Self-Stimulation of the Lateral Hypothalamus and Substantia Nigra: Excitability Characteristics of the Directly Stimulated Substrates

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ABSTRACT

Self-Stimulation of the Substantia Nigra and Lateral Hypothalamus: Recovery from Refractoriness in the Directly Stimulated Substrates

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Anatomical studies show that descending fibers link the lateral hypothalamus (LH) and substantia nigra (SN). The present series of experiments tests the hypothesis that some of these fibers are responsible for intracranial self-stimulation. Specifically, these experiments were designed to behaviourally estimate recovery from refractoriness in the neural substrate for SN self-stimulation, using the pulse-pair paradigm, and to compare it to recovery in the LH substrate. Similar refractory period estimates would be consistent with the notion of a shared subpopulation of reward-relevant neurons. In order to minimize experimenter error and to maximize the number of subjects, a computer-controlled testing setup was developed. In order to assess the performance of this set-up, refractory period estimates obtained in the newly designed, computer-controlled equipment were compared to those obtained in the hand-operated equipment used in previous experiments of this type. No meaningful differences were found. Thus, the more convenient computer-controlled setup was used to collect refractory period estimates from the SN and LH. A comparison of these values revealed differences in the slopes of the recovery curves, and in the C-T intervals bracketing their rising portions. The SN curve rose more gradually, and both began to rise and levelled off at longer C-T intervals than the LH curve. One explanation of these results is

that the distribution of excitability in the LH substrate is shifted towards higher values than the SN distribution. The overlapping portions of the recovery curves could reflect the contribution of a common bundle of reward-related fibers.

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DEDICATION

This thesis is dedicated to David, and to my family.

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Following electrical stimulation of appropriate sites in a rat's brain, the rat will modify its behaviour to receive more of the same stimulation. The phenomenon responsible for the change in behaviour is called brain stimulation reward (BSR). It appears that in order for such behaviour modification to occur, information about the hedonic consequences of brain stimulation must be processed and stored, perhaps in a cognitive map. If so, studying the characteristics and trajectories of the directly stimulated neurons subserving BSR may lead to an understanding of where and how the processing and storage of information about hedonic events takes place.

Identification of the neurons subserving BSR cannot be accomplished by visual inspection because they cannot, at least at this time, be distinguished visually from vicinal neurons that subserve functions other than BSR. A current approach to the identification of such neurons involves using psychophysical techniques to behaviourally estimate their characteristics. These characteristics are then used in electrophysiological studies to locate neurons that could be responsible for the rewarding effects (Gallistel, Shizgal and Yeomans, 1981). After a candidate neuron is located by means of electrophysiological recording, the cell can be penetrated and a dye injected, in order to trace the trajectory of its axon. By describing the trajectories of the best candidates, one obtains a draft of a circuit diagram for the directly stimulated stage of the BSR substrate.

Neural circuit diagrams have been employed in invertebrate neurobiology to explain behaviour in terms of the wiring of the nervous

system. It is hoped that a diagram of the neural circuits subserving self-stimulation in the rat will have similar explanatory power.

The substrate for BSR in the medial forebrain bundle (MFB) has been the object of much investigation. Behavioural studies have established that some of the directly driven neurons responsible for self-stimulation of the MFB project in a descending fashion through the LH and the VTN (Shizgal et al., 1980; Bielajew and Shizgal, 1982, 1984). Do these neurons terminate in the VTN, or do some or all of them continue descending? Anatomical studies have demonstrated that some descending neurons traverse the portion of the MFB between the lateral hypothalamus (LH) and the ventral tegmental area of Tsai (VTN). Some of these fibers are known to turn sharply at the VTN and travel over the substantia nigra (SN) (Nauta and Domesick, 1981). Does the substrate for MFB self-stimulation include such fibers?

The present set of experiments address this question, albeit indirectly. A psychophysical technique is employed to behaviourally estimate the refractory period of the neurons responsible for self-stimulation of the SN. This estimate is compared to an estimate of the refractory period of the neurons subserving the rewarding effects of LH stimulation. If common reward-related fibers course through the LH and SN, then it is likely that the refractory period estimates obtained from the two sites will be similar.

The refractory period is part of the excitability cycle. Thus, a general discussion of excitability cycles and their behavioural

derivation is provided below. First, the rationale for mapping the neural circuitry subserving BSR and for using the psychophysical approach to this end is discussed in more detail.

I. Rationale for studying BSR

In the self-stimulation paradigm, behaviour directed toward the lever is maintained by focal stimulation of a specific brain site. It can thus be used as a tool for identifying neurons that subserve a goal-directed behaviour. It has been argued that these neurons may also play a role in naturally occurring goal-directed, appetitive behaviours, such as eating and drinking.

Two types of studies, behavioural and electrophysiological, support the assertion that a common system processes and stores information about the reward value of brain stimulation and naturally occurring goal objects, such as food. Four lines of behavioural evidence for a link between BSR and feeding have been reviewed by Hoebel (1975). First, electrodes aimed at the LH have been shown to support both BSR and feeding. Second, postingestive factors such as high osmotic and gastric loads have been shown to depress both feeding and BSR. The third piece of evidence is that depression of self-stimulation behaviour is coupled with an increase in stimulation-escape behaviour in satiated subjects, reducing the likelihood that a food load simply disrupts operant behaviour. Fourth, appetitive suppressants such as phenylpropanolamine also suppress self-stimulation.

Electrophysiological support for a link between BSR and feeding comes from a study of neuronal activity in monkeys (Rolls, Burton and Mora, 1980). Rolls et al. (1980) discovered that food presentation altered the activity of some neurons trans-synaptically driven by electrical stimulation at BSR sites. They found that 13.6% of 764 trans-synaptically driven neurons sampled from the LH and substantia innominata increased in activity when the subject was both hungry and presented with a food stimulus. This suggests that some neurons receive inputs associated both with BSR and food reward.

In order to deduce a circuit diagram for BSR, it must be possible to distinguish the neurons that subserve BSR from other neurons. However, no anatomical features of neurons subserving BSR have been found that distinguish them from all other intertwined neurons. A four stage approach to this problem has been suggested (Gallistel et al., 1981). First, behavioural experiments are conducted to determine trade-off functions for the directly stimulated BSR substrate. These functions serve to limit the neurons that are likely to carry the reward signal. Next, an attempt is made to explain the behaviourally derived trade-off functions in terms of the physiological and anatomical properties of the neurons involved. Third, electrophysiological studies are conducted, in which recordings are made from neurons driven by stimulation at BSR sites. In the fourth and final stage of Gallistel et al.'s (1981) approach, information from the first three stages is integrated. Electrophysiological recording data from stage three are examined, and candidate BSR neurons are identified as those neurons having characteristics similar to those inferred from behavioural

trade-off experiments.

It may seem surprising that characteristics of MFB cells could be inferred from behavioural observation. The inferential logic can be illustrated with reference to the visual system where analogous inferences have played an important role. These inferences were derived from the results of trade-off experiments. In experiments of this type, the value of one parameter required to produce a criterion level of performance at each setting of another parameter is determined. The pairs of parameters that suffice to produce the criterion level of performance are plotted, resulting in a curve that describes the relationship between the parameters. The human scotopic spectral sensitivity function, which represents a trade-off between wavelength and intensity, was derived in such a manner. Flashes of dim light were shown to a dark-adapted human observer. The intensity of the flashes was adjusted until the frequency of seeing them met a constant behavioural criterion, a correct report of seeing a flash on 60% of the trials. This procedure was repeated for different wavelengths. The human spectral scotopic sensitivity curve was derived by plotting the behaviourally equivalent wavelength/intensity values. This curve, after correction for the light absorbed by non-visual pigments in the visual system, is very similar to the in vitro absorption spectrum of rhodopsin, which represents the wavelength/intensity combinations that isomerize an equal amount of rhodopsin (Cornsweet, 1970). No other visual pigment or combination of pigments has an identical absorption spectrum to that of rhodopsin. On these grounds, rhodopsin is identified as a strong candidate for the pigment responsible for human

vision in dim light, a view supported by many converging lines of evidence.

How can a psychophysically derived trade-off function, based on verbal responses, describe the action spectrum of a photopigment? All levels of the nervous system, from the rhodopsin molecules to the articulatory apparatus, must behave monotonically under the conditions of the experiment. A given level behaves monotonically when one and only one output value corresponds to each input value. In a system composed of concatenated monotonic stages, one observes a unique output from the final stage for each input to the first stage, regardless of how many stages intervene (Gallistel et al., 1981). In the visual system, behaviourally equivalent wavelength/intensity combinations appear to isomerize equivalent amounts of rhodopsin - the psychophysical method "sees through" the processes intervening between stimulus (wavelength/intensity combination) and response (verbal output).

The trade-off experiments employed in the characterization of the neural substrate subserving BSR depend in an analogous fashion on the principle of monotonicity. Because the BSR system has been shown to behave monotonically over a wide range of stimulation parameters (Edmonds, Stellar and Gallistel, 1974; Gallistel, 1978), the experimenter can "see through" the processes intervening between initial stimulus (pattern of electrical stimulation) and ultimate response (lever pressing).

The present experiment embodies the first two stages of Gallistel et al.'s (1981) four stage approach to mapping the reward-related circuitry. Stage one consists of using trade-off functions to behaviourally derive characteristics of the neurons subserving reward at sites in the SN. The derived characteristics are then explained in terms of the refractory periods of the directly stimulated neurons (stage two). If further study of the neurons subserving self-stimulation of the SN were conducted, it could include electrophysiological measurement of the refractory periods of individual neurons driven by stimulation of the SN (stage three). Stage four of Gallistel et al.'s (1981) approach would then consist of a comparison between the electrophysiologically and behaviourally derived refractory period estimates. Single neurons with electrophysiologically determined refractory periods similar to the behaviourally derived estimates would be considered likely components of the reward substrate; those with markedly dissimilar refractory periods would be removed from consideration.

II. Excitability Cycles

The excitability cycle of a single cell is determined by using a pulse-pair technique. The first pulse of the pair is called the conditioning (C) pulse, and the second pulse is called the test (T) pulse. The interval between the two pulses is called the C-T interval. If the C and T pulses are equal in amplitude, and if the T pulse is delivered before the depolarization produced by a subthreshold C pulse

has decayed to less than 50% of threshold, then an action potential will be triggered by the T pulse. This effect is called local potential summation. Because the subthreshold change produced by the C pulse decays rapidly with time, the local potential summation effect decreases quickly as the C-T interval increases.

If the C pulse current surpasses the firing threshold of a neuron, an action potential is generated and the post-stimulation excitability cycle is set in motion. The four stages of the post-stimulation excitability cycle were first established for peripheral nerves by Erlanger and Gasser (1937). These stages also have been described in central neurons (Swadlow and Waxman, 1978), and thus probably apply to the neurons subserving BSR. The four separate phases of post-stimulation excitability identified by Erlanger and Gasser (1937) were: absolute refractory period, relative refractory period, supernormal period and subnormal period. The first stage, the absolute refractory period, is evident immediately after an action potential has been triggered by the C pulse. During this stage, a second action potential cannot be elicited, regardless of the amplitude of the T pulse. The relative refractory period follows. The T pulse can now generate an action potential, but only if its amplitude exceeds the threshold value for firing the neuron when it is in its resting state. The next stage is the supernormal period, during which the neuron's threshold is somewhat depressed; it will fire in response to less current than when it is at rest. The last stage is the subnormal period in which the threshold of the neuron is somewhat elevated; more current is required to generate an action potential.

Because stimulation of the brain through macroelectrodes activates many neurons at once, it is necessary to generalize from these descriptions of the post-stimulation excitability cycle of a single neuron to the case where there are numerous axons surrounding an electrode tip. Assume that the excitability cycles of these neurons are identical. If tissue impedance is homogeneous in the plane perpendicular to the long axis of the axons, they are stimulated in a region that is circular in cross-section. Because current density decreases as a function of distance from the electrode tip (Ranck, 1975), neurons on the periphery of this region of stimulation do not receive enough current to generate an action potential in response to a C pulse. Such neurons will undergo subthreshold depolarization. Those neurons that were depolarized to at least 50% of the threshold value will fire in response to the T pulse, if the C-T interval is sufficiently short. In other words, these cells will undergo local potential summation.

All neurons that lie between the electrode tip and the region in which local potential summation occurs will fire in response to the C pulse. They cannot fire in response to the T pulse until the end of the absolute refractory period.

As the C-T interval increases, more and more neurons will be able to fire in response to the T pulse, which falls later and later in the relative refractory period. Neurons in the region of high current density, close to the electrode tip, will respond at the shorter C-T intervals. Neurons in regions of lower current density, farther away

from the electrode tip, must be in a more advanced state of recovery (i.e. have less elevated thresholds) before they can fire to the T pulse. Thus, these neurons will only fire in response to the T pulse when the C-T interval is longer. Eventually a C-T interval is reached at which all axons fired by the C pulse have recovered from refractoriness.

The effect of the supernormal period can be noted at yet longer C-T intervals, but only if the C pulse is of greater intensity than the T pulse (Yeomans, 1979). The subnormal period, the last of Erlanger and Gasser's (1937) four phases of post-stimulation excitability, may be small and difficult to detect (Yeomans, 1979).

A population recovery curve is obtained by plotting the number of neurons fired by the T pulse against the C-T interval. This recovery curve will initially fall due to decay of local potentials, then rise gradually due to recovery from refractoriness, and finally level off. Note that although ,in this example, all neurons in the region of stimulation are identical and fire in an all-or-none fashion, the recovery curve rises gradually. This is because the neurons are located at varying distances from the electrode tip. Neurons closer to the electrode tip are exposed to higher current densities than those farther away (Ranck, 1975), thus the closer neurons will receive sufficient stimulation to fire before the more distant ones.

