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The Contribution of Anterior Medial Forebrain Bundle Neurons to Self-Stimulation of the Lateral Hypothalamic and Ventral Tegmental Areas

Beverley Murray

A Thesis

in

The Department

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ABSTRACT

Contribution of Anterior Medial Forebrain Bundle Neurons to Self-stimulation of the Lateral Hypothalamic and Ventral Tegmental Areas

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Concordia University, 1992

Identification of the reward-relevant neurons activated by stimulation of the medial forebrain bundle (MFB) has been a major goal of research in the area of brain stimulation reward. In a series of experiments, the contribution of neurons in the anterior MFB to the rewarding effect of stimulating more caudal MFB sites was examined. In Experiment 1, the effect of anterior lateral hypothalamic (ALH) lesions on the frequency threshold for self-stimulation of the middle lateral hypothalamus (LH) and ventral tegmental area (VTA) was determined. In 5 out of 14 subjects, lesions to the ALH and surrounding regions resulted in long-lasting increases (0.1-0.25 $\log_{10}$ units) in the frequency threshold for self-stimulation of the LH or VTA, an effect consistent with a reduction in the rewarding effectiveness of the stimulation. In Experiment 2, the paired-pulse collision technique was used to determine whether reward-relevant neurons directly link the VTA and the site previously lesioned in the ALH. Stimulation consisted of trains of pulse pairs, with each electrode receiving one pulse from each pair. It was reasoned that if each stimulation site lay along the trajectory of the same reward-relevant axons, then collision of antidromic

and orthodromic action potentials should occur when stimulating at short inter-pulse intervals, thus resulting in a decrease in the effectiveness of the stimulation. Significant collision-like effects were obtained in 4 of the 6 subjects, supporting the notion that reward-relevant neurons directly link the AL-H and VTA. In Experiment 3, extracellular recordings were obtained from cells in the rostral bed nucleus of the MFB that were antidromically activated by stimulation of posterior MFB sites that typically support self-stimulation. The refractory periods and conduction velocities of descending MFB fibers arising from these cells were similar to those obtained for reward-relevant MFB neurons using psychophysical methods. This work suggests that neurons arising in the anterior MFB may be part of the directly-activated substrate for rewarding MFB stimulation.

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The faculty, staff, and students of the CSBN have created a unique environment in which to pursue graduate training. I am grateful to them all for their contributions to my education. A special thanks to Dena, Margaret and Sandra for being both good friends and good colleagues. Dr. John Yeomans introduced me to the field of brain stimulation reward, gave me the confidence to pursue graduate training, and has provided encouragement ever since.

My deepest gratitude goes to Tuan for his love and understanding during a long and demanding process.

Dedication

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

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Electrical stimulation delivered to several brain regions produces a powerful behavioural effect that directs the animal towards obtaining more stimulation. The vigorous manner in which animals will self-administer the stimulation, even at the expense of satisfying their physiological needs (Deutsch, Adams & Metzner, 1964; Routtenberg & Lindy, 1965), has attracted an enduring interest in brain stimulation reward (BSR) as a model for the study of goal-directed behaviour.

It has been suggested that one of the natural functions of the neurons activated by the stimulation is to steer animals toward appropriate goal objects (rewards) in their environment. For instance, it has been hypothesized that these neurons are involved in adaptive appetitive behaviours such as feeding (Gratton & Wise, 1988a, 1988b; Hoebel & Teitelbaum, 1962; Rolls, Burton, & Mora, 1980) as well as maladaptive appetitive behaviours such as self-administration of drugs of abuse (Wise, 1980). Identification of the neural circuits responsible for the rewarding effect of the stimulation would provide researchers with a means of assessing the natural function of these neurons in awake, behaving animals.

The simplest place to begin the search for the circuits that carry the reward-relevant signal is near the tip of the stimulating electrode. The directly activated neurons that transmit the reward-relevant signal to downstream stages of the circuit (the 'first stage' neurons) must pass close to the electrode tip. After almost forty years of research on the phenomenon of brain stimulation reward, the first stage neurons have yet to be identified. The complexity of the brain areas that support the most robust self-

stimulation behaviour and the use of inadequate measures to assess the rewarding value of the stimulation have been some of the impediments to progress.

The psychophysical approach to the study of BSR has addressed both of these problems by providing techniques for characterizing the first stage neurons and by developing scaling procedures that are not affected by the arbitrary choice of stimulus parameters (Gallistel, Shizgal, & Yeomans, 1981). In the last decade, an impressive body of research involving the psychophysical characterization of the first stage neurons responsible for medial forebrain bundle (MFB) self-stimulation has accumulated. Although the reward-relevant neurons activated at the electrode tip have not yet been identified, the number of candidate pathways has been considerably reduced. These more recent advances have provided hope that the neural pathways subserving MFB reward will soon be identified, providing psychologists with a cellular model for studying the neural basis of motivation and learning in vertebrates.

The Psychophysical Approach

Given that there are approximately fifty fiber pathways coursing through the MFB (Nieuwenhuys, Geeraedts & Veening, 1982), it is likely that an electrode placed within the bundle will activate many neurons besides the ones that actually contribute to the rewarding effect. Although recording the activity of cells activated by self-stimulation electrodes can provide us with their physiological characteristics, this information alone does not provide a basis for deciding whether a particular neuron actually carries the

reward-relevant signal. However, if this information is coupled with data on the physiological and anatomical characteristics of the first stage neurons, then it becomes possible to identify individual neurons that are likely to carry the reward-relevant signal. For example, if we know that the first stage neurons have absolute refractory periods in the range of 0.5 to 1.5 msec, then a neuron with a refractory period of 1.0 msec would be a likely candidate whereas a neuron with a refractory period of 5.0 msec would not.

The psychophysical approach is able to characterize those neurons responsible for the rewarding effect of the stimulation through the use of trade-off functions that describe the relationship between two stimulus parameters producing the same level of behaviour. These trade-off functions are interpreted according to the physiological and anatomical characteristics of the first stage neurons. Inferring the physiological characteristics of the directly activated reward-relevant neurons from the behaviour of the animal requires that each level of the system under study behaves in a monotonic fashion. If for every stimulus input to the first stage there is one and only one output from the final stage, then a system is considered monotonic and all of its constituent stages must also be monotonic (Gallistel et al., 1981). If the final output of a monotonic system is held constant, then the output of the first stage will also be held constant. Trade-off functions will therefore provide the combinations of two stimulus parameters that produce the same level of excitation in the first stage neurons. In this way, information about the properties of the first stage can be inferred by manipulating the pattern of the stimulation and holding constant the final behavioural output. The BSR system has been shown to possess this property

of monotonicity over a wide range of stimulation parameters (Edmonds, Stellar & Gallistel 1974; Gallistel 1978).

The anatomical and physiological characteristics of the first stage neurons are inferred from the psychophysically derived trade-off functions. These characteristics are then compared to the anatomical and physiological characteristics of identified neurons in order to determine the likelihood that they comprise part of the reward-relevant substrate. Of course, it is possible that a particular nucleus could contain many cells that have anatomical and physiological characteristics that match the first stage neurons and yet play no role in the rewarding effect of the stimulation. If it could be shown that damaging this nucleus also reduced the rewarding impact of the stimulation, then we would be more confident in proposing that it is part of the directly activated substrate. Finally, recording the activity of these cells during rewarding brain stimulation and during other appetitive behaviours would provide the most direct assessment of their role in goal-directed behaviours.

The Refractory Period Test.

The psychophysical approach to the study of BSR was initiated in 1964 when Deutsch estimated the refractory periods of the neurons involved in self-stimulation by delivering trains of paired pulses and varying the interval between each pair. He reasoned that if the second pulse in each pair was delivered while the neurons were still refractory from the first pulse, then omitting the second pulse should not influence the effectiveness of the stimulation. Deutsch used rate of responding, voltage thresholds, and

preference data as three different measures of stimulation effectiveness, primarily in an effort to dissociate the substrates for the "drive" versus "reinforcement" pathways.

Yeomans (1975) has subsequently shown that when rate of responding is used as the measure of stimulation effectiveness, the refractory period estimates thereby obtained depend upon the arbitrary choice of stimulation parameters. Yeomans introduced a frequency threshold technique for scaling the effects of varying the interval between pulse pairs that permitted a quantitative assessment to be made of changes in stimulation effectiveness. As in the experiment by Deutsch, Yeomans used trains of conditioning (C) and test (T) pulses delivered to a self-stimulation electrode and varied the interval between pulse pairs (C-T interval). Stimulation effectiveness was assessed by determining the number of pulse pairs required to produce a criterion level of behaviour (the 'required number'). Yeomans reasoned that the first stage neurons would fail to fire in response to the T-pulses at C-T intervals shorter than their refractory period. To compensate for the loss of firings, additional pulse pairs must be added to the train in order to keep the output of the directly activated neurons, and thus the behavioural output, constant. This would be reflected in a shift in the curve relating the response rate to the number of pulse pairs (rate-number curve) towards higher pulse numbers.

Yeomans also devised a scaling formula for expressing the effectiveness of the T-pulses as a proportion of the effectiveness of the C-pulses. The number of pulse pairs required for half maximal responding was determined for each C-T interval (paired pulse condition) and then compared to the

required number of pulses in a train of evenly spaced single pulses (single pulse condition). If none of the reward-relevant neurons fired in response to the T-pulses, then the required number of single pulses (N_{sp}) would be equal to the required number of pulse pairs (N_{ct}) and a T-pulse effectiveness value (E-value) of zero would be assigned to that C-T interval ($E = N_{sp}/N_{ct} - 1 = (1 - 1) = 0$). If all of the neurons fired in response to the T-pulses, then the required number of single pulses would be equal to twice the required number of pulse pairs and an E-value of 1.0 would be assigned to that C-T interval ($E = N_{sp}/N_{ct} - 1 = (2 - 1) = 1$).

Yeomans found that effectiveness values for stimulation of the posterior MFB initially declined over C-T intervals of 0.2 to 0.4 msec and then increased sharply between C-T intervals of 0.5 to 1.2 msec. A more gradual rise in effectiveness was obtained for C-T intervals between 1.2 and 5.0 msec. The initial decline in effectiveness over C-T intervals of 0.2 to 0.4 msec has been attributed to the summation of subthreshold potentials (local potential summation or LPS) in neurons along a fringe of the stimulation field that do not fire in response to the C-pulse. If the T-pulse is delivered before the subthreshold potential from the C-pulse has decayed (a few tenths of a millisecond), then the two potentials from each pulse summate and the neurons within this fringe fire.

The increase in effectiveness observed over C-T intervals of 0.5-1.2 msec has been attributed to recovery from refractoriness in the directly activated neurons mediating the rewarding effect of the stimulation. In order to test whether this increase reflected recovery from absolute refractoriness in several subpopulations of neurons or a contribution of the relative refractory

period, Yeomans used larger amplitude T-pulses in order to fire neurons closer to the end of their absolute refractory period (Yeomans, 1979). He found that larger amplitude T-pulses did not substantially hasten recovery and therefore concluded that the rise in effectiveness was primarily due to recovery in several subpopulations of neurons with a range of absolute refractory periods.

The Collision Test.

In addition to the refractory period test, the collision test has proved to be one of the most informative of the psychophysical techniques (Shizgal, Bielajew, Corbett, Skelton, & Yeomans, 1980). The technique makes use of the conduction failure that occurs when orthodromic and antidromic action potentials collide along a single axon; the equal and opposite longitudinal currents sum to zero and neither action potential conducts past the point of collision (Tasaki, 1949). The collision test has been used to infer that reward-relevant neurons directly link two self-stimulation sites and, when used in conjunction with the refractory period test, to estimate the conduction velocity of the first stage neurons (Shizgal et al., 1980; Bielajew & Shizgal, 1982, 1986).

The technique is similar to that described above for the refractory period test except that the C- and T-pulses are delivered to two self-stimulation electrodes instead of one, each electrode receiving one of the pulses in each pair. Each pulse will trigger two volleys of action potentials at each electrode; one travelling towards the terminal region (orthodromic) and one travelling towards the somata (antidromic). If the two electrodes activate

the same reward fibers at different sites along their trajectory then, at short C-T intervals, the orthodromic action potentials initiated at the electrode closest to the somata will collide with the antidromic action potentials initiated at the electrode closest to the terminals. When this collision occurs, only the orthodromic volley from the electrode closest to the terminals will reach the terminals and therefore transmit the reward-relevant signal to the next stage of the system. When the C-T interval is increased so that the first volley and its trailing refractory period zone has had time to pass by the second electrode before the T-pulse arrives, then the orthodromic volley from both electrodes will propagate successfully to the terminal region. Given the bi-directional nature of conduction along the axon, this effect will not depend upon the electrode (upstream or downstream) that receives the C-pulses.

Evidence of a collision-like effect is inferred from a decrease in the rewarding efficacy of the stimulation with C-T interval that does not depend upon the electrode receiving the C-pulses. This decrease in the rewarding effect of the stimulation is presumably due to the loss of action potentials from collision. In order to keep constant the number of firings, and thus the behavioural output, the firings lost due to collision are replaced by increasing the number of pulse pairs. Thus, as in the refractory period experiment, a decrease in the rewarding impact of the stimulation is inferred from a shift in the rate-number curve towards higher numbers of pulse pairs.

A modified version of Yeomans' effectiveness formula that takes into account any differences in the required number of single pulses at each stimulation site is used to scale the collision data in a manner similar to the

refractory period data. If all of the neurons stimulated at one site are also stimulated at the other then, at short C-T intervals, half of the action potentials generated by the two pulses will be removed due to collision. In this case, the number of single pulses required to produce the criterion level of behaviour (N_{sp}) will be equal to the required number of pulse pairs (N_{ct}) and E-values of zero will be assigned to those C-T intervals ($E = N_{sp}/N_{ct} - 1 = (1 - 1) = 0$). If none of the reward-relevant neurons are stimulated at both sites then collision cannot occur and both volleys of action potentials will arrive at the terminals. In this case, the required number of single pulses will be equal to twice the required number of pulse pairs and E-values of 1.0 will be assigned to those C-T intervals ($E = N_{sp}/N_{ct} - 1 = (2 - 1) = 1$). If only half of the fibers are common to the two sites, then only 1/4 of the action potentials will be removed due to collision. In this case, the required number of single pulses will be equal to 1.5 times the required number of pulse pairs and E-values of 0.5 will be assigned at short C-T intervals ($E = N_{sp}/N_{ct} - 1 = (1.5 - 1) = 0.5$. In all the above cases, stimulation at long C-T intervals will be twice as effective as a train of single pulses (assuming perfect summation of the rewarding effects produced by the two electrodes) and will therefore be expressed as E-values of 1.0. The difference between the effectiveness values obtained at short and long C-T intervals (assuming perfect summation at long C-T intervals) therefore gives the proportion of reward-relevant fibers common to the two sites of stimulation.

The collision interval is defined as the range of C-T intervals over which stimulation effectiveness increases. This interval can be broken down into

the time it takes for the volley of action potentials to travel between the two electrodes and the refractory period of the neurons stimulated by the T-pulse. If behavioural estimates of the refractory period are obtained and the distance between the two electrodes measured, then the collision interval can provide an estimate of the conduction velocity of the fibers undergoing collision. Since there exists a strong correlation between fiber diameter and conduction velocity (Hursch, 1939; Waxman & Bennett, 1972), the caliber of the directly activated neurons can be estimated from the collision test.

The Counter Model of Spatiotemporal Integration

Implicit in both the refractory period and collision tests is the assumption that the loss of action potentials in some or all of the directly activated neurons (due to refractoriness or collision block) can be compensated for by increasing the frequency of the stimulation and thus the number of firings in either the same or other neurons. This assumption is presented formally in the counter model of spatiotemporal integration which states that the number of action potentials produced in the first stage neurons for a train of fixed duration determines the rewarding effect of the stimulation, regardless of the temporal or spatial distribution of the activity (Gallistel et al., 1981). Thus, the rewarding effect is determined solely by some process which is able to "count" the number of firings in the directly activated substrate.

The most direct support for this model comes from the form of the curve that relates the current (a spatial variable) and the number of pulses (a temporal variable). The current-number trade-off curve can be obtained by determining the number of pulses required for a criterion level of behaviour

(e.g. half the maximum rate) at a range of currents. What one finds is that over a wide range of stimulation parameters, the relationship between current and the inverse of the required number of pulses is approximately linear. The most parsimonious explanation for this linear relationship requires the following assumptions:

1. Each 0.1 msec pulse in the train fires each reward-relevant axon at most once, therefore the number of firings in the first stage (n_f) is given by $n_f = N \times n_a$ where N = number of stimulation pulses and n_a = number of axons directly activated by each pulse.

2. The number of reward-relevant axons activated by the stimulation is a linear function of current intensity. This assumption in turn rests on the following assumptions:

   a. The square of the radius of excitation is proportional to the current.

   b. Assuming that the density of reward-relevant neurons is constant throughout the stimulation field, the number of axons passing within the radius of excitation is proportional to its square.

   Combining (a) and (b) above, it follows that current is proportional to the number of axons fired. However, due to the scar at the tip of the electrode, at currents below a certain value (I_0), no reward-relevant axons are recruited. Therefore, the curve relating current and number of axons does not pass through the origin but is rather a linear relationship of the form $n_a = k(I - I_0)$.

Combining the two equations presented above, one obtains the following expression for the current:

$$I = [n_f/k][1/N] + I_0.$$

If the number of firings is constant for each combination of I and 1/N that produces the same behavioural effect (i.e. n_f = constant), then the above expression is reduced to a linear relationship between I and 1/N.

To summarize, the simplest explanation for the (approximately) linear relationship between current and the inverse of the number of pulses includes the assumption that the rewarding effect of the stimulation is determined by the number of action potentials generated in the first stage regardless of their temporal or spatial distribution. It is this insensitivity to the temporal and spatial distribution of activity in the first stage neurons that explains why increases in the number of firings in either the same or different neurons is able to compensate for the loss of action potentials due to refractoriness or collision block.

Psychophysical Characterization of the Diencephalic Portion of the MFB

Previous psychophysical studies have provided an increasingly detailed characterization of the reward-relevant neurons directly activated by stimulation of the MFB between the levels of the lateral hypothalamus (LH) and the ventral tegmental area (VTA). For example, several studies employing the collision technique have provided evidence that reward-relevant neurons directly link the LH and VTA (Bielajew & Shizgal, 1982, 1986; Durivage & Miliaressis, 1987; Gratton & Wise, 1988; Shizgal et al., 1980).

Conduction velocity estimates derived from 19 rats in the five studies cited above range from 1.0-8.3 m/sec with a mean of 3.9 $\pm$ 0.4 m/sec (Shizgal & Murray, 1989). Based on Waxman and Bennett's (1972) analysis of the data from Hursh (1939) from peripheral nerve, these estimates (with the shortest value excluded) are consistent with myelinated fibers ranging from 0.3-1.5 um in diameter. Myelinated fibers with this range of diameters are located in the MFB (Nieuwenhuys et al., 1982).

Refractory period curves for diencephalic MFB sites have generally shown a sharp rise between C-T intervals of 0.5 to 1.5 msec and occasionally a more gradual rise between C-T intervals of 1.5 to 5 msec (Bielajew, Jordon, Ferme-Enright & Shizgal, 1981; Bielajew, Lapointe, Kiss & Shizgal, 1982; Bielajew & Shizgal, 1982, 1986; Gratton & Wise, 1985; Macmillan, Simantirakis & Shizgal, 1985; Rompré & Miliaressis, 1980; Schenk & Shizgal, 1982; Yeomans, 1975, 1979). Increasing the amplitude of the T-pulse, which should fire neurons earlier in their relative refractory period, has not been found to hasten recovery much beyond C-T intervals of 1.2 to 1.5 msec (Bielajew et al., 1982; Yeomans, 1979). It has therefore been argued that the sharp rise in effectiveness values between C-T intervals of 0.5 to 1.5 msec is primarily due to recovery from refractoriness in first stage neurons with absolute refractory periods ranging from 0.5-1.5 msec. Swadlow and Waxman (1976) found that axons in the corpus callosum with conduction velocities within the range of 1-8 m/sec had refractory periods of 0.7-1.4 msec. Therefore, the relationship between the refractory periods and conduction velocities in the first stage neurons is similar to the relationship observed in other fiber systems of the central nervous system. To

summarize, the early psychophysical data obtained for self-stimulation sites along the diencephalic portion of the MFB are consistent with a first stage comprised, at least in part, of small myelinated fibers that directly link the LH and VTA with refractory periods ranging from 0.5-1.5 msec and conduction velocities ranging from 1-8 m/sec.

Implications for Catecholamine Theories of Reward.

One of the most important issues addressed by the early psychophysical data was the nature of the involvement of catecholamine systems in the rewarding effect of MFB stimulation. Various forms of the catecholamine hypothesis of reward have dominated the literature on the neural basis of self-stimulation for almost 30 years. In its most general form, the hypothesis states that catecholamine pathways are a critical link in the reward-relevant circuitry (Crow, 1972; German & Bowden, 1974; Stein, 1969; Wise, 1978). Initial support for the hypothesis came in large part from pharmacological data showing that drugs which enhance catecholaminergic transmission increase self-stimulation rates whereas drugs which interfere with catecholaminergic transmission decrease self-stimulation rates (Olds & Travis, 1960; Poschel & Nineteman, 1963, 1964, 1966; Stein, 1962, 1964). Based primarily on the supposed strong correlation between the location of positive self-stimulation sites and the trajectory of the catecholamine pathways (e.g. Crow, 1972, German & Bowden, 1974), the strongest version of the catecholamine hypothesis proposed that it was the direct activation of catecholamine neurons that was responsible for the rewarding effect of the stimulation.

The psychophysical data that have been obtained for self-stimulation sites in the diencephalic portion of the MFB provide a serious challenge to the strong version of the catecholamine hypothesis, at least as it pertains to these sites. In comparison to the psychophysically derived estimates for the first stage MFB neurons, catecholamine neurons have longer absolute refractory periods (greater than 1.2 msec) and slower conduction velocities (less than 1.0 m/sec) (Faiers & Mogenson, 1976; Feltz & Albe-Fessard, 1972; German, Dalsass, & Kiser, 1980; Guyenet & Aghajanian, 1978; Wang, 1981; Yeomans, Maidment, & Bunney, 1988; Takigawa & Mogenson, 1977; Yim & Mogenson, 1980). Nonetheless, several studies have demonstrated that drugs which interfere with catecholaminergic transmission reduce the rewarding impact of stimulating a variety of brain regions and that this effect is not simply due to a decrease in the subject's capacity to perform the operant response (e.g. Fouriezos & Wise, 1976; Franklin, 1978; Gallistel, Boytim, Gomita, & Klebanoff, 1982; Gallistel & Freyd, 1987; Liebman & Butcher, 1974). More recent versions of the catecholamine hypothesis have therefore proposed that catecholamines, specifically dopamine, may constitute a later stage of the reward-relevant circuitry (Wise, 1978, 1980; Yeomans, 1982) or may modulate or 'gate' transmission in the system (Gallistel, 1986).

Possible Role for Descending, Anterior MFB Neurons

Although the origin and destination of the first stage neurons for MFB self-stimulation are still not known, data from a variety of studies are consistent with the hypothesis that at least some of the first stage neurons arise in the anterior MFB. Although these data do not yet provide

conclusive evidence for this hypothesis, they do support the contention that the anterior MFB is a region worthy of further study.

Description of the Anterior MFB.

In their detailed cytoarchitectonic analysis, Geeraedts et al. (1990) divided the cells of the anterior MFB on the basis of their size, shape, staining intensity, packing density, and spatial orientation. The borders of their cytoarchitectonic atlas corresponded to the following nuclear groups: the interstitial nucleus of the stria medullaris (SM), the deep layer of the olfactory tubercle (Tu), the magnocellular preoptic nucleus (MCPO), the nucleus of the diagonal band of Broca which corresponds to the horizontal limb of the diagonal band (HDB) in the Paxinos and Watson (1986) atlas, the lateral preoptic area (LPO), and the subcommissural and sublenticular substantia innominata which correspond to the ventral pallidum (VP) and substantia innominata (SI) in the atlas by Paxinos and Watson (1986). The region corresponding to the anterior LH in the Paxinos and Watson atlas (-1.3 mm from bregma) is divided into three subdivisions in the Geeraedts et al. (1990a) atlas (plate T7): a ventrolateral division that is an extension of the MCPO, a ventromedial division that is an extension of the LPO, and a dorsal division that is an extension of the SI. Although the overall borders of the anterior MFB will be defined according to the Geeraedts et al. (1990a) atlas, the nomenclature of Paxinos and Watson (1986) will be used throughout this thesis. The one exception to this rule will be the use of the term ALH to designate the region of the anterior LH in the Paxinos and Watson (1986) atlas that extends to the rostral border of the ventromedial hypothalamus

(plates -1.3 mm to -2.12 mm) according to the subdivisions of the LH used by Saper, Swanson, and Cowan (1979).

Anatomical and Electrophysiological Evidence.

The nuclear groups comprising the anterior MFB give rise to many descending fibers that pass by MFB sites that support self-stimulation (Grove, 1988; Phillipson, 1979; Saper, 1976; Saper et al., 1979; Swanson, 1976; Swanson, Mogenson, Gerfen & Robinson, 1984; Veening, Swanson, Cowan, Nieuwenhuys & Geeraedts, 1982; Zahm, 1989). Thus, on purely anatomical grounds, the nuclei of the anterior MFB are possible candidates for the origin of the first stage MFB neurons.

One prediction of the hypothesis that anterior MFB neurons give rise to the first stage is that rewarding stimulation of the MFB should activate cells in these regions. Gallistel, Gomita, Yadin, and Campbell (1985) attempted to isolate likely candidates for the first stage neurons by examining regions showing high metabolic activity during self-stimulation of the MFB. Rats were injected with [^{14}C]-2-deoxyglucose (2DG), allowed to self-stimulate for 45 minutes, and then sacrificed and their brains sliced and exposed to X-ray film. The region of the diagonal band and medial septum showed high uptake of the metabolic marker as did regions of the anterior MFB, in particular the LPO. In some of their subjects, activation of the anterior MFB appeared to be primarily confined to the region of the HDB and the MCPO (and not the LPO) as outlined in the Paxinos and Watson atlas (1986). The high metabolic activity continued caudally along the MFB to the level of the VTA.

