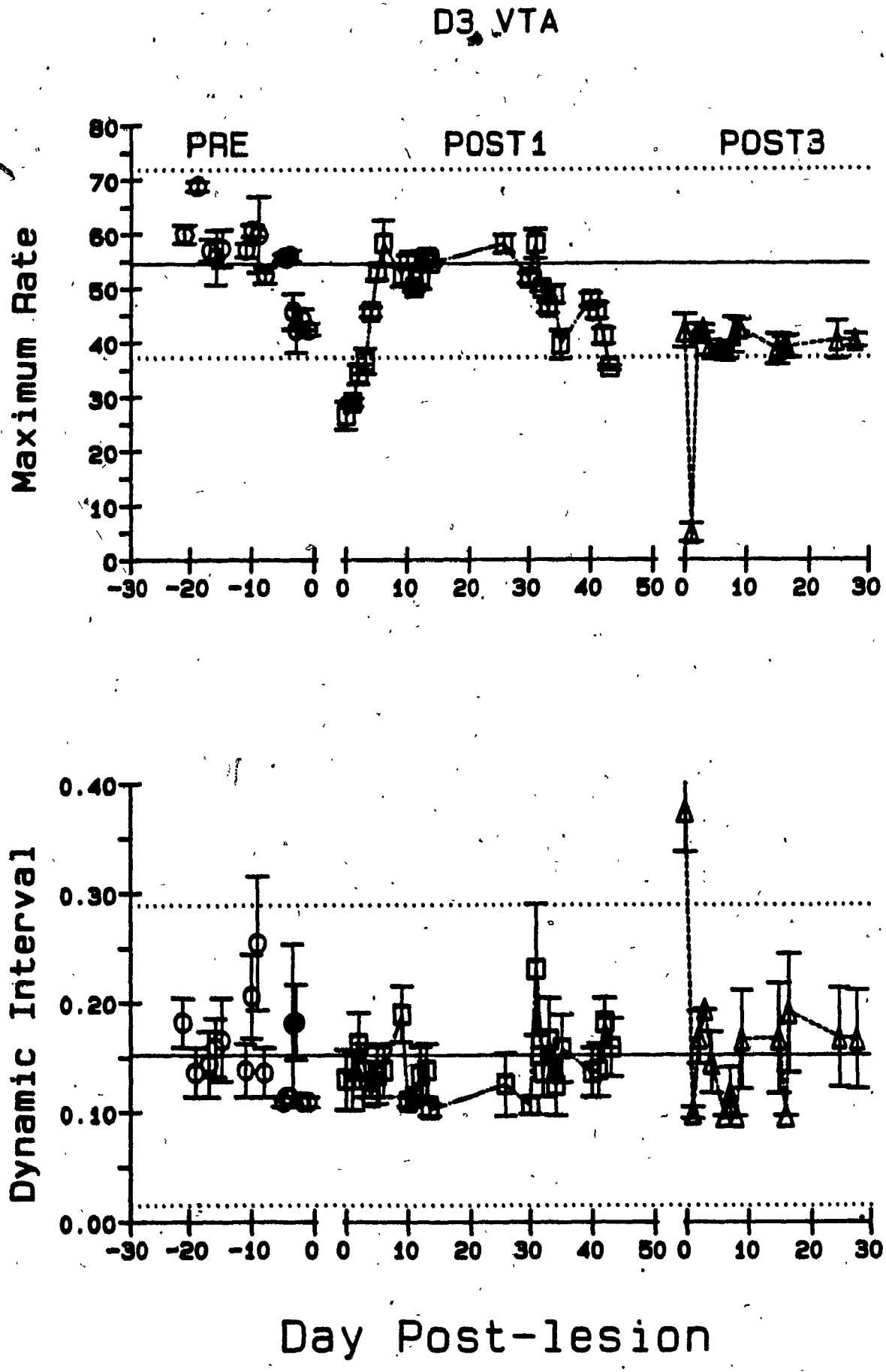

B2 LH
PRE
POST1
POST2
POST3
1.60
1.50
1.40
1.30
1.20
-30 -20 -10 0 10 20 30 0 10 20 0 10 20
B1 LH (200uA)
PRE
POST1
POST2
POST3
1.60
1.50
1.40
1.30
1.20
-20 -10 0 10 20 0 10 20 0 10 20 30
Log Number
Day Post-lesion

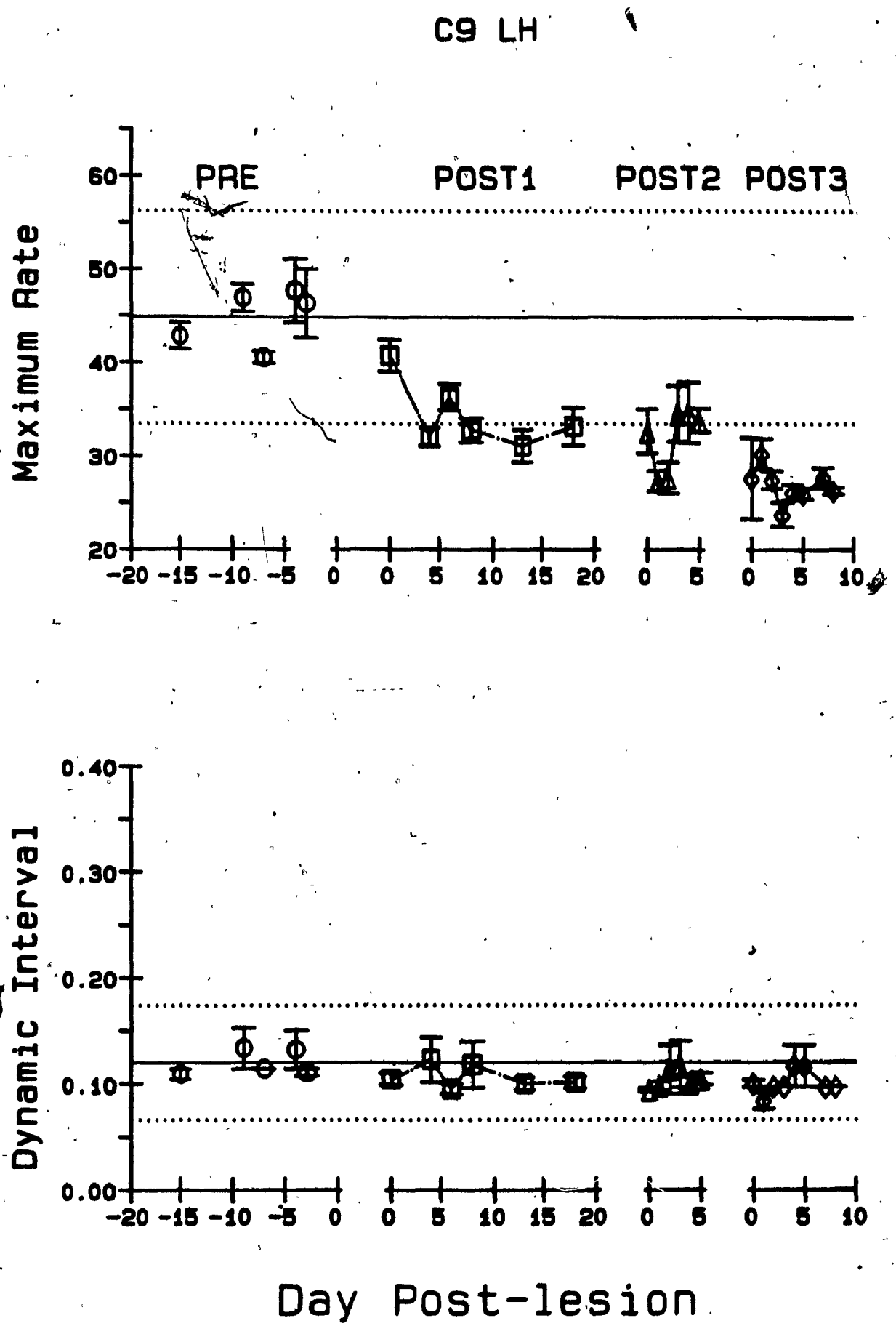
occurred after 10 days of post-lesion testing (Figure 3), preceeded the loss of the electrode assembly. In those subjects with serial lesions through the same electrode, some of the lesions produced larger shifts in the required number than other lesions. For instance, subject C9 (Figure 2, lower panel) showed a substantial shift after the third lesion, but much smaller, transient effects after the first and second lesion.

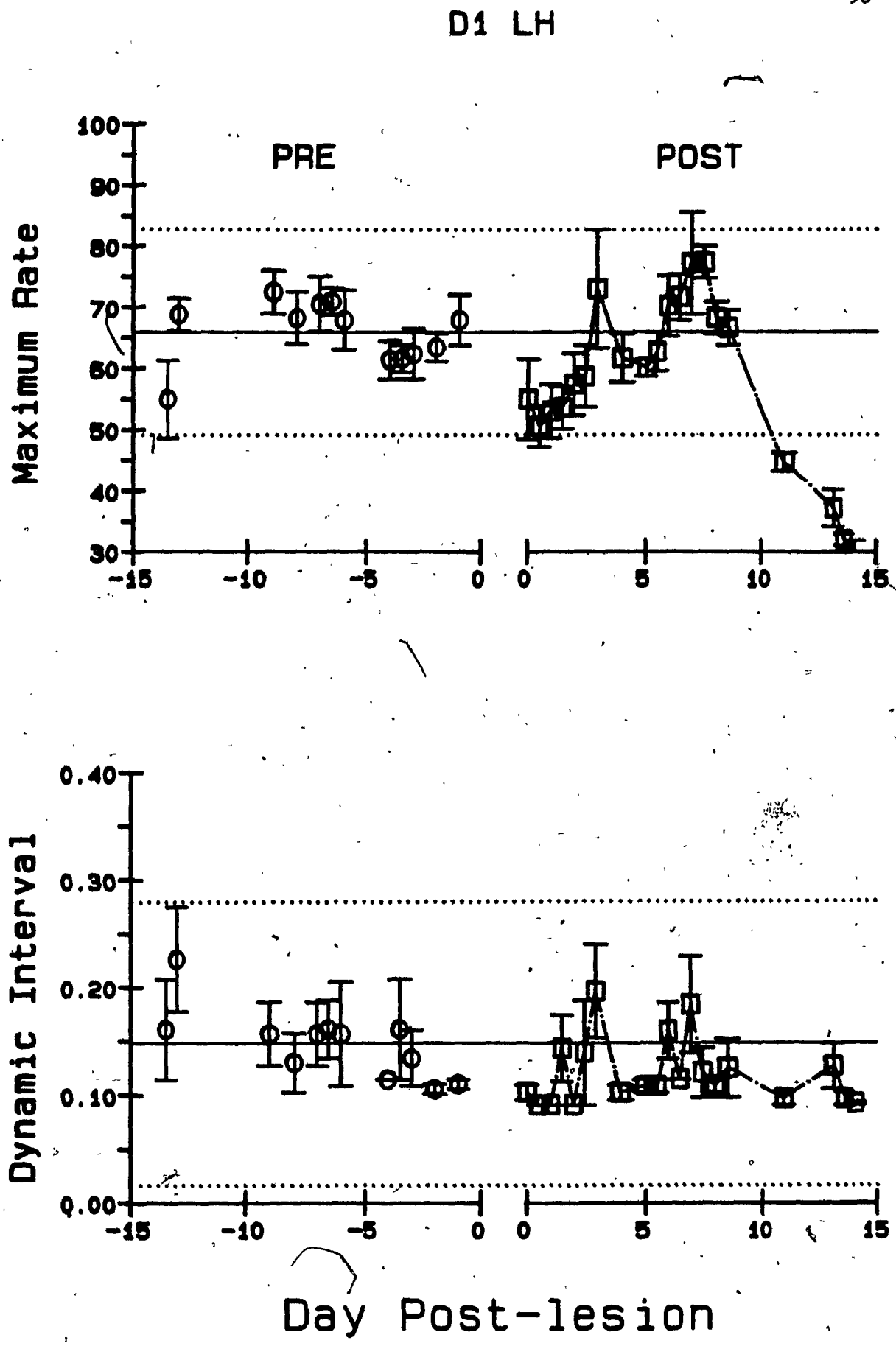
In contrast to the effects on the required number, lesions did not produce long-lasting changes in the maximum response rate as determined from the upper asymptote of the broken-line functions (Figures 5 to 10, upper panels). The depression in the maximum rate was either small (within 2 standard deviations of the baseline) or transient (lasting only a few days post-lesion). Only in the case of subject C9 (Figure 6, upper panel) was there a substantial, long-lasting decrease in the maximum rate. The decrease, however, occurred primarily after the first lesion even though the effects on the required number were seen following the third lesion. As was the case with the baseline data for the required number, the baseline for the maximum rates showed a definite trend in some subjects. This is especially prominent in the case of B1 (Figure 10, upper panel), where the maximum rate falls from 100 to 55 responses/30 sec over the course of baseline testing. Again, a better estimate of the change in post-lesion rates can be obtained from the final days of baseline testing in those subjects with unstable baseline data.

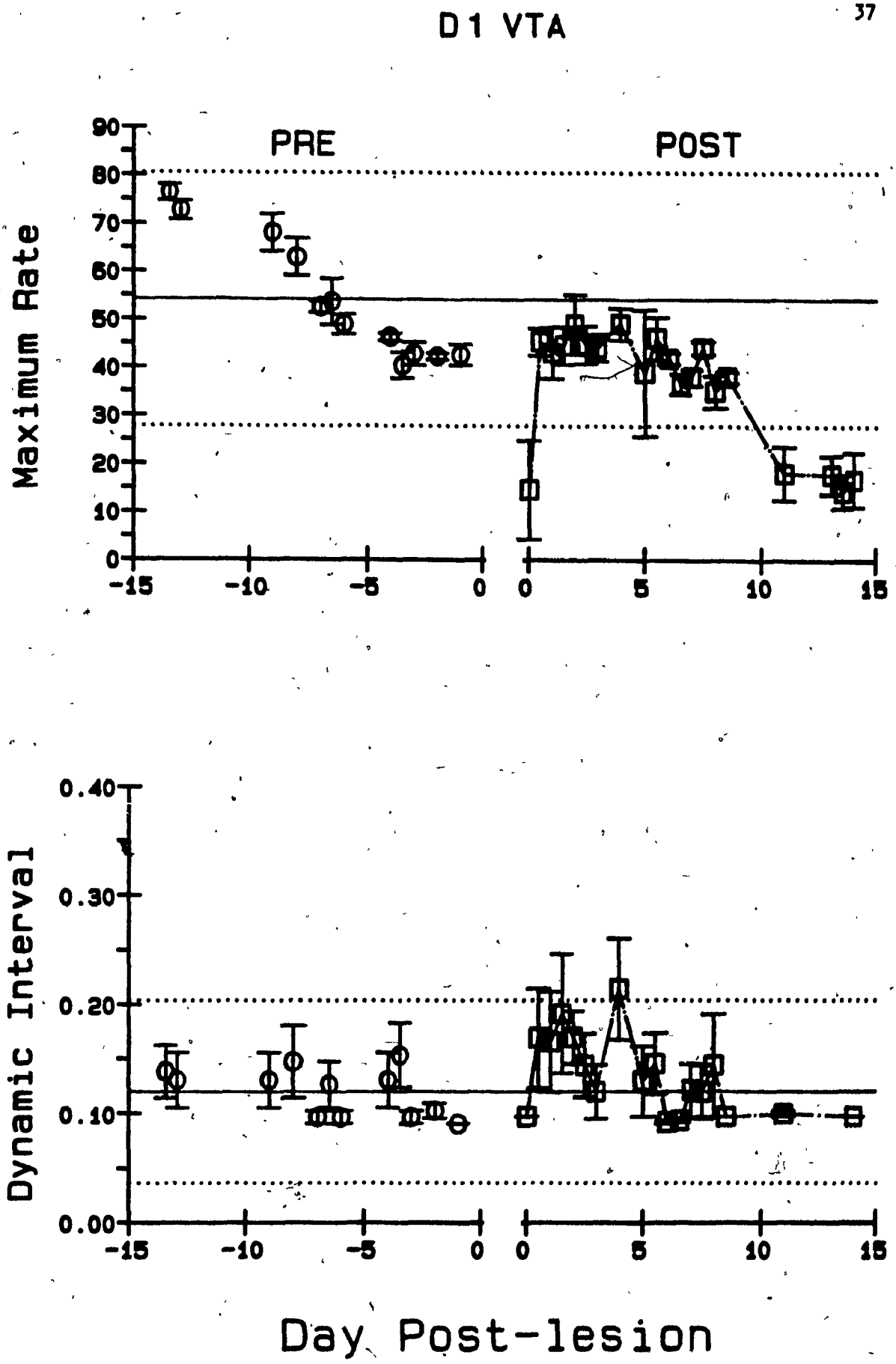
The dynamic interval of the rate-number curves, defined as the difference between the upper and lower break-points obtained from the broken-line functions, did not vary substantially from the dynamic interval of the pre-