Neurons distributed about an electrode tip may not be identical, but may be divided into classes on the basis of their diameters. How

would this influence the recovery curve? Stimulation of a group of neurons of different diameters will result in a recovery curve that is an average of the post-stimulation recovery curve for each class, weighted with respect to the proportion of the total number of stimulated fibers each class represents, with respect to the behavioural weight assigned to each class and with respect to the spatial distribution of the fibers about the electrode tip.

Neurons of different diameters are differentially excitable, large neurons being more readily excited than smaller ones. Thus, larger neurons recover from refractoriness sooner than smaller neurons, and the region in which large diameter neurons are stimulated is larger than the region is which small diameter neurons are stimulated. When the regions of stimulation for all fiber classes are superimposed on each other about the stimulating electrode tip, the periphery of the stimulation region for a group of small diameter neurons, for example, may correspond to mid-range in the stimulation region for a group of larger neurons.

In addition, neurons may be irregularly distributed at varying distances from an electrode tip. These irregularities will be reflected in the shape of the recovery curve for each class of fiber. As explained above, the population recovery curve will be a weighted average of the possibly irregular recovery curves for the individual classes.

According to the psychophysical logic developed above, it should be possible to infer from behaviour the population recovery curve for the directly stimulated substrate for self-stimulation.

Behavioural inference of excitability cycles

Deutsch (1964) pioneered the use of the pulse-pair technique in behavioural experiments designed to determine the post-stimulation excitability characteristics of BSR neurons. One of Deutsch's (1964) techniques used rate of responding as the dependent measure. Yeomans (1975) found that when this technique was employed, the stimulation parameters chosen determined the refractory period estimate obtained. He proposed a method of scaling that appears to circumvent this problem. This method relies on a constant behavioural output, an analagous measure to the 60% positive criterion used during psychophysical measurement of the human spectral scotopic sensitivity function. The number of pulse-pairs required to maintain a constant behavioural output (required number) is traded off against C-T interval. At C-T intervals where the neurons are in their relative refractory period, the T pulse generates fewer action potentials than the C pulse. More pulse-pairs must be added to the train to maintain the same level of excitation, and hence the same behavioural output. The number of pulse-pairs that must be added is directly proportional to the number of action potentials lost due to refractoriness, according to the counter model of integration (Gallistel, 1975). Yeomans' (1975) scaling formula makes use of this relationship by comparing the number of pulse-pairs required

to meet a constant behavioural criterion for a given pulse-pair interval to the number of single pulses required to meet the same criterion.

$$E = \frac{RN_{SP} - RN_{C-T}}{RN_{C-T}}$$

where E = effectiveness of the T pulse,

RN_{SP} = required number of single pulses

and RN_{C-T} = required number of pulse-pairs at a given C-T interval.

In the above equation, an E value of one results when the total number of pulses required to meet a constant behavioural criterion is identical for both single pulses and pulse-pairs. For example, if forty single pulses were required to meet a half-maximal lever pressing rate, and twenty pulse-pairs were required to meet the same criterion, the total number of pulses in each condition is equivalent and the E value will be one. Given the simplest assumptions, that all neurons and firings have the same behavioural weight, an E value of one means that the number of neurons fired by the T pulse is equal to the number of neurons fired by the C pulse. In other words, when the E value is one, all the directly stimulated neurons subserving BSR have recovered from refractoriness.

An E value of zero will result when as many pulse-pairs as single pulses are required to meet the same behavioural criterion. In this case, the T pulse fires no reward neurons because they are in their absolute refractory periods.

When effectiveness of the T pulse is plotted against C-T interval, a curve is obtained that is similar in shape to a refractory period curve collected by electrophysiological recording from nerves or fiber bundles (Yeomans, 1975).

III. Rationale for Studying SN Excitability

The present set of experiments stems directly from the findings of previous studies. These studies employed psychophysical techniques based on the collision and anodal block phenomena to determine whether reward-relevant neurons course through the LH and the ventral tegmental nucleus of Tsai (VTN) (Bielajew and Shizgal, 1982; Shizgal et al, 1980) and, if so, the direction in which these fibers conduct (Shizgal, Kiss and Bielajew, 1982; Bielajew and Shizgal, 1984). These studies show that a bundle of primarily or exclusively descending reward-relevant neurons directly links the LH and VTN.

Several anatomical studies have demonstrated that descending fibers course through the LH and the VTN at least to a region dorsal to the SN (Nauta and Domesick, 1981; Saper, Swanson and Cowan, 1979; Wolf and Sutin, 1967). Hence, it seems plausible that some of the reward-relevant neurons linking the LH and VTN continue on to the SN. Behaviourally derived refractory period estimates for the directly linked BSR substrates in the LH and VTN are very similar. Thus, finding similar refractory period estimates for the LH and SN reward substrate(s) would be consistent with the existence of reward-relevant fibers that link the two areas. Such a finding would provide impetus for undertaking the more difficult and more

decisive collision study.

Regardless of whether a collision study would demonstrate a common substrate for LH and SN self-stimulation, the refractory period results are of interest. The LH substrate has been subject to extensive psychophysical investigation (Gallistel et al., 1981); electrophysiological experiments that exploit the psychophysical data in an attempt to identify the LH substrate are well underway (Rolls, 1971; Matthews, 1978; Shizgal et al., 1982; Shizgal and Rompré, 1984). In contrast, no psychophysical study or psychophysically inspired electrophysiological study has yet been carried out on the SN substrate. If the directly-stimulated substrates for self-stimulation of the LH and SN are different, then the behaviourally derived refractory period estimates described here will be useful in the electrophysiological identification of the SN substrate.

EXPERIMENT 1

To date, refractory period estimates have been collected in this laboratory using hand-operated equipment. Collecting data in such a fashion is both tedious and error-prone, due to the many manipulations the experimenter is required to perform. In order to circumvent these problems and to increase the number of subjects that can be tested concurrently, a computer system has been developed in which test parameters are automatically determined. In addition, the computer records the subject's responses and analyzes the experimental results. To test whether refractory period estimates obtained in this manner are comparable to those obtained using hand-operated equipment, refractory period estimates were collected from seven LH sites using both systems. Unacceptable differences in the two curves are differences in refractory period estimates, either in the C-T interval at which recovery from refractoriness begins, the C-T interval at which recovery ends, or in the slope of the recovery curve.

METHOD

Subjects

Subjects were six male Long-Evans strain, "old colony" rats from Charles River Breeding Farms, weighing between 300-400 g at the time of surgery. All were individually housed, and enjoyed free access to food and water. Subjects were maintained on a 12 hr light/ 12 hr dark cycle.

Surgery

Electrodes were 0.25 mm stainless steel wire, insulated with Formvar except for the honed hemispherical tip. Under sodium pentobarbital anaesthesia (60 mg/kg, i.p.) electrodes were aimed bilaterally at the LH using an incisor bar setting of +5.0 mm and the following Pellegrino, Pellegrino and Cushman (1979) coordinates: posterior -0.4 mm, lateral 1.7 mm, and ventral 8.00 mm. The current return was a thin stainless steel wire wrapped around five jeweller's screws imbedded in the skull. Female Amphenol pins, pre-soldered to the ends of the current return wire and the flexible, insulated wires leading from the electrodes were inserted in a 9-pin connector. The connector was an externally threaded section of nylon rod that mated firmly with a matching connector mounted at the end of the stimulation cable (see below). The electrode and 9-pin connector were cemented to the skull and skull screws with dental acrylic. Subjects were given one week to recover from surgery.

Apparatus

The hand-operated and computer-controlled test chambers were basically similar, but varied in several respects. Hand-operated chambers were wooden boxes with wire mesh floors and Plexiglas fronts, while computer-controlled chambers were made wholly of Plexiglas. Both types of testing cages measured 25 cm x 25 cm x 70 cm high. Rodent levers (Lehigh Valley Electronics, 121-05) were located in diagonally opposite corners of both types of cages at a height of 6 cm. Only the rear lever was used. Over the active lever in the computer-controlled test chamber was a 1.5 cm

yellow "jewel" light, while over the inactive lever was a similar red light. The stimulation cable attached to the 9-pin connector on the rat's head was connected to the stimulator via a seven-channel slip ring commutator (Airflyte Electronics Co., CAY-652) fixed in the center of the ceiling of both types of cages. This arrangement made it possible for the rat to circle without excessively twisting the stimulation cable.

The experimenter was seated in full view of the subjects in the room with the hand-operated testing equipment, but subjects in the computer-controlled setup were isolated both from the experimenter and from each other. The computer-controlled testing chambers were encased in wooden boxes 50 cm x 50 cm x 90 cm high insulated with 2.5 cm thick Styrofoam except for the lower half of the removable hinged front. This part of the box was made of Plexiglas which made it possible to monitor the rats' activity from an adjacent room using video equipment.

The main difference between the hand and computer setups was that all experimental parameters in the computer setup were microprocessor controlled, whereas in the hand setup many parameters were controlled by hand-operated switches. All temporal parameters in the hand-operated equipment were controlled by hand-set integrated circuit pulse generators; in the computer setup temporal parameters for each rat were controlled by a dedicated microprocessor equipped with a custom built interface. A mechanical switch in the hand-operated apparatus determined which of the electrodes would be used for stimulation and which would be used as a current return; a bank of relays controlled by the parallel port of the dedicated microprocessor performed these functions in the

computer-controlled equipment.

The stimulation pulses in both setups were produced by constant current amplifiers (Mundl, 1980). In the hand-setup the amplitude of the pulses was set by a potentiometer, whereas in the computer setup the amplitude of the pulses was voltage controlled; the voltage was supplied by a digital to analog converter. Current intensity was monitored in both hand and computer setups by reading the voltage drop across a 1 kΩ, 1% resistor in series with the rat. In both setups, accumulation of charge at the brain-electrode interface was minimized by transistor switches that shorted the stimulator outputs through a 1 kΩ resistor when no pulse was present.

Procedure

Stabilization

After a one week recovery period following surgery, rats were screened for self-stimulation in the hand-operated equipment using 0.5 sec trains of rectangular, 0.1 msec duration cathodal pulses and conventional shaping procedures. Optimal values for the current intensity and number of pulses per train were determined by varying these parameters so as to maximize the vigor of self-stimulation and minimize induced disruptive movements that interfered with performance. If a rat could be shaped to press the lever for stimulation of either of its two electrodes, then it was included in the study. One rat was trained to lever press for stimulation of both electrodes, while the other five could only be induced

to lever press for stimulation of one electrode. Thus, a total of seven sites were included in the study.

After shaping, self-stimulation performance was stabilized alternately in the hand and computer-operated equipment. In both setups, stabilization consisted of repeatedly determining the number of pulses required to support a half-maximal rate of responding. In the hand operated setup, rats were allowed a five minute warm-up of vigorous self-stimulation. Then, using the same parameters, the number of lever presses in a 30 sec trial was determined and manually recorded on a data sheet. The number of pulses per train was then decreased by 0.1 $\log_{10}$ units on succeeding trials until fewer than five lever presses were recorded for two consecutive 30 sec trials.

During stabilization in the computer-controlled equipment, initial stimulation parameters identical to those in the hand-operated equipment were used. Rats were not given a five minute warm-up at the beginning of each stabilization session, but began immediately on the first required number determination. If a rat self-stimulated on the first 30 sec trial, the number of pulses per train was increased by 0.1 $\log_{10}$ units on succeeding trials until the number of lever presses during the latest trial did not exceed the number of lever presses during the previous trial by more than 10%. In this manner the maximal lever pressing rate was determined. The number of pulses per train was then reset to the number used for the first trial of the number determination; on subsequent trials it was decreased by 0.1 $\log_{10}$ units until less than 10% of the maximal bar pressing rate for that number determination was recorded for two

consecutive trials.

If a rat did not self-stimulate on the first trial of a required number determination, the number of pulses per train was increased by 0.3 $\log_{10}$ units for the next trial and the search for maximal lever pressing rate was continued from that number. The number of pulses per train was then decreased by 0.1 $\log_{10}$ units on succeeding trials until less than 10% of the maximal bar pressing rate for that number determination was recorded for two consecutive trials.

In any stabilization session in the hand-operated equipment, required number determinations were made 28 times, whereas any computer stabilization session was comprised of 29 required number determinations. For each determination, the number of pulses per train required to support a half maximal rate of responding was interpolated from the function relating rate of lever pressing to the number of pulses per train. Interpolation from the hand collected data was carried out with a programmable calculator, whereas required numbers were interpolated automatically from the computer collected data. Stabilization sessions continued in both setups until the range of required number values in a given session was either less than 0.1 $\log_{10}$ units or did not decrease significantly for three consecutive days.

Refractory Period Test

In contrast to the trains of single pulses delivered during stabilization sessions, trains of pulse pairs were delivered during the

refractory period sessions. The second pulse of the pair, called the T pulse, tested the excitability of the cells just fired by the first, or C pulse. The C-T intervals used varied from 0.15 msec to 5.0 msec and were presented in one of four counterbalanced orders for four sites, and in one of two counterbalanced orders for three sites.

The duration of each refractory period session was usually two hours, and consisted of required number determinations made at many C-T intervals. Required number determinations lasted a few minutes and were made up of several 30 sec trials. Calculation of the required number was carried out in a manner analagous to that described above for determining the required number of single pulses, except that the number of pulse pairs, not single pulses per train required to support a half maximal rate of responding, was interpolated. In order to check for shifts due to fatigue, determinations of the required number of single pulses were conducted after every four to five determinations of the required number of pulse pairs.