Electrophysiological data also provide support for the notion that rewarding stimulation of the MFB activates some anterior MFB neurons. Rompré and Shizgal (1986) and Shizgal, Schindler, and Rompré (1989) have found that some cells arising in the medial part of the anterior MFB, primarily the medial aspect of the LPO, are driven by stimulation of MFB sites that support (or typically support) self-stimulation. However, the focus of these studies was on the characterization of cells arising in the septal complex, a region that was found to show high metabolic activity during rewarding stimulation of the MFB in the study by Gallistel et al. (1985). As a result, the majority of the cells in these studies were located rostral and medial to the anterior MFB. What is intriguing about these studies in terms of the present hypothesis is that some cells localized to the anterior MFB had refractory periods consistent with the psychophysical profile for the first stage MFB neurons. In addition, both the study by Rompré and Shizgal (1986) and Shizgal et al. (1989) found that the majority of cells classified as non-candidates were localized to the septal complex (75%-87%) whereas only about half of the candidate cells were recorded in these same regions (46%-51%). Thus, a higher proportion of candidate than non-candidate neurons were found in regions that were in or bordering the nuclei of the anterior MFB. Based on these data, a more thorough electrophysiological investigation of sites caudal, lateral, and ventral to the sites examined in the studies by Rompré and Shizgal (1989) and Shizgal et al. (1989) is clearly warranted.

Psychophysical Evidence.

Although the 2DG and electrophysiological data show that anterior MFB regions are activated by rewarding stimulation of the MFB, the functional relevance of these pathways cannot be directly assessed using these techniques. The strength of the psychophysical approach is that it is able to characterize the reward-relevant pathways directly activated by the stimulation. Perhaps the most influential of the studies to propose a descending path as part of the first stage was conducted by Bielajew and Shizgal (1986). The authors reasoned that a hyperpolarized region along the MFB should only be able to block the conduction of a reward-relevant signal when it occurred downstream from the site of stimulation. Direction could therefore be inferred by noting which of two electrodes, one in the LH and the other in the VTA, was more effective at reducing stimulation effectiveness when used as the anode of the stimulation circuit.

Due to the time required for the action potentials initiated at the upstream cathode to reach the downstream anode, conduction could only be blocked if long pulse durations were used so that current was still exiting the downstream anode at the time the action potentials arrived. The experiment was therefore set-up as a trade-off between pulse duration and current intensity; anodal block was predicted to occur when using the downstream electrode as the anode and at pulse durations equal to or greater than the conduction time between the two electrodes. If anodal block was occurring at sufficiently long pulse durations, then the stimulation would be less effective and a higher current intensity would be required to produce the same criterion level of behaviour. Thus, an upward bending of the curve

relating current and pulse duration (strength-duration curve) at the longer pulse durations was taken as evidence for anodal block.

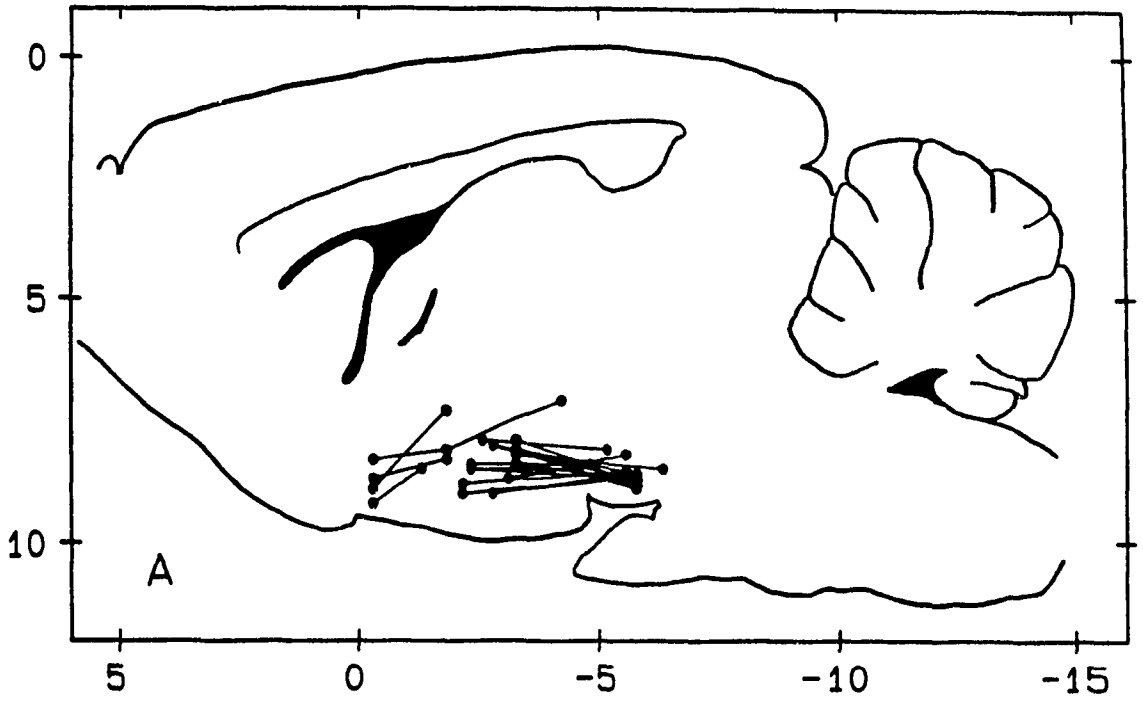
Two parameters, the rheobase and chronaxie, are able to describe the roughly hyperbolic shape of the strength-duration curve. As the pulse duration is increased, the current required to produce a criterion level of behaviour decreases until it approaches an asymptote (rheobase) at which point further increases in the pulse duration no longer result in a decrease in the required current. The chronaxie is defined as the pulse duration at which the current is equal to twice the rheobase. An upward bending of the strength-duration curve at long pulse durations, as would occur in the case of anodal block, would increase the rheobase and therefore advance the pulse duration at which current equaled twice rheobase, thus shortening the chronaxie. Because anodal block would not occur if the anode was not aligned along the trajectory of the reward-relevant bundle, Bielajew and Shizgal compared chronaxie estimates obtained with the anode and cathode placed along the MFB to chronaxie estimates obtained using the same MFB cathode and a skull screw as the anode.

Bielajew and Shizgal (1986) found that the chronaxie estimates obtained with the VTA anode and LH cathode were significantly shorter than those obtained with the skull screw anode and LH cathode, which supports the hypothesis that the normal direction of conduction in at least some of the first stage neurons is descending. However, it should be noted that other factors may have contributed to the chronaxie differences. For instance the shorter chronaxies obtained when using the VTA anode could be due to the recruitment of shorter chronaxie neurons at the VTA site (Bielajew, 1983).

Bielajew and Shizgal did find that chronaxie estimates were shorter when stimulating with a VTA cathode and skull screw anode than when stimulating with a LH cathode and skull screw anode. In addition, careful examination of the strength-duration curves obtained in an earlier self-stimulation study reveals that anodal pulses produce a slight upward bend in the strength-duration curve when compared to cathodal pulses (Matthews, 1977). This may have contributed to the shorter chronaxie estimates obtained with the LH cathode and VTA anode when compared to the LH cathode and skull screw anode. The physiological basis for the difference between the anodal and cathodal strength-duration curves in the study by Matthews (1977) is not known, but it should be noted that similar differences have been found in strength-duration curves obtained for single cells (Yeomans et al., 1988). In addition, Bielajew and Shizgal pointed out that due to the apparent difference in the density of reward-relevant fibers at the LH and VTA, their experiment was biased towards the detection of descending neurons.

If the putative descending path implicated in the study by Bielajew and Shizgal (1986) does arise in the anterior MFB, then is should be possible to demonstrate that reward-relevant neurons directly link anterior and posterior MFB sites. Previous psychophysical studies have shown that reward-relevant neurons directly link MFB sites in the mid-LH and VTA (Bielajew & Shizgal, 1982, 1986; Gratton & Wise, 1988; Durivage & Miliaressis, 1987; Shizgal et al., 1980) and in the LPO and ALH (Bielajew, Thrasher, & Fouriezos, 1987). The location of the pairs of MFB self-stimulation sites at which collision-like effects have been obtained is summarized in a schematic fashion in Figure 1 (a similar figure appears in Shizgal and Murray, 1989). Each pair of self-

Fig. 1. Schematic representation of the pairs of self-stimulation sites that have yielded collision-like effects in previous collision studies. The tips of the two stimulating electrodes for each pair have been joined by straight lines. The stimulation sites are shown in the sagittal (panel A) and the horizontal (panel B) plane. Tracings of the brain were taken from the Paxinos and Watson (1986) atlas. The numbers along the bottom of each panel give the anterior-posterior distance (mm) from bregma; numbers along the side of each panel give the dorsal-ventral distance from bregma (panel A) or the medial-lateral distance from the midline (panel B).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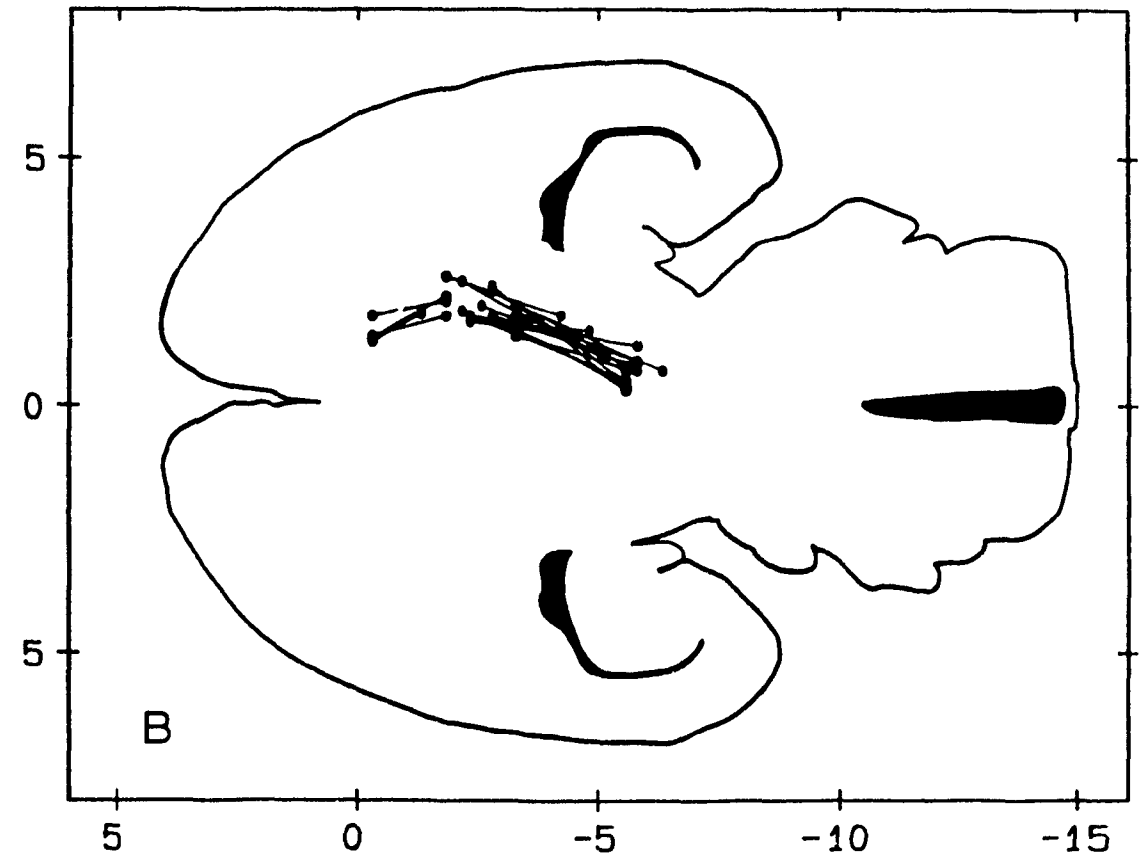
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stimulation sites has been joined by a straight line in order to visualize the gross orientation of the system; this is not to imply that the trajectory of the reward-relevant fibers follows a straight line between the two stimulation sites. Two clusters of lines are apparent in Figure 1: an anterior cluster corresponding to the sites in the LPO and LH from the study by Bielajew et al. (1987) and the larger, more posterior cluster corresponding to the sites in the LH and VTA from the remaining studies. Clearly, what remains to be determined is whether reward-relevant fibers bridge the small gap that exists between the anterior and posterior sites. The hypothesis that some of the first stage neurons arise in the rostral MFB predicts that it should be possible to obtain collision-like effects for self-stimulation sites on either side of the gap in Figure 1.

Evidence from Lesion Studies.

In order to test the hypothesis that basal forebrain regions give rise to the directly activated substrate, Waraczynski (1988) assessed the effect of knife cuts to several basal forebrain regions on the frequency threshold for MFB self-stimulation. Knife cuts that either produced substantial damage to the diagonal band/medial septum or transected the descending outflow from these nuclei were either ineffective or produced small transient increases in the frequency threshold for self-stimulation of the LH. In contrast to these minor effects following disconnection of septal outflow, some of Waraczynski's knife cuts to the LPO or ALH were able to produce substantial, long-lasting increases (up to 0.3 $\log_{10}$ units or 100%) in the frequency threshold for LH self-stimulation. In all of the remaining studies

that have investigated the effects of anterior MFB lesions, some lesions have been found to increase the frequency threshold for self-stimulation of more posterior MFB sites whereas other lesions have had no effect (Janas & Stellar, 1987; Murray & Shizgal, 1991; Stellar & Neeley, 1982).

Two findings in the study by Murray and Shizgal (1991) suggest possible explanations for some of the inconsistencies both within and across studies employing anterior MFB lesions. In the case of one subject for which rate-number curves were collected at two currents, the lesion produced a long-lasting increase in the required number for only the lower current. The arbitrary choice of current employed in the determination of the rate-frequency or rate-number curve could therefore account for at least some of the inconsistencies in previous lesion studies; some lesions may have been effective if other currents had been tested. In addition, when the lesions were reconstructed onto the Nieuwenhuys et al. (1982) atlas of the MFB, it was found that the two ineffective lesions were localized primarily to the fiber compartment c whereas the five effective lesions invaded the more lateral compartments a, d, and e. Given that only 1 current was employed in the case of the two subjects with ineffective lesions, it is possible that these lesions may have been effective had other currents been tested.

Nieuwenhuys et al. (1982) has divided the MFB into compartments based on the size and arrangement of its constituent fibers as seen in Kluver-Barrera-stained preparations. Using autoradiographic techniques, Veening et al. (1982) has demonstrated that many MFB pathways project through different compartments of the MFB. In addition to the topographic organization of the fiber components, Geeraedts et al. (1990a) has

demonstrated a correspondence between the borders of the cellular groups of the rostral MFB and the borders of the fiber compartments. Thus, the ventrolateral compartment a of the rostral MFB contains both the cells of the magnocellular preoptic area and the fibers from the olfactory tubercle, the ventromedial compartment c contains both the cells of the LPO and the septal fibers, and the dorsal compartment e contains both the cells of the SI and the ascending fibers from the parabrachial nuclei of the brainstem. Given the complex topography of both the cells and fibers of the anterior MFB, it would not be surprising if small differences in the location of anterior MFB lesions resulted in large differences in the effectiveness of the lesions. Specifically, the finding by Murray & Shizgal (1991) that their two ineffective lesions were confined to the medial compartment c, a compartment through which fibers of septal origin project, is consistent with the earlier report by Waraczynski (1988) that knife cuts transecting the descending outflow from the septum did not substantially alter the threshold for LH self-stimulation. A more thorough comparison of the anterior MFB compartments damaged by effective versus ineffective lesions could potentially aid in the identification of the reward-relevant pathways.

The Present Experiments

Following the framework of the psychophysical approach, three experiments were conducted in order to test the hypothesis that anterior MFB neurons comprise part of the directly activated substrate for MFB self-stimulation. In Experiment 1, electrolytic lesions were aimed at different regions of the ALH in an effort to determine whether damage to particular

MFB compartments reduces the rewarding impact of stimulating more posterior MFB sites. If cells in the anterior MFB give rise to some of the first stage neurons, then damage to these nuclei or to the compartments through which they project should increase the threshold for self-stimulation of the posterior MFB. Psychophysical techniques were used in Experiment 2 in order to characterize the reward-relevant neurons of the anterior and posterior MFB. The hypothesis that some first stage neurons arise in the anterior MFB predicts that it should be possible to obtain collision-like effects for self-stimulation sites in the ALH and VTA. Experiment 3 was aimed at assessing the refractory periods and conduction velocities of fibers arising from anterior MFB somata that project past posterior MFB sites that typically support self-stimulation. If some of the first stage neurons arise in the anterior MFB, then it should be possible to locate cells in this region with characteristics that match the psychophysical profile for the first stage neurons.

Experiment 1

Early efforts to identify the substrate for BSR often involved brain lesions. Unfortunately, the rate of operant responding for a fixed set of stimulation parameters served as the measure of the rewarding effect in most of the early experiments (e.g. Boyd & Gardner, 1967; Lorens, 1966; Olds & Olds, 1969). This method has been shown to confound changes in reward with changes in the subject's capacity to perform the operant response. Hodos and Valenstein (1962) were the first to show that rate of responding is not a good measure of the rewarding value of the stimulation. Rats given a choice between two levers that deliver different levels of stimulation learn to alternate their behaviour between the two levers. Hodos and Valenstein found that rats always preferred the stronger suprathreshold stimulus regardless of their rate of responding for each stimulus when presented alone.

In contrast to rate measures, the curve-shift method allows an experimenter to dissociate changes in the rewarding impact of the stimulation from changes in the subject's performance for the stimulation. The rate of lever pressing is measured for a range of frequencies such that the behaviour ranges from zero to maximum responding. Lateral shifts in the position of the rising portion of the rate-frequency curve along the frequency axis are thought to reflect changes in the rewarding impact of the stimulation. Changes in the maximum response rate are thought to indicate alterations in the strength of the priming effect (Gallistel, 1983; Gallistel,

Stellar, & Bubis, 1974) or in the subject's capacity to perform the operant response.

Validation studies have shown that the position of the rate-frequency curve along the frequency axis is relatively insensitive to manipulations of task difficulty. Making the animal run up a gradient, injection of paralytic agents, and adding weights to the lever have all been shown to produce a marked reduction in the maximum response rate while in many cases producing little or no shift in the position of the rising portion of the curve (Edmonds & Gallistel, 1974; Fouriezos, Bielajew, & Pagotto, 1990; Miliaressis, Rompré, Laviolette, Philippe & Coulombe, 1986). However, there have been instances in which manipulations of task difficulty have increased the frequency threshold by up to 0.2 $\log_{10}$ units, which is the order of magnitude of the threshold increases following lesions to the anterior MFB (Janas & Stellar, 1987; Murray & Shizgal, 1991; Waraczynski, 1988). With the exception of 1 subject in the study by Fouriezos et al. (1990), performance manipulations that have increased frequency thresholds have also produced depressions in the maximum response rate. Therefore, studies employing the curve-shift method should include an analysis of the maximum response rate in order to more clearly assess the basis for the lateral shifts in the rate-frequency curve.

In addition to distinguishing between effects on performance versus effects on reward, the curve-shift method also permits a quantitative assessment to be made of the magnitude of the changes in the rewarding effectiveness of the stimulation. For example, suppose that a lesion results in a 0.30 $\log_{10}$ unit shift to the right in the rate-number curve. This would

mean that twice as many pulses (a doubling of frequency) are required to produce the same behavioural effect (half-maximal response rates). According to the counter model, this would only occur if the lesion had effectively disconnected half of the first stage neurons; the remaining neurons would have to fire twice as often in order to produce the same total number of action potentials, and thus the same behavioural effect, as that produced prior to the lesion.

To say that half of the first stage neurons have been effectively disconnected is not to imply that the lesion has directly damaged half of the first stage neurons. One of the limitations of the lesion technique is that decreases in the rewarding impact of the stimulation cannot be linked to damage to any particular stage of the reward-relevant circuitry. Thus, a doubling in the required number of pulses could be due to the destruction of half of the first stage neurons, to downstream efferents of the first stage neurons, or pathways that somehow modulate transmission in the reward-relevant system (see Bielajew & Shizgal, 1986). The advantage of the psychophysical approach is that changes in the rewarding impact of the stimulation are interpreted according to the characteristics of the first stage neurons.

Given the relative ambiguity of the lesion technique, it would seem preferable to delineate the reward circuitry by exclusive use of the psychophysical tests currently available. However, lesions play an important corroborative role when used in conjunction with the psychophysical approach. Even if electrophysiological recording studies find a cell that possesses all the characteristics of the reward-relevant fibers as determined by the

psychophysical approach, the possibility still remains that the cell is only an 'impostor'; a cell that resembles a reward-relevant cell but in fact is not. If a lesion placed in the region of the cell body was found to decrease the rewarding impact of the stimulation, the plausibility of it being an impostor would be considerably reduced. Lesion studies could also serve as a guide for the time-consuming recording studies, pointing them towards likely candidate sites for the reward nuclei. If lesioning a particular nucleus failed to increase the frequency threshold for MFB self-stimulation, then the electrophysiologist would be well advised to search elsewhere.

Previous Lesion Studies Employing the Curve-Shift Method

Only a handful of the many studies that have examined the effects of lesions on self-stimulation have used the curve-shift method. Of these, many have found that lesions of a variety of regions known to give rise to MFB fibers do not substantially increase the threshold for MFB self-stimulation. For instance, electrolytic lesions to the amygdala (Waraczynski, Ng Cheong-Ton, & Shizgal, 1990), dorsomedial hypothalamus (Waraczynski, Conover, & Shizgal, 1992), and parabrachial nucleus (Waraczynski & Shizgal, 1992) have not resulted in significant increases in the frequency threshold for MFB self-stimulation. Similarly, Waraczynski (1988) found that knife cuts to the diagonal band/medial septum and to the medial preoptic area had no substantial or permanent effect on the frequency threshold for LH self-stimulation.

Colle and Wise (1987) found that large forebrain ablations ipsilateral to the LH stimulating electrode resulted in surprisingly modest increases

(20-30%) in the threshold for self-stimulation, increases which subsequently recovered to baseline values over several weeks of testing. These lesions removed all or part of the frontal cortex, rostral striatum, nucleus accumbens, septal area, and olfactory tubercle. Six of the 12 large forebrain ablations resulted in substantial increases (over 0.15 $\log_{10}$ units or 40%) in the frequency threshold for ipsilateral LH self-stimulation. The authors note that in addition to the regions mentioned above, these effective ablations removed rostral portions of the MFB. Ablations that were restricted to the frontal cortex had no effect on the ipsilateral frequency threshold. Individual data shown for one of their subjects with a large ablation are particularly striking. Removal of the entire hemisphere to the level of the caudal LPO resulted in a 0.16 $\log_{10}$ log unit shift in the frequency threshold for ipsilateral LH self-stimulation. The magnitude of this effect is similar to the magnitude of the effects that have been obtained with lesions restricted to the caudal LPO or ALH (see below).

Neurons arising in the MFB have long been considered a potential component of the MFB reward system (Olds, 1962; Szabo, 1972) and several studies have found that excitotoxic lesions to the MFB produce decreases in responding for self-stimulation of the MFB (Huston, Kiefer, Buscher, & Muñoz, 1987; Lestang, Cardo, Roy, & Velley, 1985; Nassif, Cardo, Libersat, & Velley, 1985; Velley, 1986; Velley, Chaminade, Roy, Kempf, & Cardo, 1983). Unfortunately, most of these studies have used measurement techniques that confound changes in the rewarding effect of the stimulation with changes in the subject's performance or alternatively have interpreted data consistent with a performance deficit as evidence for a decrease in reward. Also, it

has been shown that in addition to damaging cell bodies, excitotoxins can trigger a process that leads to demyelination of axons (Coffey, Perry, Allen, Sinden, & Rawlins, 1988; Waraczynski & Stellar, 1987). Consequently, the results of these studies cannot be unambiguously attributed to a reduction in the rewarding impact of the stimulation due to damage to neurons intrinsic to the MFB.

Two studies employing excitotoxin lesions have used threshold measures and a within-subject design to assess the effects of cell damage within or near the stimulation field on LH self-stimulation (Sprick, Muñoz, & Huston, 1985; Stellar, Hall, Waraczynski, 1991). Both of these studies have concluded that neurons intrinsic to the LH do not play a major role in the rewarding effect of MFB stimulation. Sprick et al. (1985) found no change in the weakest current required to support a maximal rate of responding after excitotoxin lesions that damaged 90% of cells in a 0.5 mm region directly above the electrode tip. Stellar et al. (1991) observed large increases in frequency thresholds following ibotenic acid (IBO) or N-methyl-D-aspartic acid (NMDA) lesions in the LH only when the zone of demyelination extended to the electrode tip. When the zone of demyelination was separated from the electrode tip, frequency thresholds in most subjects were not substantially elevated. However in 5 of their 18 subjects, excitotoxin lesions produced increases in the frequency threshold ranging from 0.07-0.16 $\log_{10}$ units even in the absence of demyelination at the electrode tip. The magnitude of these effects are comparable with those observed after some LPO or ALH lesions. Given that their lesions were aimed at different levels of the MFB ranging over a 2.5 mm distance and that the anterior-posterior spread of the

lesions varied from 1.5 to 6.4 mm, it is possible that some of these effective lesions produced damage to more rostral levels of the MFB. It can be concluded on the basis of these studies that excitotoxin lesions of the LH do not degrade the rewarding impact of stimulating most MFB sites, although they do not rule out a contribution from neurons arising in more rostral MFB nuclei.

One excitotoxin lesion study that did assess the effect of damage to anterior MFB neurons on MFB self-stimulation has suggested that neurons in this region do play a role in MFB reward. Huston et al. (1987) made ibotenic acid lesions of the preoptic area, a region that largely corresponds to the ventral pallidum and substantia innominata of the Paxinos and Watson atlas (1986), and assessed their effect on LH self-stimulation. The authors found that their neurotoxin lesions to this region reduced the rate of responding for 2 fixed currents corresponding to 25% and 100% of the minimum current required to produce a maximum rate of responding. Unfortunately, the use of fixed stimulation parameters does not permit an analysis to be made of changes in the threshold. Thus, it is possible that these effects primarily reflect a depression in maximum response rates and not a substantial increase in the threshold. In addition, these effects may be due to demyelination of fibers of passage and not to selective damage to cell bodies in this region. Nonetheless, these results are encouraging and suggest that further investigation of cells arising in the anterior MFB is warranted.