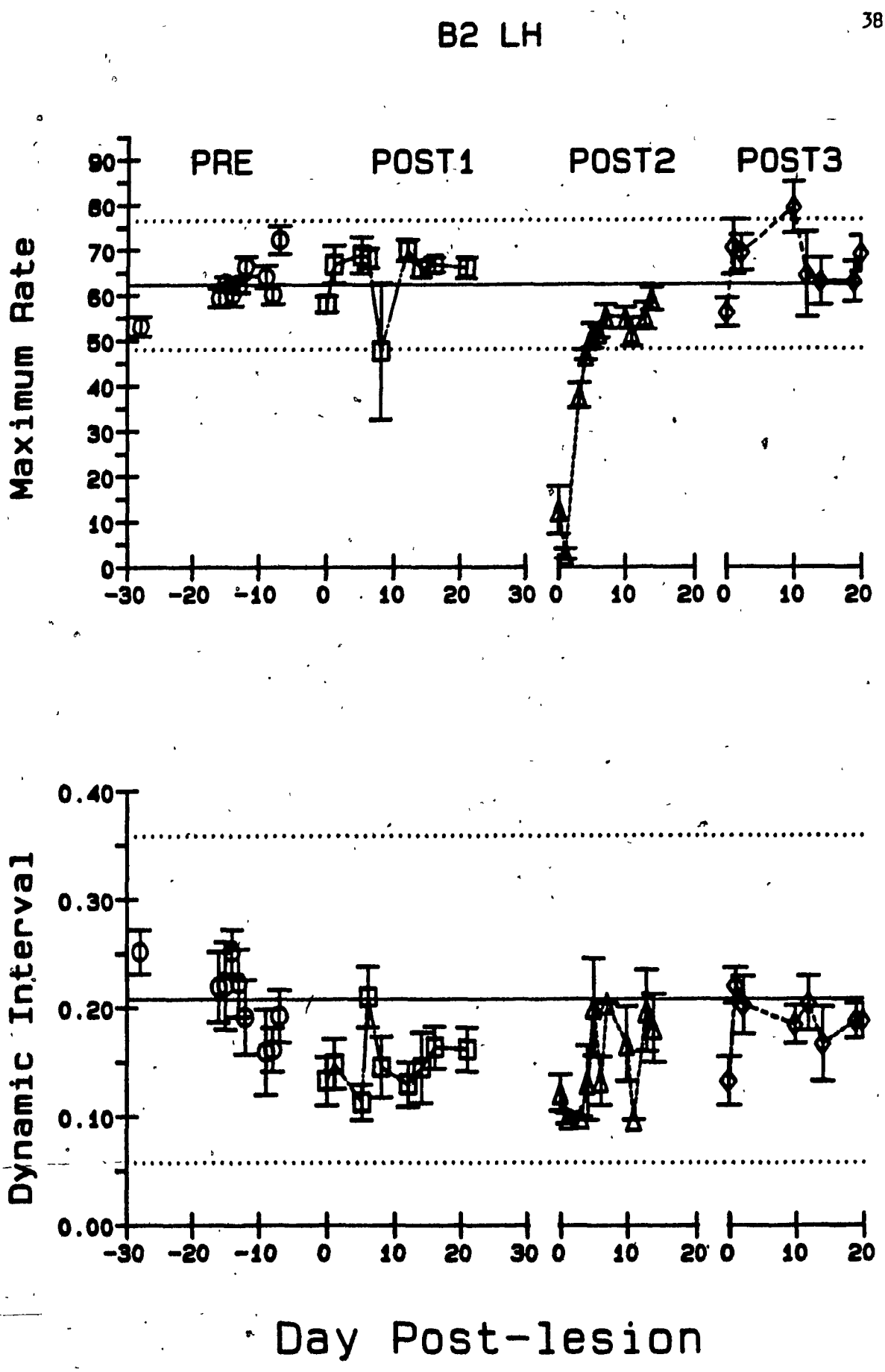
Figures 5-10. The maximum rates and dynamic intervals of the rate-number curves for the effective lesion group. The horizontal, solid lines extending across each of the graphs represent the mean for the pre-lesion data and the dotted lines indicate 2 standard deviations from the mean. Vertical lines represent the s.e.m. for that session.

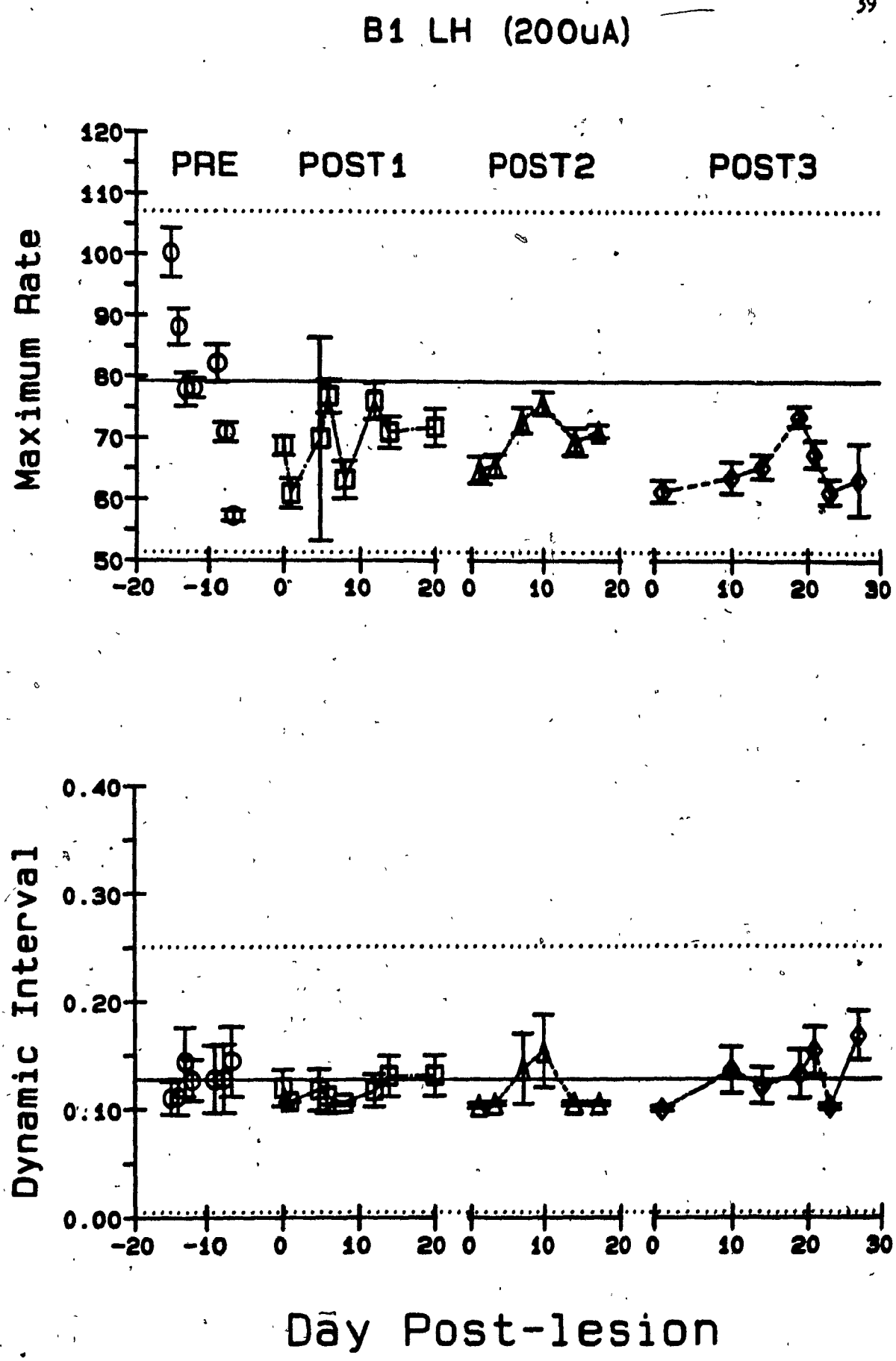

D3 VTA
PRE
POST1
POST3
Maximum Rate
80
70
60
50
40
30
20
10
0
-30 -20 -10 0 0 10 20 30 40 50 0 10 20 30
Dynamic Interval
0.40
0.30
0.20
0.10
0.00
-30 -20 -10 0 0 10 20 30 40 50 0 10 20 30
Day Post-lesion


C9 LH
PRE
POST1
POST2
POST3
Maximum Rate
60
50
40
30
20
Dynamic Interval
0.40
0.30
0.20
0.10
0.00
-20 -15 -10 -5 0 0 5 10 15 20 0 5 0 5 10
Day Post-lesion


D1 LH
PRE
POST
Maximum Rate
100
90
80
70
60
50
40
30
-15
-10
-5
0
0
5
10
15
Dynamic Interval
0.40
0.30
0.20
0.10
0.00
Day Post-lesion


D1 VTA
PRE
POST
Maximum Rate
90
80
70
60
50
40
30
20
10
0
-15
-10
-5
0
0
5
10
15
Dynamic Interval
0.40
0.30
0.20
0.10
0.00
Day Post-lesion


B2 LH
PRE
POST1
POST2
POST3
Maximum Rate
Dynamic Interval
Day Post-lesion


B1 LH (200uA)
PRE
POST1
POST2
POST3
Maximum Rate
120
110
100
90
80
70
60
50
-20
-10
0
10
20
0
10
20
0
10
20
30
Dynamic Interval
0.40
0.30
0.20
0.10
0.00
Day Post-lesion

lesion curves (Figures 5 to 10, lower panels). The only case where the post-lesion interval was outside of +/- 2 standard deviations of the baseline mean was for subject D3 (Figure 5) and this elevation occurred for only the first day after the third lesion.

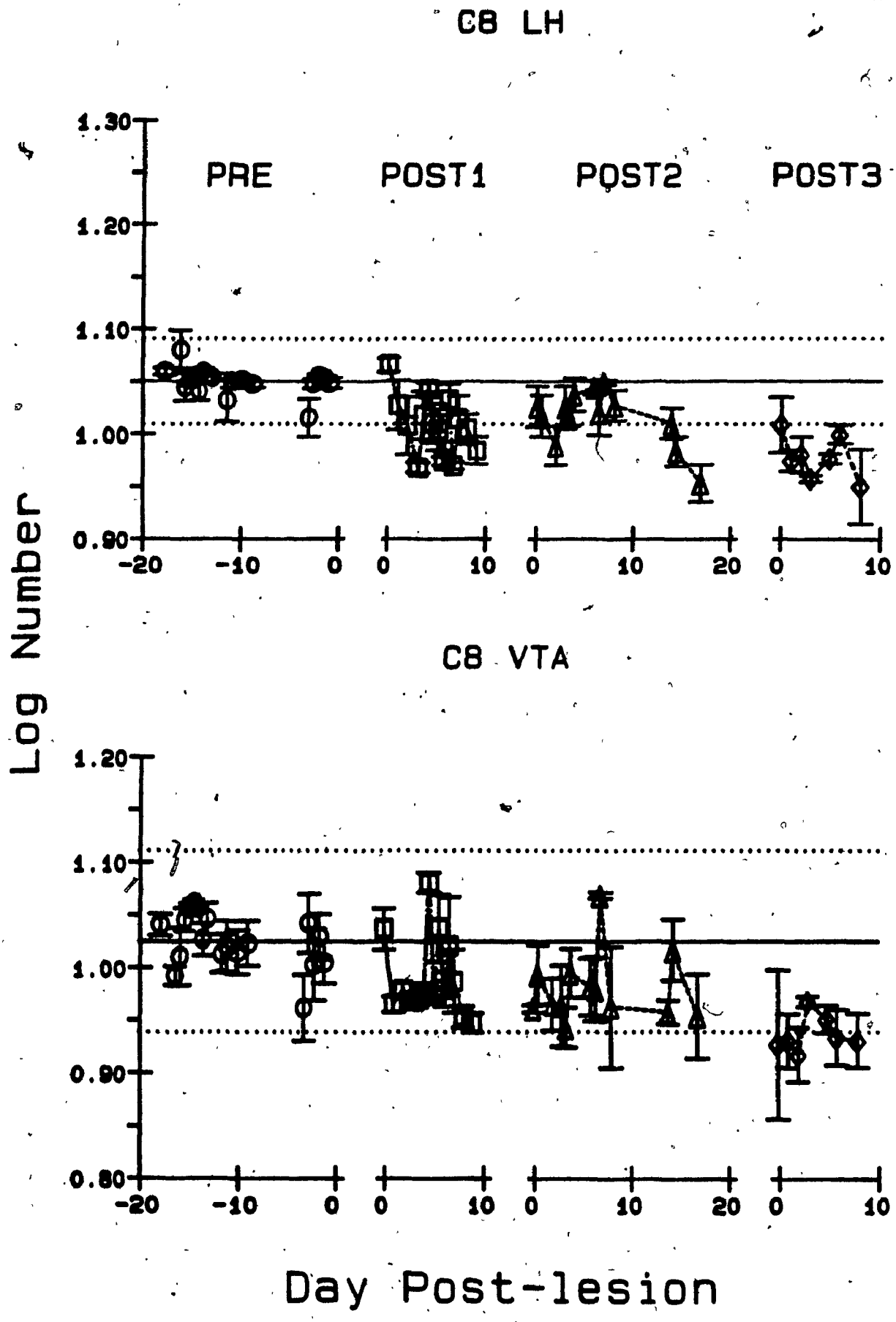
Ineffective Lesions

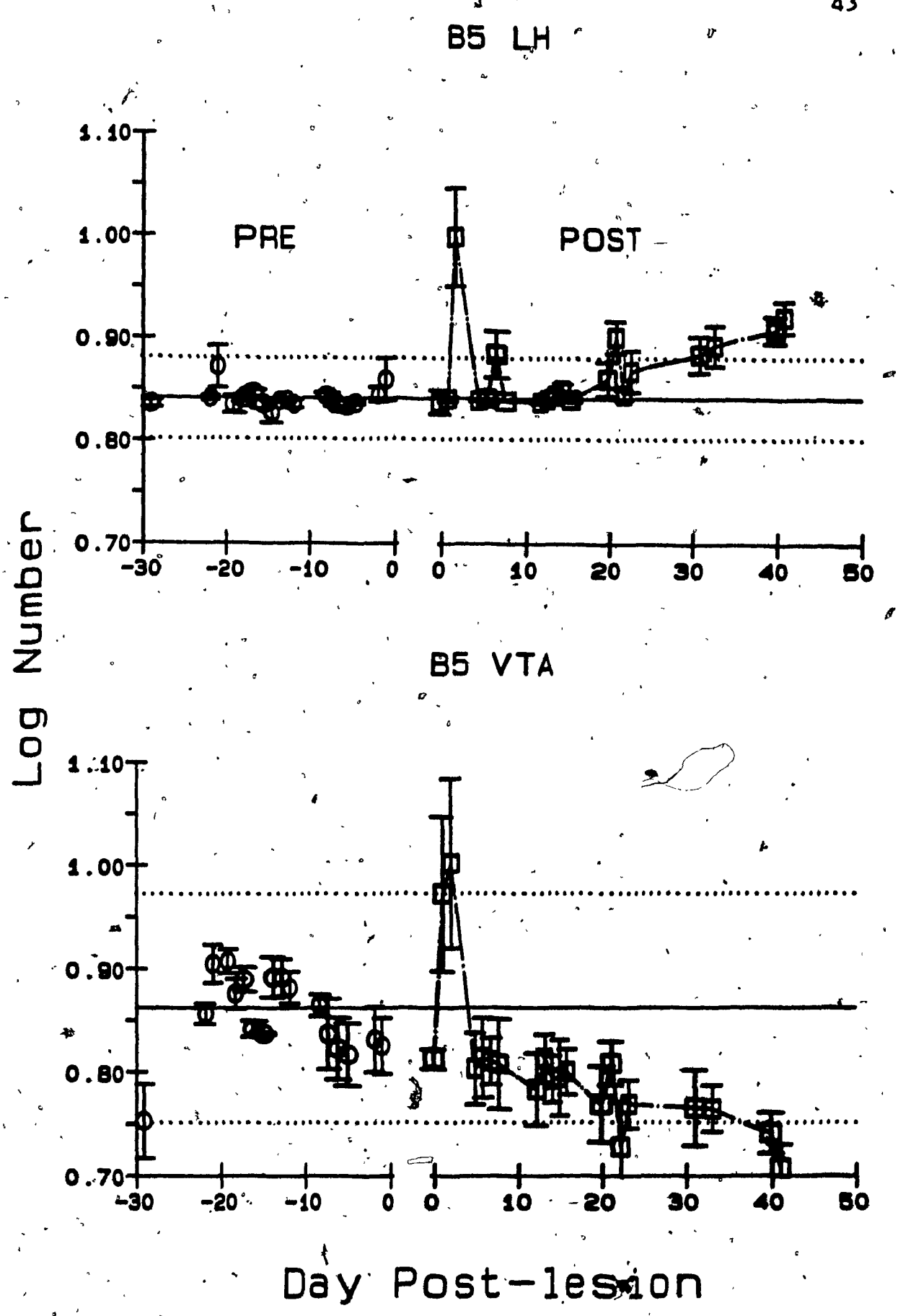
Lesions were ineffective in producing long-term increases in the required number for 2 VTA sites and 4 LH sites (Figures 11-13). In the case of subjects D3 (LH) and B1 (LH, 480uA) the lesions produced transient elevations in the required number that lasted for only a few days (Figure 13). It should be noted that subject B1 was also included in the effective lesion group described above because long-term elevations in the required number were seen at a lower current (200uA). The gradual increase in the required number seen in subject B5 (LH electrode, Figure 12) after 20 days of post-lesion testing preceeded the loss of the electrode assembly. Lesions also did not produce long-term changes in the maximum rate or dynamic interval of the rate-number curves for these subjects.

Effects on Collision

Lesions produced decreases in the size of the collision effect ranging from 27-35% in 2 of the 4 subjects tested. Figure 14 shows data from subject D1. The top panel shows the pre-lesion collision curves and the bottom panel the post-lesion curves. Given the absence of any large differences between the curves obtained in the A-P and P-A conditions, the average of the two conditions was used for all subsequent analysis. In both

Figures 11-13. The required number of pulses for the ineffective lesion group. The horizontal, solid lines extending across each of the graphs indicate the mean for the pre-lesion data and the dotted lines indicate 2 standard deviations from the mean. Vertical lines represent the s.e.m. for that session.