A total of eight to sixteen refractory period tests were made at each site; hand-operated and computer-controlled tests were interdigitated.

The effectiveness of the T pulse (E) was scaled using the formula developed by Yeomans (1975), and the E values obtained were plotted as a function of C-T interval.

Histology

Within one week following the last refractory period test, rats were deeply anaesthetized with 1 cc Somnotol (sodium pentobarbital, 60 mg/kg, i.p.) and killed by exsanguination. They were perfused with 0.9% saline solution followed by 10% Formalin. The brains were soaked in 10% Formalin for at least 48 hours. Brain slices 0.04 mm thick in which the electrode tracks were visible were mounted on gelatine coated glass slides and stained with formol thionine. Pellegrino et al.'s (1979) stereotaxic atlas was used to localize the electrode tips.

RESULTS AND DISCUSSION

Histology

Electrode tips were found to be widely dispersed in the anterior-posterior plane, from -0.8 mm to -1.6 mm posterior to bregma, with an outlying tip at -2.2 mm posterior to bregma (see Figure 1). All electrode tips between -0.8 mm and -1.6 mm posterior to bregma were located in the LH, usually just ventral to the zona incerta. The outlying electrode tip at -2.2 mm posterior to bregma was located in the MFB at the depth of the mamillary peduncle.

Refractory Periods

Computer- and hand-run refractory period curves for each site are shown in Figures 2a and 2b, left column. These curves are similar to those obtained by others in comparable sites (Bielajew et al., 1981; Yeomans,1975). The rising portion of such curves has been interpreted as a reflection of recovery from refractoriness in the directly stimulated substrate, a view which is consonant with the results of electrophysiological recording studies (Shizgal, Kiss and Bielajew, 1982; Kiss, 1982; Shizgal and Rompré, 1984).

All refractory period curves began to rise between 0.4 and 0.8 msec. Of the hand-run curves, three of the seven (sites 05, 07 and 09) began to rise between 0.4 and 0.6 msec, while the remaining four (sites C5, C6 and D8 -right and left hemispheres) began to rise between 0.6 and 0.8 msec.

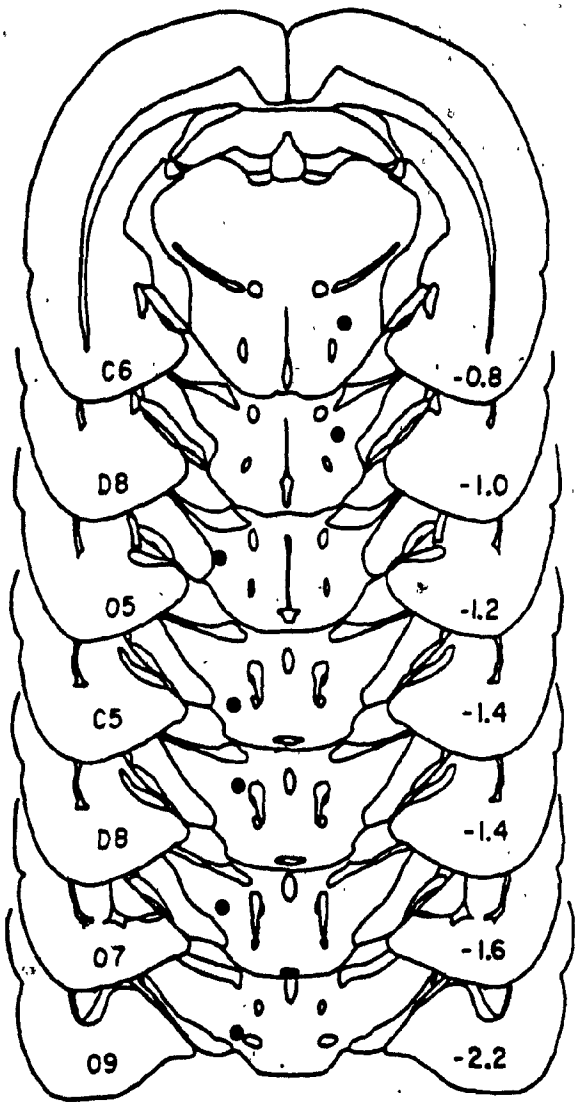

Figure 1. Electrode placements for all sites are shown on tracings from Pellegrino et al.'s (1979) stereotaxic atlas. The alphanumeric in the lower left corner of each trace refers to the subject. The left half of each trace represents the right side of the brain.

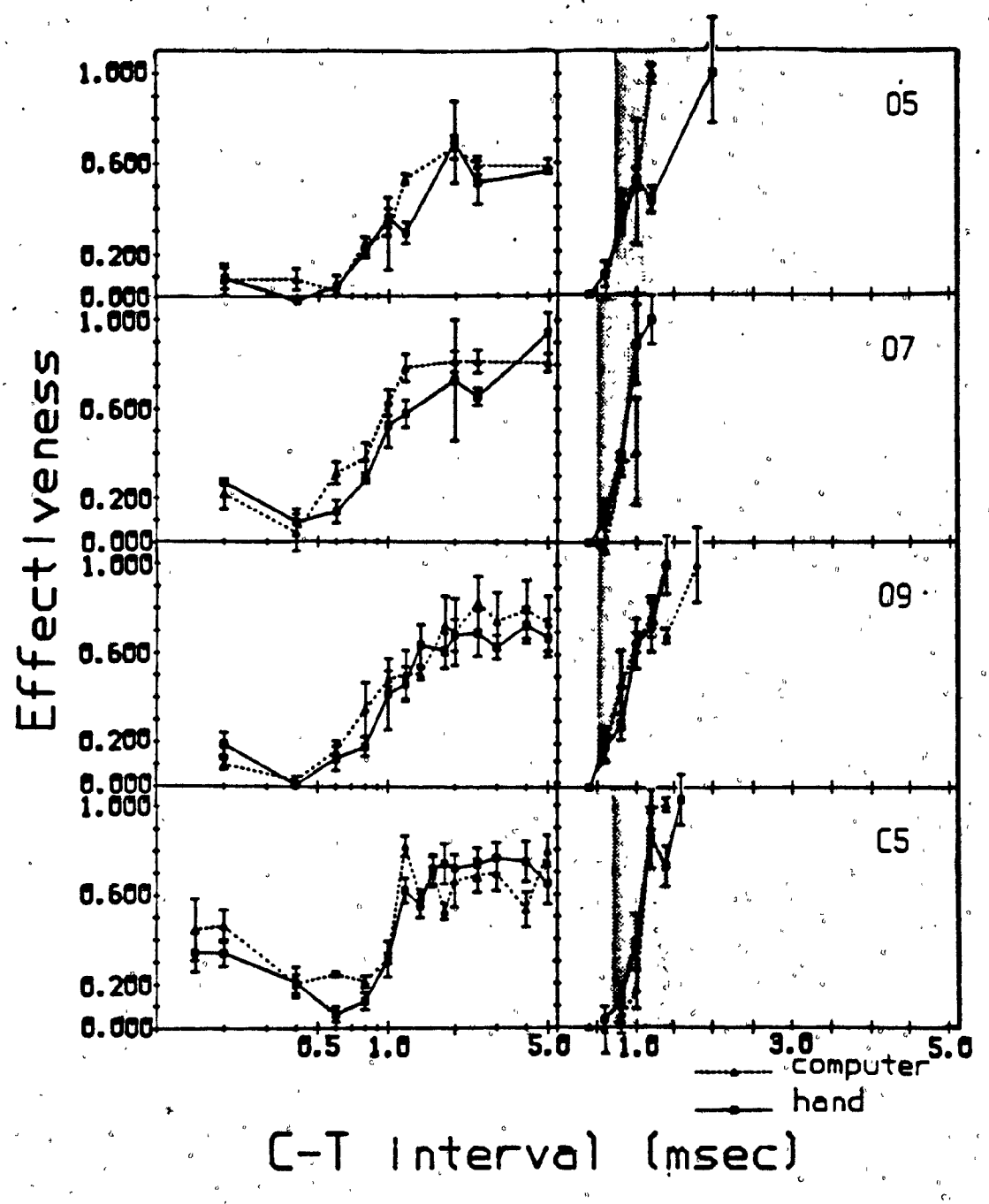

Figure 2a. Individual untransformed LH excitability curves (left column) collected in the hand- and computer-operated set-ups, and their transformed counterparts (right column) for four sites. The abscissa gives the pulse-pair interval; the interval between the conditioning pulse (C-pulse) and the test pulse (T-pulse). The ordinate represents T-pulse effectiveness as scaled by Yeomans' (1975) method. Data from the hand set-up are represented by solid lines, while data from the computer set-up are represented by dashed lines.

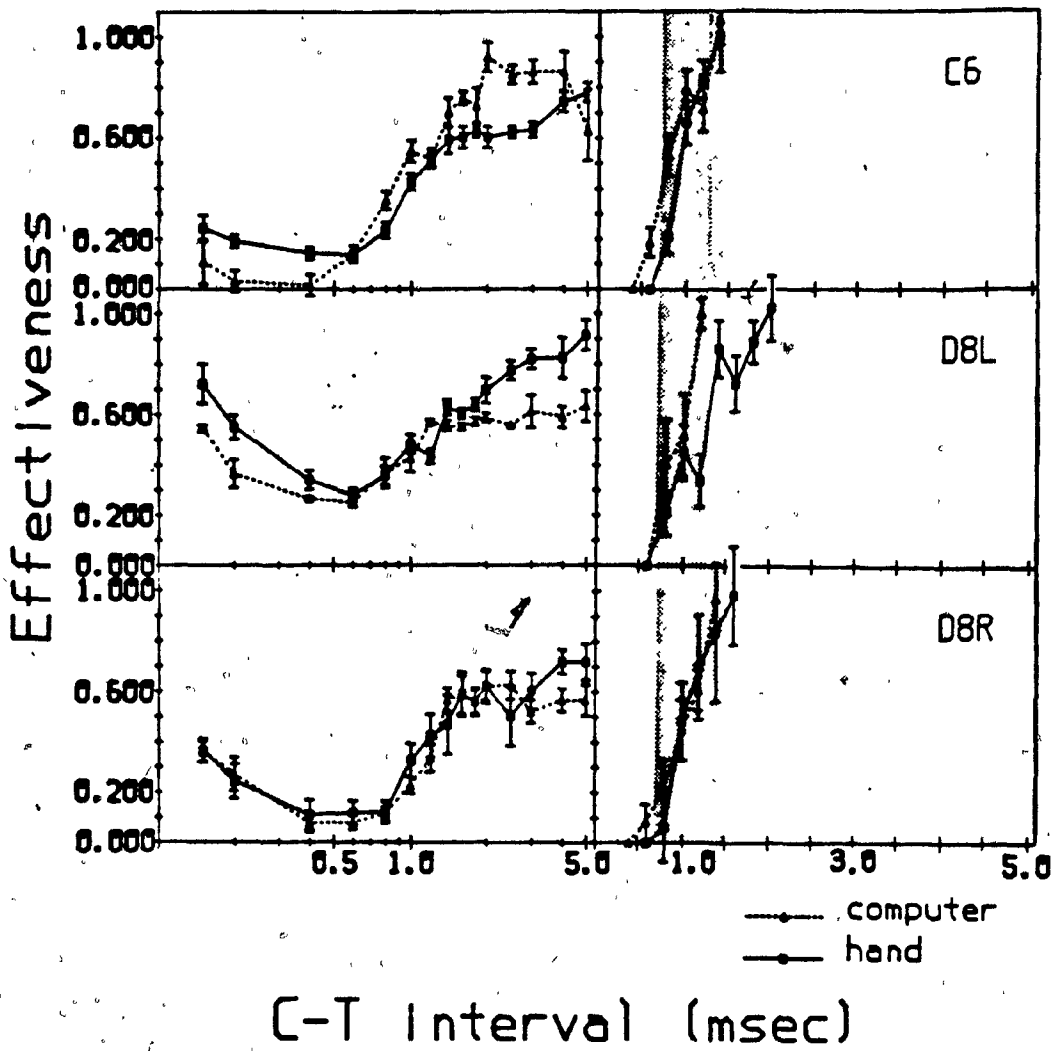

Figure 2b. Individual untransformed LH excitability curves collected in the hand- and computer-operated set-ups (left column), and their transformed counterparts (right column) for the remaining three sites. Data from the hand set-up are represented by solid lines, while data from the computer set-up are represented by dashed lines.

Five of the seven computer-determined curves began to rise between 0.4 and 0.6 msec (sites O7, O9, C5, C6, and D8 -right hemisphere), while the remaining two (sites O5 and D8 -left hemisphere) began to rise between 0.6 and 0.8 msec. Thus, the locus of rise of a particular site's refractory period curve was almost identical under both paradigms.

Both hand-run and computer-determined refractory period curves approached asymptote, as defined by Bielajew et al.'s (1981) test, between a much wider range of C-T intervals (1.2 msec to 2.0 msec) than the range in which they began to rise. Asymptotic levels of refractory period curves collected on the hand-operated equipment were approached by site O7 at 1.2 msec, by sites O9 and C6 at 1.4 msec, by sites C5 and D8 (right hemisphere) at 1.6 msec and by sites O5 and D8 (left hemisphere) at 2.0 msec. The computer-determined curves approached asymptote by 1.0 msec (site O7), by 1.2 msec (sites O5, C5 and D8 -left hemisphere), by 1.4 msec (sites C6 and D8 -right hemisphere) or by 1.8 msec (site O9). Thus, refractory period curves for a given site usually approached asymptote at similar C-T intervals under both paradigms. Hand- and computer-run refractory period curves for sites O5, O7, O9, C5, and D8R approach asymptote within one C-T interval of each other. Those for site C6 approach asymptote at identical C-T intervals. Hand- and computer-run curves for site D8L, however, approach asymptote at disparate C-T intervals. While approximately 85% of the total recovery in the computer-run curve is achieved by the hand-run curve within one C-T interval, the two curves truly appear to be different. The reason for this difference is not understood.