Perhaps the clearest effects of lesions on the threshold for MFB self-stimulation have been obtained with non-selective lesions of more posterior

MFB sites. The most consistent finding is that lesions or knife cuts to the VTA or posterior LH produce substantial and long-lasting increases in the frequency threshold for LH self-stimulation (Glimcher & Gallistel, 1988; Janas & Stellar, 1987; Stellar & Neeley, 1982; Waraczynski, 1990). The effects of lesions to the anterior MFB have been less consistent, both within and across studies. Stellar and Neeley (1982) implanted electrodes at an anterior and posterior MFB site and assessed the effect of an electrolytic lesion at one site on self-stimulation of the other. They concluded that the large anterior lesions did not degrade the reward at posterior stimulation sites and had mixed effects on performance. However, one of their subjects with an anterior lesion showed a large rightward shift in the rate-frequency curve of approximately 0.4 $\log_{10}$ units (150%) that recovered to baseline values by the eighth day post-lesion. It is not clear why this particular lesion produced such a large effect while similar lesions were ineffective.

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

Waraczynski (1988) found that knife cuts in the LPO resulted in a variety of effects on the rate-frequency curve. One group of 6 rats had appreciable shifts in the rate-frequency curve towards higher frequencies (approximately 0.1-0.3 $\log_{10}$ unit shift) that often lasted for more than a week of post-knife cut testing. A second group of subjects with similarly placed knife cuts showed either a transient or erratic rightward shift in the rate-frequency curve. A third group of animals, with knife cuts more medially situated, showed a leftward shift in the rate-frequency curve (towards lower frequencies), indicating an increase in the rewarding impact of the stimulation following the lesion. When knife cuts were situated just anterior to the LH stimulating electrode, a similar pattern of effects was seen. Only one of the anterior MFB knife cuts produced a substantial increase in threshold lasting for the 12 days of testing. Other knife cuts in this region had no effect on the rate-frequency curve, produced only small transient shifts or shifted the rate-frequency curve toward lower frequencies.

Murray and Shizgal (1991) have found that some electrolytic lesions of the ALH increase the threshold for LH or VTA self-stimulation by 0.1-0.2 $\log_{10}$ units. They also noted two additional findings that suggested a possible explanation for some of the inconsistencies in the previous literature. In one of their subjects for which rate-number curves were collected at two currents, the lesion produced a long-lasting increase in the frequency threshold for only the lower current. This suggested that the inconsistencies in previous studies may have been due to the arbitrary choice of current employed in the determination of the rate-frequency curve. In addition, when the lesions were reconstructed onto the Nieuwenhuys et al. (1982) atlas

of the MFB, it was found that the two ineffective lesions were localized primarily to compartment c whereas the five effective lesions invaded the more lateral compartments a, d, and e. Given that different fiber pathways and cellular groups are located in different compartments of the anterior MFB (Geeraedts et al., 1990a; Veening et al., 1982), this finding suggested that small differences in the location of the lesions could result in large differences in the pathways and cell groups damaged by the lesions. Thus, a comparison of the compartments damaged by effective versus ineffective anterior MFB lesions could aid in identifying the reward-relevant pathways.

Experiment 1 was aimed at extending the findings of Murray and Shizgal (1991) by making electrolytic lesions in the ALH and assessing their effect on frequency thresholds obtained at three currents for stimulation sites in the LH and VTA. It was predicted that damage to some but not all of the anterior MFB compartments would increase the required number of pulses for stimulation delivered to more posterior MFB sites. In the study by Murray and Shizgal (1991), the size of the ineffective lesions was enlarged (i.e. multi-stage lesions were made) in an effort to obtain an effect on the frequency threshold. In the present study, ineffective lesions were not enlarged in order to preserve their location. In addition, the medial-lateral coordinate for the lesioning electrode was varied in an effort to damage different portions of the MFB. It was hoped that likely candidates for the first stage could be determined by comparing the MFB compartments damaged by the effective versus ineffective lesions in a larger sample of subjects.

Method

Subjects

Sixteen male, "old colony" rats of the Long-Evans strain (Charles River Breeding Farms) served as subjects. Weight at the time of surgery varied from 400-600 grams. The animals were individually housed with unlimited access to food and water and were maintained on a reverse 12 hour light/12 hour dark cycle. All behavioral testing was carried out during the dark phase of the cycle.

Surgery

Atropine sulfate (0.5 mg/kg i.p.) was administered 20 minutes prior to anesthesia in order to reduce mucous secretions. Surgery was performed under sodium pentobarbitol anesthesia (Somnotol, 65 mg/kg i.p.) with supplements administered as required.

Stimulating and lesioning electrodes were constructed from 0.25 mm diameter stainless steel rods insulated with Formvar except for their rounded tips. Male Amphenol pins were attached to a flexible wire soldered to the electrode. Stimulating electrodes were aimed at the LH and VTA using the following level-skull coordinates: -2.8 mm from bregma, 1.7 mm lateral to the mid-sagittal sinus, and 7.8 mm below dura for the LH; -4.8 mm from bregma, 1.0 mm lateral, and 7.5 mm below dura for the VTA. Lesioning electrodes were aimed at the anterior LH using the following coordinates: -1.3 mm from bregma, 1.9-2.5 mm lateral, and 7.5-7.8 mm below dura. A

stainless steel wire wrapped around four jewelers screws imbedded in the skull served as the anode.

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

Stabilization

Apparatus.

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

Depression of the lever resulted in a 0.5 sec train of 0.1 msec, cathodal, rectangular pulses delivered to either the LH or the VTA stimulating electrodes. The temporal parameters of the stimulation were controlled by

hand-set integrated circuit pulse generators. The stimulation pulses were produced by dual constant-current amplifiers (Mundl, 1980) and their amplitude set by a potentiometer. Current was monitored by measuring the voltage drop across a 1 kohm resistor in series with the rat. Accumulation of charge at the electrode-brain interface was minimized by a circuit that shorted the stimulator outputs through a 1 kohm resistor except during delivery of a pulse.

Training.

Subjects were placed into the test boxes and non-contingent stimulation (200 uA, 40 Hz) was delivered to either the LH or VTA stimulating electrode. If the effects of the stimulation did not appear to be aversive, then the current or frequency of the stimulation was gradually increased and conventional shaping procedures were used in an effort to train the subjects to self-stimulate. On subsequent training sessions, animals were again shaped to self-stimulate and then access to the stimulation was withdrawn until lever pressing had extinguished. This process was repeated until, following an extinction trial, subjects would reliably return to the lever after 5 priming trains of stimulation. Subjects were then given access to the stimulation for 30 sec trials and the number of pulses per train was decreased over trials until the subject would no longer respond for the stimulation. This process was repeated until the subject would reliably stop responding at approximately the same number of pulses per train. Finally, the rate of responding in a 30 sec trial was determined for a range of currents and frequencies in order to obtain the stimulation parameters for

later use in the computer-operated equipment. Three currents, equally spaced in logarithmic increments, were chosen such that they resulted in reliable self-stimulation over an appreciable range of stimulation frequencies (see Appendix A for a complete list of currents used for all subjects). If possible, currents spanning a 0.60 $\log_{10}$ unit range (0.30 $\log_{10}$ unit increments) were selected. If reliable responding could not be obtained for this range, the interval between currents was reduced accordingly.

Data Collection

Apparatus.

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

Test chambers for the computer-operated setup consisted of Plexiglas boxes measuring 25 cm x 25 cm x 75 cm with hinged doors on the upper half of the front face and removable floors. Lehigh Valley rodent levers were located on opposite walls of each test box 5 cm from the floor and 5 cm from the nearest corner. A yellow jewel light measuring 1.5 cm in diameter was located 3 cm above one lever and a red jewel light was similarly placed above the other lever (which was not used in this experiment). The test chambers were mounted in 50 cm x 50 cm x 90 cm plywood boxes insulated with 2.5 cm of Styrofoam. Two, 1 inch ventilation holes were drilled into the top of the plywood boxes. Removable front

panels with Plexiglas inserts allowed viewing of the subject from an adjoining room via a remote-controlled video camera. A single 40 watt bulb illuminated the test chamber and an 11.5 cm fan provided ventilation.

Depression of the lever resulted in a 0.5 sec train of pulses, except for subject J13 (LH electrode) in which case a 0.3 sec train was used. For most of the subjects, a fixed delay was imposed at the end of each stimulation train so that subjects could self-administer no more than one train per second. A delay was not imposed in the case of subjects F3, F8, J5 and J11, so that these subjects could self-administer up to two trains of stimulation per second.

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

Procedure.

During a single testing session, 4 rate-number curves were obtained at each of 3 currents for either the LH or the VTA electrode. The procedure used to obtain a single rate-number curve was as follows: Current was held constant and the number of lever presses per 30 sec trial was recorded for a range of stimulation frequencies. Each 30 sec trial began with the overhead

light turning off for 0.5 seconds followed by 5 trains of priming stimulation. Each train of priming stimulation was identical to the stimulation that would be available to the animal during the rest of the trial. Following the priming stimulation, the yellow light located above the lever was illuminated to signal that depression of the lever would trigger a train of stimulation. The number of pulses per train available during the first 30 sec trial was chosen such that the subject would respond near its maximal rate for at least 2 more trials. On subsequent trials, the number of pulses in each train was lowered in 0.05 log unit (12%) steps until there were 10 or fewer lever presses on 2 consecutive trials.

Testing sessions in which stimulation was delivered to the LH were alternated with testing sessions in which stimulation was delivered to the VTA. Up to two testing sessions were run per day, separated by a rest period of at least one hour during which time the subject was returned to its home cage. The order in which the stimulation sites were tested was reversed across test days so that the same site was not always tested on the first session of the day.

Data Analysis

The number of pulses required for half-maximal responding (required number) was interpolated from each of the 12 rate-number curves obtained during each testing session. The required number was not calculated for a particular rate-number curve unless the number of responses on at least 2 trials exceeded half of the maximum number of responses. The first 3 rate-number curves obtained (one for each current) were used as a "warm-up" and

therefore not included in the data analysis. The mean and standard error of the mean (s.e.m.) of the required number for each current were calculated for each test session and plotted as a function of time pre and postlesion.

In order to estimate maximum response rates, each rate-number curve was fitted with a "broken-line" function, composed of a straight line joining an upper and lower asymptote, as described by Gallistel and Freyd (1987). The four parameters of the broken-line function were chosen so that the residual sum of squares was minimized. The maximum response rate (per 30 sec trial) for each rate-number curve was estimated from the upper asymptote of the broken-line function.

Electrolytic Lesions

Rate-number curves were collected for at least 10 days prior to lesioning. Baseline data were considered stable when the required number of pulses for each stimulation site did not show any apparent trend over 5 consecutive testing session. Lesions (1.0 mA for 10 sec) were made at the end of the fifth stable testing session using the ALH electrode as the anode and the skull screws as the cathode. Anesthetic was not used during the procedure. Postlesion testing began the following day.

Statistical Analysis

In order to provide a rough test of significance, 95% confidence intervals were constructed around the mean of the required number of pulses and the mean of the maximum response rates obtained for the 5 days preceding the

lesion. The standard deviation (S.D.) of the 5 means was used as an estimate of the standard error of the mean (Ferguson and Takane, 1989, p. 163). This estimate was found to be substantially larger than the estimate of the standard error obtained from the 15 individual observations obtained over the 5 days (3 per day) and therefore provided a more conservative test of significance. Confidence intervals were obtained (as per Waraczynski, 1988) by multiplying the S.D. of the 5 baseline means by the t value associated with the $p = 0.05$ level of significance for 4 degrees of freedom ($t = 2.776$). Thus, data obtained on a particular postlesion day could be considered significantly different from baseline if it differed by more than 2.776 standard deviations from the mean.

Histology

At the completion of the experiment, subjects were given an overdose of Somnotol (sodium pentobarbitol) and perfused intracardially with heparinized, phosphate buffered saline followed by 10% formalin. Jus. prior to the perfusion, a direct current of 0.1 mA was passed for 10 sec using each of the stimulating electrodes in turn as the anode and the skull screws as the cathode in order to produce a small lesion at the tip of each stimulation electrode. The brains were extracted by removing the ventral surface of the skull and gently pulling the brain away from the electrode assembly. Brains were soaked in 10% formalin for at least one week, transferred to a 20% sucrose-formalin solution for 24-48 hours, and then quick-frozen in pulverized dry ice prior to sectioning in a freezing microtome. Alternate 30 um thick sections were mounted onto 2 sets of gelatin-coated glass slides, allowed to

dry for a minimum of 24 hours, and then each set was stained separately in a formol-thionin solution.

The stained slides were examined under a tissue projector and a low-power microscope in order to determine the location of the electrode tips and lesion boundaries. Regions of tissue loss and dense gliosis were used to estimate the extent of the lesion. Landmarks located near the lesions and electrode tips were used to reconstruct their coordinates onto coronal plates from the Paxinos and Watson (1986) atlas.

Results

Stimulation Sites

Thirteen subjects responded for stimulation of both the anterior and posterior stimulation sites whereas 3 subjects responded only for stimulation of the posterior stimulation site. Eleven of the 13 anterior stimulation sites were located within the lateral hypothalamic region of the MFB between 2.3 and 3.6 mm behind bregma. The anterior stimulation site in subject J13 was medial to the intended target, located between the fornix and the anterior hypothalamic area (AHP). In the case of subject J3, the anterior stimulation site was dorsal to the LH within the subincertal nucleus (SubI). Twelve of the 16 posterior stimulation sites were located within or bordering the VTA or posterior LH between 4.16 and 5.3 mm behind bregma. Additional subjects had posterior stimulation sites in the supramammillary nucleus (J13, J7), ventral to the fields of Forel (J1), or lateral to the VTA near the

substantia nigra par compacta (SNC, subject J10). In total, 23 MFB stimulation sites were tested with an additional 6 stimulation sites located in surrounding regions.

Long-lasting Increases in the Required Number

Subjects were divided into 3 groups according to the magnitude and duration of the lesion-induced effects on the required number. In the first group of 4 rats, lesions produced substantial, long-lasting increases in the required number of pulses at one or both of the stimulation sites. A substantial and long-lasting lesion effect was defined as an immediate postlesion increase in the required number of at least 0.15 $\log_{10}$ units (41%) that did not decrease to less than 0.1 $\log_{10}$ units (26%) by the end of the testing period for that subject. In each case, these criteria exceeded the upper range of the 95% confidence intervals that were constructed around the mean of the 5 baseline days. Depending upon the stimulation site and current, the increase required to shift the required number outside of the 95% confidence interval varied from 0.011 $\log_{10}$ units (2.6%) to 0.11 $\log_{10}$ units (29%) (mean = 0.04 $\log_{10}$ units; S.D. = 0.02 $\log_{10}$ units).

The required number of pulses as a function of time pre and postlesion is shown in Figs. 2 and 3 for those subjects with substantial, long-lasting increases in the required number. The three horizontal, dashed lines extending across each graph indicate the baseline mean of the required number for each of the three currents. The range of the y-axis for each graph spans 1.4 $\log_{10}$ units so that the percent change from baseline can be directly compared across graphs.

Fig. 2. Required number data for subject J1. The lesion in this subject produced a long-lasting, substantial increase in the required number (see text for criteria). Top and bottom panels show data obtained from stimulation of the LH and VTA respectively. Prelesion data (filled symbols) for the five test days preceding the lesion have negative values along the abscissa. The three horizontal, dashed lines extending across each graph indicate the mean of the baseline data for each current. Lesions were made at the end of the last prelesion test session (day 0). Postlesion data are represented by open symbols. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.

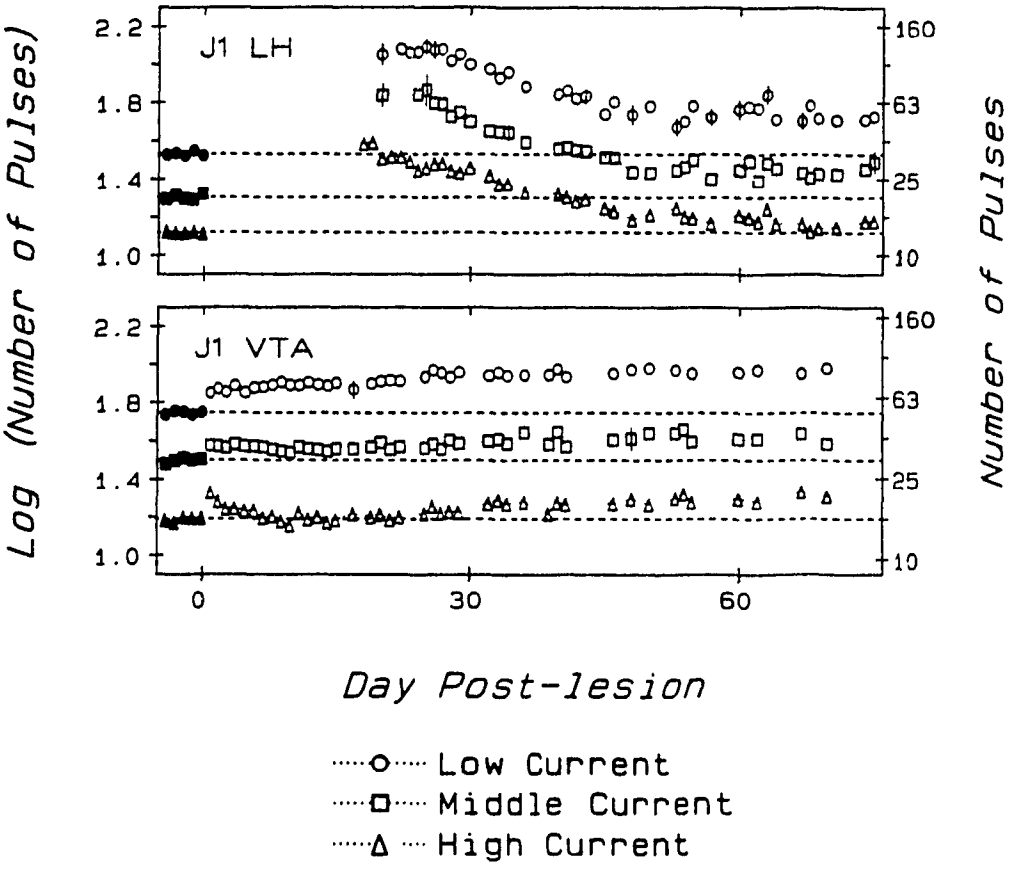

J1 LH
J1 VTA
2.2
1.8
1.4
1.0
160
63
25
10
0
30
60
Log (Number of Pulses)
Number of Pulses
Day Post-lesion
Low Current
Middle Current
High Current

Fig. 3. Required number data for the remaining subjects in which lesions produced long-lasting, substantial increases in the required number (see text for criteria). The alphanumeric in the left-hand panels identifies the subject. Left and right panels show data obtained from stimulation of the LH and VTA respectively. Prelesion data (filled symbols) for the five test days preceding the lesion have negative values along the abscissa. The three horizontal, dashed lines extending across each graph indicate the mean of the baseline data for each current. Lesions were made at the end of the last prelesion test session (day 0). Postlesion data are represented by open symbols. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.

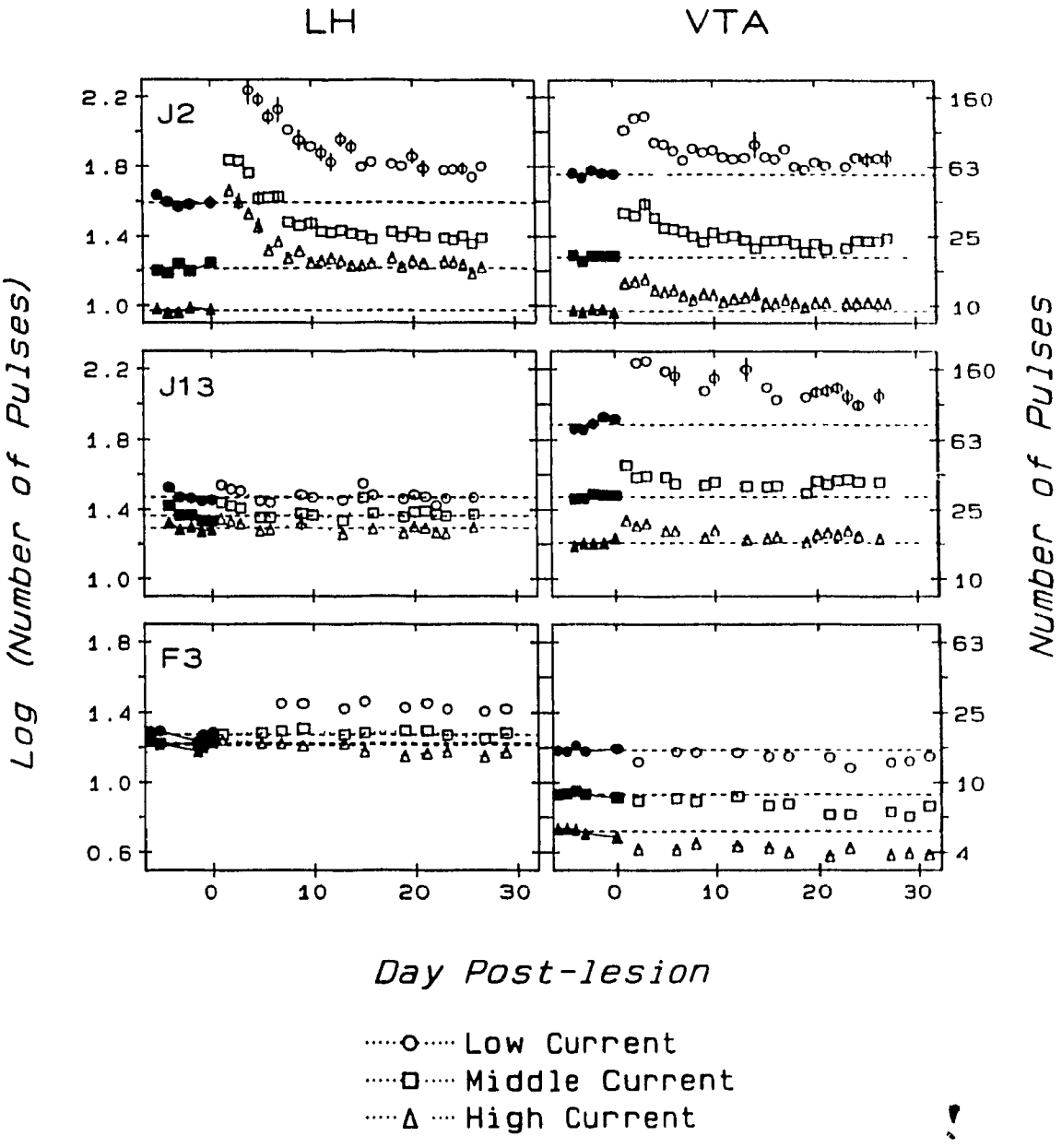
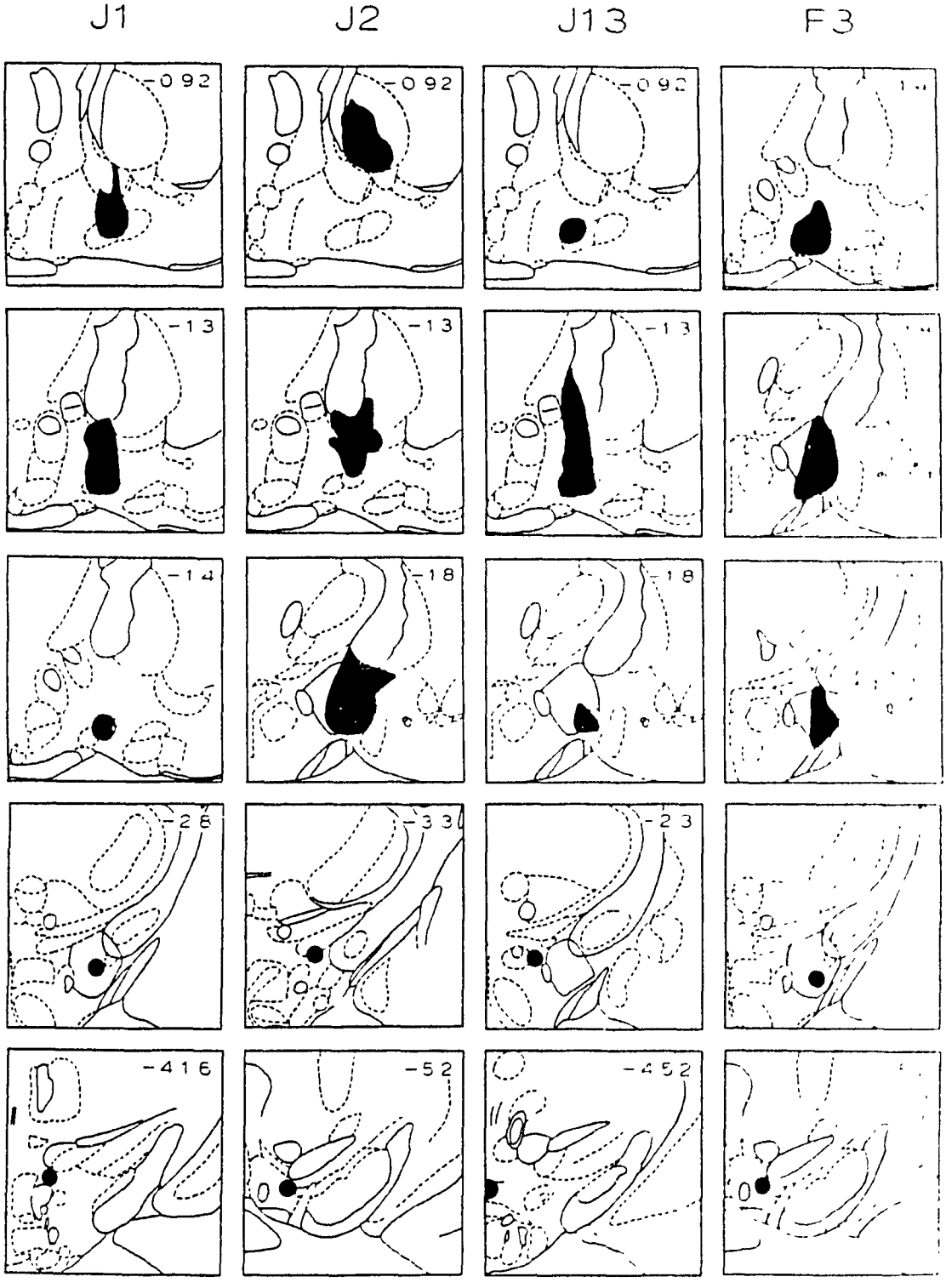

LH
VTA
J2
J13
F3
Log (Number of Pulses)
Number of Pulses
Day Post-lesion
Low Current
Middle Current
High Current

Fig. 4. Lesion and electrode tip locations for subjects with long-lasting, substantial increases in the required number (see text for criteria). Reconstructions were made onto tracings of coronal plates from the Paxinos and Watson atlas (1986). The alphanumeric at the top of each column identifies the subject. The distance of each plate from bregma is given in the upper right-hand corner. The blackened area in the top three panels of each column illustrates the extent of the lesion on three representative cross-sections. Filled circles in the bottom panels show the locations of the stimulation sites.