C8 LH
PRE
POST1
POST2
POST3
1.30
1.20
1.10
1.00
0.90
C8 VTA
1.20
1.10
1.00
0.90
0.80
-20
-10
0
0
10
0
10
20
0
10
Log Number
Day Post-lesion


B5 LH
PRE
POST
1.10
1.00
0.90
0.80
0.70
-30
-20
-10
0
0
10
20
30
40
50
B5 VTA
Log Number
Day Post-lesion

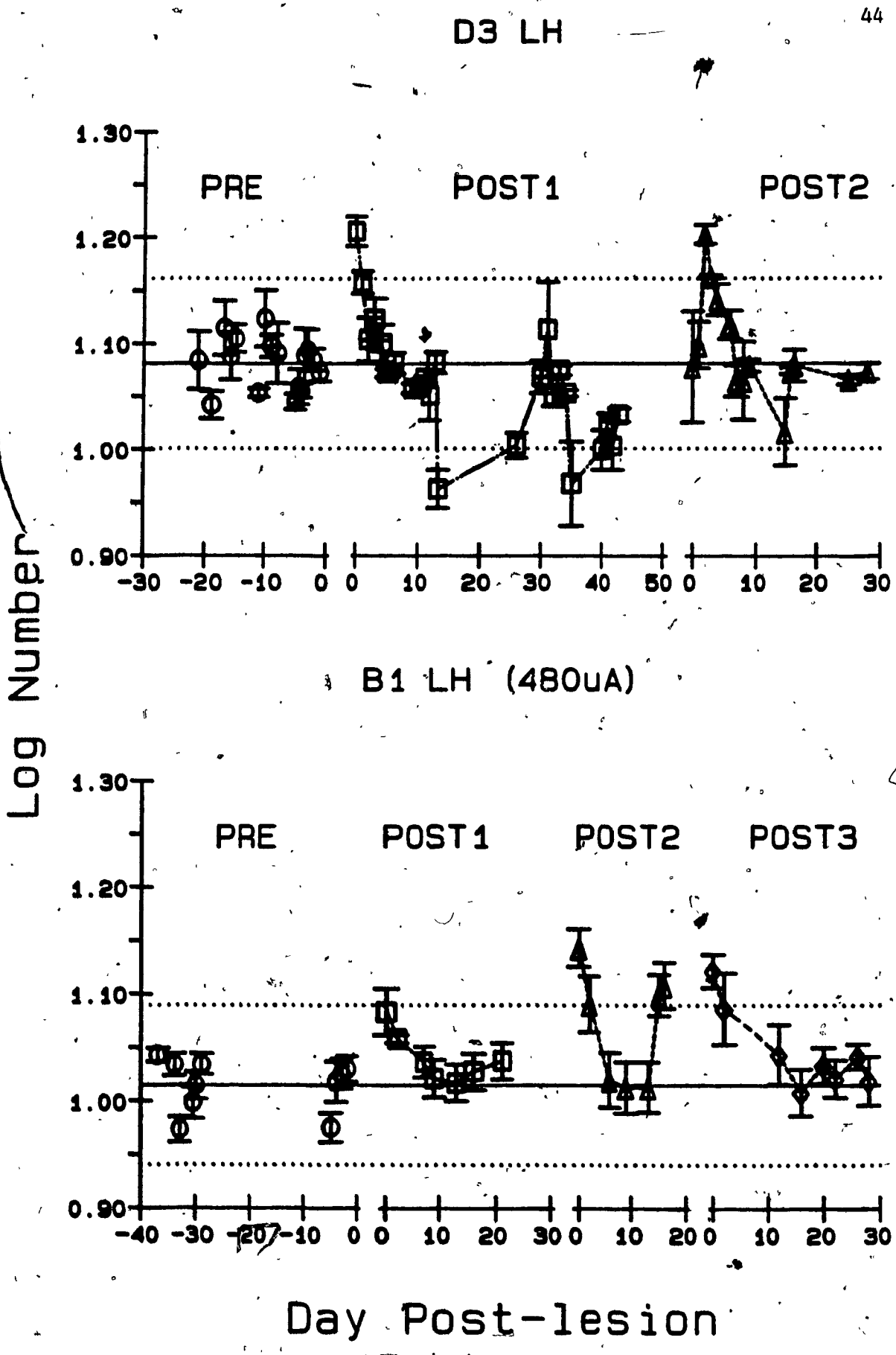
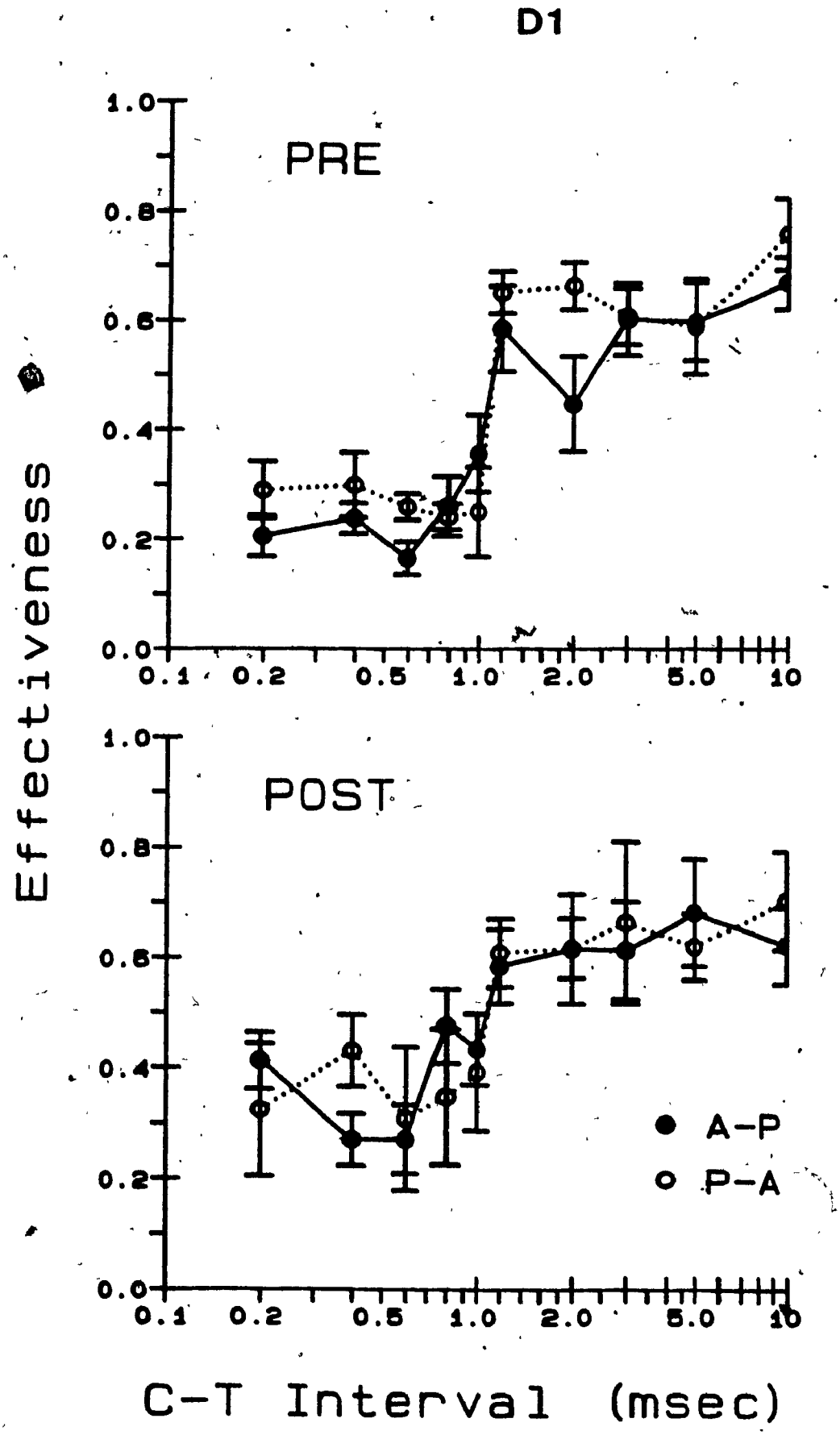

D3 LH
PRE
POST1
POST2
1.30
1.20
1.10
1.00
0.90
-30 -20 -10 0 0 10 20 30 40 50 0 10 20 30
B1 LH (480uA)
PRE
POST1
POST2
POST3
1.30
1.20
1.10
1.00
0.90
-40 -30 -20 -10 0 0 10 20 30 0 10 20 0 10 20 30
Log Number
Day Post-lesion

Figure 14. Collision curves for subject D1. The filled circles represent data collected with the C-pulse applied to the anterior (LH) electrode and the open circles represent data collected with the C-pulse applied to the posterior (VTA) electrode. Vertical lines represent the s.e.m. for each C-T interval.


D1
PRE
POST
Effectiveness
1.0
0.8
0.6
0.4
0.2
0.0
0.1
0.2
0.5
1.0
2.0
5.0
10
A-P
P-A
C-T Interval (msec)

the pre-lesion and post-lesion curves, the total residual variance of the broken-line functions was significantly less than the residual variance obtained from a horizontal line through the mean (pre-lesion: $t(116) = 6.39$, $p < .0001$; post-lesion: $t(142) = 1.96$, $p < .05$). In order to facilitate comparison of the pre-lesion and post-lesion data, the curves were forced to rise to an effectiveness value of 1.0 by dividing each data point by the upper asympote of the broken-line function (Figure 15, left panel). The re-scaled data are able to show changes in the size of the collision effect that may have been masked by changes in the overall level of summation. It should be noted that, under the simplest assumptions, the difference between the upper and lower asymptote of the re-scaled data gives the percentage of fibers undergoing collision (see Figure 15, right panel). Under these assumptions, every firing in each of the reward fibers contributes equally to the rewarding effect of the stimulation. Given this assumption, the percentage of fibers undergoing collision decreased by approximately 28% post-lesion in subject D1. Since the upper asymptote of the re-scaled data is forced to be the same before and after the lesion, decreases in the difference between the upper and lower asymptote (or the "size" of the collision effect) can be determined from differences in the lower asymptote. The lower asymptote of the pre-lesion curve was significantly different from the lower asymptote of the post-lesion curve ($t(130) = -3.12$, $p < .01$).

Data for subject D3 is shown in Figures 16 and 17. The broken-line functions fit to the average of the A-P and P-A data (before re-scaling) resulted in significantly less total residual variance than a straight line through the mean for the pre-lesion ($t(143) = 4.24$, $p < .001$) and first post-

Figure 15. The average of the A-P and P-A collision curves for subject D1. Data have been re-scaled so that both curves rise to E-values of 1.0. The right panel shows the broken-line functions fit to the pre-lesion and post-lesion curves.

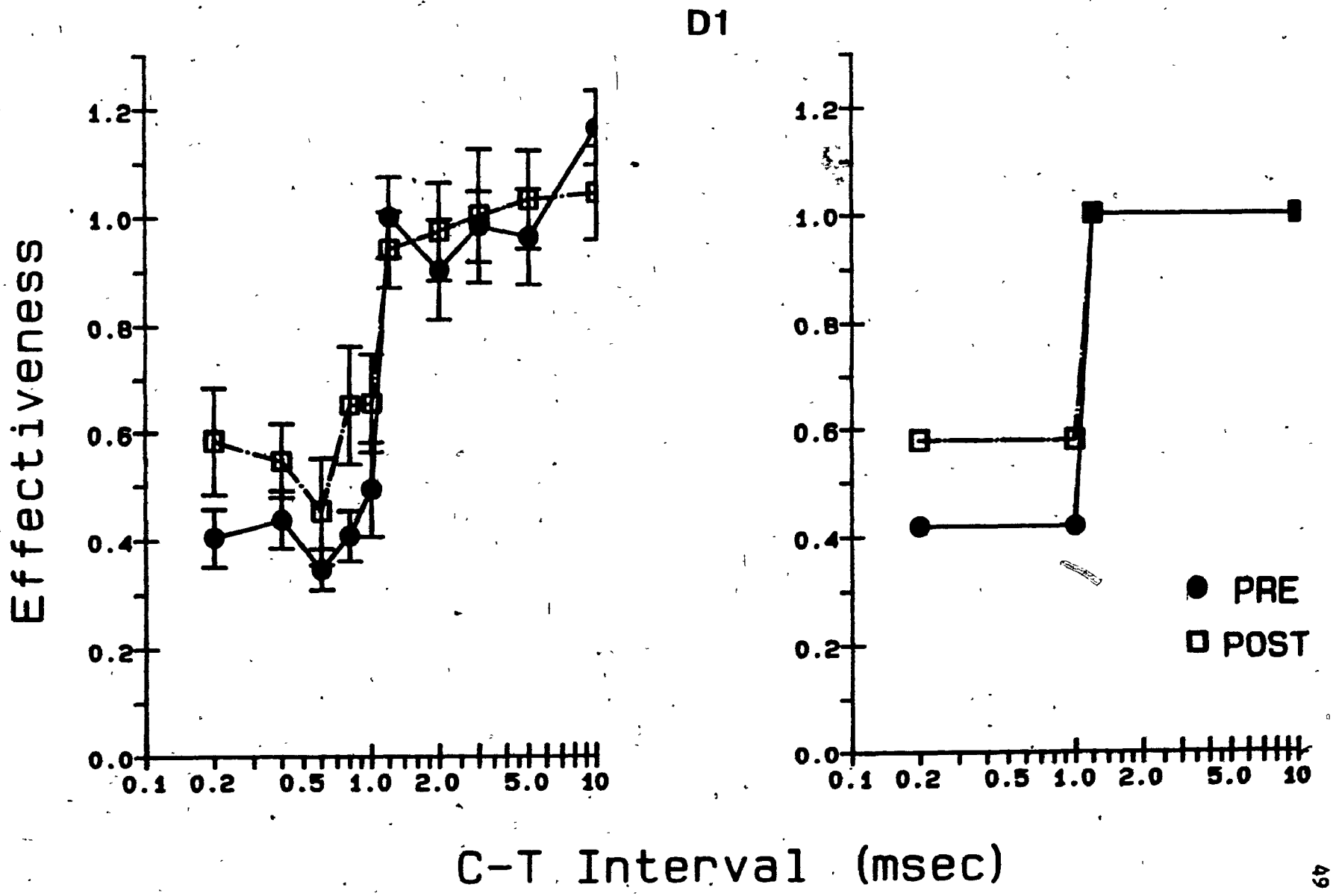

D1
Effectiveness
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.1 0.2 0.5 1.0 2.0 5.0 10
PRE
POST
C-T Interval (msec)

Figure 16. Collision curves for subject D3. The filled circles represent data collected with the C-pulse applied to the anterior (LH) electrode and the open circles represent data collected with the C-pulse applied to the posterior (VTA) electrode. Vertical lines represent the s.e.m. for each C-T interval.

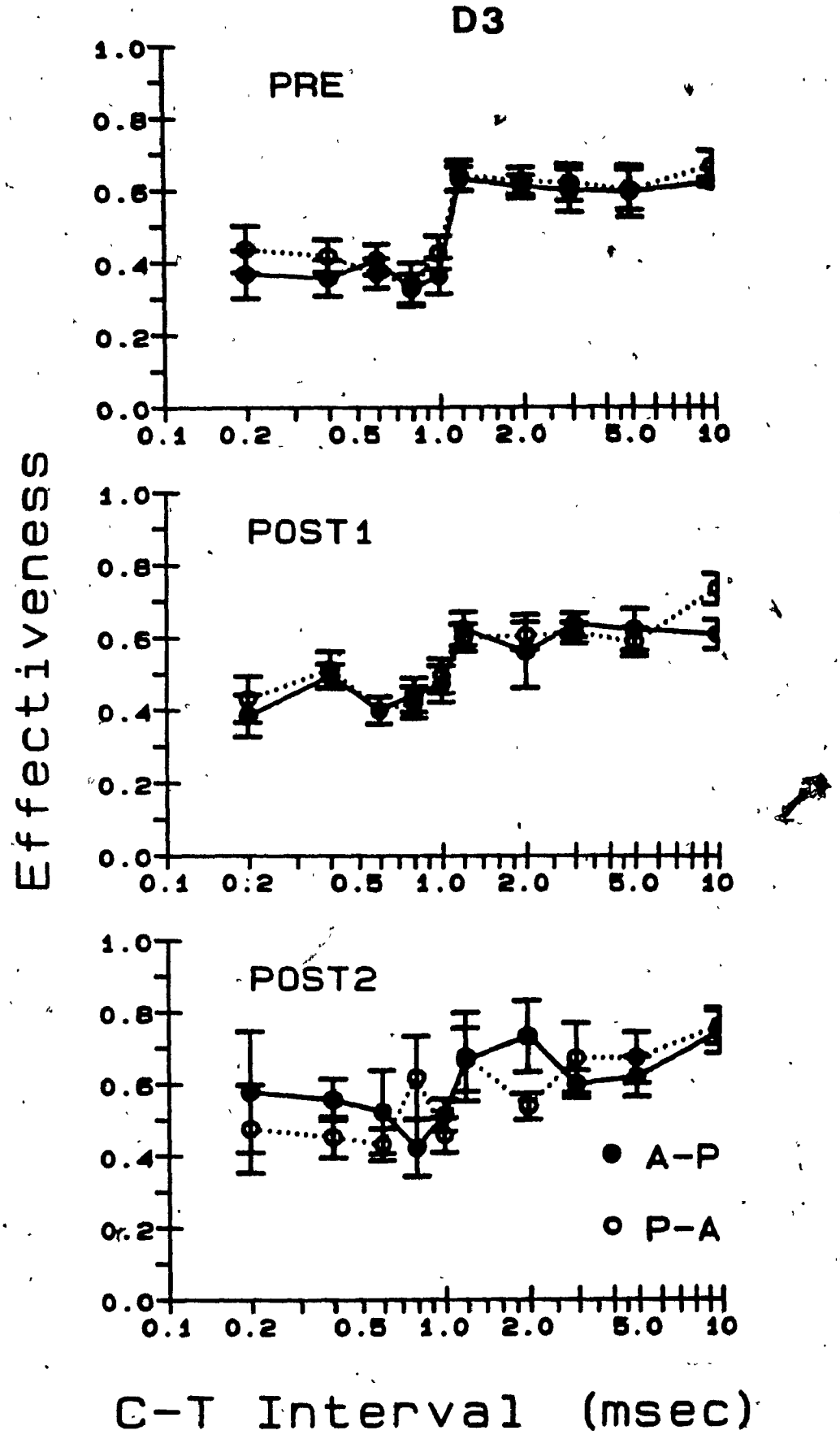
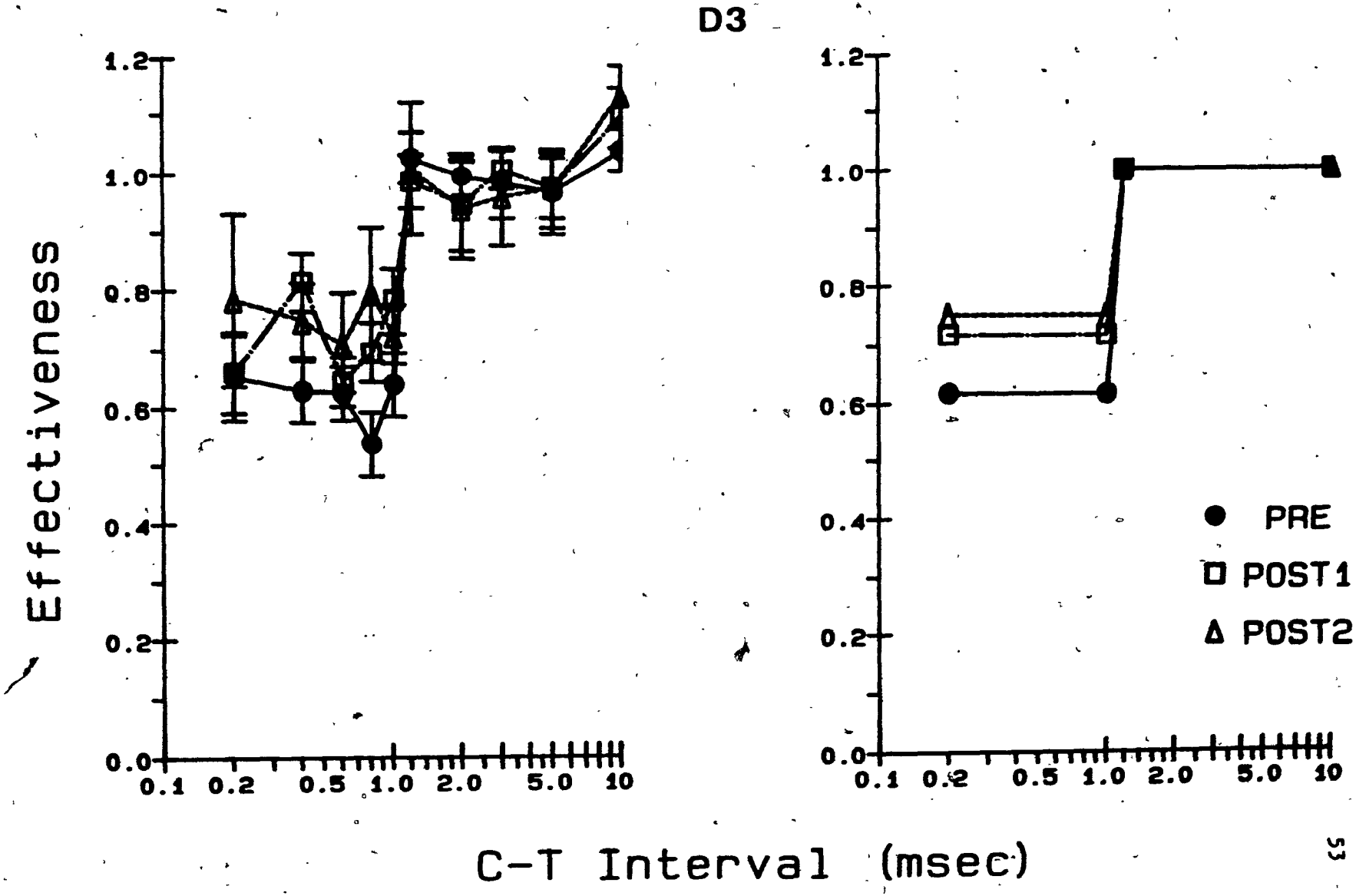