Comparison of behavioural estimates of refractory periods at different brain sites is made using curves that have been transformed such that the rising portions (i.e. the portion that has been interpreted to reflect recovery from refractoriness) span E values from zero to one (Figures 2a and 2b, right column). This transformation, introduced by Bielajew et al. (1981), serves to reduce the number of extraneous variables contributing to the slope of the excitability curve. Such variables include the amount of local potential summation present when the curve is at its lowest point and the asymptotic E values. Under ideal circumstances, the T pulse effectiveness should equal that of the single pulses at long C-T intervals, yielding an E value of one. However, this situation is not realized for most refractory period estimates (see Figures 2a and 2b, left column). If refractory period curves for two brain sites approach horizontal asymptotes at different E values, the slope of the untransformed recovery curves will be different, even if recovery begins and ends at the same C-T interval at both sites. Because the method used to test for differences in refractory period estimates relies heavily on the slopes of the curves, it is important that the slope of the transformed curves be a reliable reflection of the time course of recovery of the reward-relevant fibers.

Two-way analyses of variance were performed on the refractory period estimates made under both paradigms at each site, both for untransformed and for transformed curves. The analysis of the untransformed curves was conducted to reveal any differences in local potential summation, asymptotic E values and time course of recovery from refractoriness in the two curves. Transformation of the curves eliminates differences due to

local potential summation and asymptotic E value, thus the analysis on the transformed curves was conducted to reveal any differences solely in time course of recovery from refractoriness. The analyses of variance for transformed curves included only those E values at C-T intervals common to both hand- and computer-run curves. In addition, E values at the smallest C-T interval of the transformed curves were eliminated from the analysis because they are defined by Bielajew et al.'s (1981) asymptote test as zero and, as such, have no variance. The stippled region on the transformed curves (see Figures 2a and 2b) delineates the range of C-T intervals for which E values were compared. The results of these tests are summarized in Tables 1 and 2.

For all pairs of refractory period curves, C-T interval had the expected significant effect on E values. In a small number of cases, significant effects of paradigm or significant interactions of paradigm and C-T interval were found. For example, a significant paradigm-dependent difference was detected in the untransformed curves for site D8 -left hemisphere. Higher E values are found in the hand-run compared to the computer-run curve at very short and very long C-T intervals. Throughout the rising portion of both curves, however, E values are quite similar. After transformation, this similarity was reflected in the failure of analysis of variance to detect any significant effect of paradigm.

Several pairs of refractory period curves were found to have significant paradigm - C-T interval interactions. E values for site O5 were not found to be different before they were transformed. After

Table 1

Results of 2-way ANOVAS on untransformed curves

Site	C-T interval	Condition	C-T x Condition
O5	34.24* (8/24)	2.47 (1/3)	0.99 (8/24)
O7	40.61* (8/24)	1.24 (1/3)	1.29 (8/24)
O9	38.87*(12/48)	0.28 (1/4)	0.94 (12/48)
C5	21.99*(14/98)	0.01 (1/7)	1.32 (14/98)
C6	26.56*(14/98)	3.58 (1/7)	2.56*(14/98)
D8L	18.64*(14/98)	11.19*(1/7)	1.87*(14/98)
D8R	17.86*(14/98)	.0.02 (1/7)	0.56*(14/98)

*significant ($p < .05$)

Table 2

Results of 2-way ANOVAS on transformed curves

Site	C-T interval	Condition	C-T x Condition
O5	13.50* (3/9)	3.51 (1/3)	4.06*(3/9)
O7	26.63* (3/9)	1.16 (1/3)	0.96 (3/9)
O9	30.45* (5/20)	0.02 (1/4)	2.61 (5/20)
C5	40.28* (3/21)	0.40 (1/7)	1.16 (3/21)
C6	36.17* (4/28)	3.49 (1/7)	1.86 (4/28)
D8L	6.69* (2/14)	4.77 (1/7)	4.34*(2/14)
D8R	23.03* (3/21)	0.02 (1/7)	0.45 (3/21)

*significant ($p < .05$)

transformation, however, the dip in the hand-run refractory period curve where the computer-determined curve approaches asymptote (1.2 msec) makes the curves seem dissimilar (see Figure 2a). Nonetheless, both curves approach asymptote within 0.2 msec of each other. I suspect that the aforementionned dip in the hand-run curve is a "glitch" that is salient due to the small number of trials (four) run on this subject.

The pair of refractory period curves collected from site C6 were found to have a significant paradigm-C-T interval interaction before transformation, but not after. E values at C-T intervals longer than 2 msec are greater in the untransformed computer-determined curve than in the Hand-run curve. Bielajew's (1981) asymptote test gives identical asymptote values for both curves, however, and the resulting transformed curves are very similar. Since recovery from refractoriness is believed to be reflected by the rising part of the curves, paradigm dependent E value differences at larger C-T intervals, such as those found in site C6's pair of curves, are believed to be unimportant in characterizing the excitability of the directly stimulated cells. The basis of these differences is not understood.

The only pair of refractory period curves that showed a significant paradigm - C-T interval interaction both before and after transformation was that for site D8 -left hemisphere. Visual inspection of the untransformed curves suggests that the difference in the untransformed curves stems from higher E values obtained in the hand-operated equipment at C-T intervals less than that at which the minimum E value was reached, and greater than that at which asymptote was approached. The difference

in the curves persisted after transformation because of the dip in the hand-run curve at the C-T interval where the computer-determined curve approaches asymptote (1.2 msec). Nonetheless, the hand-run refractory period curve reaches approximately 85% of the maximum E value of the computer-determined curve within 0.2 msec. Although the curves are significantly different, the magnitude of this difference appears to be very small.

Using an F test, the variance due to error (i.e. the variance in E value at each C-T interval in the different testing sessions) was compared for each of the paradigms at each electrode placement, both before and after transformation. The results of the tests are summarized in Tables 3 and 4. More error was associated with three hand-run and three computer-determined refractory period curves before transformation, while one set of curves showed no significant difference in error variance. After transformation, one hand-run and one computer-run refractory period curve had more error variance associated with them, while five sets of curves showed no significant difference in error variance. Thus, the computer-controlled testing paradigm was at least as reliable as the hand-operated setup.

The error associated with hand-run recovery curves seems to be more dependent on the experimenter than does the error associated with computer-determined curves. Data from sites O5, O7 and O9 were collected by one experimenter, while data from the other sites were collected by another experimenter. Of the untransformed hand-run curves, data from the three sites with the most error variance (D8R, C5 and D8L) were collected

Table 3

Results of F-tests on error variance in untransformed curves

Site	MSE (hand)	MSE (computer)	F ratio	df
O5	0.0178	0.0139	1.2060	27/27
O7	0.0342	0.0111	3.0811*	27/27
O9	0.0213	0.0411	1.9571*	52/52
C5	0.0516	0.0714	1.3837*	105/105
C6	0.0217	0.0695	3.2028*	105/105
D8L	0.0390	0.0182	2.1429*	105/105
D8R	0.0660	0.0293	2.2526*	105/105

*significant ($p < .05$)

Table 4

Results of F-tests on transformed curves

Site	MSE (hand)	MSE (computer)	F-value	df
O5	0.0107	0.0843	7.8785*	12/12
O7	0.0534	0.0654	1.2247	12/12
O9	0.0446	0.0340	1.3118	24/24
C5	0.0911	0.0620	1.4693	28/28
C6	0.0689	0.0538	1.2807	35/35
D8L	0.0819	0.1683	2.0549	21/21
D8R	0.2000	0.0771	2.5940*	28/28

*significant ($p < .05$)

by one experimenter (see Table 3). Of the transformed hand-run recovery curves, data from the four sites with the most error variance (D8R, C5, D8L and C6) were collected by this same experimenter (see Table 4). No such relationship between error variance and experimenter is found in either the untransformed or transformed computer-determined curves.

The error associated with recovery curves collected in the computer-controlled apparatus is greater than that associated with curves collected by an expert experimenter working with hand-operated equipment. Hopefully, as the computer algorithm for collecting refractory period estimates is made more powerful, it will more closely mimic the success of an expert experimenter in accurately estimating refractory periods in a small number of trials.

In summary, refractory period estimates were similar regardless of the paradigm under which they were made. Consequently, I felt confident in using the more convenient computer-based procedure to compare the excitability characteristics of the substrates for LH and SN self-stimulation.

EXPERIMENT 2

Previous work has suggested that a system of reward-relevant fibers follows a descending trajectory through the LH, at least to the level of the ventral tegmental nucleus of Tsai (VTN) (Shizgal et al., 1980; Bielajew and Shizgal, 1982, 1984). Anatomical studies show that direct descending projections link these two sites (Nieuwenhuys et al., 1982). Some of the fibers in this bundle terminate at the VTN (Phillipson, 1979), while others continue beyond it, branching both medially and laterally. The lateral branch of fibers passes through or over the substantia nigra (SN) (Nauta and Domesick, 1981). Using psychophysical inference, the present study compares the refractory periods of the LH and SN reward substrates. If these two sites are directly linked by the same reward-related fibers, then it is likely that refractory period estimates obtained for the two sites will be similar.

METHOD

Subjects

Subjects were ten male Long-Evans strain, "old colony" rats from Charles River Breeding Farms, weighing between 300-400 g at the time of surgery. All were individually housed and enjoyed free access to food and water. Subjects were maintained on a 12 hr light/dark cycle.

Surgery

Electrodes were 0.25 mm stainless steel wire, insulated with Formvar except for the hemispherical tip. Under sodium pentobarbital anaesthesia (60 mg/kg, i.p.), electrodes were aimed bilaterally at the LH in three of the rats. The remaining seven rats had a fixed electrode aimed at the LH and a movable electrode aimed approximately 2 mm above the pars compacta of the SN. With the incisor bar set at +5.0 mm, the following Pellegrino, Pellegrino and Cushman (1979) coordinates were used: LH - posterior 0.4 mm, lateral 1.7 mm, ventral 8.00 mm; SN - posterior 3.2 mm, lateral 2.5 mm, ventral 6.5 mm.

The moveable electrode employed was very similar in design to that developed by Wise (1976). One end of a 1.25 cm section of a 2/56 stainless steel screw was filed to a 0.25 cm truncated cone. A small hole was drilled in the center of this cone, and a 1 cm length of 0.25 mm stainless steel electrode wire was soldered into the hole. To insure concentric placement of the wire, the screw was rotated in a jeweller's lathe during this operation. Two locked 2/56 nuts were soldered to the opposite end of the screw, allowing the electrode to be lowered with a compatible screwdriver. The electrode wire and solder were insulated, then the screw was inserted in a sleeve constructed of a 1 cm section of a number ten plastic screw, threaded internally by a 2/56 tap. Before surgery, white grease was applied to the bottom of the threaded sleeve, around the electrode wire, to prevent dental cement from entering this region and freezing the electrode.

Design of the electrode assembly was constrained by the need to periodically lower the moveable electrode and by the need for a strong, reliable connection between the stimulation cable and the electrodes. After testing several designs, the one described below was chosen. The current return was a thin stainless steel wire wrapped around five jeweller's screws imbedded in the skull. Male Amphenol pins were presoldered to one end of the current return wire, one end of the flexible, insulated wire leading from the LH electrode and to both ends of a short, insulated wire. The Amphenol pin at one end of the short insulated wire was set in dental cement close to the side of the moveable electrode. The Amphenol pin at the other end of this wire and the Amphenol pins attached to the current return and LH electrode were inserted in a nine-pin connector, which was then cemented to the skull and skull screws with dental acrylic. During experimentation, an electrical connection between the stimulator and the moveable electrode was maintained by wrapping a "jumper" around the Amphenol pin set in dental cement beside the moveable electrode and the moveable electrode itself. Electrode gel was smeared on and around the jumper to ensure constant electrical contact. Thus, the jumper routed stimulating currents from the nine-pin connector to the moveable electrode.

Subjects were given one week to recover from surgery.

Procedure

Adjustment of Moveable Electrodes

On the basis of histological findings and behavioural observations obtained in pilot experiments, the following protocol was developed for moving the electrodes. Rats were screened for self-stimulation at varying current intensities and numbers of pulses per train for 15 to 30 minutes each day. The moveable electrode was lowered roughly 0.25 mm (one half turn), if stimulation elicited signs suggestive of aversion (i.e. vocalization, attempts to escape from the test cage). If stimulation evoked signs suggestive of neither aversion nor interest (i.e. sniffing, exploring) the moveable electrode was lowered roughly 0.13 mm (one quarter turn). If the behaviour evoked by the stimulation did not clearly fall into the categories above, movement of the electrode was postponed until additional screening had been conducted one day later. To allow sufficient time for the tissue to stabilize around the electrode tip, 24 hours were allowed to pass after moving the electrode before behavioural testing was carried out.

Once the rat appeared interested in the stimulation, it was trained to press the lever using conventional shaping procedures. If these did not prove effective, as was usually the case, the rat was food deprived to 85% of its *ad lib.* weight. Food deprived rats explore their environments more than other rats, and are particularly attracted by desirable food. In order to take full advantage of these attributes, melted chocolate was spread on the lever during training.

Once lever pressing was established, the rat was lightly anaesthetized with ether and the moveable electrode was cemented in position with dental cement to prevent accidental movement during testing.