J1
J2
J13
F3
-092
-092
-092
-13
-13
-13
-14
-18
-18
-28
-33
-23
-416
-52
-452

The postlesion increase in the required number was particularly dramatic in the case of the LH site of subject J1 (Fig. 2, top graph). Rate-number curves were not collected for stimulation delivered to the LH site in this subject for over 2 weeks postlesion; this subject was tested every few days in the hand-operated setup but would not self-stimulate for the stimulation parameters offered. During this period of time, rate-number curves were obtained for stimulation delivered to the VTA site. Thus, it seems unlikely that the inability to obtain self-stimulation for the LH site was due to a global behavioural deficit. The required number for the VTA site increased by approximately 0.10 $\log_{10}$ units immediately postlesion (Fig. 2, lower graph) with the largest increase occurring at the lowest current. Over the next 7-10 days, the required number declined at the middle and high currents and then began to increase again at around 20 days postlesion. This later increase was probably unrelated to the effects of the lesion (see section below on maximum response rates for this subject). Rate-number curves were obtained for the LH site beginning 18 days postlesion. Immediate increases in the required number ranged from 0.47 to 0.56 $\log_{10}$ units with the largest increase occurring at the lowest current. The required number declined over the next month, stabilizing around 50 days postlesion at values ranging from 0.035 to 0.2 $\log_{10}$ units above baseline values.

The largest immediate increase in the required number was obtained at the highest current delivered to the LH site of subject J2 (Fig. 3, top left-hand graph). A substantial increase in the required number, ranging from 0.63 to 0.70 $\log_{10}$ units, was obtained for each current. This was followed by a rapid decline in the required number over the next 10-12 days, at which

time the required number re-stabilized at values ranging from 0.17 to 0.25 $\log_{10}$ units above baseline. The largest, long-lasting increase was obtained at the highest current. Smaller increases in the required number were also obtained for the VTA site in this subject. Immediate increases in the required number ranged from 0.18 $\log_{10}$ units for the high current to 0.3 $\log_{10}$ units for the low and middle currents which declined to within 0.05 and 0.09 $\log_{10}$ units respectively.

Lesions in two additional subjects produced substantial, long-lasting increases in the required number for one of the two stimulation sites tested in each subject. In the case of the posterior stimulation site of subject 315, the required number increased by 0.13-0.35 $\log_{10}$ units immediately after the lesion and then recovered to within 0.05-0.16 $\log_{10}$ units of baseline levels. The largest increase in the required number was obtained using the lowest current. The lesion in subject F3 produced a 0.15 $\log_{10}$ unit increase in the required number for the LH site using the lowest of the three currents. This subject was unusual in that the required number determined prior to lesioning was approximately the same for each of the three currents (300uA, 450uA, 675uA) yet the postlesion increase in the required number only occurred when using the lowest current. The implications of this finding will be discussed later. Also, there was a small decrease in the required number ranging from 0.07 to 0.12 $\log_{10}$ units for the VTA site in this subject (Fig. 4, bottom right-hand panel) with the largest decrease occurring at the highest current.

Location of Lesions.

Damage to portions of the anterolateral LH was evident in all subjects with substantial, long-lasting increases in the required number. Histological reconstructions of the lesions (top three panels of each column) and stimulation sites (bottom two panels) for these rats are shown in Fig. 4. Of the long-lasting effects, the largest increase in the required number was obtained following the lesion in subject J2. In addition to the ALH, the lesion in this subject also damaged the substantia innominata (SI), ventromedial portions of the globus pallidus, and the magnocellular preoptic area (MCPO). Degeneration of fibers in the ventromedial GP extended into more rostral sections than those shown in Fig. 4. Lesions in the other 3 subjects with substantial, long-lasting increases in the required number were largely confined to regions within the anterior MFB. These areas included the horizontal limb of the diagonal band (HDB), MCPO, SI, and the anterior LH. In the case of subject J13, the lesion extended into the internal capsule (ic).

Transient or Small Increases in the Required Number

In a second group of 4 rats (F8, J9, J10, and J3), lesions produced transient increases or small but long-lasting increases in the required number (Fig. 5). A transient increase was defined as an immediate increase in the required number of at least 0.15 $\log_{10}$ units that recovered to less than 0.10 $\log_{10}$ units by the end of the testing period for that subject. Small, long-lasting increases in the required number were defined as immediate increases

Fig. 5. Required number data for the subjects in which lesions produced transient or small, long-lasting increases in the required number (see text for criteria). The alphanumeric in the left-hand panels identifies the subject. Left and right panels show data obtained from stimulation of the LH and VTA respectively. Prelesion data (filled symbols) for the five test days preceding the lesion have negative values along the abscissa. The three horizontal, dashed lines extending across each graph indicate the mean of the baseline data for each current. Lesions were made at the end of the last prelesion test session (day 0). Postlesion data are represented by open symbols. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.

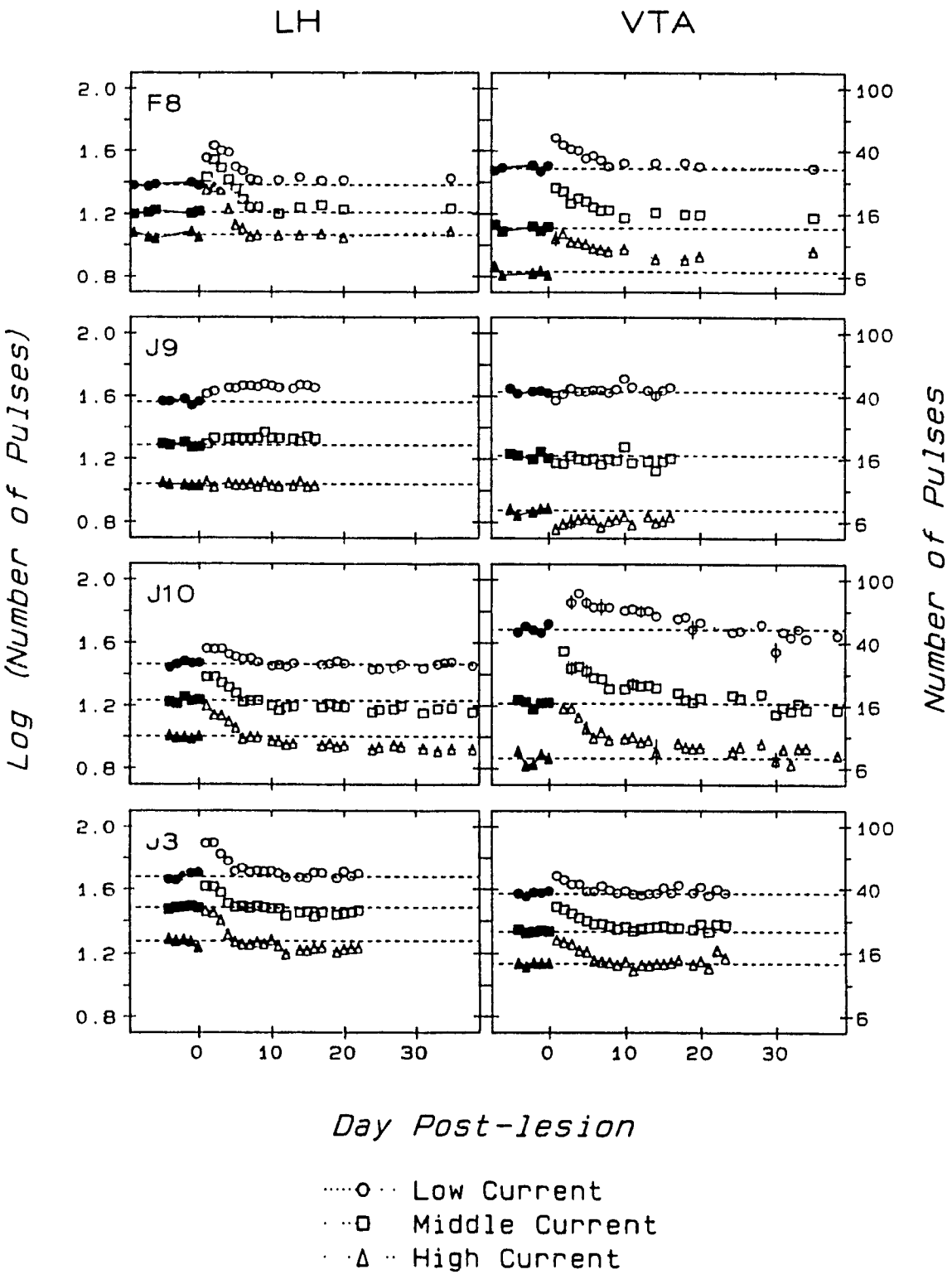
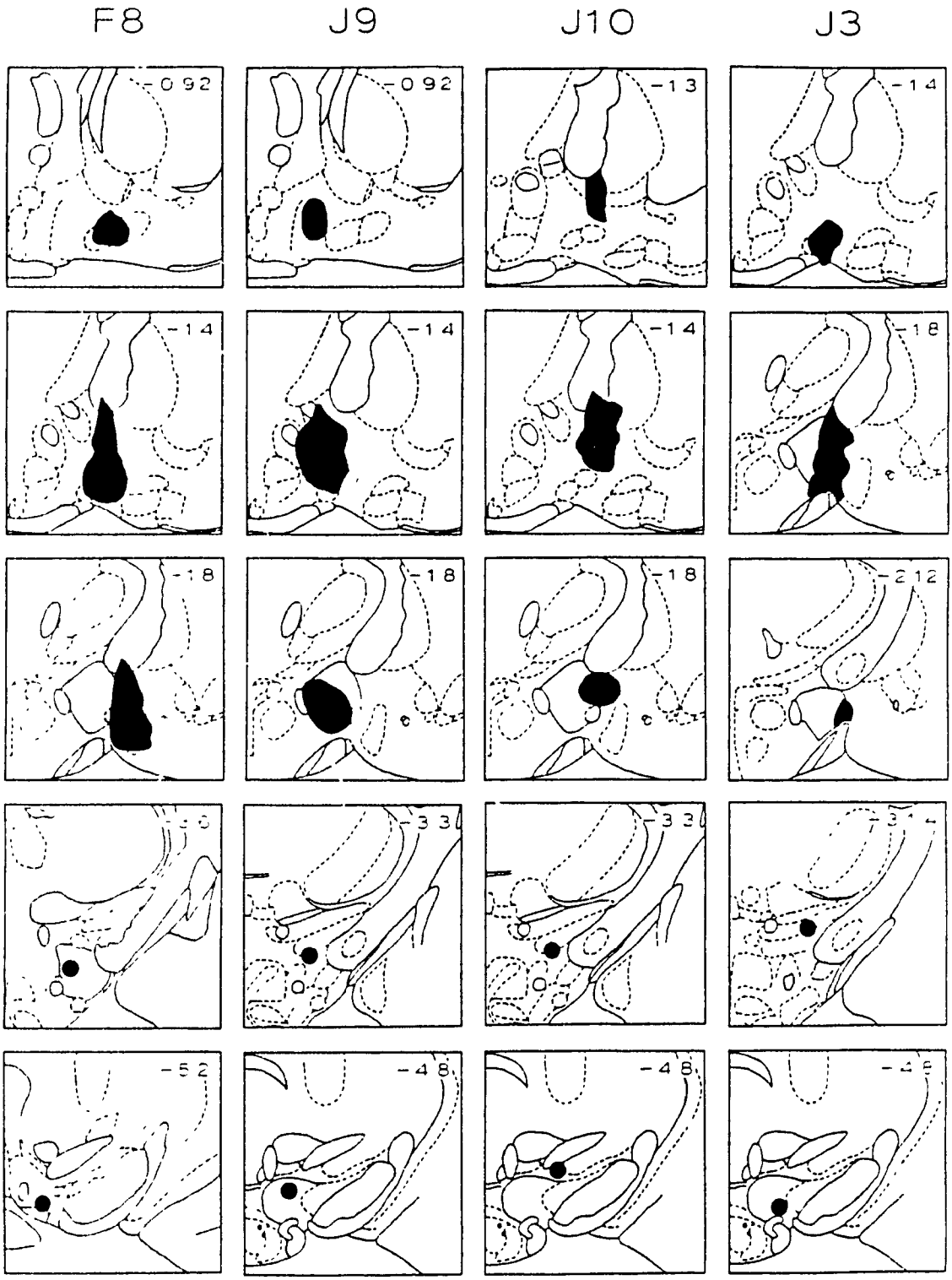

LH
VTA
F8
J9
J10
J3
2.0
1.6
1.2
0.8
100
40
16
6
0
10
20
30
Log (Number of Pulses)
Number of Pulses
Day Post-lesion
Low Current
Middle Current
High Current

Fig. 6. Lesion and electrode tip locations for the group of subjects with transient or small, long-lasting increases in the required number (see text for criteria). Reconstructions were made onto tracings of coronal plates from the Paxinos and Watson atlas (1986). The alphanumeric at the top of each column identifies the subject. The distance of the plate from bregma is given in the upper right-hand corner of each panel. The blackened area in the top three panels of each column illustrates the extent of the lesion on three representative cross-sections. Filled circles in the bottom panels show the locations of the stimulation sites.


F8
J9
J10
J3
-.092
-.092
-.13
-.14
-.14
-.14
-.14
-.18
-.18
-.18
-.18
-.212
-.33
-.33
-.48
-.48
-.48

of at least 0.10 $\log_{10}$ units but less than 0.15 $\log_{10}$ units that lasted for the entire duration of testing.

In several cases, lesions produced transient increases in the required number that recovered back to baseline values within 7 days (e.g. subject F8, LH site; subject J10, LH site; subject J3). Recovery was more prolonged in the case of the VTA site in subject J10; required number values did not return to prelesion levels for at least 3 weeks. Required number values for the VTA site of subject F8 recovered to baseline levels at the low current but not at the middle or high current. The lesion in subject J9 produced a small increase in the required number (0.10 $\log_{10}$ units) at the low current that did not decline over the testing period for this subject.

Decreases in the required number, consistent with an increase in rewarding effectiveness, were obtained for 3 subjects in this group. In 2 of these subjects, a transient increase in the required number was followed by a decline that plateaued below baseline values (J10 and J3, LH site). In the third subject, a 0.10 $\log_{10}$ units decrease in the required number occurred immediately after the lesion and required number values remaining depressed (by, on average, 0.065 $\log_{10}$ units) for the duration of testing (J9, VTA site). In each of these subjects, the largest decrease in the required number was obtained when using the highest current.

Location of Lesions.

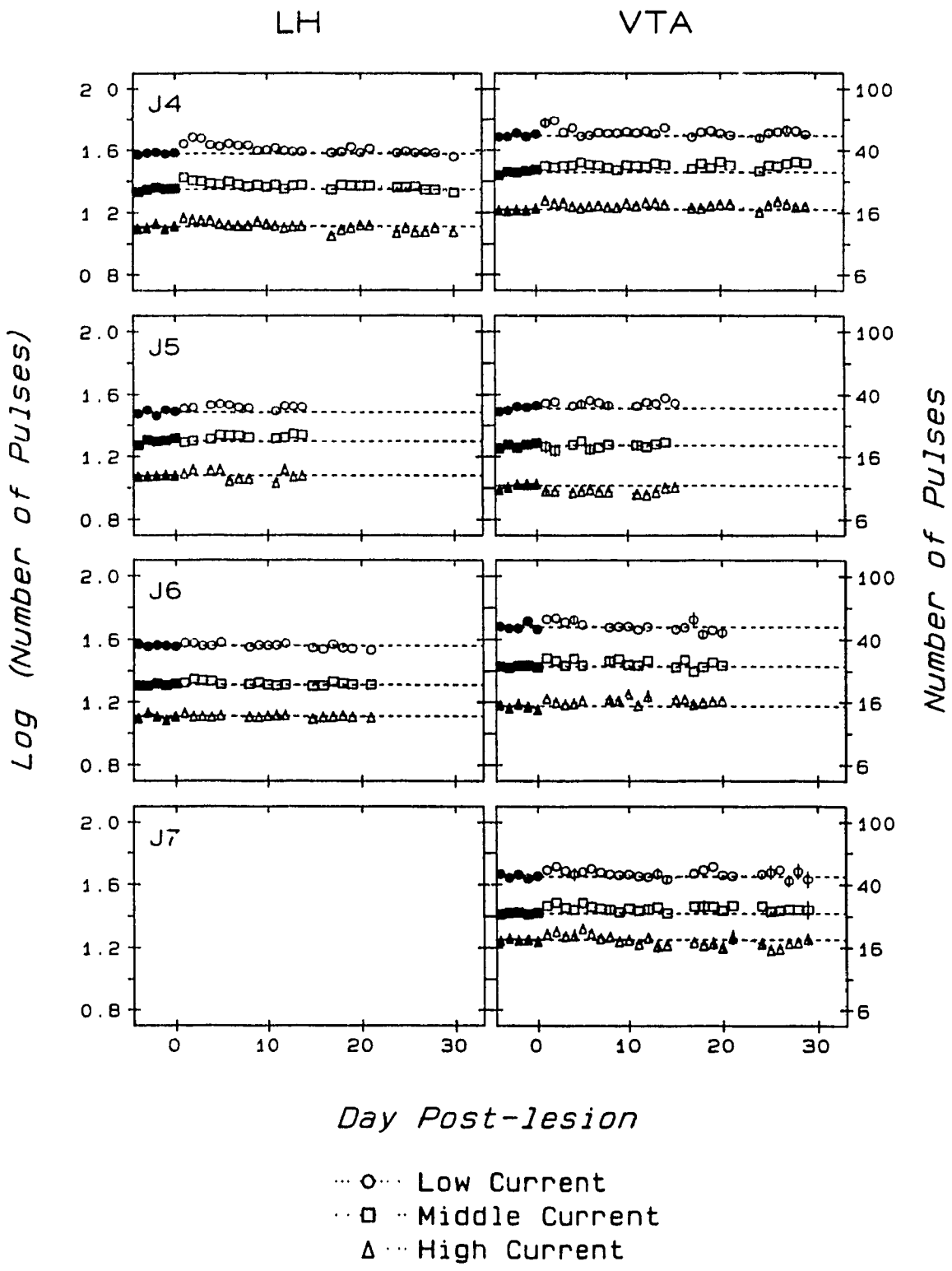
Lesions that resulted in transient or small increases in the required number damaged many of the same regions damaged by lesions that produced

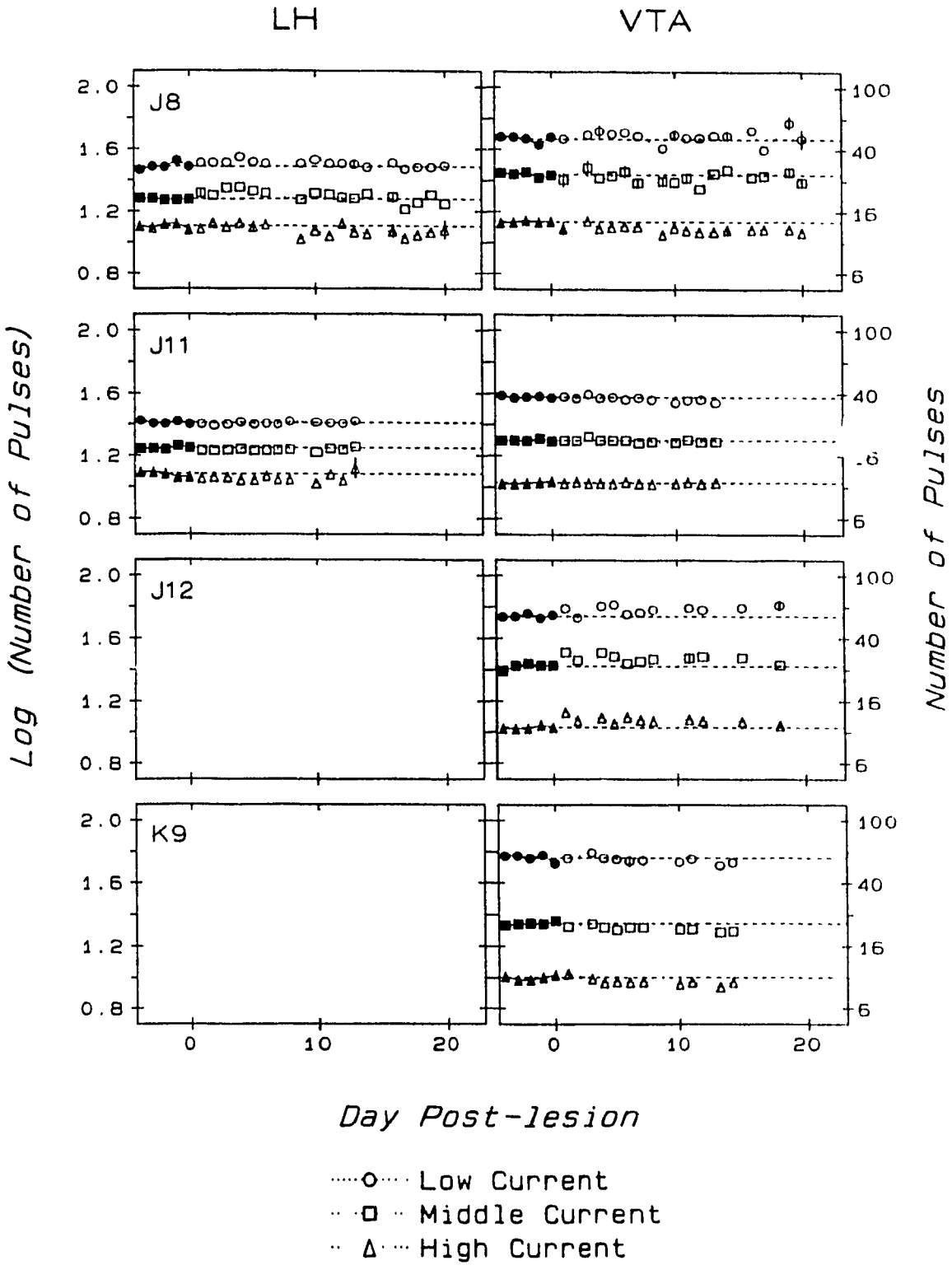
substantial, long-lasting increases. Histological reconstructions of the lesions (top three panels of each column) and stimulation sites (bottom two panels) for these rats are shown in Fig. 6. F8's lesion extended into more lateral regions, destroying the medial amygdaloid nucleus (MeA) in addition to parts of the caudal HDB/MCPO complex and the anterolateral LH. Subject J9's lesion was centered in the medial half of the LH and also damaged the caudal pole of the lateral preoptic area (LPO), the nucleus of the stria medullaris (SM), the bed nucleus of the stria terminalis (medial division, posterolateral part, BSTMPL), and the fornix.

No Substantial Increases in the Required Number

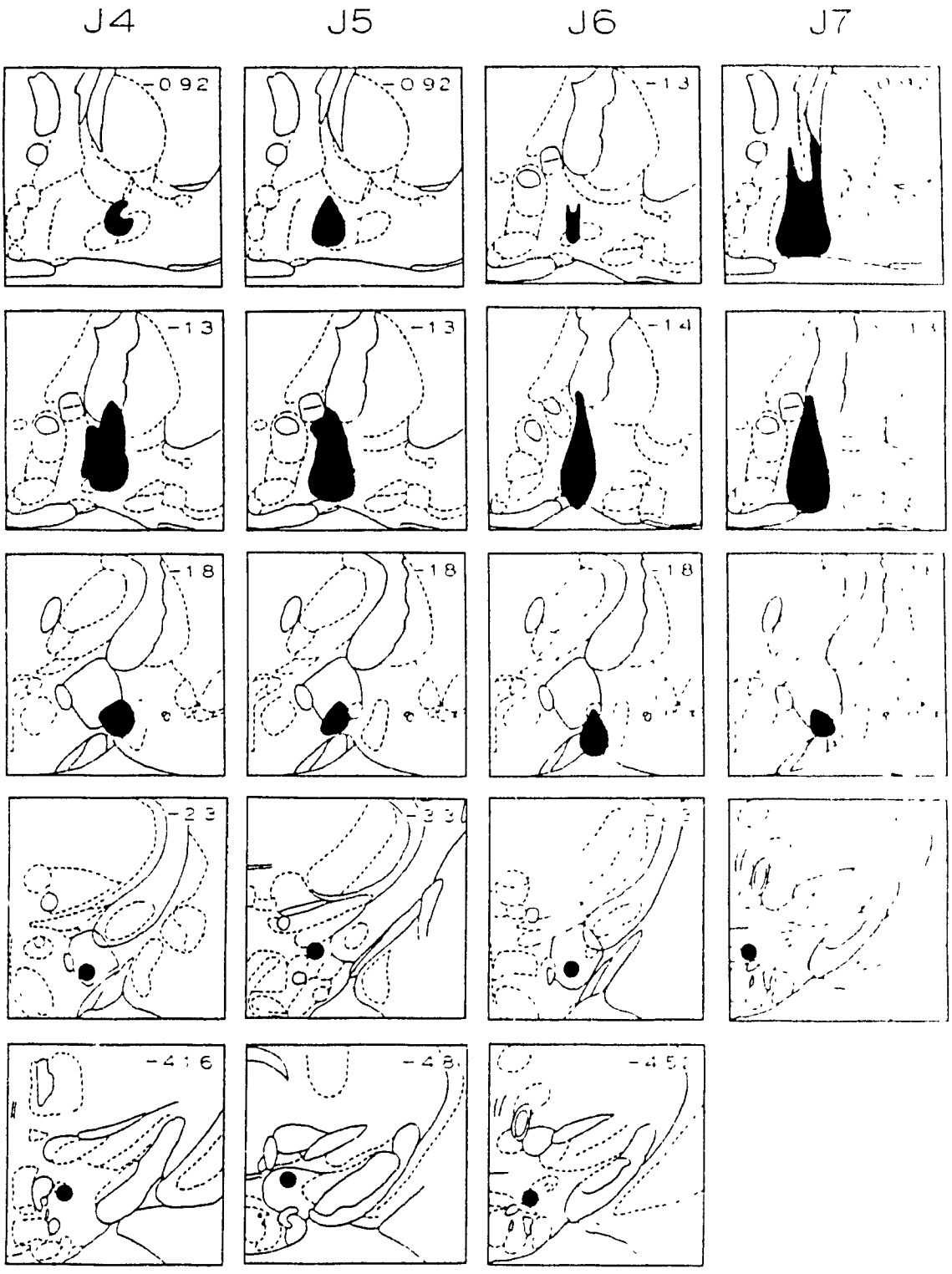
In the third group of 8 rats, lesions did not produce immediate increases in the required number of at least 0.15 $\log_{10}$ units or small, long-lasting increases of at least 0.10 $\log_{10}$ units (Figs. 7 and 8). Small increases in the required number were seen in the case of some subjects included in this group. The largest increase, 0.10 $\log_{10}$ units, occurred in the case of subject J4 although required number values returned to baseline levels within several days. Small, long-lasting increases in the required number ranging from 0.03-0.05 $\log_{10}$ units (7-12%) were also obtained in the case of subjects J5 (low current, LH and VTA), J6 (VTA, high current), J7 (VTA, middle current), and J12 (VTA, all currents). Small decreases (0.033-0.046 $\log_{10}$ units) in the required number were also evident for subjects J5 (VTA, high current) and J8 (VTA, high current).