D3
PRE
POST1
POST2
1.0
0.8
0.6
0.4
0.2
0.0
0.1
0.2
0.5
1.0
2.0
5.0
10
Effectiveness
A-P
P-A
C-T Interval (msec)

Figure 17. The average of the A-P and P-A collision curves for subject D3. Data have been re-scaled so that both curves rise to E-values of 1.0. The right panel shows the broken-line functions fit to the pre-lesion and post-lesion curves.


D3
Effectiveness
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.1 0.2 0.5 1.0 2.0 5.0 10
PRE
POST1
POST2
C-T Interval (msec)

lesion data ($t(198) = 2.17$, $p < .05$) but not after the second lesion ($t(108) = .88$, $p < .4$) The first lesion produced a 27% decrease in the size of the collision effect and the second lesion resulted in a 35% decrease when compared to the pre-lesion curves. The lower asymptote of the first post-lesion curve (re-scaled) was significantly different from the lower asymptote of the pre-lesion curve ($t(172) = -2.82$, $p < .01$). The lower asymptote of the second post-lesion curve was not significantly different from the lower asymptote of the first post-lesion curve ($t(153) = -0.72$, $p < .47$).

The collision data for subject C8 is shown in Figure 18. Broken-line functions fit to the average of the A-P and P-A data (before re-scaling) resulted in significantly less total residual variance than a straight line through the mean in the case of the pre-lesion data ($t(158) = 2.08$, $p < .05$) but not after the first lesion ($t(123) = 1.37$, $p < .2$) or the second lesion ($t(97) = .47$, $p < .7$). The re-scaled data are shown in Figure 19. There was no significant difference between the lower asymptotes of the pre-lesion and first post-lesion curves ($t(127) = -0.51$, $p < .61$) or the lower asymptotes of the first post-lesion and second post-lesion curves ($t(97) = -0.91$, $p < .37$).

The collision data for subject B5 is shown in Figures 20-22. The A-P and P-A conditions were not averaged for B5 because of differences in the two curves post-lesion (see Figure 20, lower panel). After re-scaling and fitting the data, it became clear that the major differences between the post-lesion P-A and A-P curves were the level of summation and the range over which the curves rose. Re-scaling eliminated the differences in summation (Figure 21 and 22) and revealed no significant difference between the lower

Figure 18. Collision curves for subject C8. The filled circles represent data collected with the C-pulse applied to the anterior (LH) electrode and the open circles represent data collected with the C-pulse applied to the posterior (VTA) electrode. Vertical lines represent the s.e.m. for each C-T interval.

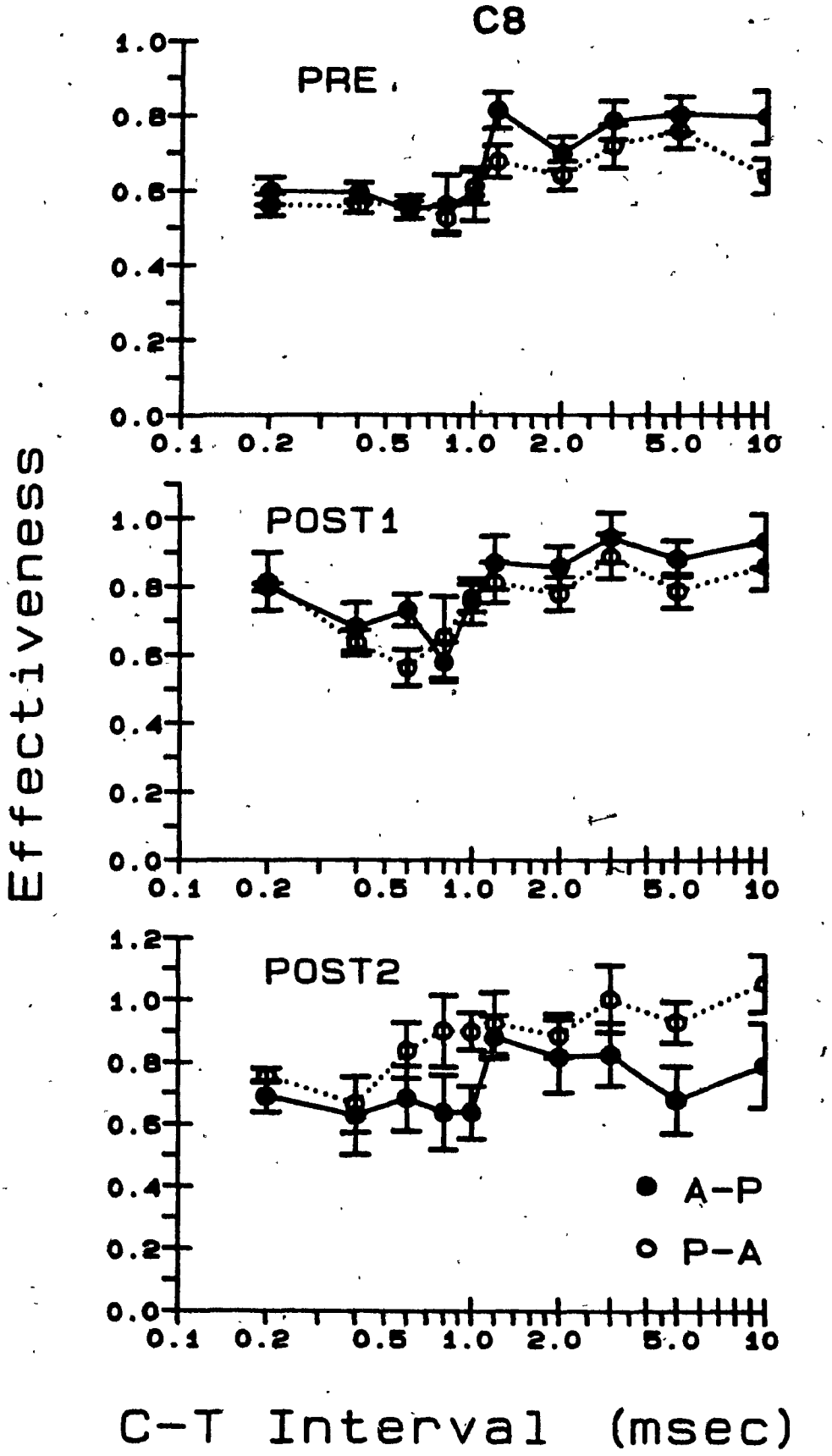

C8
PRE
POST1
POST2
Effectiveness
C-T Interval (msec)
A-P
P-A
0.1
0.2
0.5
1.0
2.0
5.0
10

Figure 19. The average of the A-P and P-A collision curves for subject C8. Data have been re-scaled so that both curves rise to E-values of 1.0. The right panel shows the broken-line functions fit to the pre-lesion and post-lesion curves.

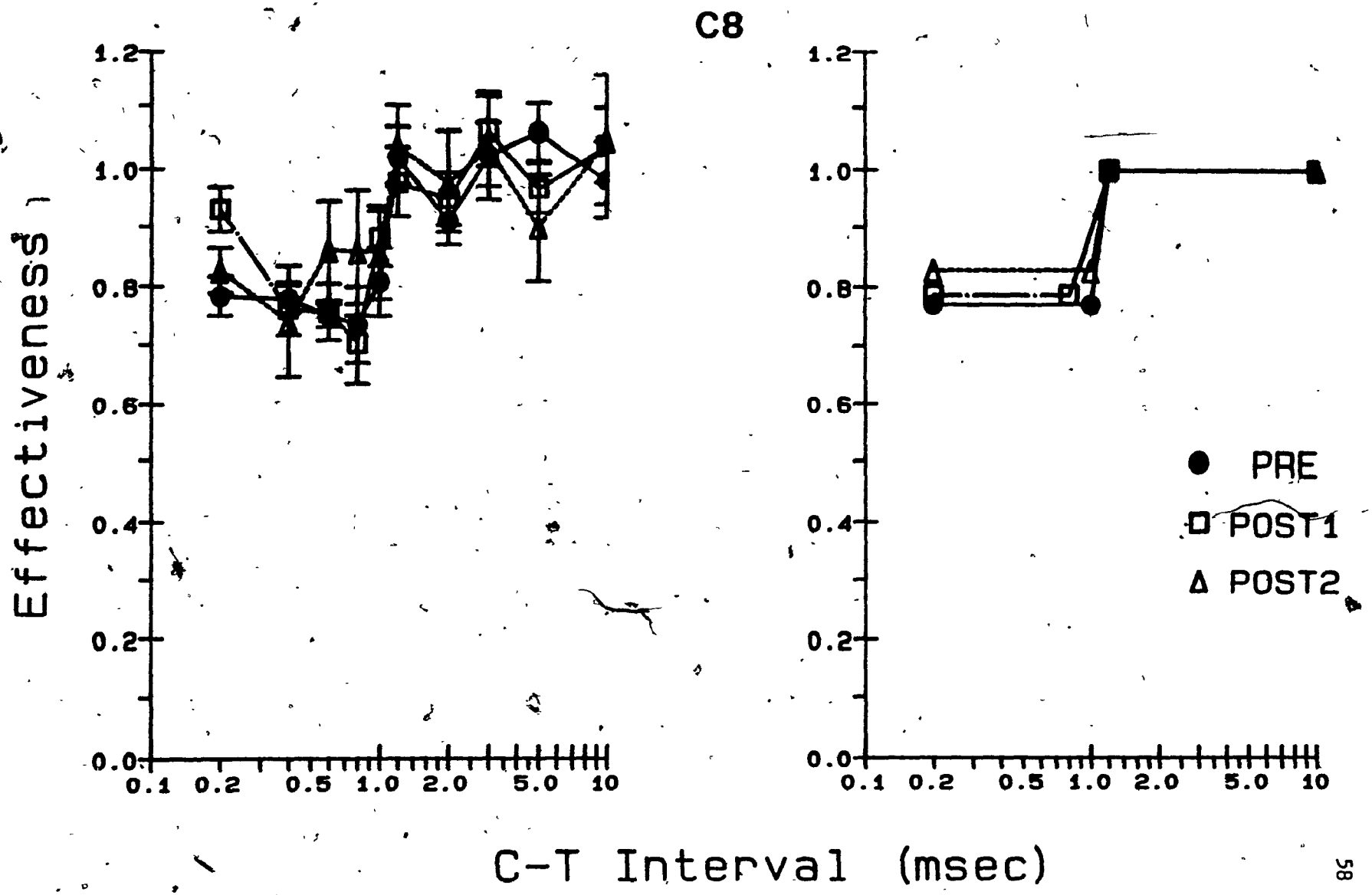
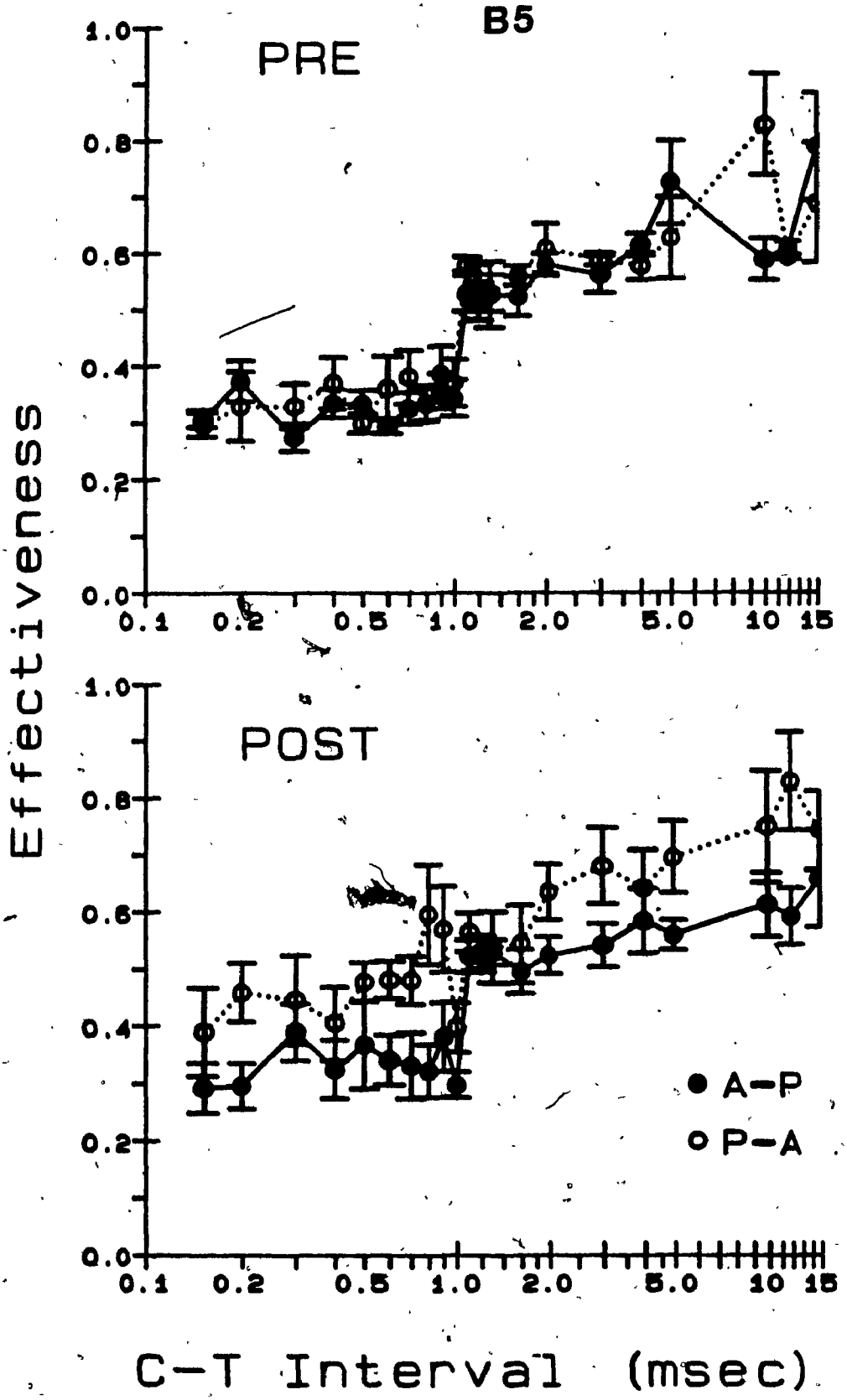
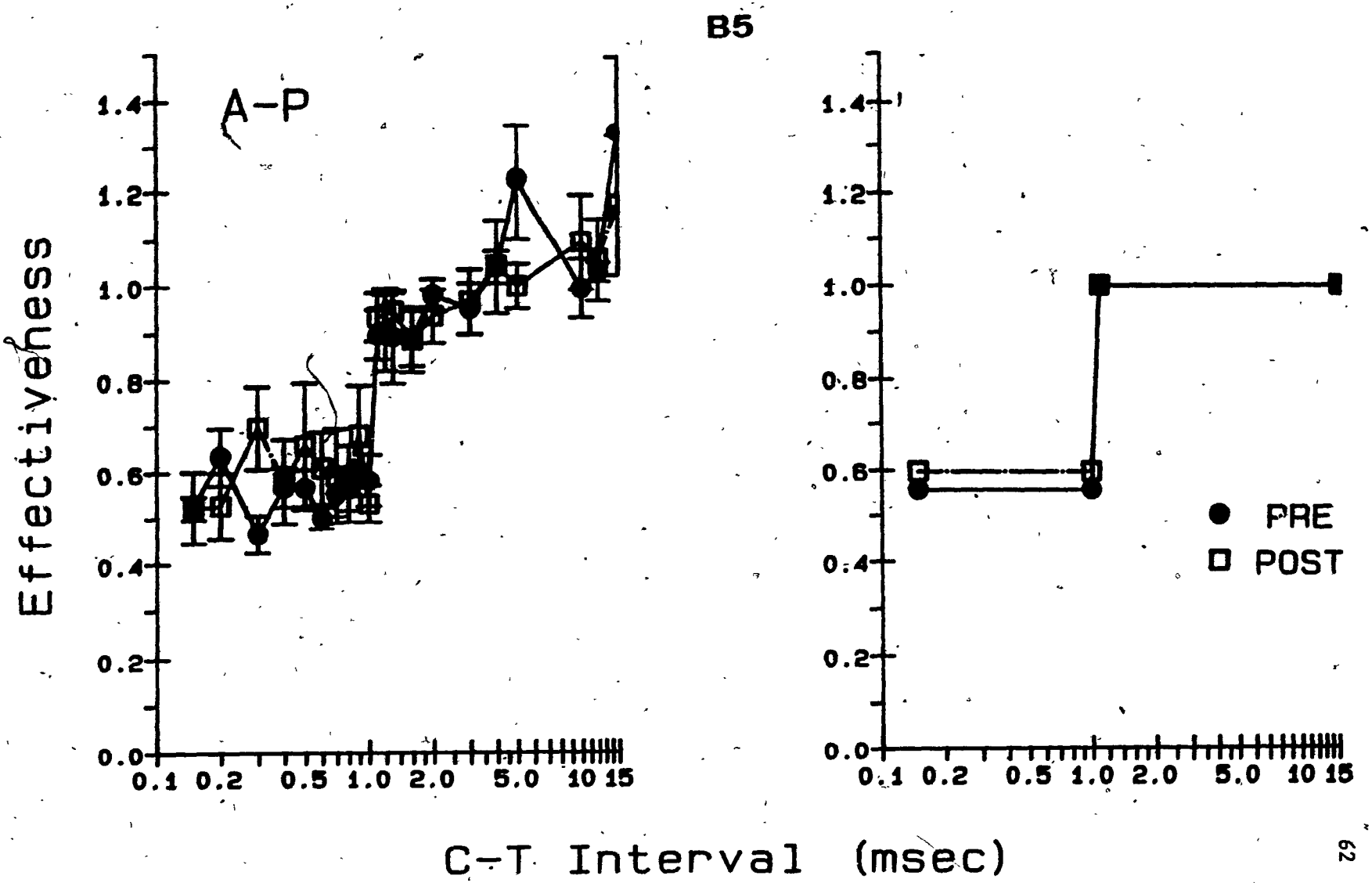
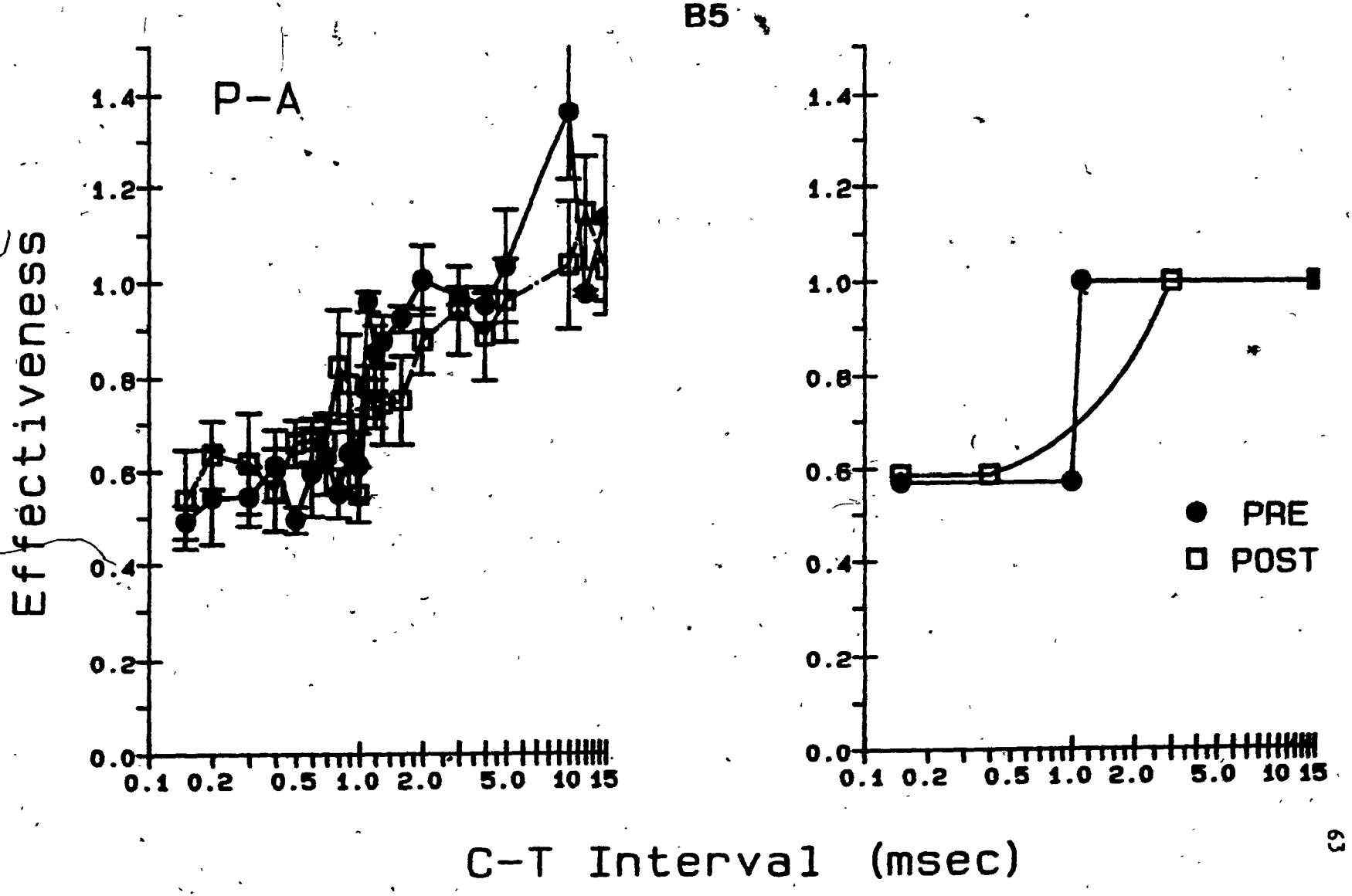