Refractory Period Test

Of the three rats with bilateral LH electrodes, one was trained to self-stimulate on both electrodes, while the other two were trained on one electrode. Three of the seven rats with electrodes in both the LH and SN were trained on both electrodes, while the other four were trained only on the SN. Thus, a total of seven LH sites and seven SN sites were studied.

Stabilization sessions and refractory period tests were carried out as described in Experiment 1: computer-controlled equipment. Between five and eight refractory period determinations were collected for each of the LH sites, whereas between six and twelve determinations were collected for each of the SN sites. The E values were again scaled using the formula developed by Yeomans (1975). E values were plotted as a function of C-T interval.

Histology

These procedures were the same as in Experiment 1.

RESULTS AND DISCUSSION

Histology

LH electrode placement and final location of SN electrodes are shown in Figures 3 and 4. All electrode tips aimed at the LH (Figure 3) were located in the LH, although they were widely dispersed in the anterior-posterior plane from bregma to 1.4 mm posterior to bregma. Electrode tips C4 and C5 were located at the level of the fornix, tips B2, B8, C6 and D8 -right hemisphere were located just ventral to the zona incerta and electrode tip D8 -left hemisphere was located at the level of the zona incerta.

Electrode tips aimed dorsal to the SN were all found dorsal to the far lateral edge of the SN (Figure 4) although they too were scattered in the anterior-posterior plane, from 2.6 mm to 3.6 mm posterior to bregma. Two electrode tips were located at the level of the medial lemniscus, more dorsal to the SN than the others.

The black circles in Figure 4 represent the location of the electrode tips. Unfortunately, estimates of current-distance relationships, from which the size of the region of stimulation can be calculated, are available only for the LH (Fouriezos, 1981). Thus, the focus of the histological discussion below will be on the electrode tip and surrounding anatomical structures, although the region of stimulation may be larger than the black circles. The currents used to stimulate the SN were large; stimulating currents used ranged from 0.8 to 1.0 mA. Nonetheless, there

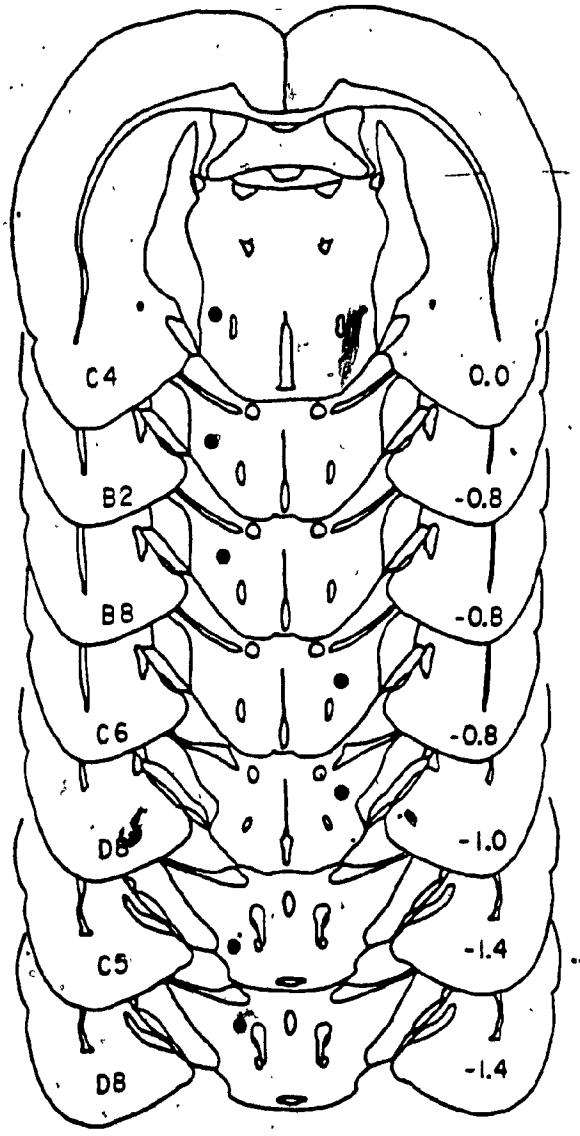

Figure 3. Electrode placements for LH sites are shown on tracings from Pellegrino et al.'s (1979) stereotaxic atlas. The alphanumeric in the lower left corner of each trace refers to the subject. The left half of each trace represents the right side of the brain.

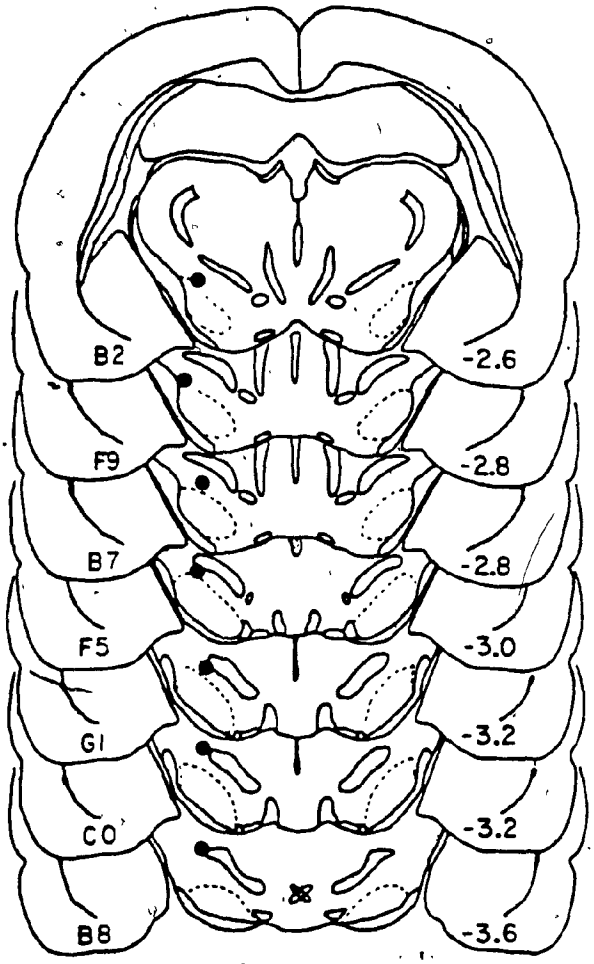

Figure 4. Electrode placements for SN sites at which refractory period estimates were obtained are shown on tracings from Pellegrino et al.'s (1979) stereotaxic atlas. The alphanumeric in the lower left corner of each trace refers to the subject. The left half of each trace represents the right side of the brain.

is evidence that the region of stimulation was small. Electrode movements as small as 0.13 mm served to abolish self-stimulation.

In the process of screening rats with moveable electrodes implanted dorsal to the SN, many sites other than those from which refractory period estimates were obtained were found to support self-stimulation. One of several reasons precluded drawing estimates from these additional sites: self-stimulation at some sites was not sufficiently vigorous to permit refractory period testing, some sites ceased to support self-stimulation, and some rats became ill and died during training. However, the majority of sites for which there is a record of self-stimulation but no refractory period estimate were in rats that lost their electrode assemblies before training was complete. These sites, as well as the sites from which refractory period estimates were obtained, are illustrated in Figure 5. The majority of electrode tips represented in this figure are located within the boundaries of the region found to support self-stimulation in Corbett and Wise's (1979) and Wise's (1981) mapping studies. Descending fibers that have been traced from the LH pass through this region (Saper et al., 1979; Wolf and Sutin, 1967). The two sites found to support self-stimulation but of which the locations are not reported in any of the abovementionned studies (i.e. the two lateral electrode tips at 3.4 mm posterior to bregma in Figure 5) are located more dorsal to the far lateral edge of the SN. Descending fibers, the trajectories of which have been traced from the LH by other investigators (Nauta and Domesick, 1981), appear to pass through this region.

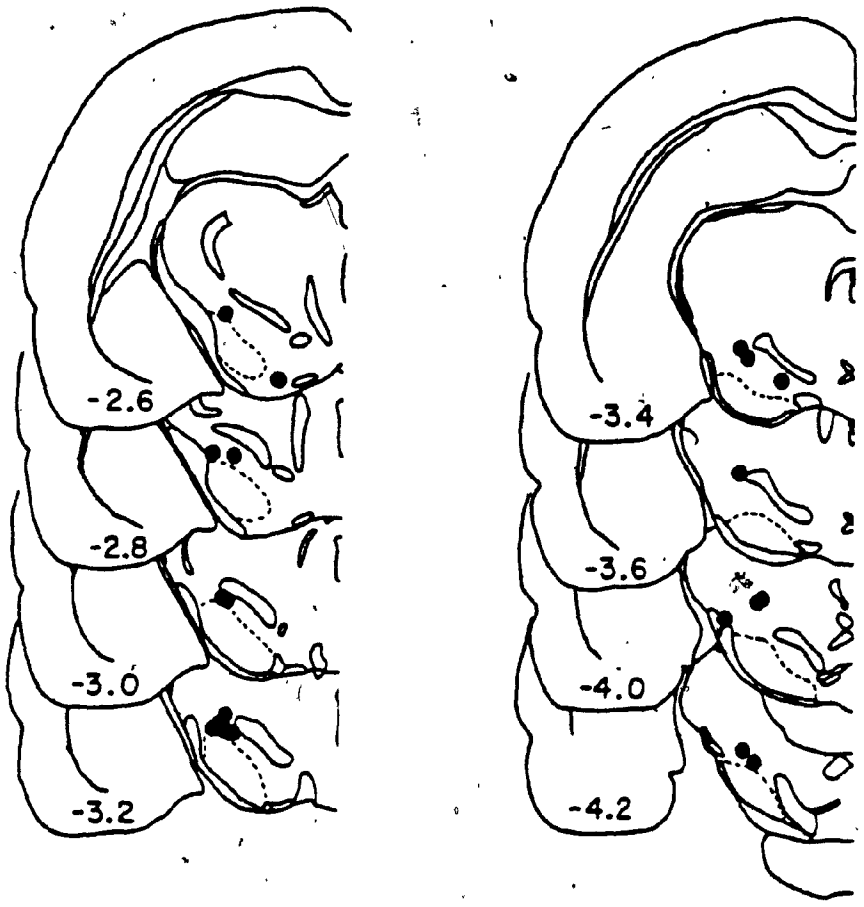

Figure 5. Electrode placements for all SH sites at which self-stimulation was supported are shown on tracings from Pellegrino et al.'s (1979) stereotaxic atlas. The left half of each trace represents the right side of the brain.

While attempting to find sites dorsal to the SN that would support self-stimulation, many sites were found that would not. The final locations of electrode tips that passed through these sites are represented by black circles in Figure 6. The average travel of electrode tips before reaching their final position was 2 mm, thus above each final position there is an additional column of negative sites approximately 2 mm in height. The majority of sites not supporting self-stimulation fell outside the trajectories of descending neurons traced from the LH by Nauta and Domesick (1981), Saper et al. (1979) and Wolf and Sutin (1967). However, the location of sites in the region of the SN found not to support self-stimulation is not entirely consistent with the results of Wise's (1981) mapping study. The tracks of some electrodes that never supported self-stimulation (i.e. the most lateral electrode tracks at 2.6 and 2.8 mm posterior to bregma in Figure 6) pass directly through areas reported to support self-stimulation in some rats (Wise, 1981). Nonetheless, not all rats in Wise's (1981) study could be induced to self-stimulate for stimulation of these sites. It is noteworthy that the discrepancy between these results and Wise's do not exceed the internal inconsistancy in the results of the present study. In other words, some of the negative results (Figure 6) were obtained with electrodes that passed through structures found to support self-stimulation in other rats (Figure 5).

These discrepancies could be due to the size of individual electrode movements. In some rats, the brain region capable of supporting self-stimulation may have been traversed in one movement. Support for this notion stems from the observation that the distance travelled by the

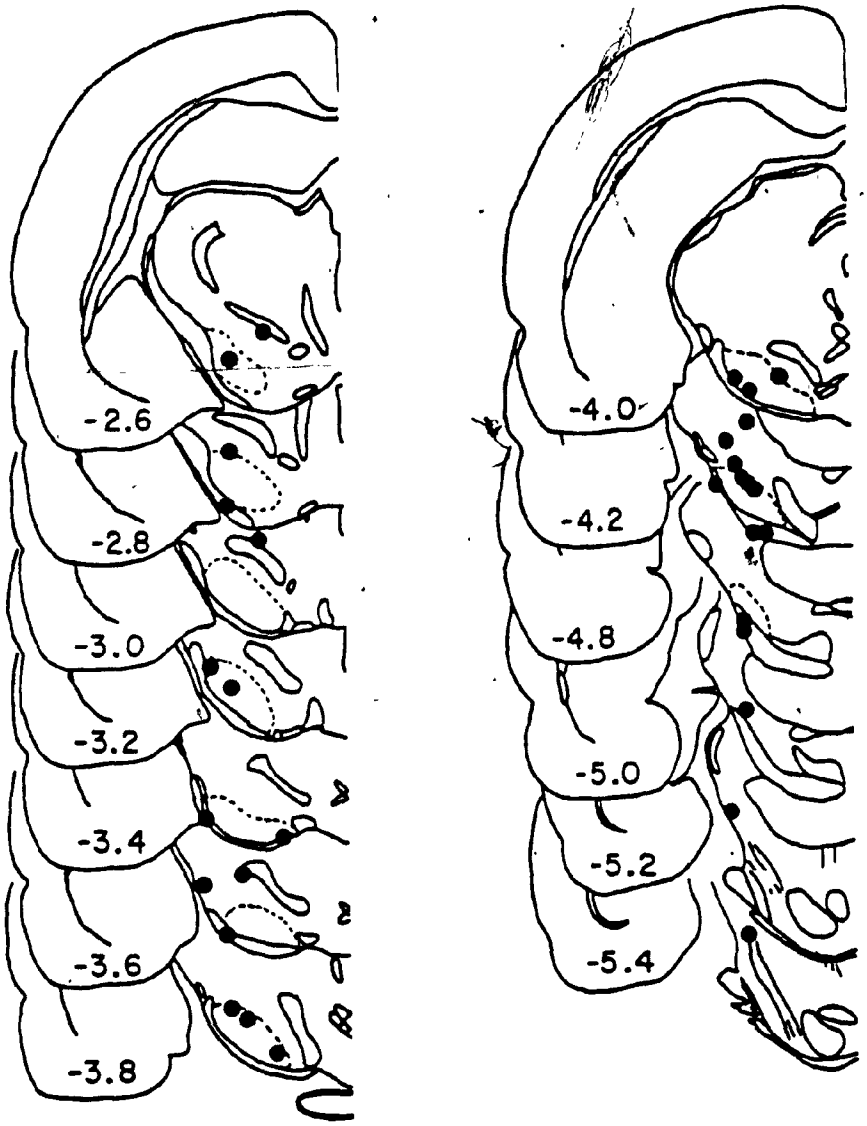

Figure 6. Final electrode locations for all SN sites at which self-stimulation was not supported are shown on tracings from Pellegrino et al.'s (1979) stereotaxic atlas. The left half of each trace represents the right side of the brain.

electrode in moving from a site where self-stimulation could not be established to a positive site was often roughly 0.13 mm; an additional electrode movement of roughly the same size abolished self-stimulation. This suggests that the layer of reward-relevant neurons was very thin.