Figs. 7-8. Required number data for the subjects in which lesions did not produce substantial increases in the required number (see text for criteria). The alphanumeric in the left-hand panels identifies the subject. Left and right panels show data obtained from stimulation of the LH and VTA respectively. Prelesion data (filled symbols) for the five test days preceding the lesion have negative values along the abscissa. The three horizontal, dashed lines extending across each graph indicate the mean of the baseline data for each current. Lesions were made at the end of the last prelesion test session (day 0). Postlesion data are represented by open symbols. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.


LH
VTA
J4
J5
J6
J7
2.0
1.6
1.2
0.8
100
40
16
6
0
10
20
30
Log (Number of Pulses)
Number of Pulses
Day Post-lesion
Low Current
Middle Current
High Current


LH
VTA
J8
J11
J12
K9
2.0
1.6
1.2
0.8
100
40
16
6
0
10
20
Log (Number of Pulses)
Number of Pulses
Day Post-lesion
Low Current
Middle Current
High Current

Figs. 9-10. Lesion and electrode tip locations for the group of subjects with no substantial increases in the required number (see text for criteria). Reconstructions were made onto tracings of coronal plates from the Paxinos and Watson atlas (1986). The alphanumeric at the top of each column identifies the subject. The distance of the plate from bregma is given in the upper right-hand corner of each panel. The blackened area in the top three panels of each column illustrates the extent of the lesion on three representative cross-sections. Filled circles in the bottom panels show the locations of the stimulation sites.


J4
J5
J6
J7
-0 92
-0 92
-1 3
-1 3
-1 3
-1 4
-1 8
-1 8
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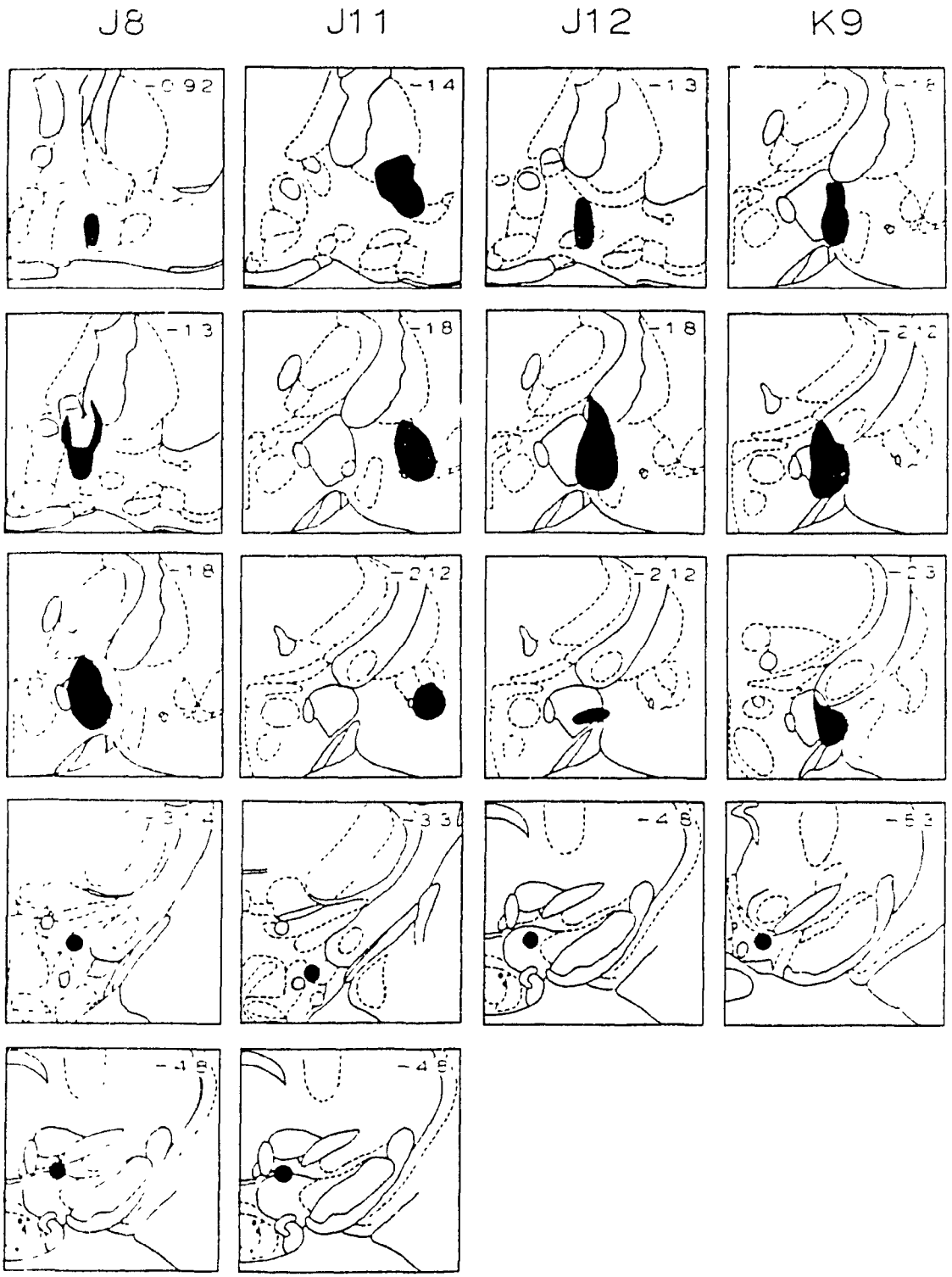
-3 3
-4 1 6
-4 8
-4 5


J8
J11
J12
K9

Location of Lesions.

Histological reconstructions of the lesions and stimulation sites for the third group of 8 rats in which lesions did not produce substantial immediate increases or small, long-lasting increases in the required number are shown in Figs. 9 and 10. In these subjects, the regions damaged by the lesions overlapped with areas destroyed in the first two groups of rats. The only exception to this was subject J11 whose lesion was lateral to the MFB and destroyed parts of the GP, substriatal area (SStr), and portions of the central amygdaloid nucleus. Subject J8's lesion primarily damaged the medial aspect of the anterior LH and was similar to J9's lesion except that it did not produce as much damage to surrounding structures such as the LPO, BSTMPL, or SM.

Effects on Maximum Response Rates

Data for subjects in which the average maximum rate on two consecutive postlesion days was outside of the 95% confidence interval or was shifted by more than 0.10 $\log_{10}$ units (whichever was greater) are shown in Figs. 11-14. Depending upon the stimulation site and current, the increase required to shift the maximum response rate outside of the 95% confidence interval varied from 0.01 $\log_{10}$ units (2.6%) to 0.29 $\log_{10}$ units (95%) (mean = 0.09 $\log_{10}$ units; S.D. = 0.05 $\log_{10}$ units).

Two of the 4 subjects with substantial, long-lasting increases in the required number also had some depression in the maximum response rate (Figs. 11 and 12, J1 and J15). As was the case with the increases in the

Fig. 11. Maximum response rates at each of three currents for subject J1. Top and bottom panels show data obtained from stimulation of the LH and VTA respectively. Prelesion data (filled symbols) for the five test days preceding the lesion have negative values along the abscissa. Lesions were made at the end of the last prelesion test session (day 0). Post-lesion data are represented by open symbols. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.

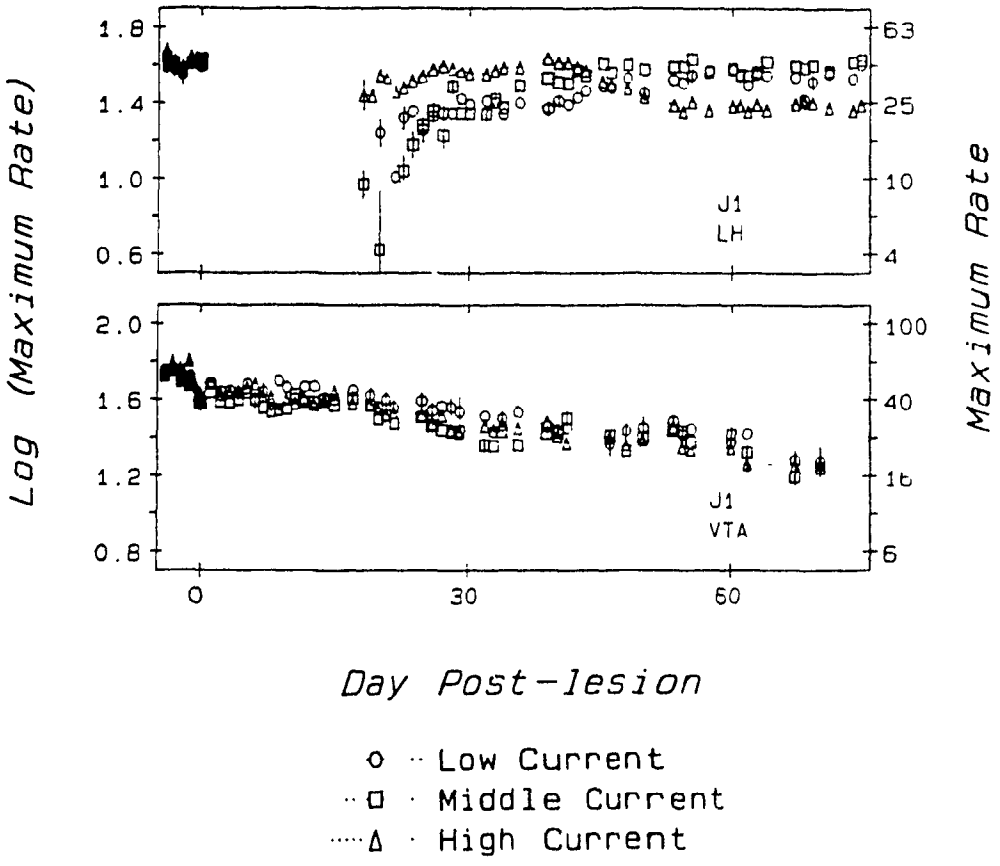

Log (Maximum Rate)
1.8
1.4
1.0
0.6
63
25
10
4
J1
LH
2.0
1.6
1.2
0.8
100
40
16
6
J1
VTA
0
30
60
Maximum Rate
Day Post-lesion
Low Current
Middle Current
High Current

Fig. 12. Maximum response rates for selected subjects (see text) in which lesions produced substantial, long-lasting increases in the required number. The subject and stimulation site are given in the lower right-hand corner of each graph. Prelesion data (filled symbols) for the five test days preceding the lesion have negative values along the abscissa. Lesions were made at the end of the last prelesion test session (day 0). Postlesion data are represented by open symbols. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.

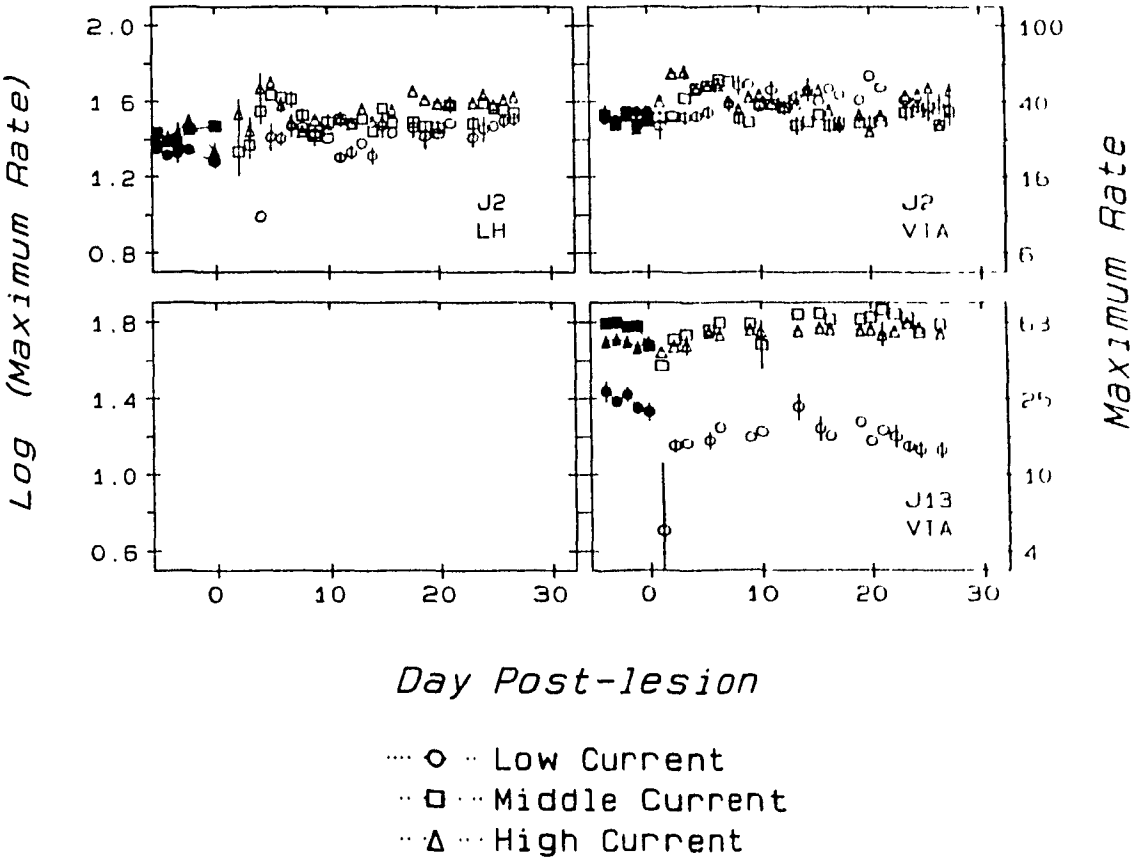

Log (Maximum Rate)
Maximum Rate
2.0
1.6
1.2
0.8
1.8
1.4
1.0
0.6
100
40
16
6
25
10
4
J2
LH
J2
V1A
J13
V1A
0
10
20
30
Day Post-lesion
Low Current
Middle Current
High Current

Fig. 13. Maximum response rates for selected subjects (see text) in which lesions produced transient or small, long-lasting increases in the required number. The subject and stimulation site are given in the lower right-hand corner of each graph. Prelesion data (filled symbols) for the five test days preceding the lesion have negative values along the abscissa. Lesions were made at the end of the last prelesion test session (day 0). Postlesion data are represented by open symbols. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.

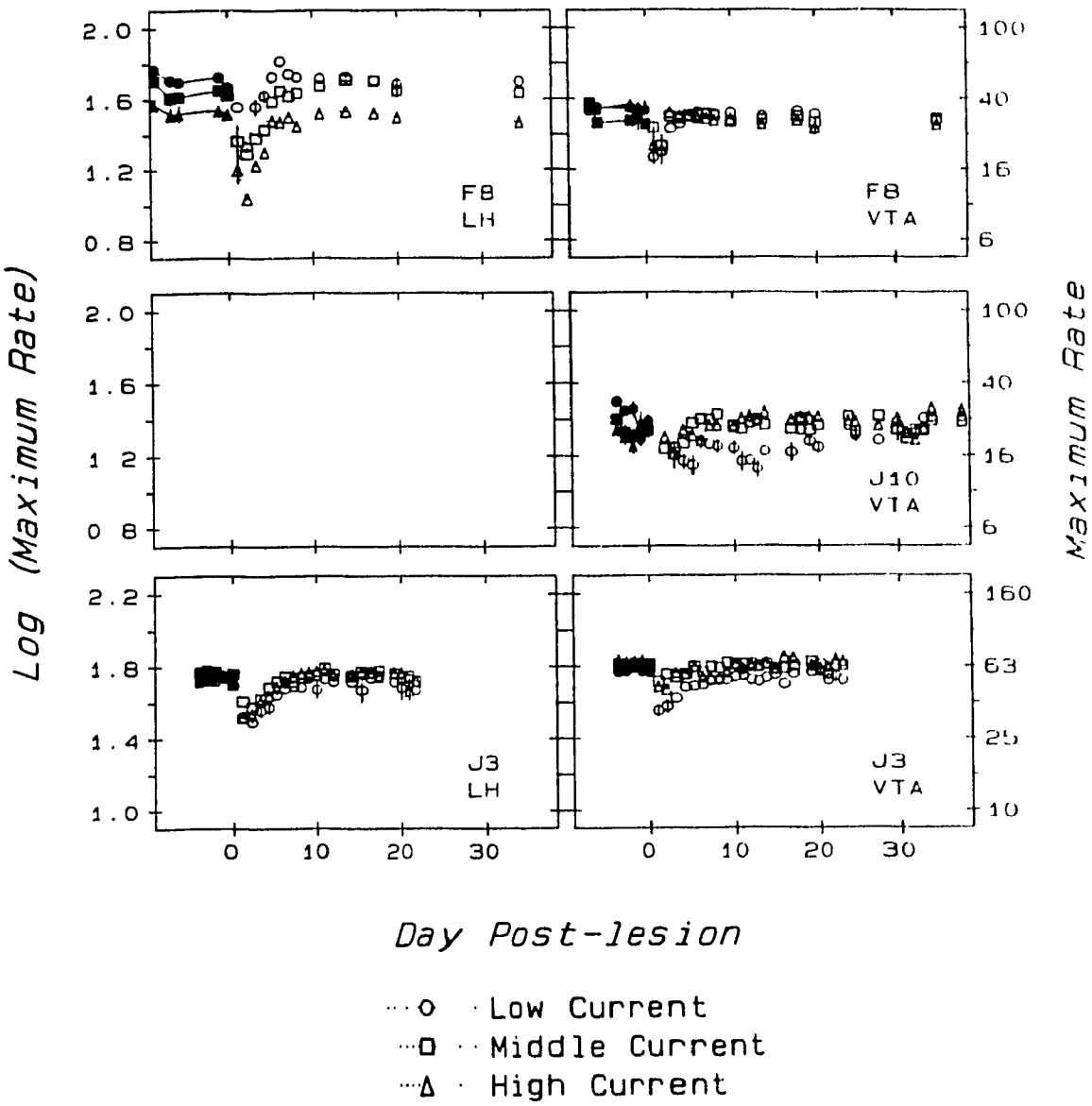
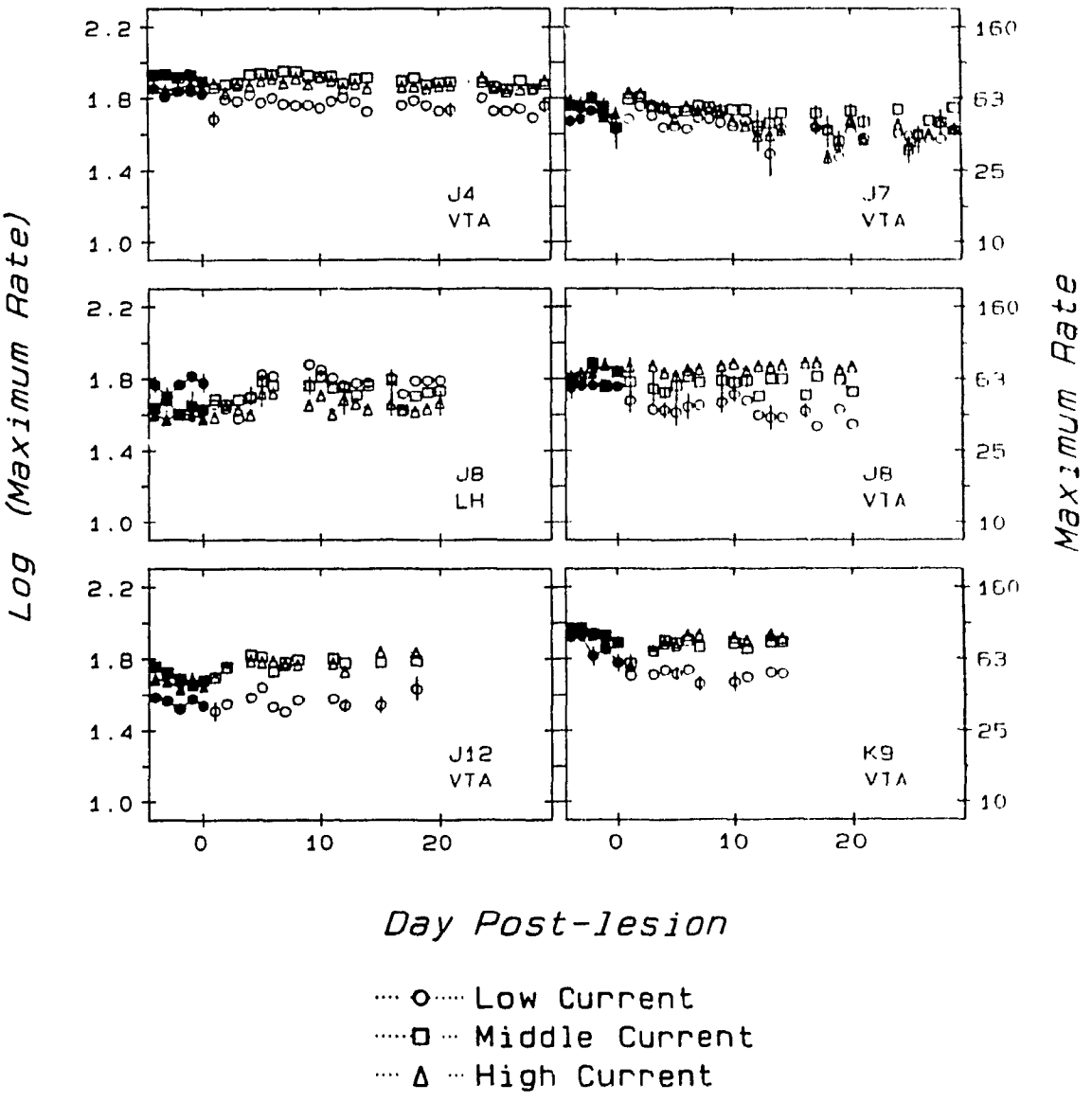

Log (Maximum Rate)
Maximum Rate
F8
LH
F8
VTA
J10
VTA
J3
LH
J3
VTA
Day Post-lesion
Low Current
Middle Current
High Current

Fig. 14. Maximum response rates for selected subjects (see text) in which lesions did not produce substantial increases in the required number. The subject and stimulation site are given in the lower right-hand corner of each graph. Prelesion data (filled symbols) for the five test days preceding the lesion have negative values along the abscissa. Lesions were made at the end of the last prelesion test session (day 0). Postlesion data are represented by open symbols. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.


Log (Maximum Rate)
Maximum Rate
2.2
1.8
1.4
1.0
160
63
25
10
J4
VTA
J7
VTA
J8
LH
J8
VTA
J12
VTA
K9
VTA
0
10
20
Day Post-lesion
Low Current
Middle Current
High Current

required number, the decreases in the maximum response rate were dependent upon the stimulation site and current. The maximum response rate was not depressed for the LH site of subject J13 or for the VTA site when stimulating at the high and middle currents. The maximum response rates for the VTA site were decreased on average by 0.20 $\log_{10}$ units when stimulating at the low current and remained depressed for the entire postlesion testing period. When stimulating at the high current however, maximum response rates for the VTA site were slightly elevated (0.05 $\log_{10}$ units) after approximately 4 days postlesion.

Current-dependent decreases in the maximum response rate were also seen in the case of the LH site of subject J1. The decreases were more pronounced at the middle and low currents immediately postlesion. Decreases were also seen when stimulating at the high current but response rates recovered to within 0.10 $\log_{10}$ units of baseline within 8 days. Starting at around 45 days postlesion, this pattern reversed itself; response rates at the middle and low currents increased back to baseline values and response rates at the high current decreased by approximately 0.20 $\log_{10}$ units. In the case of the VTA site, maximum response rates, which did not appear to be current-dependent in this subject, gradually decreased over the testing period while the required number gradually increased over the same period. Given that a downward trend in the maximum response rate was evident during baseline testing, it is not clear whether either of these trends were related to the effects of the lesion.

The largest, long-lasting decrease in the required number was obtained following the lesion in subject J2. Except for the first postlesion day,

maximum response rates were not depressed for this subject. In fact, greater than 0.10 $\log_{10}$ unit increases in the maximum response rate were obtained at each current. Similarly, there was a tendency for response rates to increase postlesion at the VTA site.

Significant decreases in the maximum response rate were obtained on at least 2 consecutive days in 3 of the 4 subjects that had transient or small postlesion increases in the required number (Fig. 13). In all cases, the decreases in the maximum rate recovered to within 0.10 $\log_{10}$ units of the baseline within several days (F8, J3) or weeks (J10, VTA site, low current). In 2 of these subjects, the depressions in the maximum response rate were largest at the lowest current (J10, VTA site; J3, VTA site).

Maximum response rates for the group of subjects with no substantial or long-lasting increases in the required number are shown in Fig. 14. In several cases, significant decreases in the maximum response rate occurred only at the lowest current tested (e.g. J4, VTA site; J8, VTA site; K9, VTA site). Maximum response rates were also decreased in subject J7 (high current, VTA site) although the response rates decreased gradually after the lesion and decreases did not exceed 0.10 $\log_{10}$ units until several days postlesion. Increases in the maximum response rate exceeded 0.10 $\log_{10}$ units for several of the postlesion testing days at both the middle and high currents in subject J8 (LH site) and at the high current in subject J12 (VTA site).