C8
Effectiveness
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.1 0.2 0.5 1.0 2.0 5.0 10
PRE
POST1
POST2
C-T Interval (msec)

Figure 20. Collision curves for subject B5. The filled circles represent data collected with the C-pulse applied to the anterior (LH) electrode and the open circles represent data collected with the C-pulse applied to the posterior (VTA) electrode. Vertical lines represent the s.e.m. for each C-T interval.


B5
PRE
POST
Effectiveness
1.0
0.8
0.6
0.4
0.2
0.0
0.1
0.2
0.5
1.0
2.0
5.0
10
15
● A–P
○ P–A
C–T Interval (msec)

Figures 21-22. A-P and P-A collision curves for subject B5. Data have been re-scaled so that all curves rise to E-values of 1.0. The right panels shows the broken-line functions fit to the pre-lesion and post-lesion curves.


B5
A-P
Effectiveness
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.1 0.2 0.5 1.0 2.0 5.0 10 15
PRE
POST
C-T Interval (msec)


B5
P-A
Effectiveness
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.1 0.2 0.5 1.0 2.0 5.0 10 15
PRE
POST
C-T Interval (msec)

asymptotes of the pre-lesion and post-lesion curves for either the A-P condition ($t(119) = -0.21$, $p < .83$) or the P-A condition ($t(113) = -0.38$, $p < .7$).

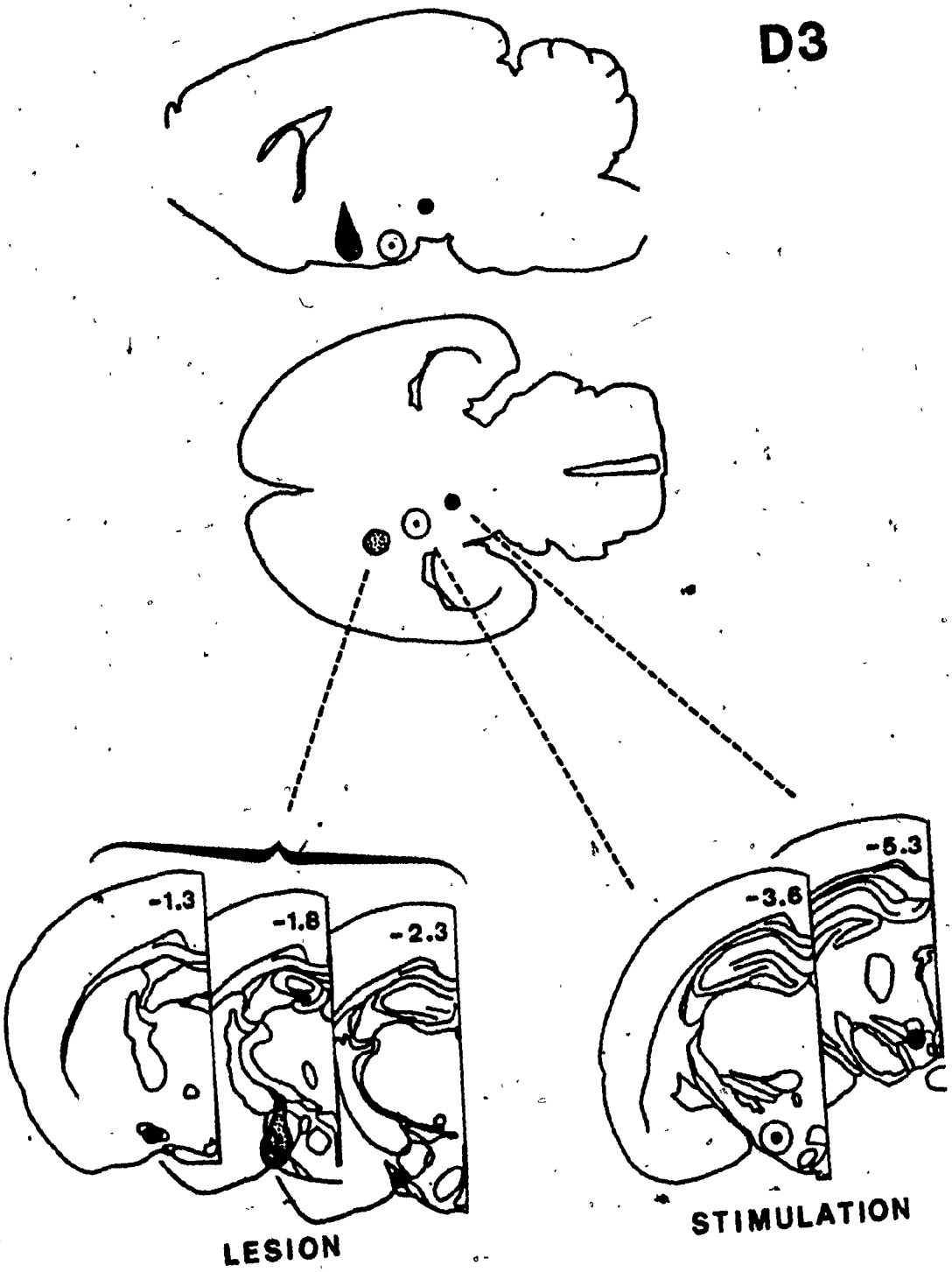
Histology

Histology for the 7 subjects is shown in Figures 23 to 29. The first sign, largest cross-section, and last sign of the lesion as well as the electrode locations were reconstructed onto tracings from the Paxinos and Watson (1986) atlas of the rat brain in the sagittal, horizontal and coronal planes. Lesions were also traced onto plates from the Nieuwenhuys et al. (1982) atlas of the MFB (Figures 30-31). Estimates of the current-distance relationship obtained at the LH by Fouriezos and Wise (1984) were used to provide estimates of the current spread for each of the electrodes. Although these estimates were obtained for current spread along the medial/lateral axis and are therefore valid only in this plane, the stimulation fields were assumed, for the sake of simplicity, to be symmetrical in all planes.

Lesions were located in the anterior LH between -0.8 and -2.3 from bregma. Lesions that were ineffective in producing shifts in the required number were located almost exclusively in the medial compartment 'c' of the MFB (Figure 30) while lesions that produced shifts in the required number also encroached on the more lateral compartments 'a', 'd', and 'e' (Figure 31). In the case of subject D1 (Figure 25) the lesion may have encroached on the LH stimulation field. Electrodes aimed at the LH were located in or bordering the LH between -2.56 and -3.8 mm from bregma. Electrodes aimed at the VTA were located in or bordering the VTA and were found between -4.8 to -5.3 mm from bregma.

Figures 23-29. Lesion and electrode locations on tracings from the Paxinos and Watson (1986) atlas of the rat brain. Sagittal (top) and horizontal (middle) reconstructions are on a "representative" sagittal and horizontal plate. Coronal reconstructions show the first sign, largest cross-section, and last sign of the lesion (bottom left) and the location of the electrode tips (bottom right). Numbers on the coronal sections give the distance of the plate from bregma. Circles drawn around the electrode tips provide an estimate of the stimulation field. The approximate increase (if any) in the required number for each stimulation site is given below the coronal section for that electrode. The decrease (if any) in the size of the collision effect is given in the lower right-hand corner.