Behaviour of Rats Receiving Substantia Nigra Stimulation

The behaviour of rats stimulated in the SN was highly consistent across rats, yet different from that of rats stimulated in the LH. Rats with electrodes implanted in the SN were much more difficult to shape than were rats with electrodes in the LH, because SN stimulation often elicited movements that interfered with lever pressing. When the SN on the right side of the brain was stimulated, the rats' bodies tended to roll to starboard with the left forepaw in extension. Usually the effect of this roll was to leave the rats approximately one-quarter turn anti-clockwise from their original position. Thus, if a rat were to experimentally press the lever during shaping, it would find itself facing the front of the cage after the train of stimulation had finished. Any post-stimulation exploration aimed at discovering the source of stimulation would continue from this orientation. Such exploration often appeared slow and confused. It was never as frantic and determined as that seen in rats with electrodes implanted in the LH. Rompré (1983) showed that adding contingent stimulation-elicited movements to rewarding stimulation resulted only in small changes in number threshold as calculated in the present experiment (i.e. half-maximum). If number threshold, from which the refractory period estimate is derived, is not significantly affected

by stimulation-induced movements, then behaviourally derived refractory period estimates should not be affected by stimulation-induced movements.

Another peculiarity of behaviour of SN rats following stimulation was their tendency to search and explore to port following stimulation. A rat already turned 90 degrees away from the lever following stimulation was thus inclined to search in a direction even further removed from it. This turning away from the stimulated side of the brain has been noted by others (Vaccarino and Franklin, 1982) in response to stimulation of the lateral SN pars compacta. Only after a long period of training (a common training interval was two weeks) did such a rat learn to make a quarter turn clockwise after a train of stimulation to position itself for the next lever press. During this training period melted chocolate smeared on the lever and food deprivation to 85% ad lib weight served to speed the rats' learning considerably.

For several days following the successful shaping of a rat with an electrode implanted in the SN, it was usually necessary to retrain it at the start of each experimental session. Retraining took anywhere from 5 to 20 minutes before the rat would continue to press the lever on its own. Approximately one-half of the rats never lever-pressed spontaneously when put in the testing chamber; only after priming did they begin to press. In contrast, once trained to lever press for LH stimulation, rats rarely needed retraining at any time.

In view of these observations, it is understandable that the absolute pressing rate of rats receiving stimulation in the SN was much lower than

that of rats receiving stimulation in the LH, and that the post-stimulation excitability curves of rats with electrodes in the SN had considerably more error associated with them. The long training period necessary for rats stimulated in the SN and the retraining necessary at the start of experimental sessions for several days after acquisition of the lever pressing response may result from priming and reward effects of stimulation that are not as strong as those for rats stimulated in the LH. If either of these two effects were relatively weak, lengthier and more vigorous training would be necessary to induce and maintain self-stimulation (Gallistel, 1973).

Untransformed Curves

Behaviourally derived LH and SN refractory period curves are presented in Figure 7. Each curve is an average of all the individual curves for all seven sites. The LH curve declines at short C-T intervals, rises at moderate C-T intervals and approaches asymptote at long C-T intervals. The SN curve behaves similarly to the LH curve at short and moderate C-T intervals, but appears to still be rising at the longest C-T interval tested. In general, this pattern of change in E value as C-T interval increases resembles a post-stimulation excitability cycle. The decline at short C-T intervals is interpreted as a decrease in summation due to decay of local potentials, the rise at moderate intervals is interpreted as recovery from refractoriness and the levelling off at long C-T intervals is interpreted as the completion of recovery (Yeomans, 1979; Yeomans et al., 1979). The behaviourally derived LH refractory period

curve lies above the SN refractory period curve at all C-T intervals (Figure 7); the LH curve begins to rise and level off earlier than the SN curve.

Individual refractory period curves for the seven LH snd SN sites are found in Figures 8a and 8b. Most individual LH curves begin to rise and level off earlier than the individual SN curves. There appears to be less error associated with the LH curves than the SN curves. Some E values less than zero are expected due to random variation, but a significant portion of the SN curve for site B8 lies below zero. According to Yeomans' (1975) formula for scaling E values, an E value of less than zero at a certain C-T interval means that more pulse pairs than single pulses were required to achieve the same behavioural output. The reasons for this phenomenon are not understood.

Transformed Curves

Averaged transformed LH and SN curves are presented in Figure 9. They were produced in the following manner: individual curves for each test session were transformed using Bielajew et al.'s (1981) method. A statistical test was applied to find the E-value at which the untransformed curves approached asymptote. This E-value was assigned a value of one. A value of zero was assigned to the lowest E-value on each untransformed curve, and the E-values at C-T intervals between these two extremes were rescaled accordingly. E values of zero were assigned to C-T intervals less than the x-intercepts of the transformed curves, while E

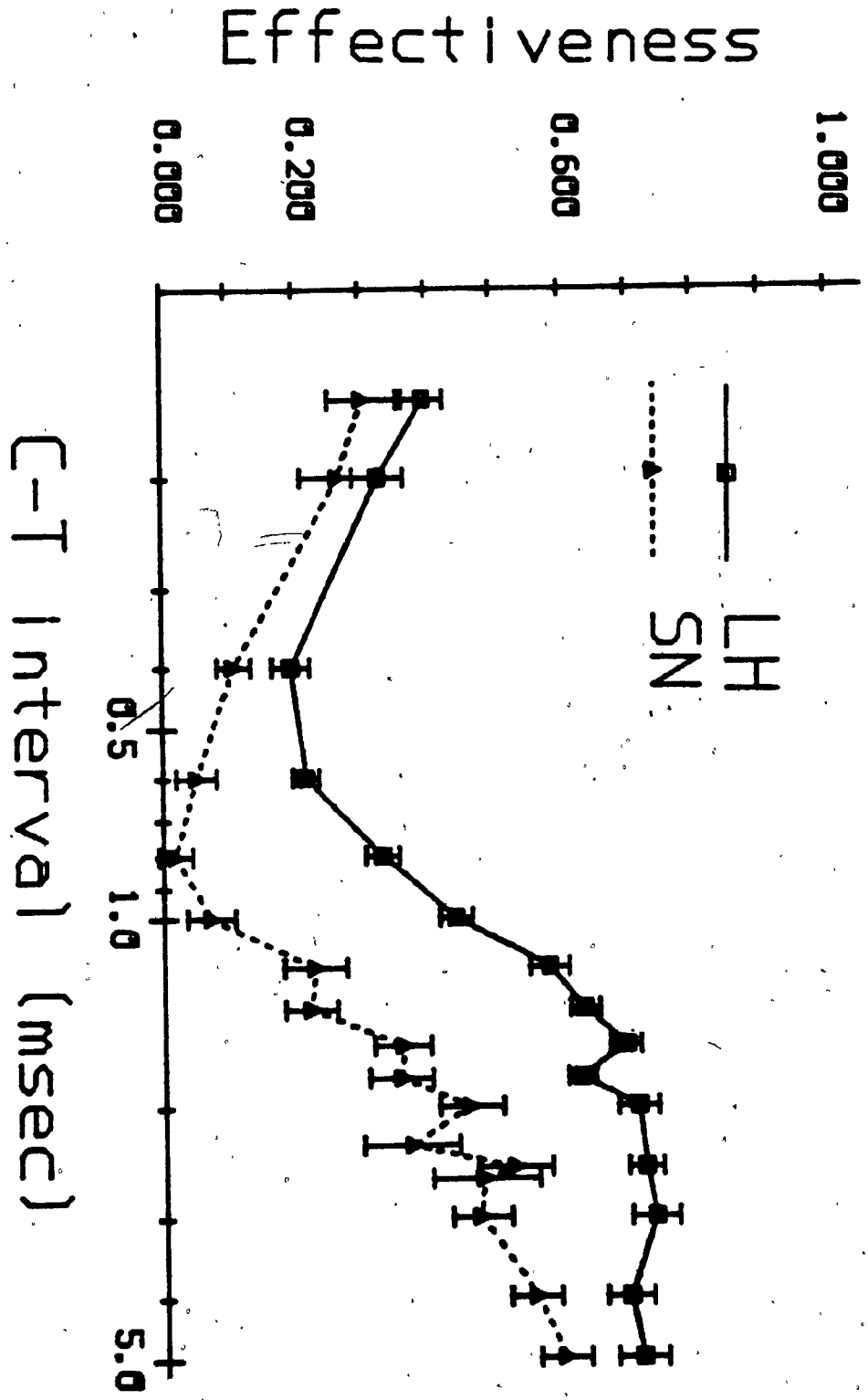

Figure 7. Untransformed excitability curves. The solid line is the average of the LH data, while the dashed line is the average of the SN data.

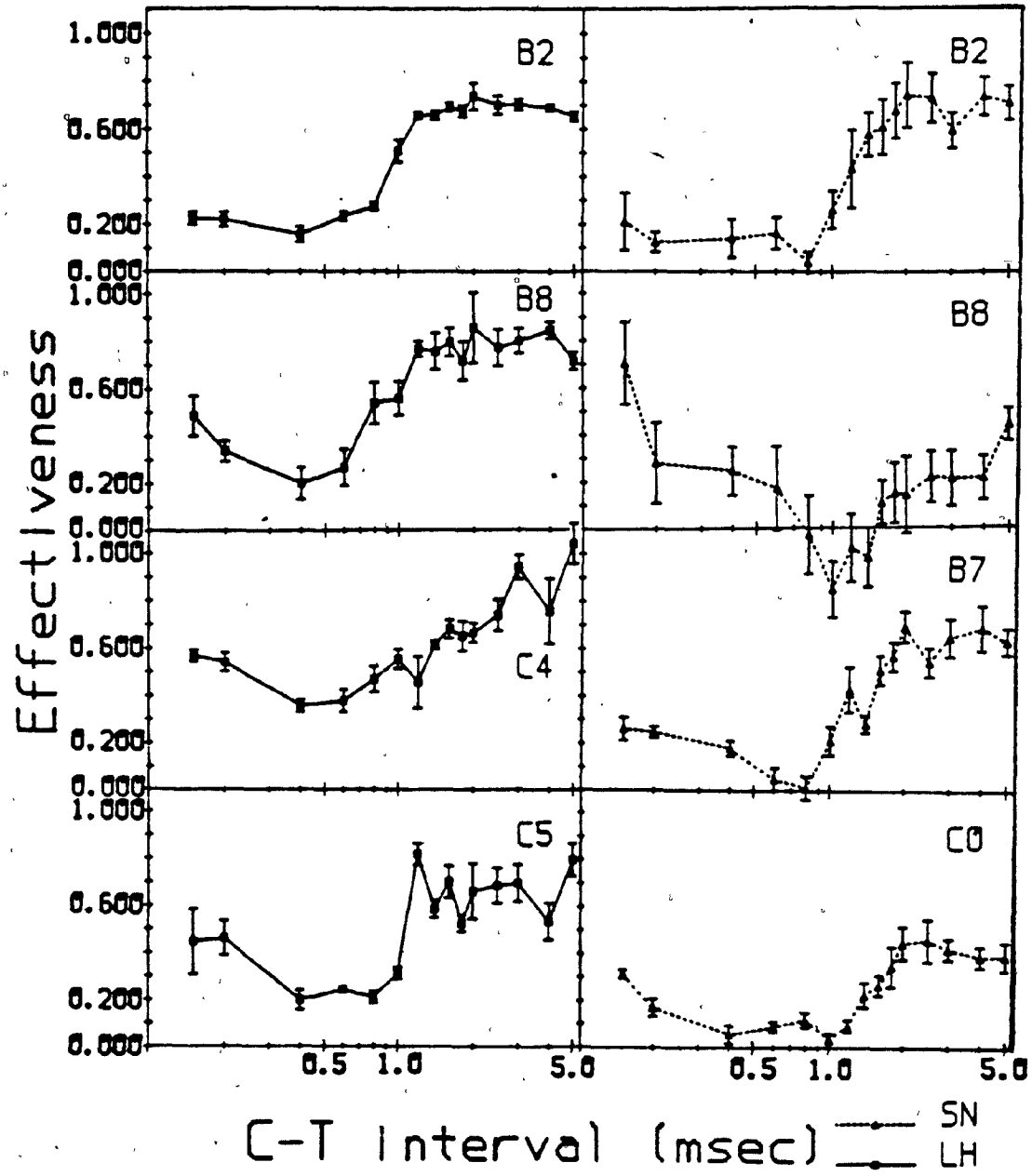

Figure 8a. Individual untransformed excitability curves. The left column is composed of solid lines representing LH curves, while the right column is composed of dashed lines representing SN curves.