Discussion

In agreement with the earlier study by Murray and Shizgal (1991), the present study found that some electrolytic lesions to the ALH and surrounding regions resulted in long-lasting increases in the threshold for self-stimulation of the LH and VTA. Lesions of the ALH in 5 of the 14 subjects with MFB stimulating electrodes produced long-lasting increases in the required number of pulses ranging from 0.10-0.25 $\log_{10}$ units. These data are consistent with the notion that neurons in the ALH contribute to the rewarding effect of stimulating more posterior MFB sites. Under the assumptions of the counter model of spatiotemporal integration in the reward substrate, the magnitude of these effects is equivalent to the destruction of 20-44% of the directly activated substrate.

Comparison of the Effective and Ineffective Lesion Sites

One of the goals of this study was to compare the MFB compartments damaged by the effective and ineffective lesions in an effort to identify likely candidate pathways responsible for the lesion effects. In the earlier study by Murray & Shizgal (1991), the effective lesions had damaged the more lateral compartments a, d, and e whereas the two ineffective lesions were confined largely to the medial compartment c. It had been hoped that the MFB compartments critical for the efficacy of ALH lesions could be further differentiated in a larger sample of subjects tested at a range of stimulation currents.

A comparison of the ineffective and effective lesions in the present study reveals that the medial/lateral distinction did not hold in all cases. Many lesions that primarily damaged lateral MFB compartments had little or no effect on the required number obtained at each of 3 currents at both MFB stimulation sites (e.g. J4, J5, J6). Also, one lesion centered in the anteromedial LH (J9) produced a small (0.10 $\log_{10}$ units) yet long-lasting increase in the required number at the lowest current tested for the LH site. Thus, the relative position of the lesions in the medial/lateral plane could not be used to predict their relative efficacy.

One procedural difference between the earlier study by Murray and Shizgal (1991) and the present one is the use of multi-stage versus one-stage lesions. Many of the initial lesions made in the Murray and Shizgal (1991) study produced little or no effect on the threshold for self-stimulation. It was only when subsequent lesions were made in the same subject, through the same lesioning electrode but using a higher current, that significant or long-lasting increases in the required number were obtained. Although the size and location of the earlier, ineffective lesions could not be determined, it is reasonable to assume that they were also centered in the anterolateral LH. Thus, the finding that many of the anterolateral LH lesions in the present study were ineffective is not necessarily inconsistent with the early study by Murray and Shizgal (1991).

Multi-stage lesions were not employed in the present study in order to preserve the location of the ineffective lesions. In general, lesions that were ineffective in substantially increasing the required number were strikingly similar to lesions that produced large increases. Some of these comparisons

are all the more remarkable in that the stimulation sites for some of these subjects were also very similar. For example, there is good overlap between the regions damaged by the lesions in subjects J1, J4, J5 and J6 (see Fig. 4 and Fig. 9) and the locations of some of the stimulation sites are also similar. In the case of subject J4, only a small postlesion increase (0.10 $\log_{10}$ units) in the required number was obtained at the lowest currents which recovered back to baseline values within several days. The lesions in subjects J5 and J6 produced little or no change in the required number at both stimulation sites and all currents. In contrast to these minor effects on the required number, the lesion in subject J1 produced a substantial and long-lasting increase in the required number that lasted for many weeks postlesion.

Regardless of whether the postlesion increase in the required number obtained for subject J1 was due to a decrease in the rewarding efficacy of the stimulation, the contrast between the data obtained for these subjects given the similarity of their lesions and stimulation sites is perplexing. It is consistent in some respects with the earlier findings of Murray and Shizgal (1991). They found that the second or third lesions produced in some subjects resulted in increases in the required number that were not obtained after the first lesion. It is unlikely that Murray and Shizgal (1991) were substantially increasing the volume of damaged tissue with each additional passage of current given that their lesions were comparable in size to the one-stage lesions that were produced in this study using less current. Nonetheless, the additional damage produced by the second- or third-stage lesions was in some cases sufficient to result in an increase in the required

number. If it were possible to compare the damage produced by the early, ineffective lesions with the later, effective lesions, the similarity between the lesion sites may have been as perplexing as the comparison between the effective and ineffective lesion sites in this study.

Despite the similarity between the effective and ineffective lesions, there are discernible differences between the stimulation and/or lesion sites for these subjects and these differences, or other differences not discernible on the basis of the histology, may account for the differences in the efficacy of the lesions. Depending upon the assumptions made about the reward-relevant substrate, there are several ways in which differences in the location of the lesions and or stimulation sites might be used to explain their relative efficacy. The simplest model assumes that stimulation of any MFB site recruits some portion of a relatively homogeneous bundle of reward-relevant neurons travelling through the MFB. Some psychophysical studies that have characterized MFB stimulation sites support this view of the reward-relevant substrate. For example data from collision studies are consistent with the view that the same reward-relevant neurons link self-stimulation sites in the LH and VTA (Bielajew & Shizgal, 1982, 1986; Shizgal et al., 1980). In addition, the approximately linear relationship between the current and the inverse of the required number of pulses over such a wide range of currents is consistent with a relatively homogeneous bundle of reward-relevant neurons occupying a considerable volume of the MFB.

According to the "homogeneous bundle" model, the relative alignment of the stimulating and lesioning electrodes within the bundle of reward-relevant neurons is what determines the efficacy of a particular lesion. Lesions that

damage portions of the bundle that project through the stimulation site (or efferents of reward-relevant neurons projecting through the stimulation site) will reduce the rewarding impact of the stimulation. Alternatively, lesions that damage portions of the reward-relevant bundle that do not happen to project through the effective stimulation field will not reduce the rewarding impact of the stimulation.

It is difficult to reconcile the above model of the reward-relevant substrate with the lesion data presented in this study. Every effort was made to maximize the probability of aligning the lesion and stimulation sites. Multiple stimulation sites were tested in most subjects and 3 currents were tested for each stimulation site. Nonetheless, many lesions that damaged substantial portions of the anterolateral LH had little or no effect on the threshold for self-stimulation at any of the 3 currents tested at both MFB stimulation sites. In contrast, other lesions that destroyed similar regions of the anterolateral LH had substantial and long-lasting effects on the threshold for MFB self-stimulation.

One possibility that must be considered is that the histological reconstructions of the lesions and stimulation sites are not an accurate representation of the tissue damaged by the lesions or activated by the stimulation. An unknown relationship exists between the location of the electrode tip and the location of the reward-relevant neurons that are recruited by the stimulation. In addition, the reconstructions of the lesions were made several weeks after their induction. Wolf and DiCara (1969) have shown that the apparent volume of the lesion changes dramatically over the first few weeks after lesion induction. Based on their data, at the time the

subjects from the present study were sacrificed the lesions had probably undergone considerable contraction of the central cavity. This contraction would lead to distortions in the topographic relationship between surrounding structures. Wolf and DiCara concluded that in large, morphologically homogeneous structures or in regions of indistinct nuclear differentiation, precise reconstructions of the lesion boundaries may not be possible. Although an effort was made to base the lesion reconstructions on the structures showing loss of tissue or dense gliosis and not on the apparent volume of the lesion, it is likely that these reconstructions underestimate the actual volume of tissue damaged by these lesions.

In addition to possible inaccuracies in the histological reconstructions, alternate models or assumptions about the reward-relevant substrate may have to be considered in order to explain the apparent inconsistencies in the lesion data presented here. One possibility is that stimulation of the MFB recruits different populations of reward-relevant neurons that pool their outputs to varying degrees. In the extreme case, the outputs from the two populations may not summate at all so that stimulation of both populations is no more effective than stimulation of the more "dominant" population. In such a case, damage to the "recessive" population will not affect the threshold for self-stimulation due to the fact that stimulation of this population did not affect the threshold prior to the lesion. If the dominant population is damaged, the balance between the two populations may be altered such that the activity in the recessive population is now able to influence the threshold for self-stimulation. This might result in a smaller postlesion increase in the threshold than would be predicted if only the

dominant population was being activated by the stimulation. According to such a view, a lesion would be able to produce a substantial increase in the threshold for self-stimulation if it produced substantial damage to both the dominant and recessive populations or if it damaged only the dominant population and the threshold for the remaining recessive population was substantially higher. Of course, MFB stimulation could recruit multiple populations of reward-relevant neurons whose outputs summate to varying degrees, further complicating the relationship between the location of the lesion and stimulation sites and lesion efficacy.

Several studies support the notion that there exist populations of reward-relevant neurons whose outputs do not show a high degree of summation. For example, the rewarding effects of stimulating the medial prefrontal cortex (MPFC) does not summate well with the rewarding effects of stimulating the LH (Conover & Shizgal, 1992; Schenk & Shizgal, 1982). Also, Fouriezos and Wise (1984) found that there can be relatively poor summation between the rewarding effects of stimulating adjacent MFB sites. In an effort to determine the current-distance relationship for reward-relevant MFB neurons, paired-pulse stimulation was delivered through two MFB electrodes separated by approximately 0.4 mm in the medial-lateral plane. Summation between the rewarding effects of stimulating the two MFB sites was surprisingly low in the case of one of their subjects, effectiveness values at long C-T intervals ranged from approximately 0.5-0.6 depending upon the current. Effectiveness values were not given for the other 4 subjects. Given the proximity of the stimulation sites for this subject (0.35 mm), these

data provide the strongest support for the notion that stimulation of a single MFB site could recruit independent populations of reward-relevant neurons.

In addition to this notion of independent reward-relevant systems, there might also exist antagonistic systems that oppose the rewarding effects of the stimulation. Damage to such a system could increase the rewarding impact of the stimulation and result in lower thresholds for self-stimulation. Depending upon the relative damage done to each system, thresholds could increase, decrease, or remain relatively stable postlesion. Waraczynski (1988) found that some of her knifecuts to the lateral preoptic area produced significant increases in the threshold for self-stimulation of the LH, whereas other knifecuts to this region produced significant decreases in threshold. She also noted that the threshold-decreasing lesions tended to extend into more medial regions than the threshold-increasing lesions. These findings lend support to the hypothesis that reward-relevant and reward-inhibitory systems may be located in nearby MFB sites.

Small decreases in the threshold for self-stimulation were also noted in the present study. The largest decrease in thresholds was obtained for stimulation of the VTA site in subject F3, a subject for which the same lesion produced a substantial increase in the threshold for the LH site. The finding that this lesion produced opposing effects at two different MFB sites is consistent with the hypothesis that both reward-relevant and reward-inhibitory systems were damaged in this subject.

The hypothesis that systems antagonistic to the reward-relevant system co-exist within the MFB would also explain another peculiar result from

subject F3. The effect of the lesion on the required number was found to be current-dependent in this subject despite the fact that the current had little if any effect on the required number prior to the lesion. In order to clarify this point, consider two possible explanations for the finding that the required number was roughly the same for the 3 currents prior to the lesion. One explanation assumes that increasing the current did not reduce the required number because additional reward-relevant neurons were not recruited by increasing the current and therefore increasing the size of the stimulation field. This explanation predicts that the effect of the lesion will not depend upon current because the same reward-relevant neurons are being activated at each current. However, the effect of the lesion did depend upon the current; increases in the required number were only obtained when stimulating with the low and not the middle or high current. The second explanation for the finding that the required number was the same for all 3 currents prior to the lesion assumes that additional reward-relevant neurons were recruited at the higher currents. However, if reward-inhibitory neurons were also recruited at the higher currents (but not the lower) then it is still possible that the threshold would not have decreased due to cancellation of the effects from the reward-inhibitory and the reward-relevant neurons. If the lesion damaged both reward-relevant (recruited at the low and high currents) and reward-inhibitory neurons (recruited at only the higher currents) then the lesion may have increased thresholds at the low current (due to damage to the reward-relevant neurons) and had no effect at the higher currents (due to cancellation of the effects of damaging reward-relevant and reward-inhibitory neurons). Although this second explanation is more

complicated, it does adequately account for both the pre and postlesion data for this subject.

Dependence of Lesion Efficacy on the Stimulation Current

In agreement with the earlier study by Murray and Shizgal (1991), some of the postlesion increases in the required number were found to be dependent upon the stimulation current. In some cases, the increase in the required number was largest at the lowest current tested (e.g. J1, LH and VTA sites; J13, VTA site; F3, LH site) but in other cases it was largest at the highest current tested (e.g. F8, VTA site; J2, LH site). One possible explanation for this dependence on stimulation current is that it reflects the alignment of the lesioned neurons within the stimulation field. For instance, if the lesion damaged neurons that project (or their efferents project) close to the electrode tip, then the increase in the required number would be greatest at the low current because increasing the current will recruit proportionately fewer lesioned fibers. In contrast, if the lesion damaged neurons that project farther from the electrode tip, then the increase in the required number would be evident only at high currents that bring these fibers within the effective stimulation field.

In general, the dependence on stimulation current was less dramatic for those cases in which the increase in the required number was largest at the highest current. In those two cases, increases in the required number were evident at all three currents so that in order to use alignment to explain these effects it would be necessary to assume that the lesion had damaged neurons both far and close to the electrode tip but that proportionately more

neurons were damaged far from the electrode tip. In the case of subject J2 (LH site), the increase in the required number was greatest at the low current immediately after the lesion if one compares the first day on which data were obtained for all three currents (postlesion day 3). Thus, although at the end of testing the required number was most elevated at the highest current, immediately postlesion the largest increase may have been at the lowest current. Current dependence in this subject may therefore reflect differences in the amount of recovery occurring in the neural circuits activated at each stimulation current rather than the relative amounts of damage done to neurons at varying distances from the electrode tip.

It is rather surprising to note that none of the subjects in this study had a postlesion increase in the required number that was only evident at the highest current. If the alignment of the lesion and stimulation sites is as crucial as suggested by the low numbers of subjects with effective lesions, then it would seem more likely that lesion effects would be obtained at the highest currents since the larger effective stimulation field would increase the probability of aligning the lesion and stimulation sites. In fact, the largest increases in the required number were most frequently seen at the lowest stimulation currents. This increased sensitivity at low currents to the threshold-increasing effects of lesions is not peculiar to the present study. Waraczynski, Conover, and Shizgal (1992) also found in one of their subjects with a lesion to the perifornical LH that self-stimulation thresholds for the posterior LH were increased by almost 0.8 $\log_{10}$ units at the lowest current but only by 0.1 $\log_{10}$ units at the highest current.

If we assume that this dependence upon current reflects the relative alignment of the lesioned neurons within the stimulation field, then the finding that the largest increases in threshold were usually obtained at the lowest current is consistent with the greatest damage occurring to neurons (or their efferents) projecting close to the electrode tip. However, it seems unlikely that such a high proportion of the effective lesions would only disconnect neurons projecting close to the electrode tip. An alternative explanation of the dependence on current is that the lower currents were closer to the "current wall", the current below which self-stimulation cannot be obtained at any pulse frequency. As the current approaches the current wall, the counter model of spatiotemporal integration begins to break down so that the relationship between current and the inverse of the required number of pulses is no longer linear. For example, at currents above the current wall, halving the current might result in approximately a doubling in the required number of pulses. At currents approaching the current wall, halving the current might result in a tripling in the required number of pulses. Thus, the same proportional change in current has a greater effect on the required number at low currents than it does at higher currents.

A lesion can reduce the number of directly activated reward-relevant neurons in much the same way as a reduction in current. Therefore, the same proportional change in the number of activated neurons produced by a lesion could result in a larger proportional change in the required number at low currents than at higher currents. It is likely that this explanation can account for the larger increase in threshold observed at the low current for the VTA site of subject J13. In this particular subject, maximum response

rates were depressed at the low current prior to the lesion and were further depressed at the low current after the lesion. Several studies have demonstrated that at currents approaching the current wall, maximum response rates fall below those obtained at higher currents and lower pulse numbers (Gallistel, Leon, Waraczynski, & Hanau, 1991; Malette & Miliaressis, 1990; Waraczynski & Kaplan, 1990). Thus, there is some evidence, at least for this subject, that the larger increase in threshold observed at the low current was due to the fact that this current was approaching the current wall.

Another possible explanation for the increased sensitivity to the effects of lesions at low currents is that at higher currents there is a greater likelihood of recruiting other reward-relevant systems that are able to compensate for the loss of the lesioned systems. Thresholds obtained for the higher currents tended to be more sensitive to the threshold-decreasing effects of some lesions. By whatever mechanism, damage to reward inhibitory systems may also have been more likely to decrease the threshold at the higher currents, thus cancelling any additional threshold-increasing effect of the lesions.

Effects of Lesions on Maximum Response Rates

In the group of subjects with substantial, long-lasting increases in the required number, maximum response rates either increased slightly (J2), decreased (J13), or stayed the same (F3). In the case of subject J1, both a decrease in the maximum response rate and an increase in the threshold were obtained for the LH site. However, maximum response rates for this subject eventually recovered to baseline values whereas the required number

of pulses remained elevated at the low and middle currents. Decreases in maximum rates were also obtained in the absence of any long-lasting increases in the required number of pulses (e.g. J4, J8). It is therefore clear from these data that maximum response rates can vary independently from the threshold for self-stimulation.

It should also be noted that changes in the maximum response rate were dependent upon the stimulation site and current. This finding calls into question the use of a second self-stimulation electrode to control for performance-related deficits when rate-measures are used as an index of changes in the rewarding efficacy of the stimulation. Several self-stimulation studies have employed such a design based on the assumption that performance-related deficits will be global in nature or at least confined to the hemisphere ipsilateral to the experimental manipulation and therefore, if present, can be detected by depressions in the maximum response rate obtained for other self-stimulation sites.

The variables that determine the maximum response rate, which have been collectively labelled performance-related variables, are poorly understood but are known to include variables that alter task difficulty (Edmonds & Gallistel, 1974; Fouriezos et al., 1990; Miliaressis et al., 1986). Given the dependence of maximum rates on stimulation site and current, it would also appear to depend upon the properties of the tissue being stimulated. Even in the absence of any obvious rate-limiting motor effects of the stimulation, maximum response rates can vary widely across stimulation sites. It has also been found that maximum response rates decrease at currents approaching the current wall, a finding that may be related to the concurrent decrease in

the maximum obtainable reward that also occurs at low currents (Gallistel et al., 1991). The fact that the decrease in maximum response rates at low currents may be related to a reduction in the maximum possible reward does not imply that maximum response rates are an adequate measure of changes in the rewarding impact of the stimulation. Unlike the threshold for self-stimulation, which is extremely sensitive to changes in current, maximum response rates can remain remarkably stable over a wide range of currents and are therefore not a good index of the rewarding impact of the stimulation.

In some studies of the effect of performance variables on the rate-frequency curve, manipulations of the task difficulty have been found to increase the frequency threshold by as much as 0.2 $\log_{10}$ units (Edmonds & Gallistel, 1974; Fouriezos et al., 1990; Miliaressis et al., 1986). Since the magnitude of the long-lasting increases in the required number observed in this study ranged from 0.10-0.25 $\log_{10}$ units, one must consider the possibility that these shifts were due to performance deficits and not to a decrease in the rewarding effectiveness of the stimulation. The relative lack of effect of the lesions on maximum response rates suggests that the increases in the required number were not due to performance deficits. With the exception of 1 subject from the study by Fouriezos et al. (1990), performance manipulations that have increased frequency thresholds have also produced depressions in the maximum response rate. In most of the subjects with long-lasting increases in the required number, depressions in the maximum response rate were either not present or lasted for only a few days (J2; F3; J9; F8, VTA site). In the case of subject J1, maximum

response rates were initially depressed but then recovered to baseline values whereas the required number remained elevated above baseline at the low and middle currents. This lesion may therefore have produced both a performance deficit and a decrease in the rewarding effectiveness of the stimulation.

Summary

In 5 of the 14 subjects with self-stimulation electrodes localized to the MFB, lesions to the ALH and surrounding regions resulted in long-lasting increases in the required number ranging from 0.10-0.25 $\log_{10}$ units. Under the assumptions of the counter model, the magnitude of these effects is equivalent to the destruction of 20-44% of the first stage neurons. Given the relative lack of effect of the lesions on the maximum response rate, it is unlikely that these increases in the threshold were due to performance deficits. These data are therefore consistent with the hypothesis that neurons in the ALH contribute to the rewarding effect of stimulating more posterior MFB sites. The effects of the lesions on the required number were found to be current-dependent in some cases; the largest increases were usually observed when stimulating at the lowest currents. It is likely that this increased sensitivity to the effects of the lesions at low currents reflects changes in the spatiotemporal integrating properties of the reward-relevant substrate at low values of current and not on the relative alignment of the lesion and stimulation sites. It was not possible on the basis of the histological analysis carried out in this study to differentiate between the critical areas of the MFB damaged in the effective versus ineffective lesions.

Given the complex topography of the MFB, more sophisticated anatomical techniques may be required to determine the relationship between the pathways damaged by the effective lesions and the pathways activated by the stimulation.

Experiment 2

The results from Experiment 1 support the notion that neurons in the ALH contribute to the rewarding effect of stimulating more posterior MFB sites. A more precise question that can be addressed by the collision test is whether the same or different reward-relevant neurons are recruited at the anterior and posterior MFB sites. The collision test has been used to infer that reward-relevant neurons directly link self-stimulation sites in the anterior MFB between the LPO and ALH (Bielajew et al., 1987) and sites in the posterior MFB between the LH and VTA (Shizgal et al., 1980). What is not yet known is whether reward-relevant neurons directly link the anterior MFB at the level of the ALH with the posterior MFB at the level of the VTA.

Psychophysical Characterization of Anterior versus Posterior MFB Sites

Collision-like effects have been reported most extensively for stimulation sites in the MFB at the levels of the LH and VTA and conduction velocity estimates based on these data range from 1.0-8.3 m/sec (Bielajew & Shizgal, 1982; Bielajew & Shizgal, 1986; Durivage & Miliaressis, 1987; Gratton & Wise, 1988; Shizgal et al., 1980). Given the large number of LH and VTA sites that have been tested with the collision technique, these data have

become the standard by which to compare collision effects obtained from other brain regions. For the most part, the collision curves that have been obtained from posterior MFB sites have shared certain characteristics: the increase in stimulation effectiveness has occurred over a narrow range of C-T intervals (often 0.5 msec) and in all cases the rise in effectiveness has been complete by C-T intervals of 5.0 msec.

The collision data that have been obtained from anterior MFB sites differ in several respects from the data obtained from posterior sites. Bielajew et al. (1987) obtained collision-like effects for 5 subjects with self-stimulation electrodes in the LPO and ALH. The rise in effectiveness values occurred over a wide range of C-T intervals, beginning to rise in some cases between C-T intervals of 0.5 and 1.0 msec and reaching maximum effectiveness values at C-T intervals as long as 10 to 15.8 msec. Thus, the collision interval for these curves spanned several milliseconds compared to the 0.5 msec range of many of the LH-VTA collision curves. Conduction velocity estimates based on these data also spanned a wider range of values from 0.24-11.0 m/sec. These data are consistent with the notion that a more heterogeneous population of reward-relevant neurons is recruited at anterior MFB sites including a population of slowly conducting neurons with conduction velocities less than 1.0 m/sec that has not been evident from the collision curves obtained for the posterior MFB.

The differences obtained between the anterior versus posterior MFB collision curves suggests that the slowly conducting population evident at the anterior sites either arises in the ALH and projects rostrally or arises in more rostral sites but terminates in the region of the ALH. Either

interpretation predicts that the collision curves obtained for stimulation of ALH and VTA sites should more closely resemble the collision curves obtained for LH and VTA sites than for LPO and ALH sites. If the fast conducting neurons detected in both the anterior and posterior sites are not continuous across the junction of the ALH, then collision-like effects would not be obtained. This would imply that the neurons mediating the collision-like effects at the posterior sites arise from regions caudal to the ALH and the neurons mediating the collision-like effects at the anterior sites do not project to the caudal MFB.

Fouriezos, Walker, Rick, and Bielajew (1987) estimated the refractory periods of neurons supporting self-stimulation in several basal forebrain regions including the nucleus accumbens, caudate nucleus, vertical and horizontal limbs of the diagonal band, and LPO. Refractory period estimates obtained from anterior MFB sites ranged from 0.6-5.0 msec. Increasing the amplitude of the T-pulse, which should fire neurons closer to the end of the absolute refractory period, did not substantially hasten recovery suggesting a heterogeneous population with a range of absolute refractory periods. The recovery range for the anterior sites overlaps with, but is not identical to, the recovery range of 0.5-1.5 msec obtained for many posterior MFB self-stimulation sites. Thus, both the refractory period and conduction velocity estimates for the anterior MFB are consistent with a fast-conducting population common to posterior MFB sites and a more slowly conducting population of neurons evident at only the anterior sites.

The goal of Experiment 2 was to assess whether reward-relevant neurons directly link the anterior MFB at the level of the ALH with the posterior

MFB at the level of the VTA. If the fast-conducting neurons evident at the anterior and posterior sites directly link these regions, then it was predicted that the collision curves obtained for sites in the ALH and VTA should resemble the collision curves obtained for sites in the LH and VTA. Refractory period estimates were also obtained for sites in the ALH and VTA in order to estimate the range of conduction velocities in the reward-relevant neurons undergoing collision. In some cases, unequal pulse refractory period tests (T-pulse larger than C-pulse) were conducted in order to assess whether the relative refractory period of the first stage neurons contributed to the rise in the equal-pulse refractory period curve. Refractory period and conduction velocity estimates obtained from the ALH and VTA sites were also used to determine whether the electrophysiological estimates obtained in Experiment 3 for neurons arising in the rostral MFB match the psychophysical profile for the first stage neurons at these stimulation sites.

Method

The procedures and equipment used for Experiment 2 were similar to those described in Experiment 1. Only those aspects of Experiment 2 that differ from Experiment 1 will be described below.

Subjects and Surgery

Stimulating electrodes were implanted into 10 male rats weighing between 455-654 grams at the time of surgery. Electrodes were aimed at the ALH

and VTA using the following level-skull coordinates: -1.3 mm from bregma, 2.2 mm lateral to the mid-sagittal suture, and 7.6 mm below the dura mater for the ALH; -4.8 mm from bregma, 1.0 mm lateral, and 7.5 mm below dura for the VTA.

Temporal Parameters of the Stimulation

Depression of the lever resulted in a 0.5 sec train of pulses (or pulse pairs) for all subjects except K8 in which case a 0.3 sec train was used. Trial duration was set to 30 sec for subjects K5 and K6 and to 40 sec for the remaining subjects. A fixed delay of 0.5 sec was imposed after each stimulation train during which time the light above the lever was extinguished and lever presses were counted but did not trigger a stimulation train. No delay was imposed after each stimulation train for subjects K2 and K4.