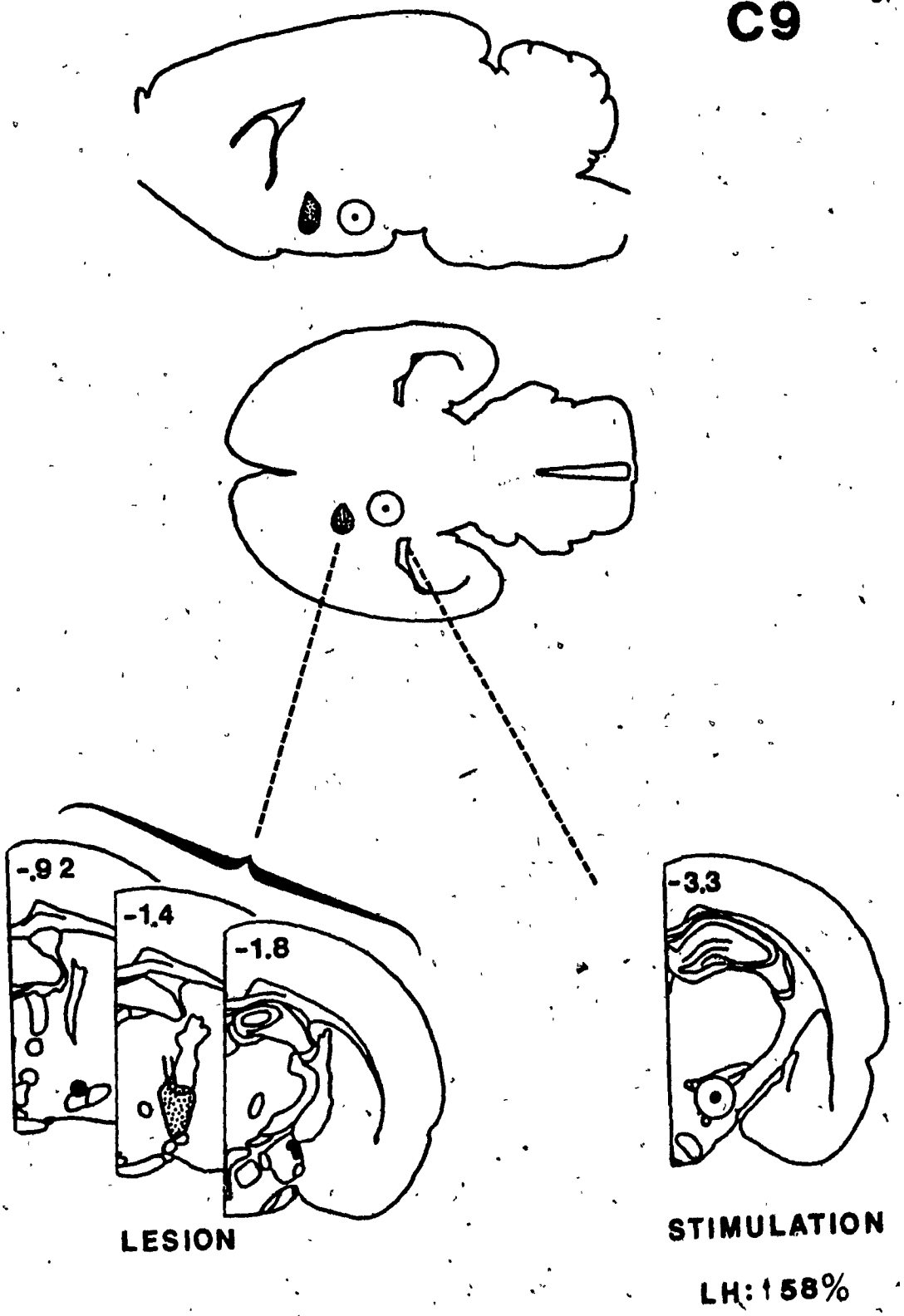
D3



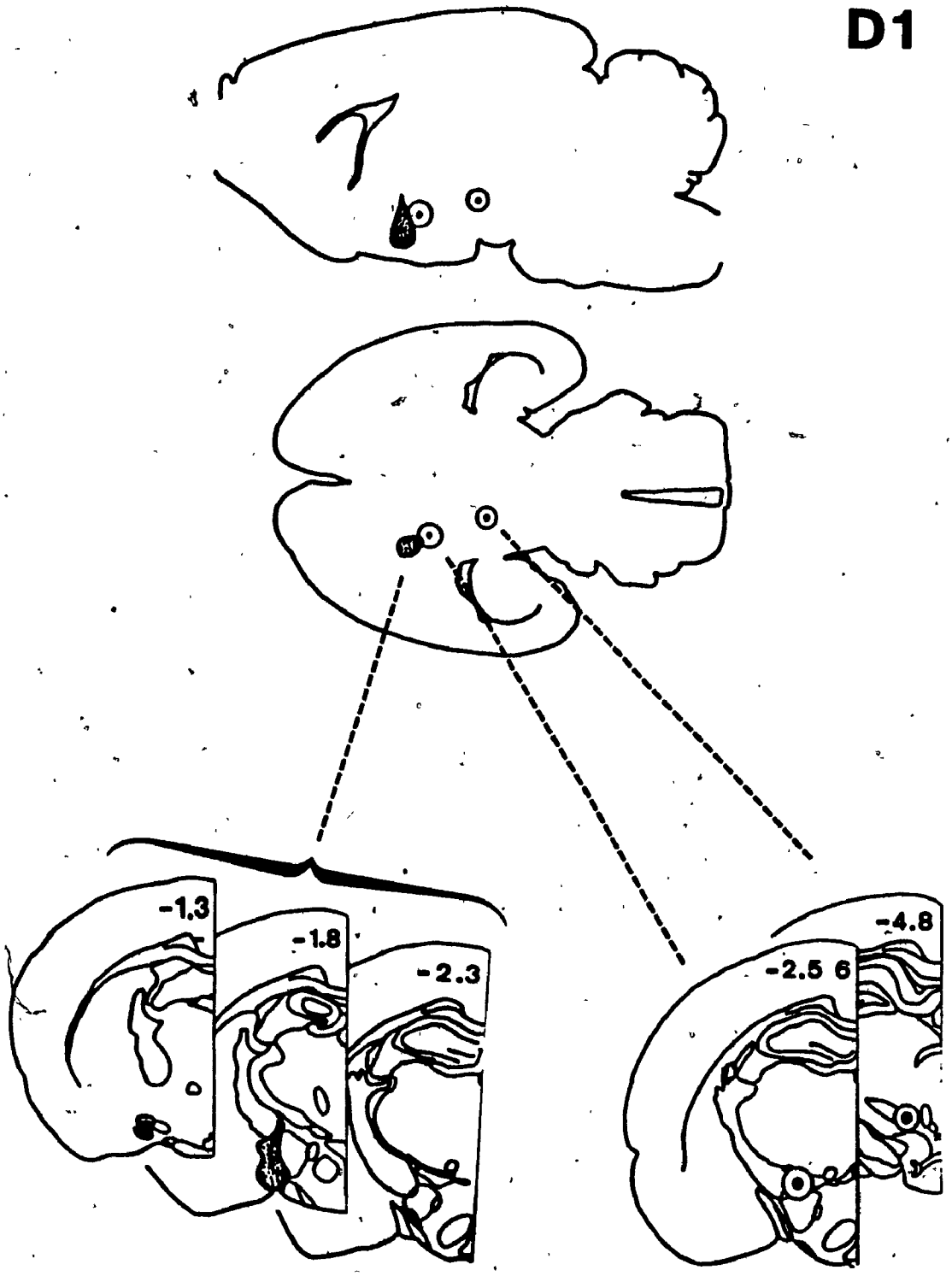
LH: N/C VTA: 158%

COLLISION: 135%

C9



D1



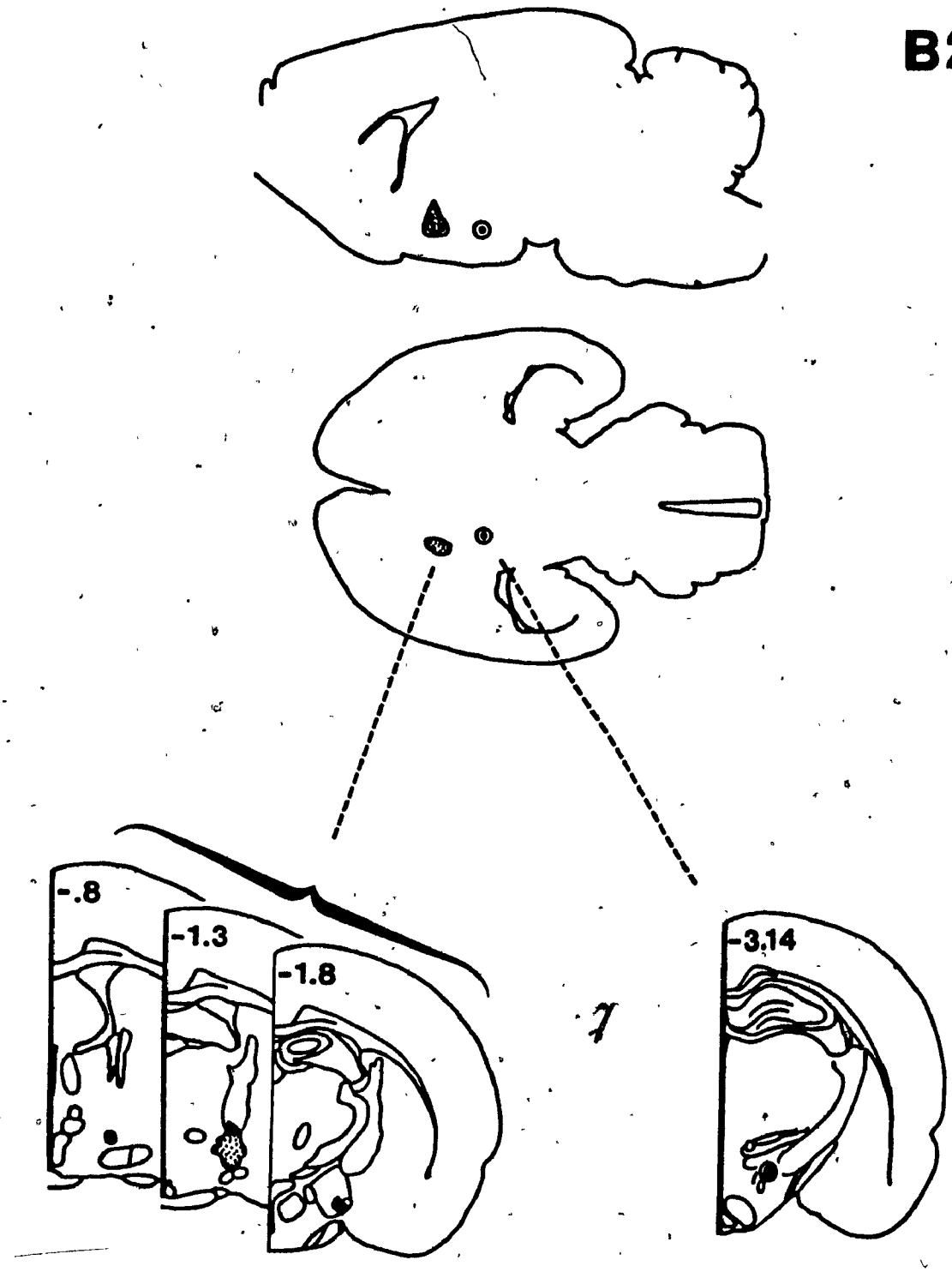
LESION

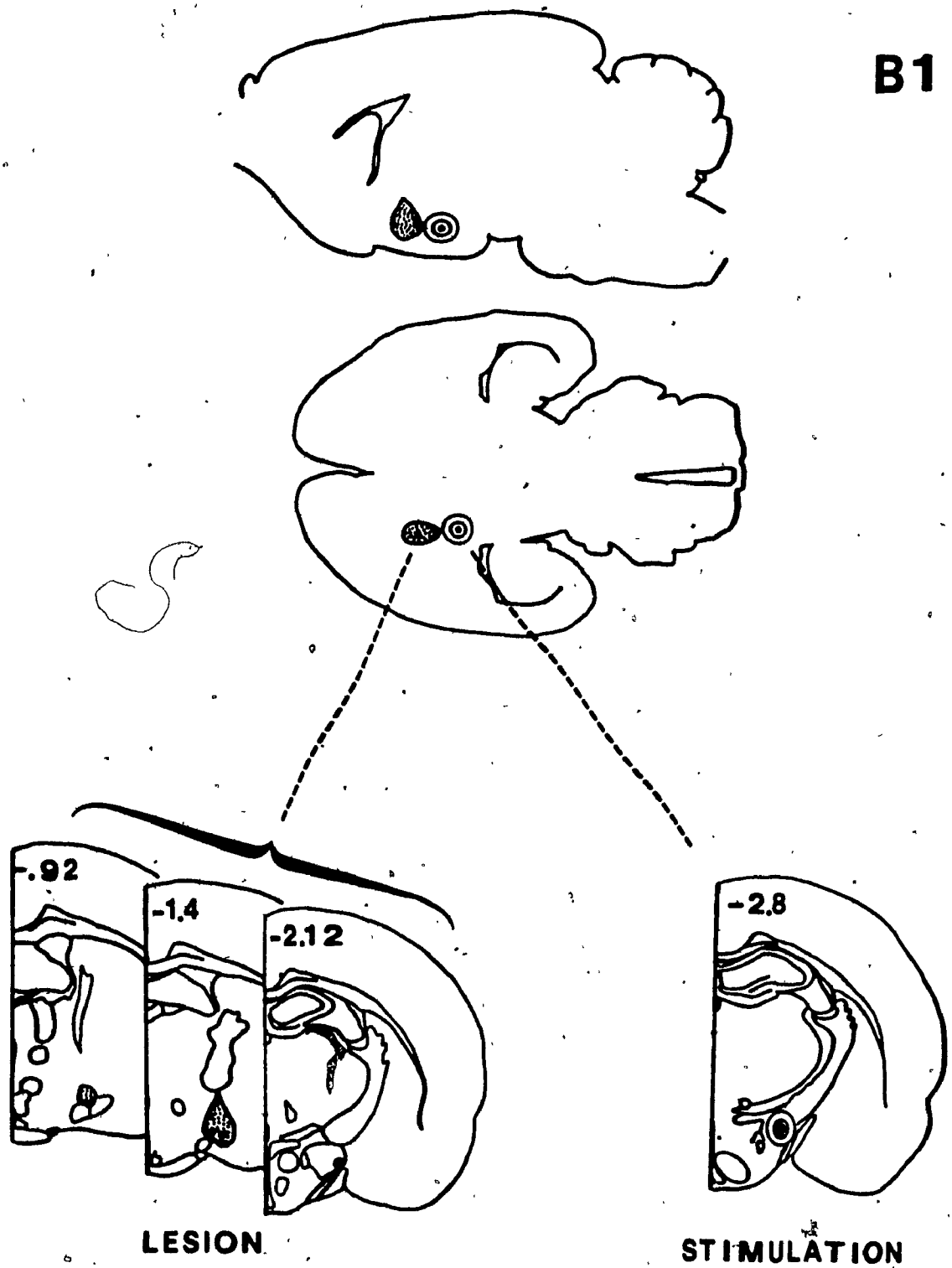
STIMULATION

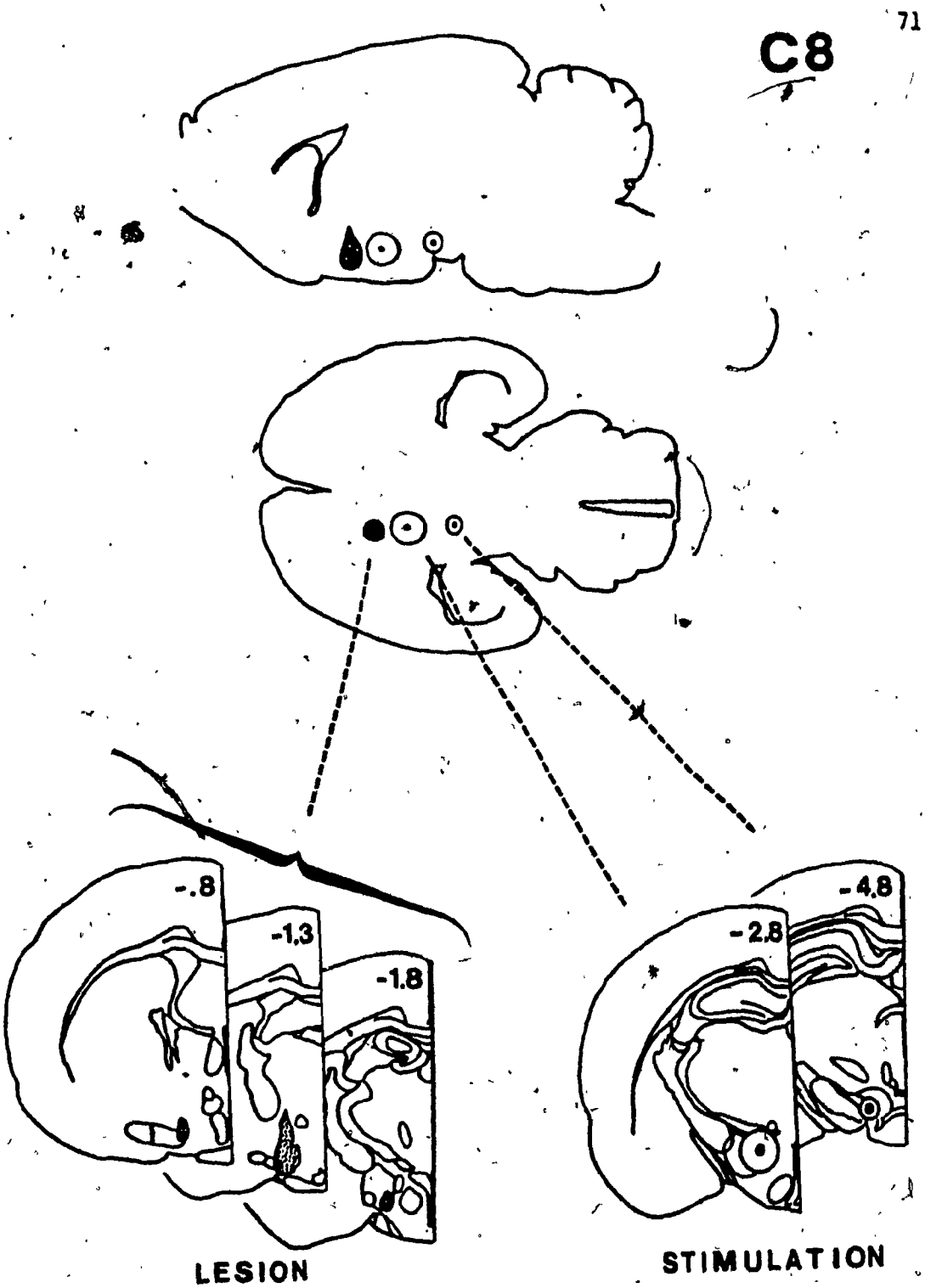
LH: ↑26% VTA: ↑41%

COLLISION: ↓28%

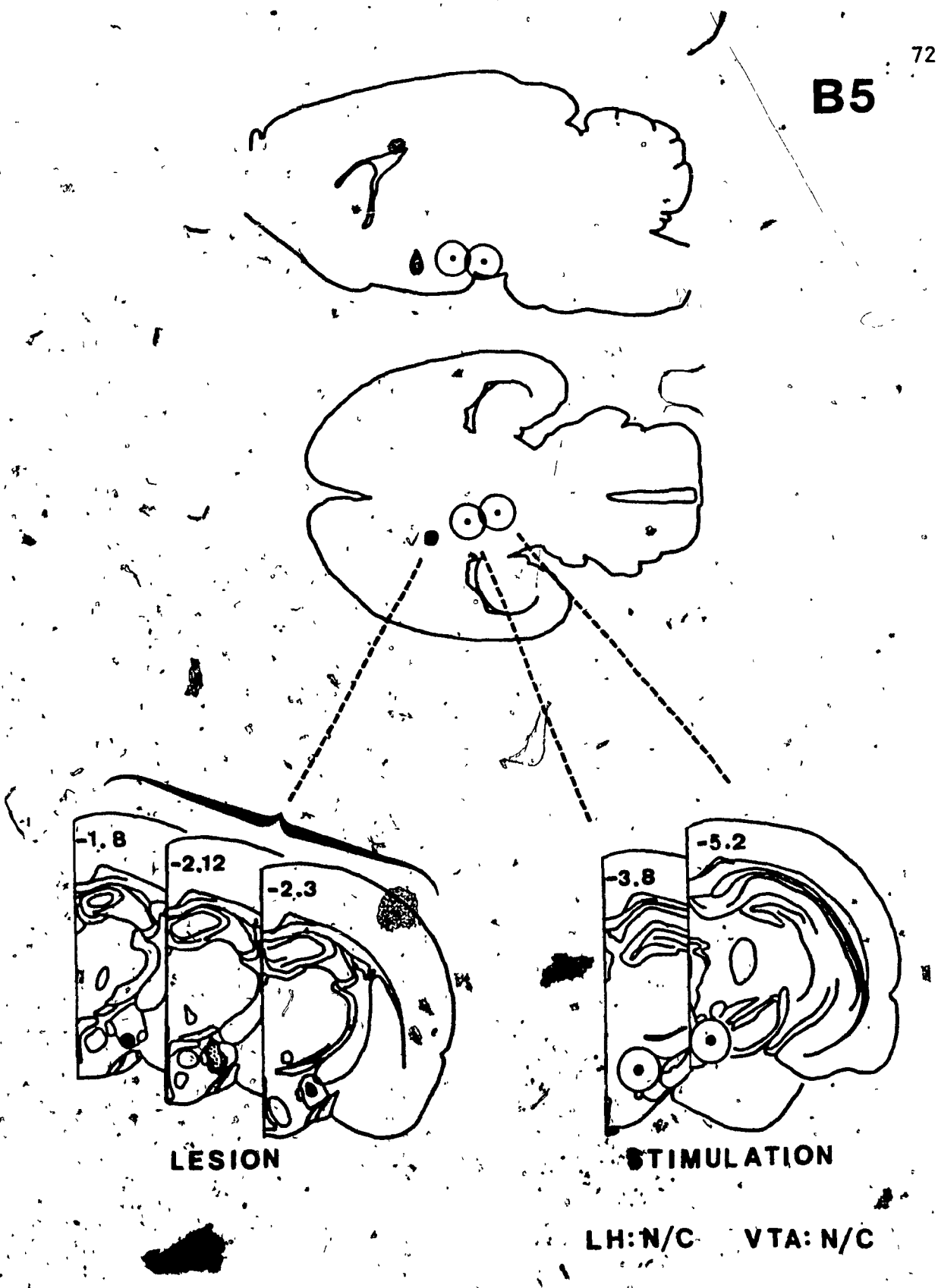
B2




B1
-.92
-1.4
-2.12
-2.8
LESION
STIMULATION
LH: 141%


C8
-.8
-1.3
-1.8
-2.8
-4.8
LESION
STIMULATION
LH:N/C VTA:N/C
COLLISION: N/C

B5

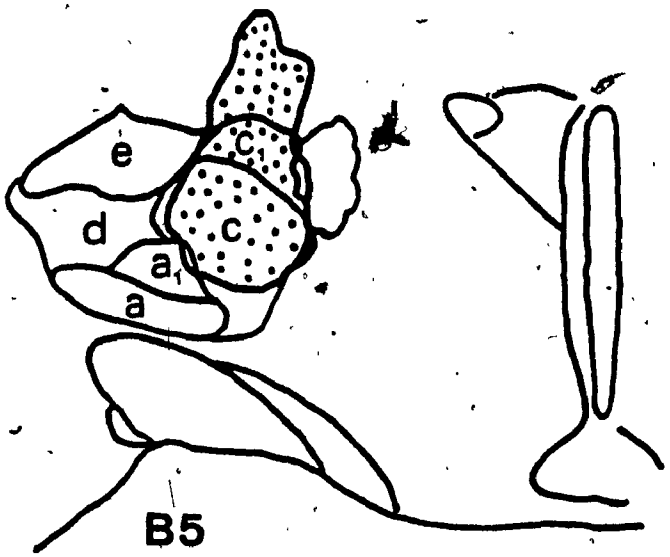


LH: N/C VTA: N/C

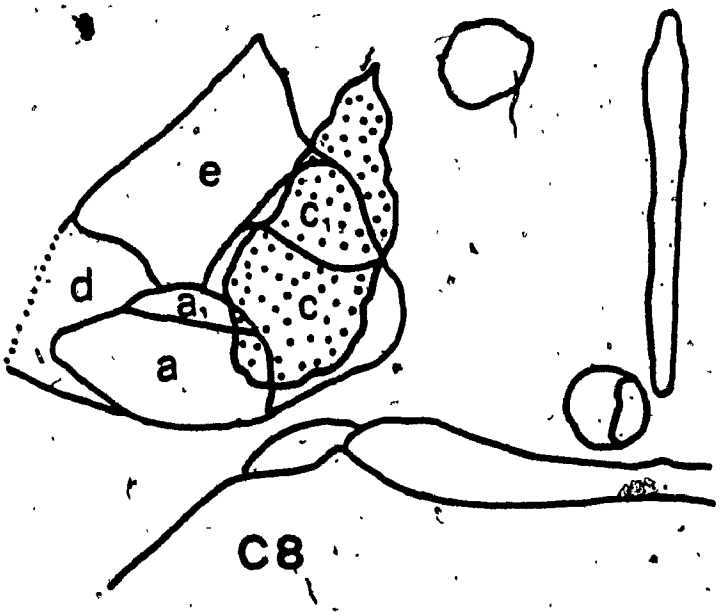
COLLISION: N/C

Figures 30-31. Reconstructions of the ineffective and effective lesions onto tracings taken from the Nieuwenhuys et al. (1982) atlas of the MFB.

INEFFECTIVE LESIONS

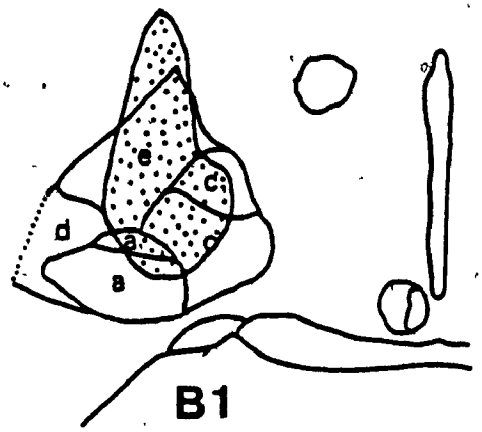


B5

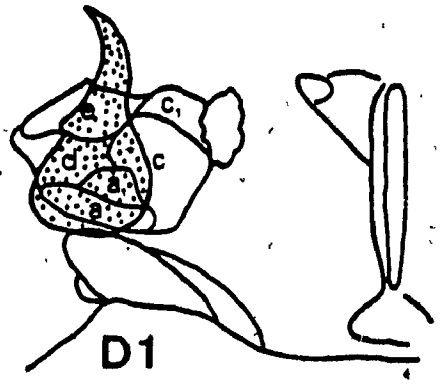


C8

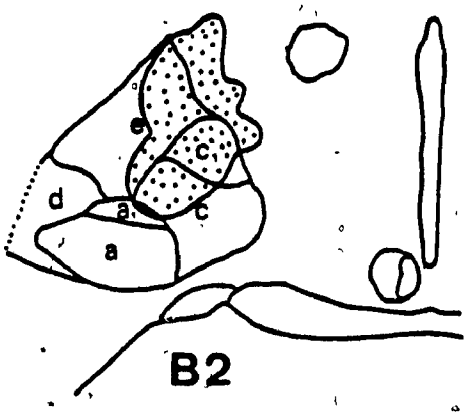
EFFECTIVE LESIONS



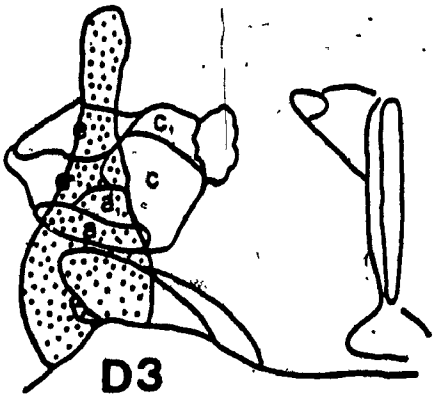
B1



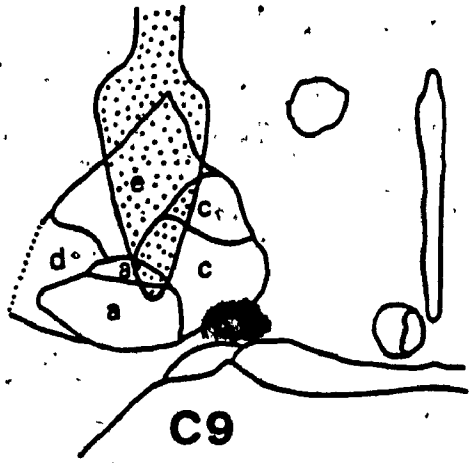
D1



B2



D3



C9

Discussion

Single Pulse Effects

Lesions to the antero-lateral portion of the MFB produced stable increases in the required number at both the LH and VTA stimulation sites. Lesions affecting more medial portions of the MFB (compartment 'c') did not produce increases in the required number at either the LH or the VTA site. In the case of the VTA site, the decrease in the rewarding impact of the stimulation cannot be attributed to the encroachment of the lesions on the field of stimulation as can be seen from the sagittal and horizontal reconstructions. Damage to neurons within the stimulation field, especially in the case of subject D1, may have contributed to some of the shifts in the required number at the LH electrode. Despite the close proximity of the lesions to the LH site, the size of the shifts in the required number are rather small, a finding consistent with the results of previous studies (Janas & Stellar, 1987; Waraczynski, 1988). This could indicate that the contribution of the anterior MFB to LH and VTA reward is a relatively minor one. However, it also possible that the small size of the shifts in the required number is due to misalignment of the lesioning and stimulating electrode. When searching for collision between the LH and VTA, small movements of the VTA electrode of around 200 microns in the dorsal-ventral plane produce dramatic changes in the collision effect. There is no reason to believe that alignment would be any less important when looking at the effects of lesions on the required number. Indeed, in the present study, very small differences in the location of the lesion resulted in large differences in

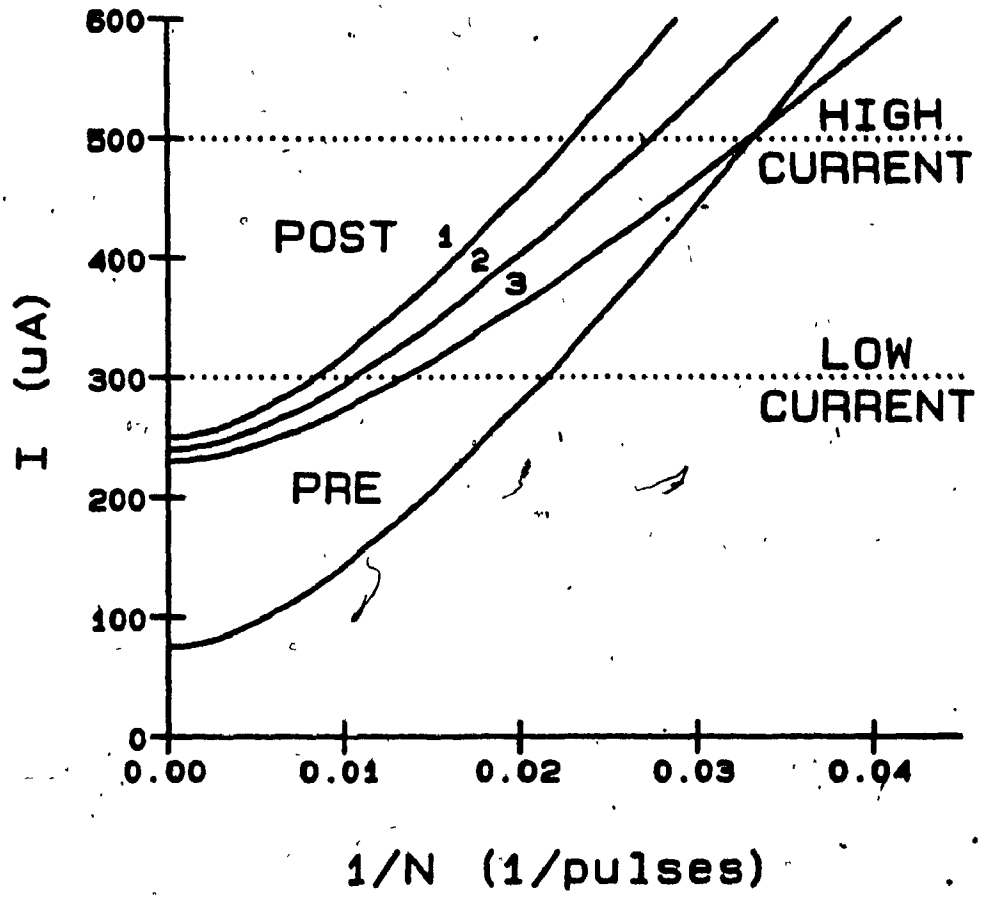
the effectiveness of the lesion in degrading MFB reward. For instance, in the case of subject C9 the first and second lesion (0.3 and 1.0 mA for 10 sec) produced very little change in the required number while the third lesion (2.0 mA for 10 sec) produced one of the largest shifts seen in this study (0.2-0.3 log units). Across animals the same impact of lesion location on effectiveness was seen. Lesions that were localized to the medial compartment 'c' of the MFB were ineffective while lesions just slightly lateral and encroaching on compartments 'a', 'd' and 'e' were effective. An obvious solution to the problem of aligning the lesion within the bundle of reward neurons is to lesion the entire cross-sectional area of the MFB. Unfortunately, this approach to the problem does not seem to work. Waraczynski (1988) found that some of her large knifecuts to the anterior MFB did not produce any decrease in the rewarding effect of MFB stimulation even though they encompassed the area damaged by smaller, effective cuts. This phenomenon of large lesions producing less extensive deficits when compared to smaller lesions has recently been discussed by Irle (1987). She suggests that the partial functioning of a damaged level in a hierarchical system may be more disruptive to the system than the complete destruction of that level. Whatever the explanation, it is clear that there is no simple relationship between the the efficacy of anterior MFB lesions and their relative size. Systematic variations in the location of small lesions, perhaps with the aid of a moveable lesioning electrode, would be better able to determine whether the small size of the shifts is due to misalignment or due to the small contribution of anterior MFB neurons to MFB reward.

The small size of the required number shifts found in the present study raises a more serious question than that of the relative contribution of the anterior MFB. Two studies that have examined the effect of performance variables on the rate-frequency function have found that manipulations of the task difficulty are able to produce shifts of up to 0.2 $\log_{10}$ units in the required number (Edmonds & Gallistel, 1974; Miliaressis et al., 1986). Since the shifts observed in this study were around 0.1-0.2 $\log_{10}$ units, it is possible that these shifts were due to performance deficits and not due to decreases in the rewarding effect. There are two reasons for doubting this interpretation. One reason is that there was no marked depression of the maximum response rate after the lesion except for subject C9. In both of the studies mentioned above, performance manipulations always produced depressions in the asymptotic rate. A second reason is that there was no change in the dynamic interval of the rate-number curves. Miliaressis et al. (1986) found that performance manipulations produced changes in the asymptotic response rate and the range over which the rate-frequency curves rose. The changes in the slope of the rate-number curves were largely responsible for the changes in the half-maximum point used as the behavioural criterion. They concluded that a better index of changes in the rewarding efficacy of the stimulation could be obtained from the intercept of the rate-number curve with the number axis since the curves pivotted around this point following performance manipulations. Since the rising portion of the pre-lesion and post-lesion rate-number curves obtained in this study were parallel, the change in the required number was not due to changes in the slope of the curves.