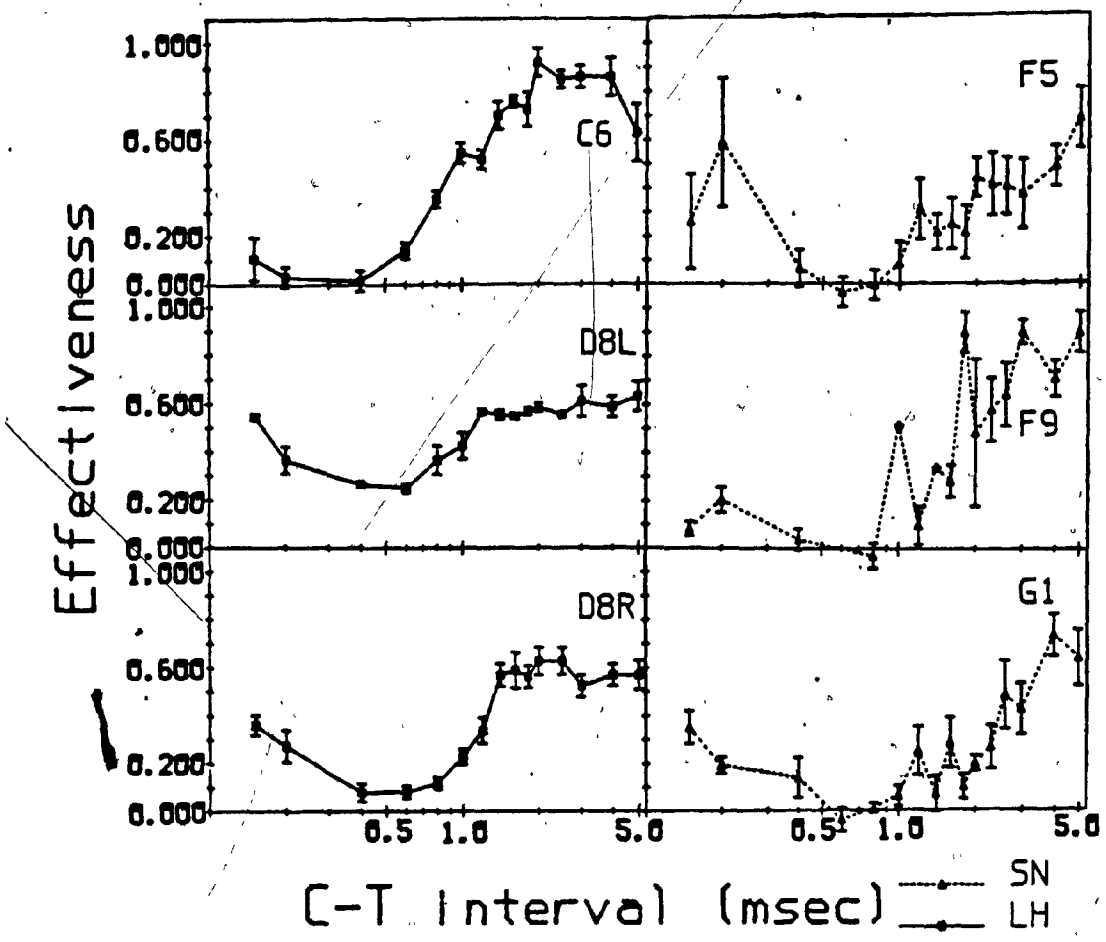

Figure 8b. Individual untransformed excitability curves. The left column is composed of solid lines representing LH curves, while the right column is composed of dashed lines representing SN curves.

values of one were assigned to C-T intervals greater than those at which the untransformed curves approached asymptote. The transformed E values for all test sessions were then averaged, yielding the curves in Figure 9. However, these values were not used for statistical analysis because the assigned E values of zero and one artificially reduce the variance of the E values at the shortest and longest C-T intervals. The E values used for statistical analysis were those at C-T intervals between the x-intercepts of the transformed curves from individual test sessions and the asymptotes of the corresponding untransformed curves. The averages of these values over all test sessions are plotted as a function of C-T interval in Figure 10.

Statistical analysis was restricted to the transformed curves for two reasons. First, the transformed curves represent only the rising portions of the untransformed curves. Both behavioural (Yeomans, 1979; Yeomans et al., 1979) and electrophysiological evidence (Yeomans et al., 1979; Kiss, 1982; Shizgal and Rompré, 1984) supports the notion that the rising portion of behaviourally derived curves reflects recovery from refractoriness in the directly stimulated reward substrate. If so, the excitability of the directly stimulated neurons responsible for LH and SN self-stimulation can be compared by analyzing the rising portions of the behaviourally derived curves. Second, Bielajew et al.'s (1981) asymptote test, which was used to transform the curves, reduces the number of extraneous variables contributing to the slope of the rising portion (see Results and Discussion for Experiment 1: Refractory Periods).

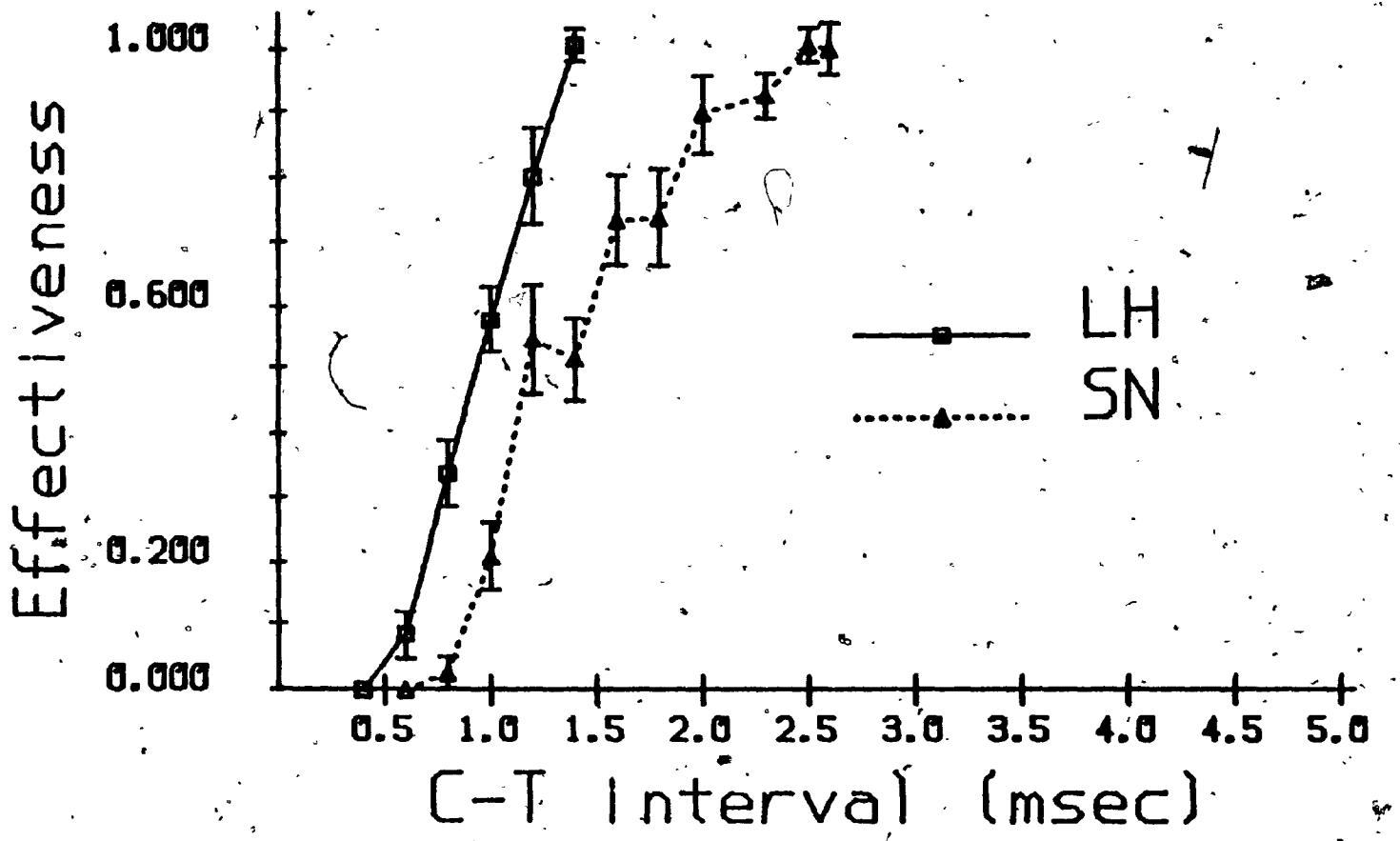

Figure 9. Transformed excitability curves. The solid line is the average of the LH data, while the dashed line is the average of the SN data. Effectiveness values represent the contribution of an equal number of sites. Each curve is transformed so that the rising portions span a range of effectiveness values from 0.0 to 1.0.

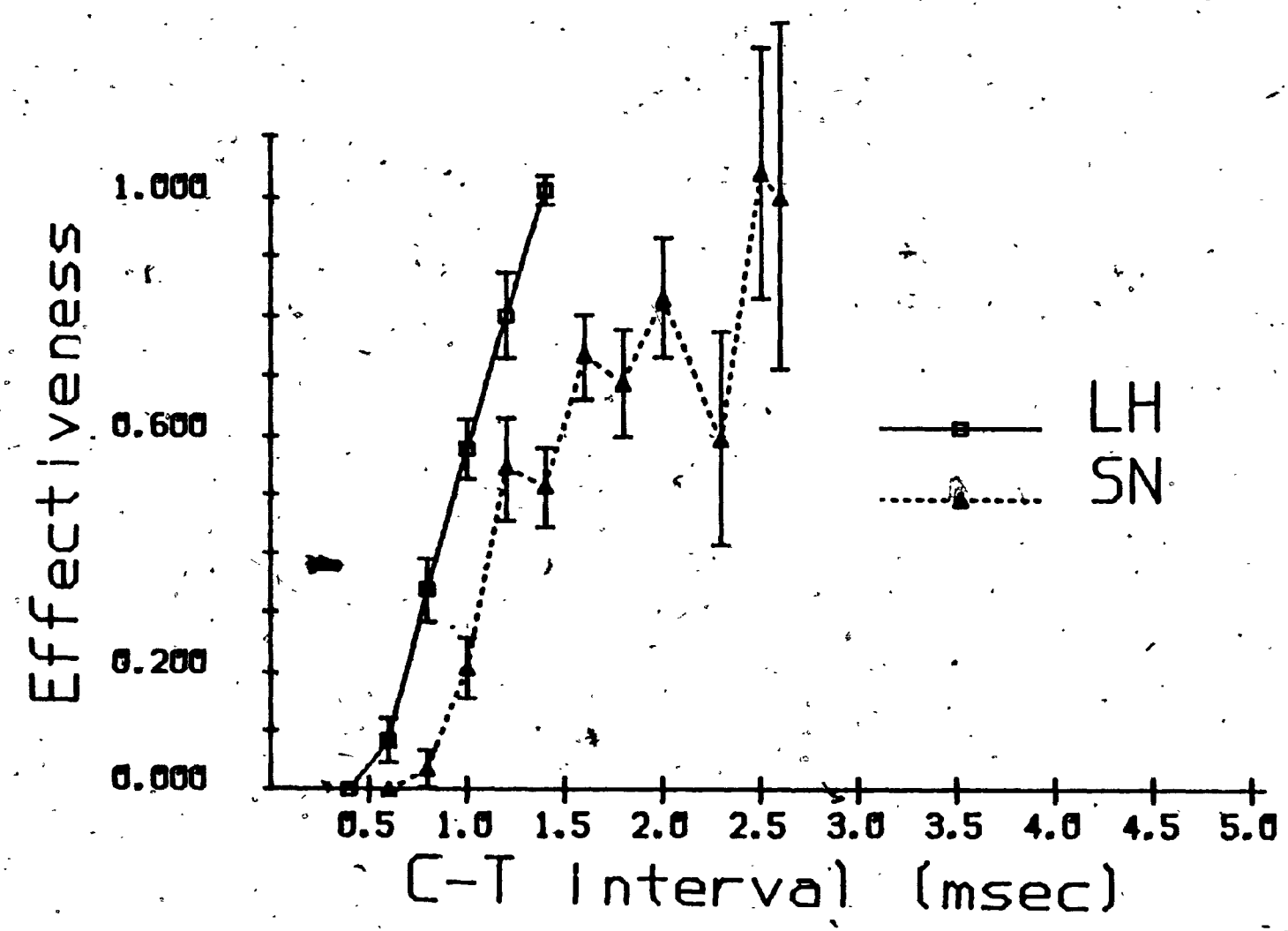

Figure 10. Transformed excitability curves used for statistical analysis. The solid line is the average of the LH data, while the dashed line is the average of the SN data.