Selection of Currents

For each subject, the current employed during the collision test was chosen so that the required number of pulses was approximately the same for each of the two stimulation sites. Initially, rate-number curves were obtained for each of 3 currents delivered to either the ALH and VTA sites. If possible, the 3 currents were chosen such that 0.30 $\log_{10}$ units separated each current (e.g. 200, 400 and 800 uA). If reliable rate-number curves could not be obtained for this range, the interval between currents was reduced accordingly. The resulting curve relating the required number of

pulses to the current was used as a guide in selecting the currents to be used in the collision tests.

Collision Test

In the collision test, trains of C- and T-pulses were delivered to the two stimulation sites, with each electrode delivering one of the pulses from each pair. The C-T interval was varied and a rate-number curve was collected for each of the C-T intervals tested (paired pulse condition). The procedure for determining the rate-number curve in the paired pulse conditions was essentially the same as that described in Experiment 1 except that the number of pulse pairs per train was decreased across trials instead of the number of single pulses. The number of pulse pairs delivered in the first 40 sec trial of each paired pulse condition was the same for all C-T intervals. Between 6 and 9 C-T intervals, ranging from 0.2 msec to 25.6 msec, were tested per subject. The C-T intervals were presented in one of two orders, the second being the reverse order of the first. The order of presentation was chosen so that long and short C-T intervals were interdigitated.

Interspersed amongst the paired pulse conditions were single pulse conditions in which rate number curves were obtained using evenly spaced pulses delivered to one of the electrodes. Four single-pulse rate-number curves were obtained at the start of each testing session (2 per stimulation site), 2 single-pulse curves in the middle of the session (1 per site) and 2 single-pulse curves at the end of the test session (1 per site). The first single-pulse rate-number curve obtained for each stimulation site at the start of each test session was discarded from the data analysis.

During each testing session, C-pulses were delivered to either the ALH electrode (ALH-VTA condition) or to the VTA electrode (VTA-ALH condition). Each condition (ALH-VTA or VTA-ALH) was run once per day with a rest period of at least one hour between sessions. The four possible combinations of conditions (ALH-VTA or VTA-ALH and 2 C-T schedules) were tested in a counterbalanced fashion so that each combination was tested equally often in the first and second sessions of each day. Seven to 11 replications (usually 8) of each condition (ALH-VTA and VTA-ALH) were obtained for all subjects except K6, in which case 4-11 replications were obtained.

If evidence of collision-like effects were obtained for a particular set of currents, refractory period data were then collected (see section below) at the same current intensities. Collision tests were repeated at several sets of currents (at least 3) using the same procedure as outlined above. Current was increased across collision tests in the case of 2 subjects (K8 and K10), decreased across collision tests in the case of 3 subjects (K4, K5, K6), and increased and then decreased across collision tests in the case of 2 subjects (K2 and K7).

Data Analysis.

The required number of pulse pairs for half-maximal responding was interpolated for each C-T interval from the rate-number curve obtained using that interval in the paired pulse condition. Similarly, the required number of single pulses was obtained for each of the stimulation sites in the single pulse conditions. In order to obtain a measure of relative stimulation effectiveness (E-value) for each C-T interval, the required number of pulse

pairs for that C-T interval was compared to the required number of single pulses using a modified version of Yeomans' effectiveness formula designed to take into account any differences in the required number of pulses at each stimulation site:

$$E = [N_{low}/N_{ct} - 1] \times N_{high}/N_{low}$$

where

E = effectiveness of paired-pulse stimulation.

N_{low} = average of the required number of single pulses for the stimulation site yielding the lower required number.

N_{high} = average of the required number of single pulses for the stimulation site yielding the higher required number.

N_{ct} = required number of pulse pairs for a given C-T interval.

The average effectiveness value for each C-T interval was plotted as a function of C-T interval and condition (ALH-VTA versus VTA-ALH).

Refractory Period Test

The refractory period test was similar to the collision test with the exception that both pulse pairs were delivered to a single stimulation site. Only those aspects of the refractory period test that differ from the collision test will be described below.

Each stimulation site was tested in separate sessions that were separated by a rest period of at least one hour. The current was the same for both the C- and T-pulses and was identical to the current delivered to that

stimulation site during the preceding collision test. Within each session, 9-11 C-T intervals ranging from 0.2-12.8 msec were tested. Four single-pulse conditions were interspersed throughout the session in the same manner as the collision test. The order in which the stimulation sites were tested and the two schedules of C-T intervals were counterbalanced across testing days. A total of 4-8 rate-number curves were obtained for each C-T interval across the testing sessions. In some cases, additional refractory period tests were carried out using currents not employed in the collision tests.

Effectiveness values were obtained for each C-T interval using Yeomans' formula:

$$E = [N_{sp}/N_{ct} - 1]$$

where

E = effectiveness of the T-pulse.
N_{sp} = average of the required number of single pulses for that session.
N_{ct} = required number of pulse pairs for a given C-T interval.

Unequal-pulse Refractory Period Test.

Additional refractory period tests were conducted for the ALH site in 4 of the subjects using unequal intensity C- and T-pulses. The procedure was identical to that outlined above with the exception that during the paired-pulse stimulation, the intensity of the T-pulse was set to a value equal to 1.2-1.4 times that of the C-pulse. Four single-pulse rate number curves were collected at each current (C-pulse current and T-pulse current) and

were interspersed throughout the session in the same manner as that described previously. Equal-pulse refractory period tests were alternated with the unequal-pulse refractory period test using the same stimulation site and the same C-pulse current. The four possible combinations of conditions (equal-pulse versus unequal-pulse and two schedules of C-T intervals) were tested in a counterbalanced fashion so that each combination was tested equally often in the first and second sessions of each day.

Effectiveness values were calculated for each C-T interval using the formula:

$$E = [N_{low}/N_{ct} - 1] \times N_{high}/N_{low}$$

where

E = effectiveness of the T-pulse.

N_{low} = average of the required number of single pulses for the current yielding the lower required number.

N_{high} = average of the required number of single pulses for the current yielding the higher required number.

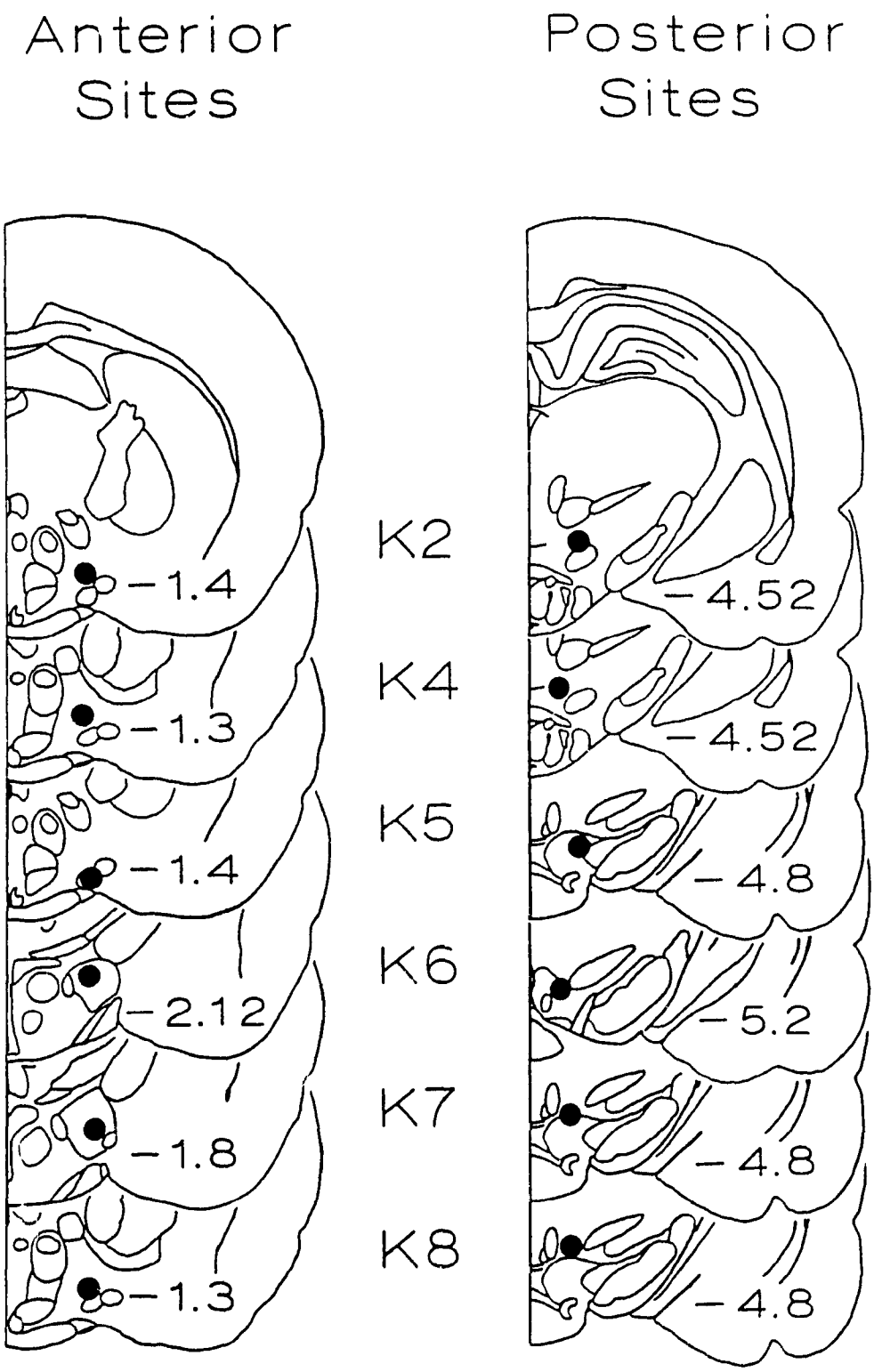
N_{ct} = required number of pulse pairs for a given C-T interval.

Results

Seven subjects that could be trained to respond for stimulation delivered to both sites were tested in the collision and refractory period tests. It was later confirmed that the anterior stimulation site in one of the collision subjects was located in the mid-LH and data for this subject were therefore included in another study on collision-like effects between LH and anterior VTA self-stimulation sites (Murray and Shizgal, 1992). One subject (K3) could only be trained to respond for stimulation delivered to the ALH and was therefore only tested in the refractory period experiment. An additional 2 subjects that could not be trained to self-stimulate for stimulation delivered to the anterior stimulation site were discarded from the experiment.

Electrode tip locations for the 6 subjects tested in both the collision and refractory period experiments are shown in Fig. 15. Anterior stimulation sites were localized to the region of the ALH between -1.3 and -2.12 mm from bregma based on the Paxinos and Watson atlas (1986). Subject K3's anterior stimulation site (see Fig. 26) was located near the ventral border of the VP. Stimulation of the ALH produced seizures in several of the subjects (K2, K4, K5, and K7). In general, a seizure would occur near the beginning of the test session at which time the subject was disconnected from the stimulator and allowed to recover. The experiment was then re-started and in most cases no further seizures occurred during that testing session. An effort was made to reduce the incidence of the seizures by increasing the

Fig. 15. Electrode tip locations reconstructed onto tracings of coronal plates from the Paxinos and Watson atlas (1986) for the subjects in Experiment 2. The anterior stimulation sites are shown in the left-hand column and the posterior stimulation sites in the right-hand column. The distance (mm) of the plate from bregma is given in the lower right corner of each section.


Anterior Sites
Posterior Sites
K2
K4
K5
K6
K7
K8
-1.4
-1.3
-1.4
-2.12
-1.8
-1.3
-4.52
-4.52
-4.8
-5.2
-4.8
-4.8

fixed delay after each stimulation train to 0.5 sec or, if possible, reducing either the current or number of pulses.

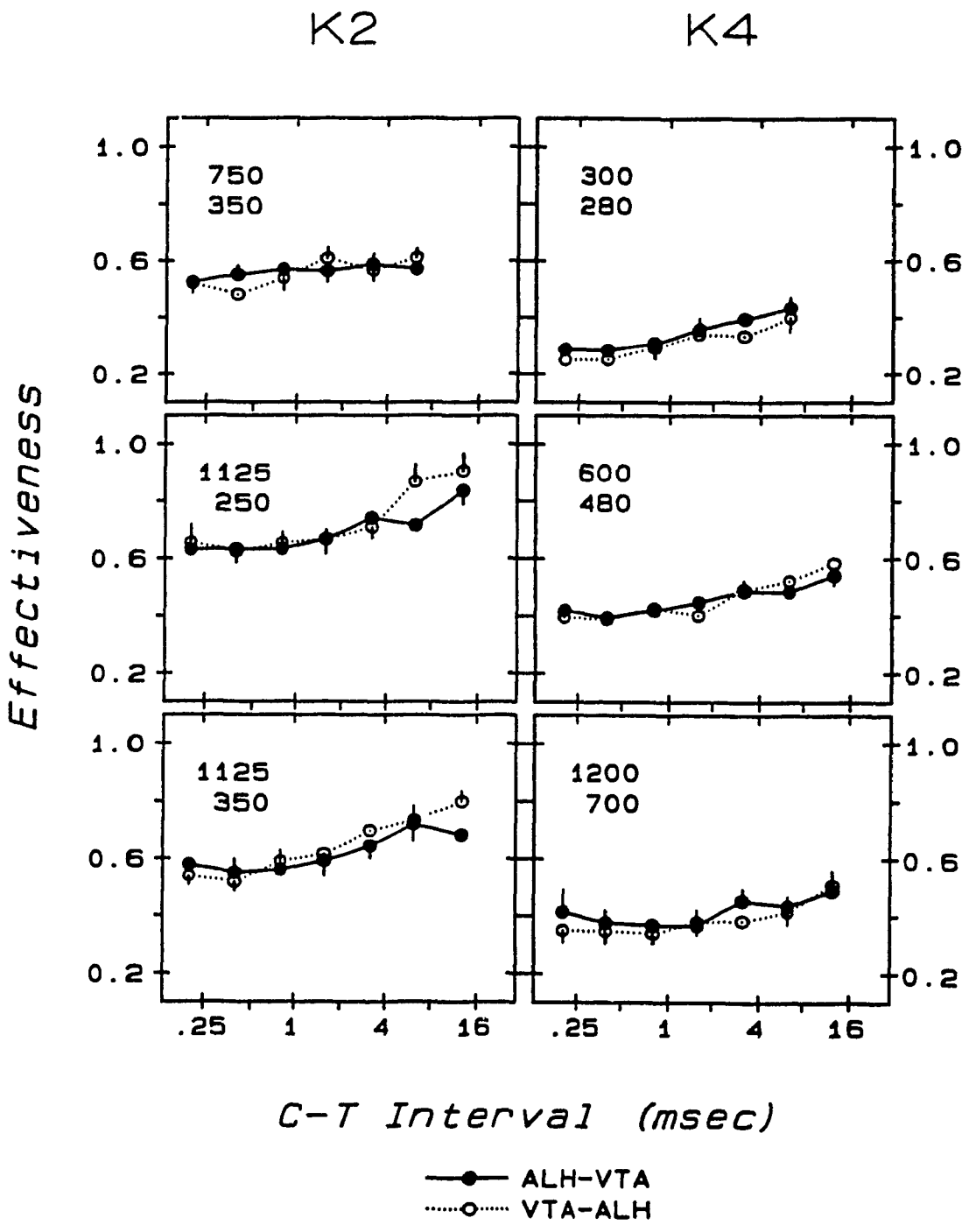
Four of the 6 posterior stimulation sites were in or bordering the VTA between -4.8 and -5.2 mm from bregma. The posterior stimulation site for subject K2 was located along the dorsal border of the rostral VTA (VTAR) at approximately -4.52 mm from bregma. Subject K4's posterior stimulation site was dorsomedial to the rostral VTA near the mammillotegmental tract.

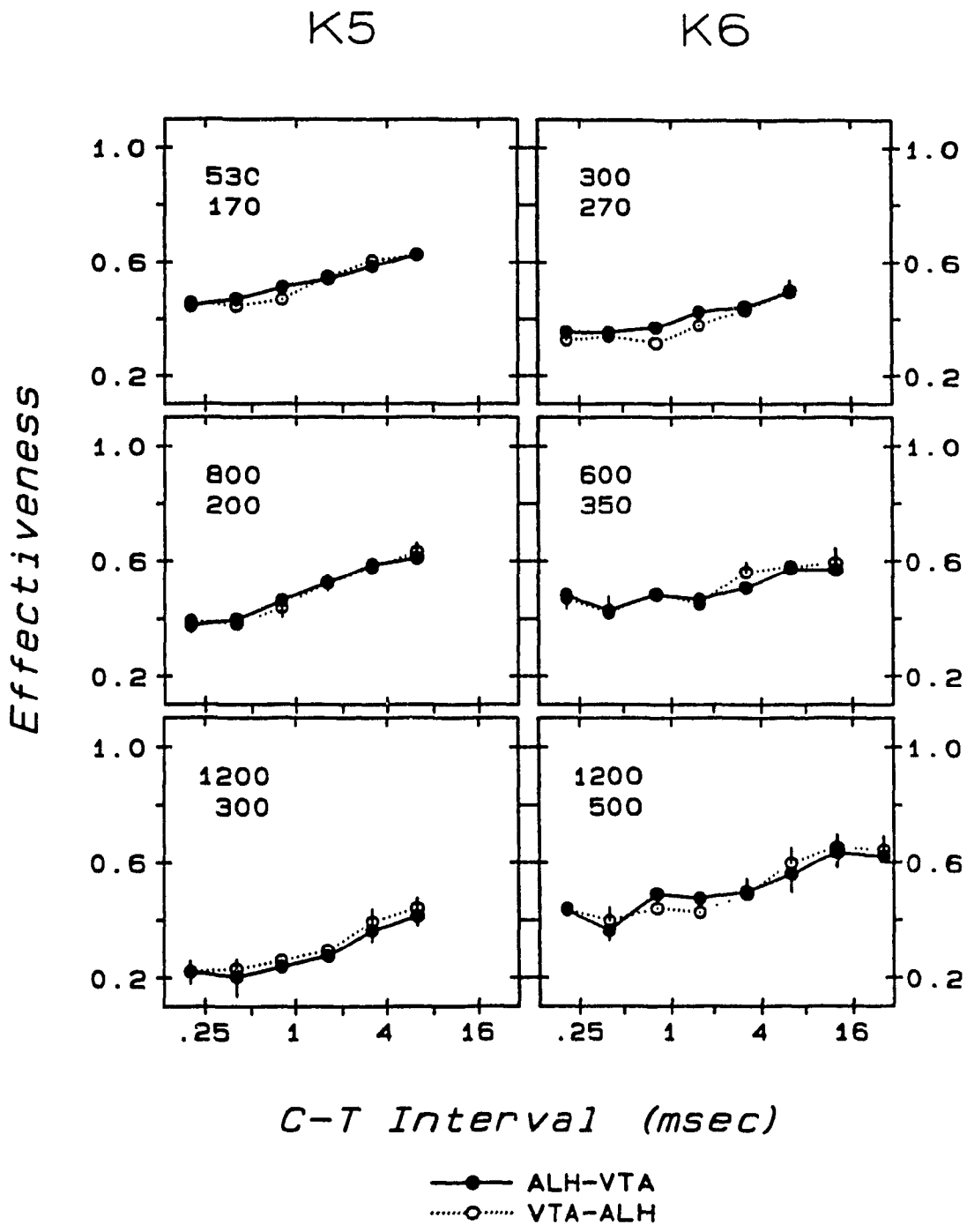
Collision Data

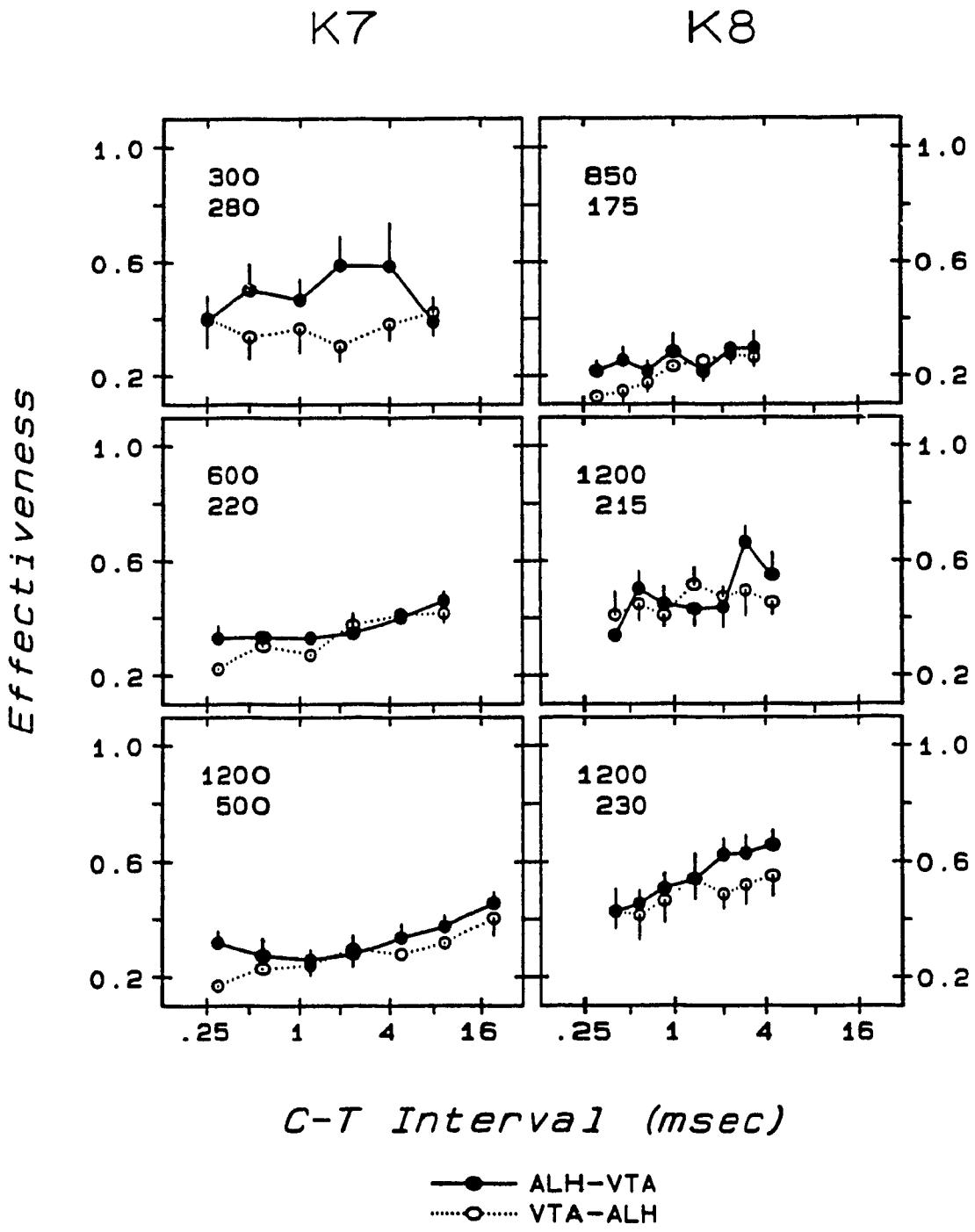
Effectiveness values as a function of C-T interval at each of 3 sets of currents are shown in Figs. 16-18 for each of the 6 subjects. The data collected with the C-pulses delivered to the anterior site (ALH-VTA condition, filled symbols) are shown separately from the data collected with the C-pulses delivered to the posterior site (VTA-ALH condition, open symbols). A two-way analysis of variance was performed on the ALH-VTA and the VTA-ALH data.

[It is generally agreed that t- and F-tests are inappropriate for single subject designs if the data are serially dependent (Hartmann, 1974; Thoresen and Elashoff, 1974). The effect of any time-dependent correlations that may exist in these data would be minimized by the fact that testing sessions alternated between the two conditions (ALH-VTA versus VTA-ALH) and that two pseudo-random C-T schedules were used. Nonetheless, the statistical analysis presented here should only be considered as a rough guide to the significance of these effects.]

Figs. 16-18. Effectiveness values as a function of C-T interval obtained at each of 3 sets of current for the six subjects in the collision experiment. The alphanumeric on the top of each column identifies the subject. The current (uA) delivered to the ALH (top number) and VTA (bottom number) sites are shown in the upper left-hand corner of each graph. Data obtained in the ALH-VTA condition versus VTA-ALH condition are represented by filled and open symbols respectively. Data points without error bars correspond to cases where the s.e.m. was less than the radius of the symbol.


K2
K4
750
350
300
280
1125
250
600
480
1125
350
1200
700
1.0
0.6
0.2
.25
1
4
16
Effectiveness
C-T Interval (msec)
ALH-VTA
VTA-ALH


K5
K6
530
170
300
270
800
200
600
350
1200
300
1200
500
1.0
0.6
0.2
.25
1
4
16
Effectiveness
C-T Interval (msec)
ALH-VTA
VTA-ALH


K7
K8
300
280
850
175
600
220
1200
215
1200
500
1200
230
1.0
0.6
0.2
.25
1
4
16
Effectiveness
C-T Interval (msec)
ALH-VTA
VTA-ALH

An increase in effectiveness with increasing C-T interval that did not depend upon which electrode delivered the C-pulse of each pair was considered evidence for a collision-like effect. This would be reflected in a significant main-effect of C-T interval with no main-effect of condition (ALH-VTA versus VTA-ALH) and no interaction. In order to bias the results of this study against the finding of collision-like effects, a stringent criterion (p less than 0.01) was chosen for an effect of C-T interval and a less stringent criterion (p less than 0.1) for an effect of condition or an interaction.