The findings of Miliaressis et al. (1986) and Edmonds and Gallistel (1974) raise serious questions about the use of the curve-shift paradigm in assessing relatively subtle effects on the rewarding efficacy of BSR. In those cases where the shifts are small (less than 0.2 $\log_{10}$ units) and there is a concurrent decrease in the maximum response rate, there is no way of determining whether or not these changes reflect both a performance deficit and a reward deficit or just a performance deficit. Fortunately, the parallel nature of the shifts seen in this study coupled with the lack of effect on the maximum response rate suggest that the lesion-induced changes in the rate-number curves represent real decreases in the rewarding efficacy of the stimulation.

Data from subject B1 suggests that the effects of lesions on the rate-number curve may be dependent on the current intensity. At the low current used (200uA, Figure 4), the shifts in required number were stable over time while at a higher current (480uA, Figure 13), the shifts were transient and recovered to baseline values within a few days. A possible explanation for these effects follows from the demonstration that number-current trade-off functions change as recovery occurs after a lesion (Shizgal, Howlett & Corbett, 1979). Figure 32 illustrates this point with hypothetical number-current functions taken before and after lesioning through the stimulating electrode. The lowest curve, labelled "PRE" is the function obtained before the lesion and the three higher curves ("POST" 1, 2, and 3) were collected at three different times after the lesion. The recovery process changes the slope of the post-lesion curves, pivoting then around a common point on the current axis. According to these curves, required

Figure 32. Hypothetical number-current curves taken before and after lesioning through the stimulating electrode (based on data from Shizgal et al., 1979). The three post-lesion curves show the changes that occur in the slope as a function of time post-lesion. The intersection of the two horizontal, dotted lines with the four curves gives the required number values that would have been obtained at high verus low current.


600
500
400
300
200
100
0
I (uA)
HIGH
CURRENT
LOW
CURRENT
POST
1
2
3
PRE
0.00
0.01
0.02
0.03
0.04
1/N (1/pulses)

number determinations collected at a current of 500uA would show complete recovery to baseline values while data collected at a current of 300uA would show almost no recovery. If the change in slope caused the post-lesion curve to cross the pre-lesion curve then data collected at high enough currents could result in decreases in the required number following recovery from the lesion; an outcome usually attributed to increases in the rewarding impact of the stimulation. This example is based on data collected following lesions through the stimulating electrode; whether the same kinds of changes would be seen with lesions away from the stimulating electrode would depend on the position of the lesioned fibers in the stimulation field. It would be a good idea for future lesion studies to obtain the complete number-current function in order to more fully characterize the post-lesion changes and to test whether changes in slope occur during recovery.

Collision Effects

Lesions of the anterior LH that produced increases in the required number also produced significant decreases in the size of the collision effect between the LH and VTA. Lesions that had no effect on the required number also had no significant effect on the size of the collision effect. The simplest interpretation of these data is that the effective lesions to the antero-lateral MFB damaged reward neurons linking the LH and VTA.

In the case of subject D1, an increase in the required number of approximately 0.1 log unit (26%) was seen at both the LH and VTA while a 28% decrease in the size of the collision effect was also seen. Based on these results, an important question to ask is: What proportion of the

lesioned reward fibers were also collision fibers? To illustrate the relationship between changes in the required number and changes in the size of the collision effect consider the following hypothetical example in which each stimulated neuron contributes equally to the rewarding effect: 1000 neurons are stimulated by the two electrodes and 500 of these neurons undergo collision. The collision curve in this example would rise between E-values of 0.5 and 1.0. After lesioning, the number of reward neurons is reduced to 800. In this case, the required number would increase by approximately 0.1 log units ($1000/800 = 1.25 \cong 10^{0.1}$). If all of the lesioned neurons were also collision neurons, then the number of neurons undergoing collision would be 300 (500 - 200) and the proportion of fibers undergoing collision would be 0.375 (300/800). The collision curve would now rise between E-values of 0.625 and 1.0. This would represent a 25% reduction in the size of the collision effect ((0.5 - 0.375)/0.5). Based on a 25% increase in the required number, we would therefore expect a 25% decrease in the size of the collision effect if all of the lesioned neurons were also collision neurons. Thus, under the simplest assumptions, the results from subject D1 are consistent with the hypothesis that all of the reward neurons damaged by the lesion were also collision neurons.