Caseweights were determined by computing the inverse of the variance of the E values at each C-T interval. They were assigned to the averaged transformed curves in order to control for the heteroscedasticity of of the error. The analysis of variance approach to regression (Neter and Wasserman, 1974) was applied. This analysis fits seperate regression lines to the LH and SN curves, and fits a single line to all of the data. An F-test is then performed to determine whether more variance is accounted for by one or two lines. The data were better fit by two lines, one for the LH curve and one for the SN curve, than by one line for all the transformed E values combined ($F=24.893$, $df=2/539$, $p<.00001$). This result shows that the regression lines fit to the transformed data from the LH and SN differed, either in slope, y-intercept or some combination of these two. Post-hoc t-tests were conducted to determine which of these three possibilities was the case. The y-intercepts of the transformed LH and SN curves were not different ($t=0.055$, $df=541$, $p>.05$), however a difference was found in the slope of recovery ($t=24.682$, $df=541$, $p<.001$); the SN refractory period curve was less steep than that of the LH.

The main finding of the analysis of variance approach to regression is that the rising portions of the LH and SN refractory period curves differ in slope. Nonetheless, a t-test conducted on the mean of the C-T intervals just less than the interval at which recovery from refractoriness was first evident (Table 5) yielded a significant difference in onset of recovery at the two sites ($t=5.461$, $df=6$, $p=.01$). Recovery from refractoriness was first evident in the SN curves somewhat later than in the LH curves. This difference may not have been detected by the analysis of variance approach to regression due to differences in

the linearity of the curves. The LH curve is nearly linear (see Figure 10), while the SN curve deviates substantially from linearity at C-T intervals greater than 1.2 msec. The nonlinearity in the SN curve draws the x-intercept of the regression line closer to that of the LH curve. The marked changes in the SN curve as C-T interval increases may indicate the recruitment of additional fiber types.

The inferred difference in onset of recovery from refractoriness in the LH and SN could have several causes. On the average, the LH electrode tips may have been relatively closer to the behaviourally relevant neurons than the SN tips. Because current density decreases as a function of distance (Ranck, 1975), neurons in the periphery of the effective stimulation field are fired later in their relative refractory periods than neurons nearer to the electrode tip. In other words, since the stimulation they receive is weaker, neurons in the periphery fire only once their thresholds have recovered to a near normal value. Hence, recovery would occur later if the electrode were relatively far from the intended target. If placement errors were random, such an effect should have disappeared as more subjects were added to the study. No such trend has been noted.

Another explanation for the difference in the refractory period estimates is that the substrates for self-stimulation of the LH and SN have different fiber spectra. Specifically, the LH substrate may include a subpopulation of highly excitable cells that is absent from the SN site.

Table 5

Beginning of recovery C-T values

LH sites	C-T value (msec)	SN sites	C-T value (msec)
B2	0.4	B2	0.8
B8	0.4	B8	1.0
C4	0.4	B7	0.8
C5	0.4	C0	1.0
C6	0.4	F5	0.6
D8L	0.6	F9	0.8
D8R	0.4	G1	0.6

In addition to differences in locus of rise of the LH and SN curves, significant differences were found in the C-T interval at which the curves levelled off ($t=6.823$, $df=6$, $p=.001$). In every case, the SN curves levelled off later than the LH curves (Table 6). This inferred difference in completion of recovery from refractoriness in the LH and SN could have several causes. This may reflect another difference in fiber spectra: the SN substrate may include a subpopulation of neurons with long absolute refractory periods that is absent from the LH site. Another explanation for the difference in the refractory period estimates is that stimulaton of the SN recruits a population of fibers in which relative refractory period contributions are more pronounced. The slope of recovery would be less steep under this condition.

Despite the inferred differences in both onset and completion of recovery from refractoriness, the overlapping portions of the recovery curves could reflect the contribution of a common bundle of reward-related fibers. Nonetheless, these differences make it unlikely that all the fibers responsible for the rewarding effects of brain stimulation at both sites are the same.

Assumptions Underlying Comparison of Refractory Periods

Yeomans' (1975) method of behaviourally estimating the refractory period of the neurons subserving reward makes two assumptions: that the rate of operant responding is monotonically related to excitation of the reward-related fibers, and that the reward system has a linear frequency

Table 6

Asymptotic C-T values

LH sites	C-T value (msec)	SN sites	C-T value (msec)
B2	1.2	B2	1.6
B8	1.2	B8	2.5
C4	1.4	B7	1.8
C5	1.2	C0	2.0
C6	1.4	F5	2.0
D8L	1.2	F9	1.8
D8R	1.4	G1	2.6

response over the range of frequencies used during testing. Numerous studies (Huston et al., 1972; Edmonds et al., 1974; Gallistel, 1978; Schindler, 1983) have provided evidence in support of the first assumption over a wide range of stimulation parameters.

If the frequencies used in the present experiment were outside the range in which the frequency response of the substrate(s) for the rewarding effects of self-stimulation was linear, then the refractory period estimates obtained would reflect both the refractory periods of substrate neurons and their nonlinear frequency response. These estimates would be poor constraints for analyzing electrophysiologically measured refractory periods. In addition, one would have to consider the possibility that the differences found in the LH and SN refractory period curves may have been due to differences in the frequency response of the two substrates, rather than differences in the refractory periods of the substrate neurons. The empirical and theoretical arguments presented below indicate that it is unlikely that the curves are distorted in such a manner.

Hawkins et al. (1983) provide evidence that the frequency threshold method yields LH refractory period estimates that are stable over a wide range of stimulating frequencies. That is, a family of refractory period curves was collected using different single pulse frequencies. Curves obtained using single pulse frequencies from 25 to 200 Hz were virtually superimposable. The stimulating frequencies used to obtain the LH refractory period curves in the present experiment are within this range. Thus, it is unlikely that the LH curves are distorted by inability of the

neural circuitry subserving reward to follow these frequencies.

Psychophysical studies that traded off current and frequency (Gallistel, 1978; Schindler, 1983) also suggest that the frequency response of the directly stimulated LH neurons is roughly linear, at least over an appreciable range. In these experiments, the number of pulses per train was systematically varied and the current was then adjusted to yield half-maximal performance. Over an order of magnitude of currents, the relationship between the current and the inverse of the required number of pulses was roughly linear. The simplest explanation of this finding is that the distribution of the directly stimulated neurons about the electrode tip was roughly homogeneous, and that the frequency response of the substrate was roughly linear.

At extreme current intensities there is a breakdown in the linearity of the current-frequency relationship. At low current intensities, a number of pulses is found beyond which further increases in number of pulses do not require current decrements to maintain a half-maximal criterion. It has been hypothesized that this breakdown is due to inability of the directly stimulated neurons to follow the high frequencies used at low current intensities (Gallistel et al., 1981; Schindler, 1983), but it is possible that the breakdown in linearity is in part due to spatial factors. At best, determining the frequency at which this breakdown is first evident provides a first approximation of the firing limits of the neurons. The decrease in the effectiveness of high frequency stimulation is called the "high frequency roll-off".

In a pilot study involving three rats (Simantirakis, 1984), the current-frequency trade-off has been used to investigate the frequency response of the SN substrate. The frequency at which the high frequency roll-off began was estimated from the breakdown of the trade-off between the current and the inverse of the required number of pulses. The range of estimates of obtained by visual inspection of pilot data was between 50 and 200 Hz. The frequencies used to stimulate three SN sites in the present study (B7, B8, C0) clearly fall below the critical frequencies determined by Simantirakis (1984). The frequencies used to stimulate another three SN sites (F5, F9, G1) lie within the range of critical frequencies reported by Simantirakis (1984), while the frequencies used to stimulate the final SN site (B2) lie on the lower border of this range.

If the refractory periods of the directly stimulated substrates for LH and SN self-stimulation were the same, and if a high frequency roll-off was responsible for the differences between the LH and SN curves in Figure 8, then the following results should have been obtained: 1) the three curves in which high frequency roll-off is unlikely to contribute (B7, B8, C0) should have been similar to the LH curves, 2) the three curves in which high frequency roll-off is likely to contribute (F5, F9, G1) should have differed from those in which it is not, and 3) the remaining SN curve should have fallen between these two extremes. As can be seen in Figure 8, none of these predictions is validated. The SN curves for F5, F9 and G1 are no more similar to the LH curves than are the other SN curves. Furthermore, no differences between the SN curves appear to be correlated with frequency.

Theoretical modelling of the effects of high frequency roll-off on refractory period curves (Shizgal, 1984) suggests that the C-T interval at which onset of recovery is first evident, and the C-T interval at which the curve levels off ought to be unaffected by the roll-off; the refractory period curve merely undergoes bowing between these two points. It is clear from Figure 7 and the results of t-tests described above that the SN curve begins to rise and levels off at longer C-T intervals than the LH curve. If Shizgal's model is valid, then these differences cannot be due to a difference in the high frequency roll-off in the LH and SN substrates.

Although the contribution of high frequency roll-off to the SN curves cannot be ruled out, it is unlikely that this phenomenon makes an important contribution to the differences in the LH and SN curves.

Another possible explanation for the differences in the LH and SN recovery curves is that the motor neurons involved in SN stimulation had shorter refractory periods than the reward neurons. If the motor neurons in the SN had recovered from refractoriness prior to the reward-related neurons, the beginning of the recovery from refractoriness of the reward-related neurons could be masked. Thus, the refractory period curves for LH and SN self-stimulation could appear dissimilar despite identical distributions of excitability in the reward substrates activated at the two sites. The likelihood of this possibility is minimal. An argument analogous to one presented above for limiting the contribution of a high frequency rolloff to the SN recovery curve is also valid in the present case. If the refractory periods of the directly stimulated

substrates for LH and SN self-stimulation were the same, and if earlier recovery of motor fibers was responsible for the differences between the LH and SN curves in Figure 8, then the following results should have been obtained: 1) the curves obtained from subjects in which the motoric effects of stimulation were weak should have been similar to the LH curves, 2) the curves in which the motoric effects were strong should have differed from those in which they were weak. Neither of these predictions is supported by the data.

An additional argument against attributing the differences in the LH and SN recovery curves to motor fibers with shorter refractory periods than the reward fibers stems from the work of Rompré (1983). For each pulse delivered to a reward site in the LH, he delivered a stimulation pulse to a region from which powerful motoric effects were elicited. He found that the addition of this contingent motoric stimulation decreased both the slope and the asymptote of the rate-frequency functions used to determine number thresholds. Thus, depressed slopes and asymptotes can be regarded as the signature of motoric contamination. If the rate-frequeny functions at C-T intervals between that at which the LH curve begins to rise and that at which the SN curve begins to rise could be shown to have depressed slopes and asymptotes, then motor fibers with refractory periods shorter than those of the reward-related neurons could account for the differences observed between the LH and SN curves. Examination of data from three subjects failed to reveal depression of the maximum rate that was specific to the appropriate C-T intervals. Furthermore, there was no evidnce of slope changes. Thus, although it is possible that the motoric effects contributed to the difference between the LH and SN curves, they

cannot be regarded as having made a very large contribution.

The Role of Dopaminergic Neurons

It has been argued (Corbett and Wise, 1980) that self-stimulation in the ventral tegmentum is obtained only in those regions in which the concentration of dopaminergic neurons is high. Do these neurons constitute the directly stimulated stage of the substrate for SN self-stimulation? Cells in the SN identified as dopaminergic have an electrophysiologically estimated absolute refractory period of 2.6 msec (Wang, 1981). This value exceeds the range of refractory periods that is consistent with the rapidly rising portion of the behaviourally derived SN curve. However, Wang's (1981) estimate of the refractory period of SN dopaminergic neurons is based on a method that may reflect the characteristics of the cell body and initial segment of the axon (Swadlow, 1982), rather than those of the neuronal site of stimulation. The refractory period estimation method developed by Swadlow (1982) ensures that recovery at or near the site of stimulation is reflected. Because the refractory period of the cell body is longer than that of the neuronal site of stimulation, refractory period estimates made with Swadlow's (1982) technique are shorter than those made with the technique employed by Wang (1981). However, the difference between refractory period estimates for dopaminergic fibers obtained using Swadlow's (1982) technique and the technique employed by Wang (1981) would have to be greater than any found to date to render the electrophysiologically derived refractory period estimate consistent with the behaviourally

derived curves presented here. Hence, it is unlikely that the substrate for SN self-stimulation is exclusively dopaminergic.

Two pieces of evidence suggest that dopaminergic neurons cannot exclusively account for the behaviourally derived LH recovery curve. First, dopaminergic neurons ascend through the MFB (Ungersted, 1971), whereas psychophysical estimates of the direction of conduction in the directly stimulated substrate for self-stimulation implicate descending fibers (Shizgal et al., 1982; Bielajew and Shizgal, 1984). Second, the conduction velocities of dopaminergic neurons are much too slow (German, Dalsass and Kiser, 1980; Guyenet and Aghaganian, 1978; Yim and Mogenson, 1980) to correspond to psychophysical estimates for MFB reward fibers (Bielajew et al.,1981).

Conclusions

The behaviourally inferred estimates of recovery from refractoriness in the neurons subserving self-stimulation of the LH and SN differ both in onset and completion. These results may reflect differences in fiber spectra. The LH substrate may include a subpopulation of highly excitable fibers that is not present in the SN substrate, while the SN substrate may include a subpopulation of fibers with long absolute refractory periods that is absent from the LH substrate. Nonetheless, because the recovery curves overlap at some C-T intervals, some fibers could be common to both substrates. It is unlikely that the fibers responsible for self-stimulation of the SN are exclusively dopaminergic because of the disparity between the behaviourally and electrophysiologically derived estimates of recovery from refractoriness at this site.

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