The correlation between the single pulse effects and the collision effects is not as consistent in the case of subject D3. Recall that the shifts in the required number occurred after the second lesion and were only long-lasting for the VTA electrode. Based on this finding, we might predict that non-collision fibers had been damaged by the lesion (fibers stimulated at the VTA but not at the LH) and therefore the collision effect should have gotten

larger after the second lesion. The collision effect in fact was 27% smaller after the lesion and this decrease occurred after the first lesion not the second. The second lesion also produced a small decrease in the size of the collision effect (12% compared to the first post-lesion curve) but the lower asymptote of the first post-lesion curve was not significantly different from the lower asymptote of the second post-lesion curve. This discrepency between the change in the required number and the change in collision raises some concern about the use of the collision technique in assessing the effects of lesions on reward. Should we "believe" the shifts in required number or should we believe the changes in the collision effect? There are several reasons for considering the collision data more reliable than the single pulse data. One reason long-term changes in the required number may not have been seen at the LH in the case of D3 is because of the arbitrary choice of current used for that electrode. The data for the LH electrode for subject D3 (Figure 13, top panel) suggests that this is the case. Shifts in the required number of up to 0.2 $\log_{10}$ units were seen after both of the lesions but they recovered to baseline values within a few days. Perhaps, if a lower current had been used, less recovery would have been seen for the reasons stated earlier. A transient shift in required number was not seen following the first lesion for the VTA electrode so that even if this argument holds, an increase in the collision effect still should have been seen after the first lesion. There is also the possibility that compensatory effects on the required number could act to cancel one another out so that even though reward neurons had been damaged, no shifts are seen in the rate-number curve. The basis for such an argument comes from data collected by Waraczynski (1988). She found that many of her knifecuts produced

decreases in the locus of rise (required number) indicative of an increase in the rewarding efficacy of the stimulation. If a lesion damaged an area that normally would produce a decrease in the required number as well as damaging an area that alone would produce an increase, the net effect may be no change in the required number. Synaptic recovery mechanisms (e.g., increased neurotransmitter release, receptor supersensitivity, etc.) and damage to inhibitory mechanisms, while greatly affecting the rate-number curves, would be less likely to affect the collision curves. The only way a decrease in collision could be observed following a lesion is if there was a decrease in the number of collision fibers, their efferents, or neurons that gate their efferent signals. Recovery could only occcur if there was some regeneration of the neurons (or efferents) linking the LH and VTA or if there was an increase in the behavioural weighting of neurons efferent to the fibers still undergoing collision.

One way of testing the predictions we have made about changes in the collision effect would be to lesion through one of the stimulating electrodes used in the collision test. It would first be necessary to judge the relative distance between the collision fibers and the electrode tips. For instance if collision was found at low currents, then the field of stimulation would be small and the collision fibers would have to be close to the electrode tip. If collision was only found at high currents, then the collision fibers would be located far from the electrode tip. Of course, the currents passed through each electrode could be unbalanced so as to determine whether collision fibers were located close to one electrode but far from the other. If the collision fibers were located far from the tip of one electrode, then a small

lesion through the tip of the electrode would only damage non-collision fibers and the collision effect should get larger. Conversely, if the fibers were located close to the tip of one electrode then a small lesion would be expected to damage many collision fibers and the collision effect would get bigger. This experiment would test the predictions we have made and give us greater confidence in interpreting the effects of lesions on collision, especially if these effects are not in accordance with the effects on the required number.

Candidate Pathways

Lesions that were ineffective in producing increases in the required number were found to be localized to the medial compartment 'c' of the MFB as described by Nieuwenhuys et al. (1982). The five effective lesions encroached on the more lateral compartments 'a', 'd' and 'e'. Using autoradiography, Veening, Swanson, Cowan, Nieuwenhuys, and Geeraedts (1982) examined the position and topographic relationship of several structures known to contribute fibers to the MFB. They found that descending fibers from the olfactory tubercle, nucleus accumbens, and central amygdaloid nucleus project primarily through the lateral compartments 'a', 'd' and 'e' while descending fibers of septal origin appear most concentrated in the ventro-medial MFB. Since in all cases there was some scattered fibers labelled in other compartments, it is not possible to eliminate any given projection based on the histology of this study. Even though the majority of septal fibers were found in the medial MFB, it is possible that the lesion effects were due to damage to the few septal fibers found in the lateral

compartments. Similarly, it is possible that the lesion effects were due to damage to diffuse MFB projections, e.g. from lateral preoptic area (LPO), if all of the reward fibers from this nucleus project through the lateral MFB. Nonetheless, the differences between the location of the effective and ineffective lesions is intriguing and raises the possibility that smaller lesions, perhaps using a moveable lesioning electrode, would further distinguish the relative importance of the MFB compartments in MFB reward. These findings also suggest that the inconsistencies found in previous lesion studies could be due to subtle differences in lesion location that were not detected in the histological analysis.

Fibers Arising in the Amygdaloid Nuclei

Several studies point to a possible involvement of amygdaloid fibers in MFB reward. The effectiveness of lateral MFB lesions found in the present study is consistent with damage to central amygdaloid fibers that project predominantly through the lateral MFB (Veening et al., 1982). Anatomical studies have found that amygdaloid projections pass by the LH and VTA and some continue on to the periaqueductal grey (PAG, Hopkins & Holstege, 1978; Krettek & Price, 1978) which has been shown to contain reward-relevant fibers common to the VTA and posterior LH (Boye & Rompré, 1987). Rolls (1972) recorded from units in the baso-lateral amygdaloid nucleus and nearby pyriform cortex and found that the cells were antidromically activated by LH stimulation. The refractory periods of these cells were within the range of estimates for MFB reward neurons. Bilateral, electrolytic lesions to the amygdala have been found to produce large increases in the current threshold for LH self-stimulation although these

increases were found to recover over time (Kelly, 1974). One study that is not in keeping with a role for the amygdala in MFB reward has found that subthreshold LH stimulation influences the performance for, but not the rewarding impact of, threshold amygdala stimulation (Kane, Coulombe, & Miliaressis, 1988).

Fibers Arising in the Septal Nuclei

The lack of effect of the two lesions located in the medial MFB, where the highest concentration of fibers originating in the septum are found, is not consistent with their playing a large role in MFB reward. Knifecuts directly in the septum have also failed to produce substantial decreases in the rewarding efficacy of LH stimulation (Waraczynski, 1988). These data are disappointing in that septal units, driven by LH or VTA stimulation, have been found to possess refractory periods that agree with the estimates obtained from psychophysical tests (Rompré & Shizgal, 1986). The ineffectiveness of septal lesions suggests that these septal neurons are probably "imposters": cells that resemble MFB reward-relevant cells but in fact play no role in MFB reward.

The Path Neurons of the MFB

The effect of lesions on LH and VTA self-stimulation seen in the present study may have been due to damage to cell bodies located in the antero-lateral MFB. The intrinsic neurons of the MFB, the so-called path neurons, are scattered throughout the lateral preoptic-hypothalamic zone and possess ascending and descending axons (Millhouse, 1969). The path neurons have

long been considered a potential component of the MFB reward system (Olds, 1962; Szabo, 1972) and none of the psychophysical data collected to date has suggested that they are incompatible with the characteristics of the first-stage neurons. Autoradiographic studies have found that the path neurons located in the LPO and LH send projections past the VTA and PAG (Arbuthnott, Mitchell, Tulloch, & Wright, 1976; Hosoya & Matsushita, 1981; Saper, Swanson, & Cowan, 1979; Swanson, 1976) thus providing anatomical support for their possible role in MFB reward.

Huston and his co-workers have shown that lateral hypothalamic self-stimulation in the guinea pig is possible after removal of the ipsilateral telencephalon and transection of all forebrain commissures down to the level of the brachium conjunctivum (Mueller, Huston, and Pritzel, 1981). This study suggests that the intrinsic neurons of the diencephalon are sufficient for MFB self-stimulation to occur. A more interesting question from the perspective of the psychophysical approach is whether the path neurons transmit part or all of the reward signal in the intact animal and if so do they give rise to the descending reward axons that link the LH and VTA.

If path neurons are an important component of MFB self-stimulation, then their selective destruction should result in shifts in the rate-frequency function towards higher frequencies. Most of the studies of MFB self-stimulation that have injected neurotoxins into the MFB have used ibotenic acid (Lestang, Cardo, Roy, & Velley, 1985; Nassif, Cardo, Libersat & Velley, 1985; Velley, 1986; Velley, Chaminade, Roy, Kempf & Cardo, 1983). Ibotenic acid is a neurotoxin that was thought to kill neurons while sparing fibers of passage (Schwarcz, Hokfelt, Fuxe, Jonsson, Goldstein, & Terenius, 1979)

however recent evidence has shown that ibotenic acid also causes demyelination as well as neuronal depletion (Waraczynski & Stellar, 1987; Coffey, Perry, Allen, Sinden, & Rawlins, 1988). It is therefore not possible to attribute their effects solely to the destruction of path neurons. These studies have also either failed to use curve-shift scaling or have interpreted scalar decreases in the rate of responding at different current intensities for decreases in the rewarding impact of the stimulation. Edmonds and Gallistel (1974) and Miliaressis et al. (1986) have shown that scalar decreases in the response rate at different levels of stimulation strength occurs after manipulation of performance variables. Sprick, Munoz and Huston (1985) used a procedure closer to curve-shift scaling. They found that ibotenic acid or the neurotoxin kainic acid injected through a cannulae located .5 mm above the electrode tip, failed to alter the weakest current required to support a maximal rate of responding. Although this study does not implicate path neurons located near the electrode tip, there remains a possible role for path neurons with somata at other levels of the MFB.

The results of the Mueller et al. (1981) ablation study suggest that the intrinsic neurons of the MFB are sufficient for MFB self-stimulation. Unfortunately, the effect of the selective destruction of path neurons on MFB self-stimulation is not clear due to the methodological problems of studies employing ibotenic acid. The knifecut studies described in previous sections (Janas & Stellar, 1987; Waraczynski, 1988) and the present study could also be interpreted as implicating path neurons in MFB reward since the changes in threshold were relatively small given the proximity of the lesions to the stimulating electrode. As soon as a more specific neurotoxin

is found, studies examining the effect of lesions to path neurons found along the entire extent of the MFB will be the next crucial step in the resolution of this issue.

Fibers Arising in the Lateral Preoptic Area

The path neurons located at the level of the LPO are a particularly attractive candidate for the origin of the descending MFB reward neurons. Injection of labelled amino acids into the junction between the LPO and LH has revealed projections that pass near the LH, VTA and PAG (Swanson, 1976). The LPO also shows an increase in uptake of 2-deoxyglucose during self-stimulation of the ipsilateral MFB (Gallistel, Gomita, Yadin, & Campbell, 1985). Lesions in the present study encroached on the posterior LPO and may have damaged fibers projecting from the LPO, through the lateral compartments of the MFB, to the LH and VTA. Janas and Stellar (1987) found that knifecuts in the posterior LPO produced increases in the frequency threshold for self-stimulation of the LH ranging from approximately 0.2 to 0.4 $\log_{10}$ units. Waraczynski (1988) also found that some of her knifecuts in the LPO produced long-term increases in the threshold for LH self-stimulation but her results were more erratic and there was considerable variation across subjects that could not be readily explained from the histology. Radio-frequency lesions centered in the LPO have produced decreases in the rate of self-stimulation for three fixed currents corresponding to 25, 50 and 100 per cent of maximum response rates (Munoz, Kellar & Huston, 1985). The use of three fixed currents makes it difficult to ascertain the change in the threshold for self-stimulation but for three

animals self-stimulation could not be obtained at the pre-lesion currents so thresholds were inadvertently obtained. In these 3 subjects currents 3.5-6.7 times (0.54-0.83 $\log_{10}$ unit increase) the prelesion currents were required to reinstate self-stimulation. Since the lesions encroached on many structures besides the LPO (including the nucleus accumbens, diagonal band of Broca, anterior hypothalamus, ventromedial hypothalamus, dorsomedial hypothalamus, lateral hypothalamus and caudate nucleus), it is impossible to ascertain whether the LPO was the critical structure.

In order to determine whether the intrinsic neurons of the LPO play a role in LH reward, Huston, Keifer, Buscher and Munoz (1987) examined the effects of ibotenic acid lesions of the LPO on LH self-stimulation. Huston et al. found that ibotenic acid lesions centered in the LPO produced decreases in the rate of responding at a high and low current intensity for LH stimulation. As was the case with the study by Munoz et al. (1985), lesions encroached upon structures other than the LPO and the use of two fixed currents prevents an analysis of threshold changes. The effects on self-stimulation seen in this study cannot be unequivocally attributed to damage to LPO neurons since the neurotoxin ibotenic acid was used. An interesting finding of the Huston et al. study was that a decrease in the rate of LH self-stimulation was seen for the low current condition but not the high current condition in a group of animals with small lesions. This finding is consistent with the hypothesis that greater recovery is evident at high current intensities.

Some electrophysiological studies have found LPO cells with characteristics within the range of the psychophysical estimates for the MFB

reward neurons. Barone, Wayner, Tsai, and de Coronado (1981) found 3 cells in or near the LPO that were antidromically driven by PAG stimulation. The conduction velocities of 1.02, 2.45, and 2.52 m/sec recorded for the 3 cells are within the lower end of the behavioural range (1-8 m/sec). Rompré and Shizgal (1986) have also recorded from 6 cells near or in the LPO that were antidromically activated by LH and/or VTA stimulation. They reported that 3 of the cells found near the border of the medial and lateral preoptic area had refractory periods within the behavioural range for MFB reward neurons (< 1.5 msec).

A recent psychophysical study aimed at the characterization of reward neurons in the basal forebrain has found that refractory period estimates for reward neurons stimulated at the LPO overlap with, but are not identical to, the refractory period estimates for MFB reward neurons (Fouriezos, Walker, Rick, & Bielajew, 1987). Refractory period curves began to rise at C-T intervals of 0.6 and 0.8 msec but did not level off until 5.0 msec. These results indicate that some LPO reward neurons recover more slowly than MFB reward neurons and others are of similar caliber to MFB reward neurons. Bielajew, Thrasher and Fouriezos (1987) have found evidence of collision between self-stimulation sites in the LPO and the anterior LH indicating a direct axonal link between the two areas. All but one of the collision curves show a double step; an initial rise in effectiveness compatible with the collision curves obtained from the LH-VTA segment of the MFB and a later rise compatible with a population of slowly conducting neurons. The contribution of these slowly conducting fibers is, for the most part, absent

from data collected from MFB electrodes, suggesting that these fibers synapse somewhere between the anterior and posterior LH.

The knifecut studies of Janas and Stellar (1987) and Waraczynski (1988) as well as the present study have provided evidence that LPO neurons may play a role in MFB reward and recording studies have confirmed that at least some LPO cells exist with characteristics that match those of MFB reward neurons. Some of the reward fibers linking the LPO and anterior LH have characteristics similar to the reward fibers connecting the LH and VTA but the two bundles cannot be identical. Together, this information suggests that the LPO is a promising candidate for the origin of the descending MFB reward fibers. Collision studies, bridging the present gap between the anterior and middle LH, will help to determine whether there exists a direct connection between the LPO and posterior LH or VTA.

Ascending Dopamine Fibers from the VTA

Ascending dopamine fibers from the VTA pass through the areas damaged by the effective lesions in this study (Lindvall and Bjorklund, 1974). Since injection of dopamine receptor blockers has been found to attenuate the rewarding effect of MFB stimulation (Liebman & Butcher, 1974; Fouriezos and Wise, 1976; Franklin, 1978; Gallistel, Boytim, Gomita, Klebanoff, 1982), it is possible that the shifts in the required number seen in the present study were due to damage to ascending dopamine fibers in the lateral MFB. The reduction in the size of the collision effect, if due to damage to the directly activated collision fibers, is not consistent with damage to dopamine fibers since their conduction velocities are too slow to account for the

psychophysical data (Guyenet & Aghajanian, 1978; Yim & Mogenson, 1980; German, Dalsass, & Kiser, 1980). If the reduction in the collision effect was due to damage to the efferents of the cells undergoing collision, then it is possible that dopamine neurons were involved. This explanation requires that the efferents of the collision neurons be spatially segregated from the efferents of the non-collision neurons.

Conclusions

Lesions in the antero-lateral portion of the MFB produced stable increases (26-58%) in the required number at both the LH and VTA stimulation sites. These changes were not accompanied by corresponding increases in the dynamic interval of the rate-number function or systematic decreases in the asymptotic rate of responding. Thus it would appear that fibers or cell bodies in the damaged areas contributed to the rewarding value of the stimulation. Based on the size of the increases in the required number and the assumptions of the counter model of spatio-temporal integration in the reward substrate (Gallistel, 1978), lesions destroyed approximately 20-37% of the neurons stimulated at the electrode sites. Subtle differences in the location of the lesions produced dramatic differences in the effect on the required number: ineffective lesions were localized to the medial compartment 'c' of the MFB while effective lesions were slightly lateral and encroaching on compartments 'a', 'd' and 'e'. The importance of the location of the lesion within the MFB may be one reason previous studies have found seemingly inconsistent results from similarly positioned lesions or knifecuts. Future lesion studies, again employing small, well-localized lesions, will help determine the relative importance of the lateral MFB compartments ('a', 'd', and 'e') and perhaps further narrow down the possible candidate pathways involved. Based on the differences in the amount of recovery seen at high versus low current for subject B1, future studies should also employ several current intensities in order to maximize the likelihood of seeing long-term effects on the required number.

Lesions to the anterior LH that produced increases in the required number also produced significant decreases (27-35%) in the size of the collision effect between the LH and VTA. Lesions that had no effect on the required number also had no significant effect on the size of the collision effect between the LH and VTA. The simplest interpretation of these data is that the effective lesions to the antero-lateral MFB damaged 27-35% of the reward neurons linking the LH and VTA. Based on data reviewed and the results of this experiment, likely candidate sites for the origin of the descending MFB reward neurons include the amygdala and path neurons of the MFB.

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