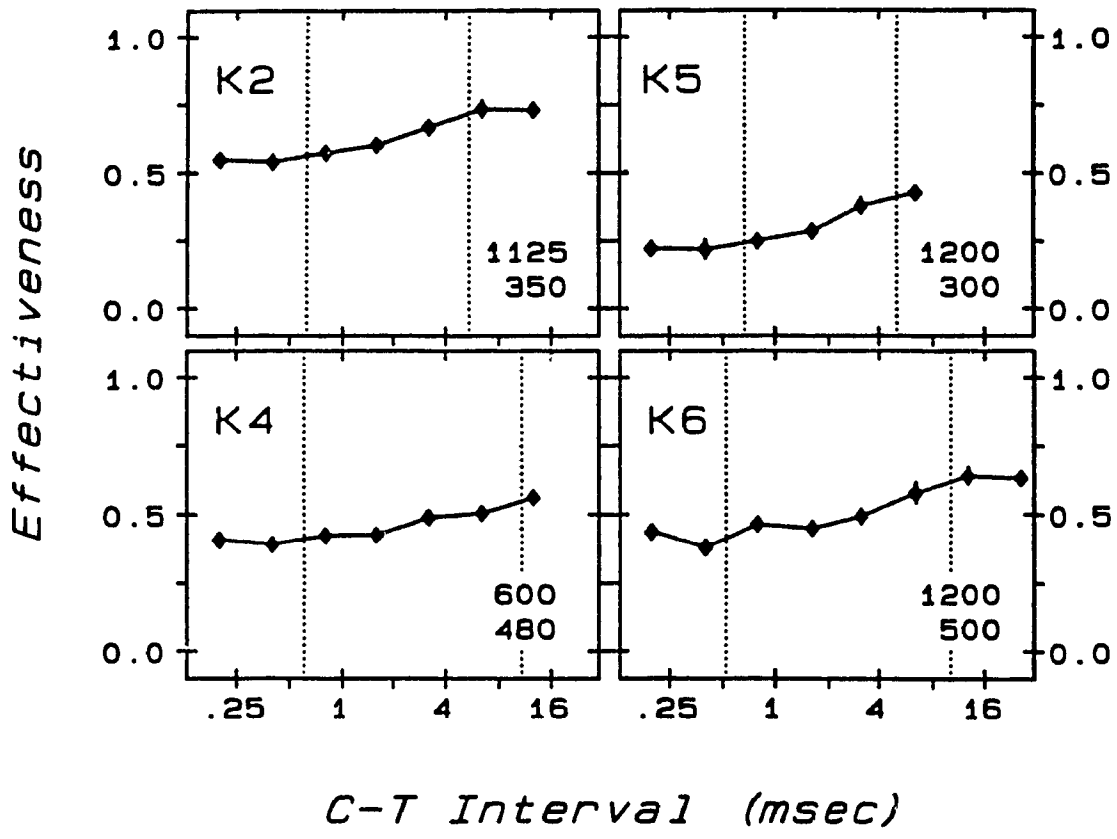
Using these criteria, collision-like effects were obtained in 4 of the 6 subjects (K2, K4, K5, and K6). (If a more conventional criterion for significance ($p = 0.05$) had been used, collision-like effects would have been obtained in all 6 subjects.) In the case of subjects K2, K4, and K6, collision-like effects were obtained at the 2 higher sets of currents but not for the lowest set of currents. Significant collision-like effects were obtained at each of the 3 sets of currents for subject K5. Increasing the current produced an increase in both maximum effectiveness values and the magnitude of the collision effect for subjects K2 and K6. In contrast, increasing the currents in subject K5 reduced the overall effectiveness of the stimulation at the longest C-T intervals tested but nonetheless increased the magnitude of the collision effect. The lowest currents produced the largest percent collision effect for subject K4, however there was also a significant main effect of condition at these currents. Increasing the current in subject K4 produced first an increase, then a decrease in maximum effectiveness values and the percent collision decreased with current. In the case of

subjects K7 and K8, there was either a significant effect of condition, no significant main effect of C-T interval, or both. There were no significant interactions between C-T interval and condition in any of the collision data.

In those subjects with significant collision-like effects (K2, K4, K5 and K6), the increase in effectiveness with C-T interval was always gradual, in no case did we see the step-like rise characteristic of collision curves from previous studies (Bielajew and Shizgal, 1982; 1986; Gratton and Wise, 1988; Shizgal et al., 1980). In order to estimate the range of C-T intervals over which effectiveness increased, data from the two conditions (ALH-VTA versus VTA-ALH) were averaged together for the currents yielding the highest percent collision (% collision = $(E_{high} - E_{low})/E_{high}$) in each subject (Fig. 19). In the case of subject K2, the middle (1125, 250 uA) and high (1125, 350 uA) set of currents yielded roughly the same percent collision effect (27.8% versus 26.5 %). However, the higher set of currents was used to estimate the collision interval because the effect of condition approached significance ($p = 0.15$) in the case of the middle set of currents. The C-T intervals corresponding to 10% and 90% of the total recovery were interpolated from the averaged collision curve and used to estimate the beginning and end of the collision interval (vertical, dotted lines in Fig. 19).

In each of the 4 subjects with collision-like effects, the collision curves began to rise between C-T intervals of 0.4 and 0.8 msec. Due to the fact that C-T intervals between 0.4 and 0.8 msec were not tested, more precise estimates of the beginning of the collision interval cannot be made with any confidence. Therefore, the beginning of the collision interval might have been as late as 0.8 msec. The end of the collision interval occurred

Fig. 19. Effectiveness values as a function of C-T interval in the four subjects with significant collision-like effects (see text for criteria). Data obtained in the two conditions (ALH-VTA versus VTA-ALH) have been averaged together for the currents (given in uA) that yielded the highest percent change in effectiveness. The C-T intervals corresponding to 10% and 90% of the total recovery are denoted by the vertical, dotted lines. Data points without error bars correspond to cases where the s.e.m. was less than the radius of the symbol.


K2
1125
350
K5
1200
300
K4
600
480
K6
1200
500
1.0
0.5
0.0
.25
1
4
16
Effectiveness
C-T Interval (msec)

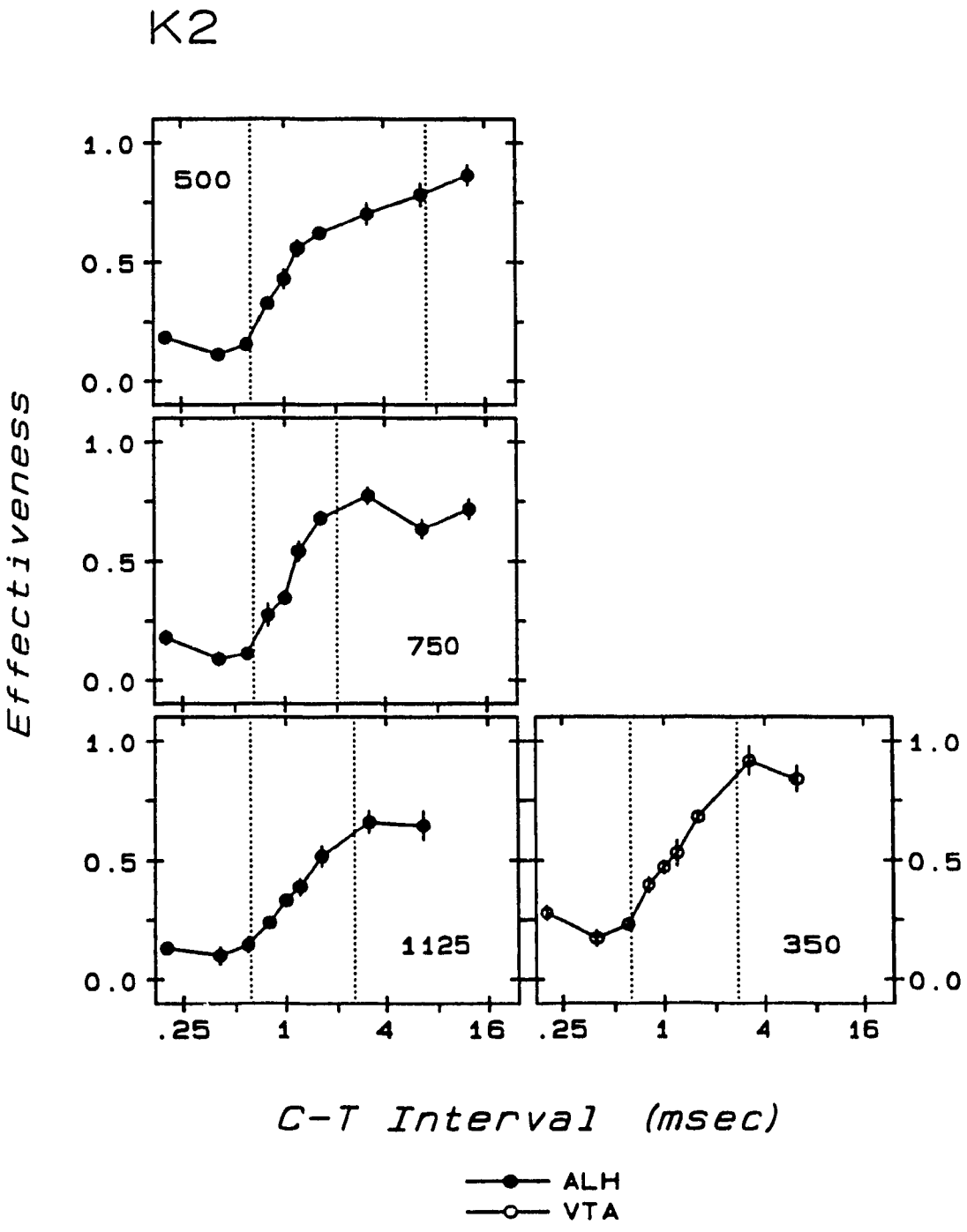
between C-T intervals of 6.4 and 12.8 msec in 2 subjects (K4 and K6) and between C-T intervals of 3.2 and 6.4 msec in the remaining 2 subjects (K2 and K5). It is not clear whether effectiveness values had reached their maximum value at the longest C-T intervals tested in the case of subjects K4 and K5. It was not possible to test longer C-T intervals in these subjects due to the fact that the C-T interval cannot be greater than half the interval between C-pulses. In these two cases, the end of the collision interval may have been underestimated.

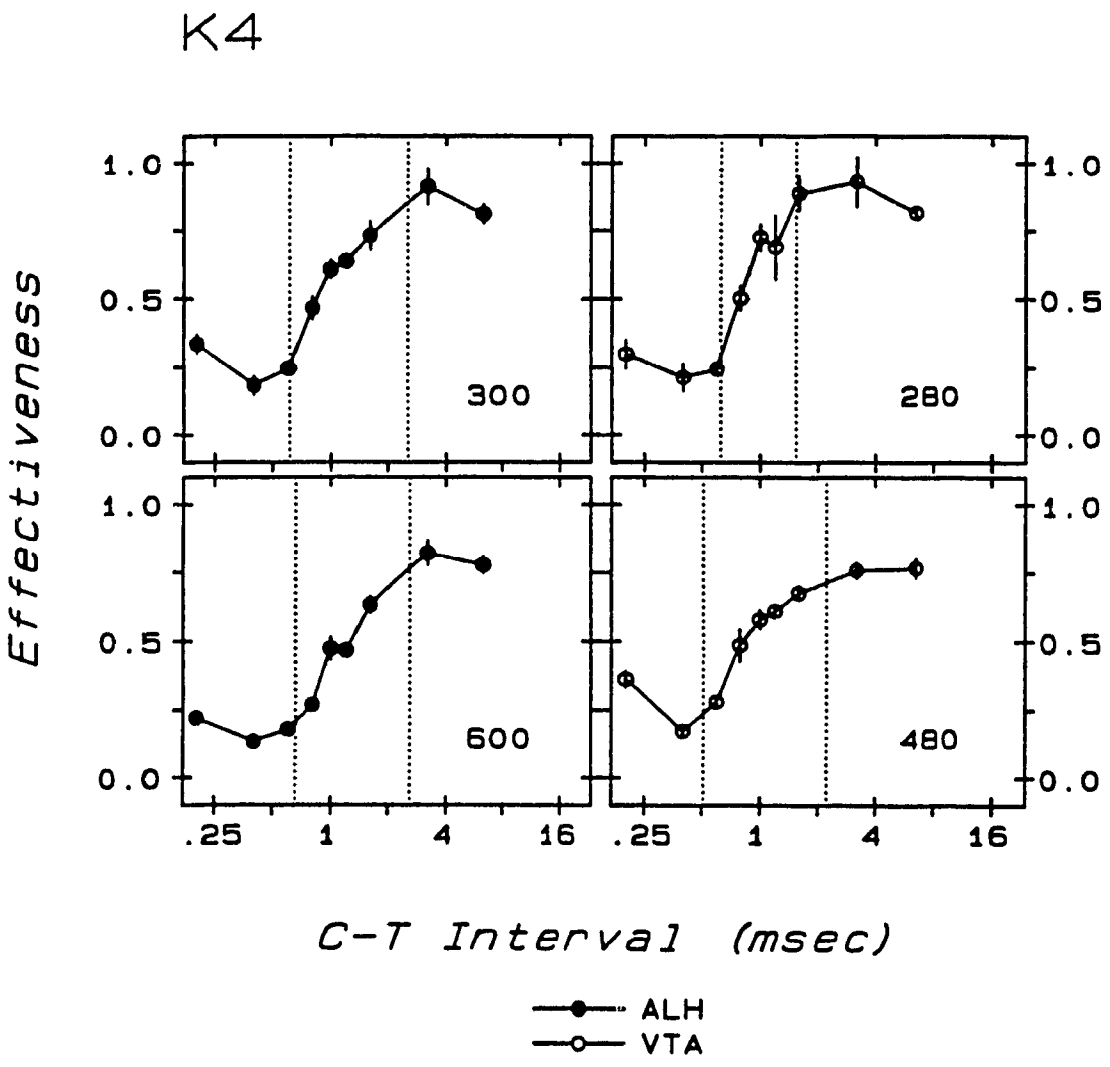
In those subjects with significant collision-like effects, the highest percent collision obtained for each subject varied from 27% (K2) to 49% (K5). Maximum effectiveness values were lowest in the subject with the highest percent collision (K5, E_{max}=0.43) and highest in the subject with the lowest percent collision (K2, E_{max}=0.74).

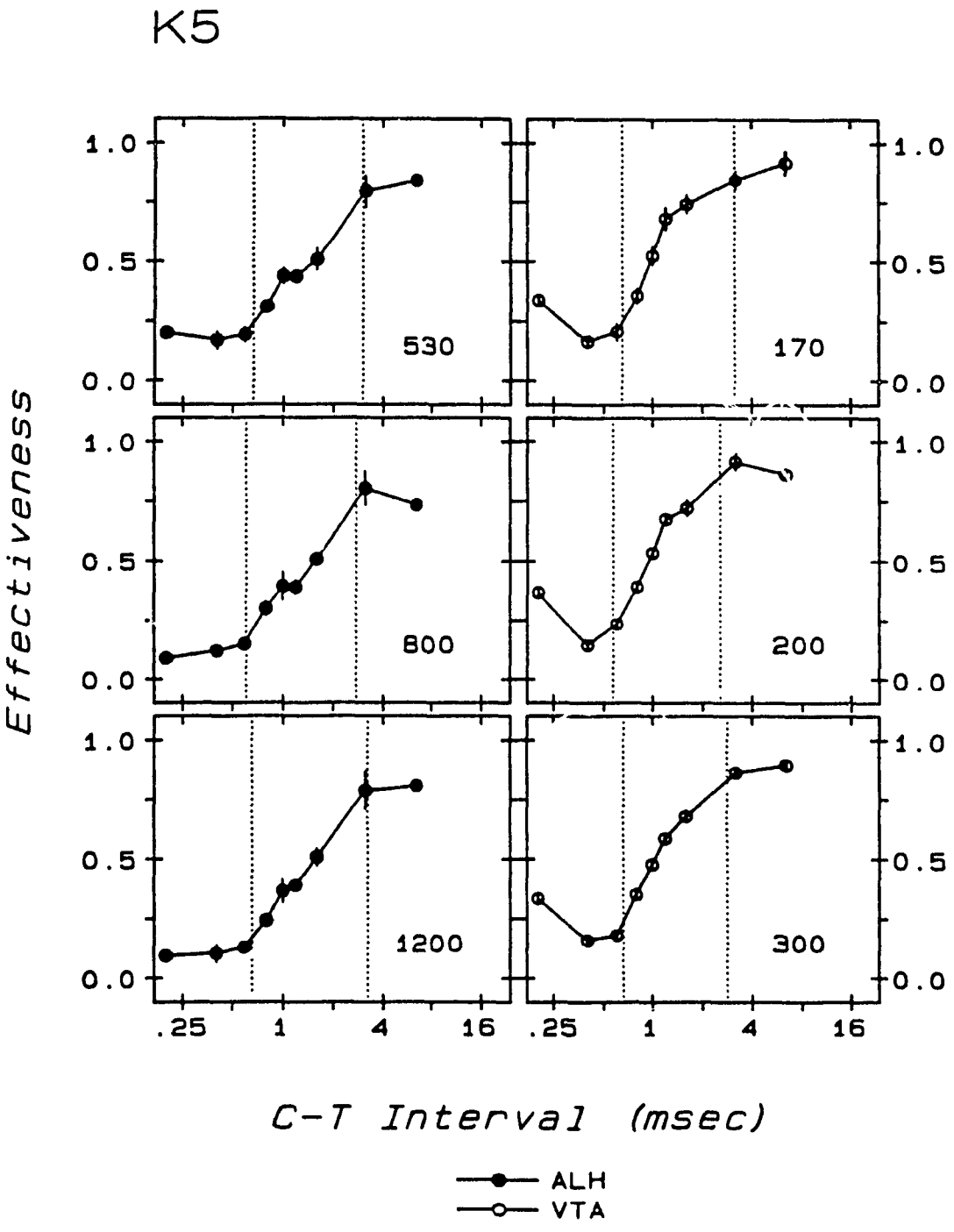
Refractory Period Data

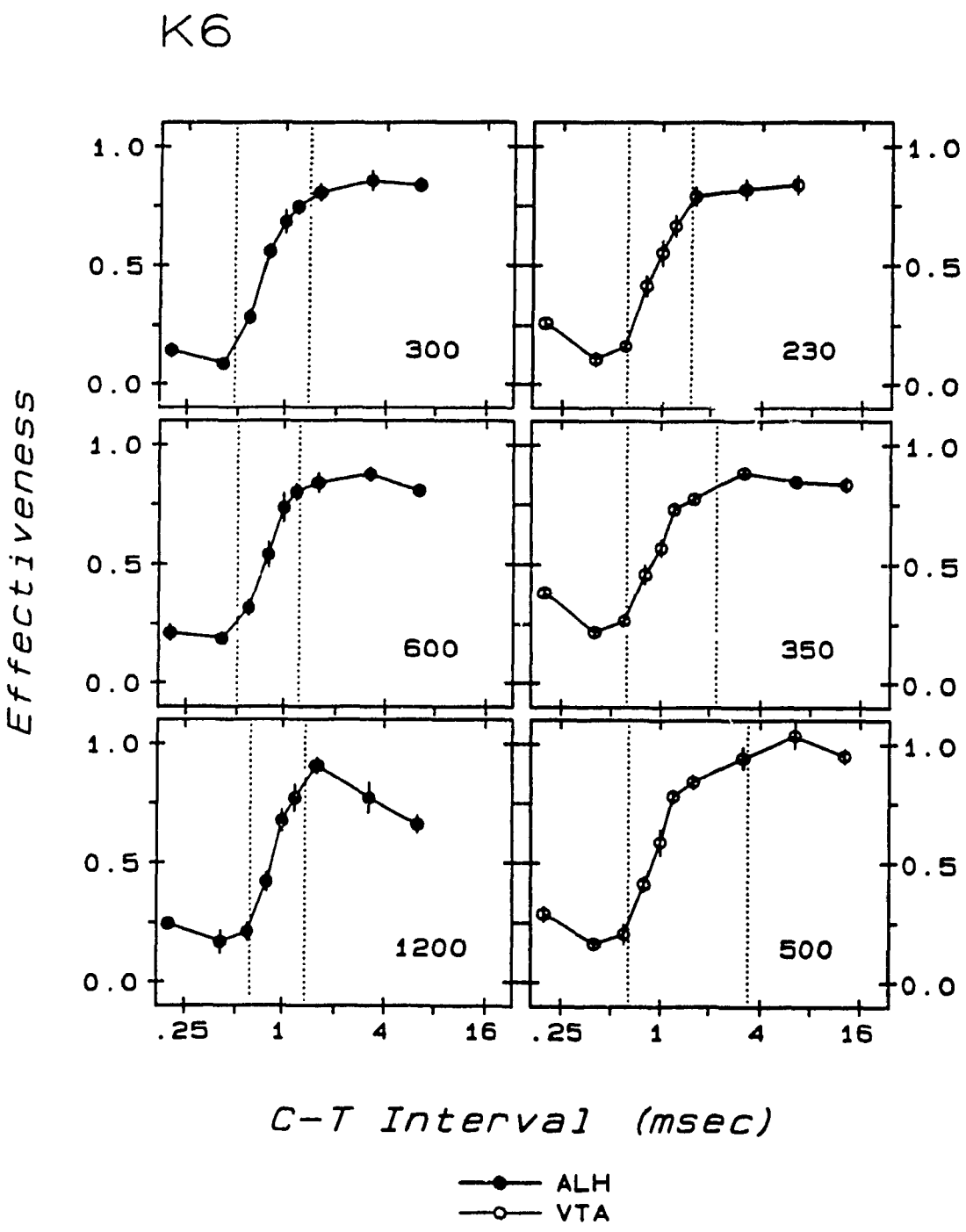
Refractory period data for the 6 subjects tested in the collision experiment are shown in Figs. 20-25. An additional subject, K3, was only tested in the refractory period experiment and these data are shown in Fig. 26. Refractory period estimates were obtained from a total of 7 ALH sites (filled symbols) and 6 VTA sites (open symbols) using equal amplitude C- and T-pulses. Vertical, dotted lines in Figs. 20-26 denote the C-T intervals corresponding to 10% and 90% of the total recovery. All but one of the stimulation sites were tested using more than one current which is given (in uA) in the upper left- or lower right-hand corner of each graph.

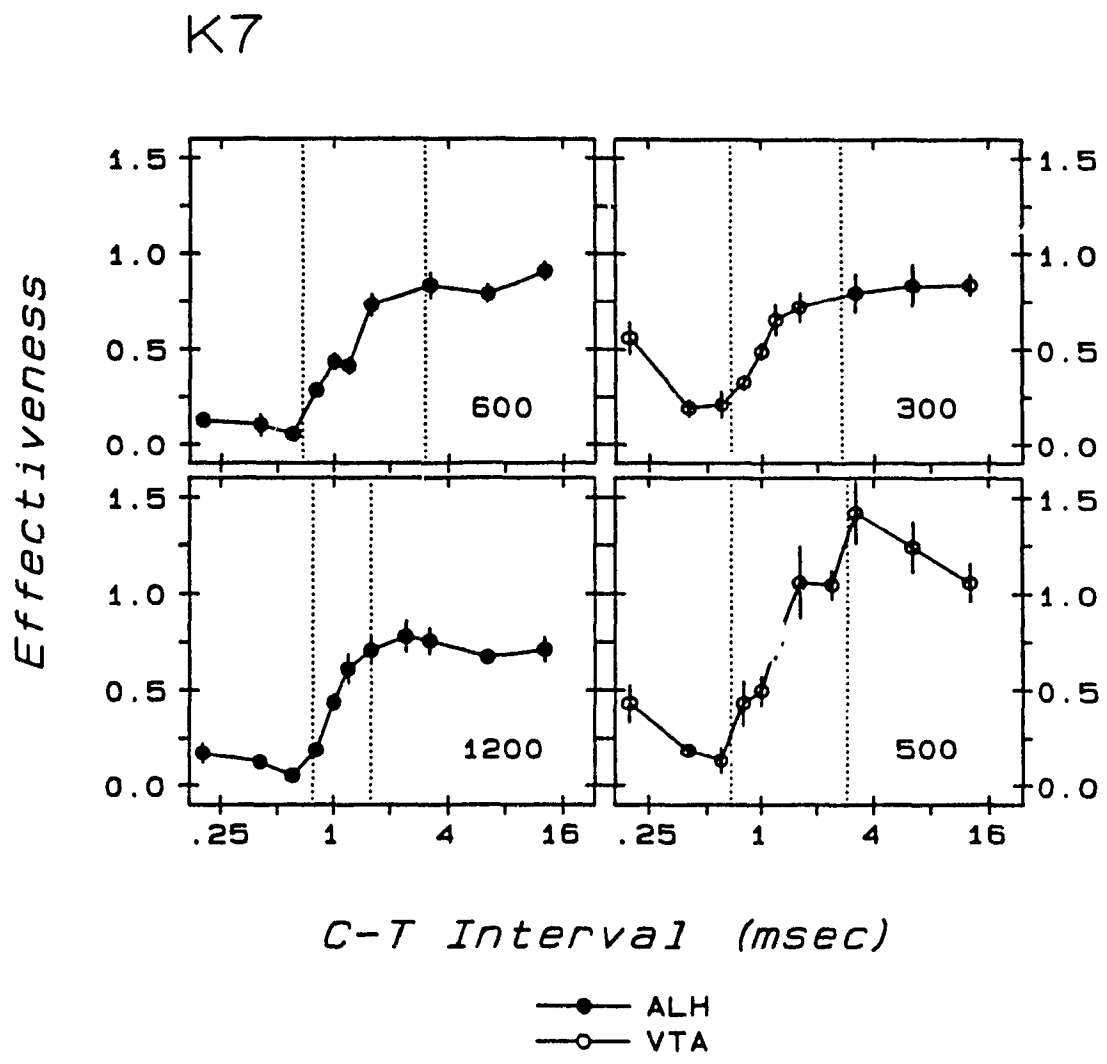
Figs. 20-25. Refractory period data for the six subjects tested in the collision experiment. The alphanumeric on the top of each figure identifies the subject. Data collected from stimulation of the ALH (filled symbols) are shown on the left and data collected from stimulation of the VTA (open symbols) are shown on the right. The current (uA) is given in the upper left- or lower right-hand corner. Data points without error bars correspond to cases where the s.e.m. was less than the radius of the symbol.


K2
500
750
1125
350
1.0
0.5
0.0
.25
1
4
16
Effectiveness
C-T Interval (msec)
ALH
VTA


K4
Effectiveness
1.0
0.5
0.0
300
280
600
480
.25
1
4
16
C-T Interval (msec)
ALH
VTA


K5
Effectiveness
1.0
0.5
0.0
530
170
800
200
1200
300
.25
1
4
16
C-T Interval (msec)
ALH
VTA


K6
1.0
0.5
0.0
300
230
600
350
1200
500
.25
1
4
16
Effectiveness
C-T Interval (msec)
ALH
VTA


K7
Effectiveness
1.5
1.0
0.5
0.0
600
300
1200
500
.25
1
4
16
C-T Interval (msec)
ALH
VTA

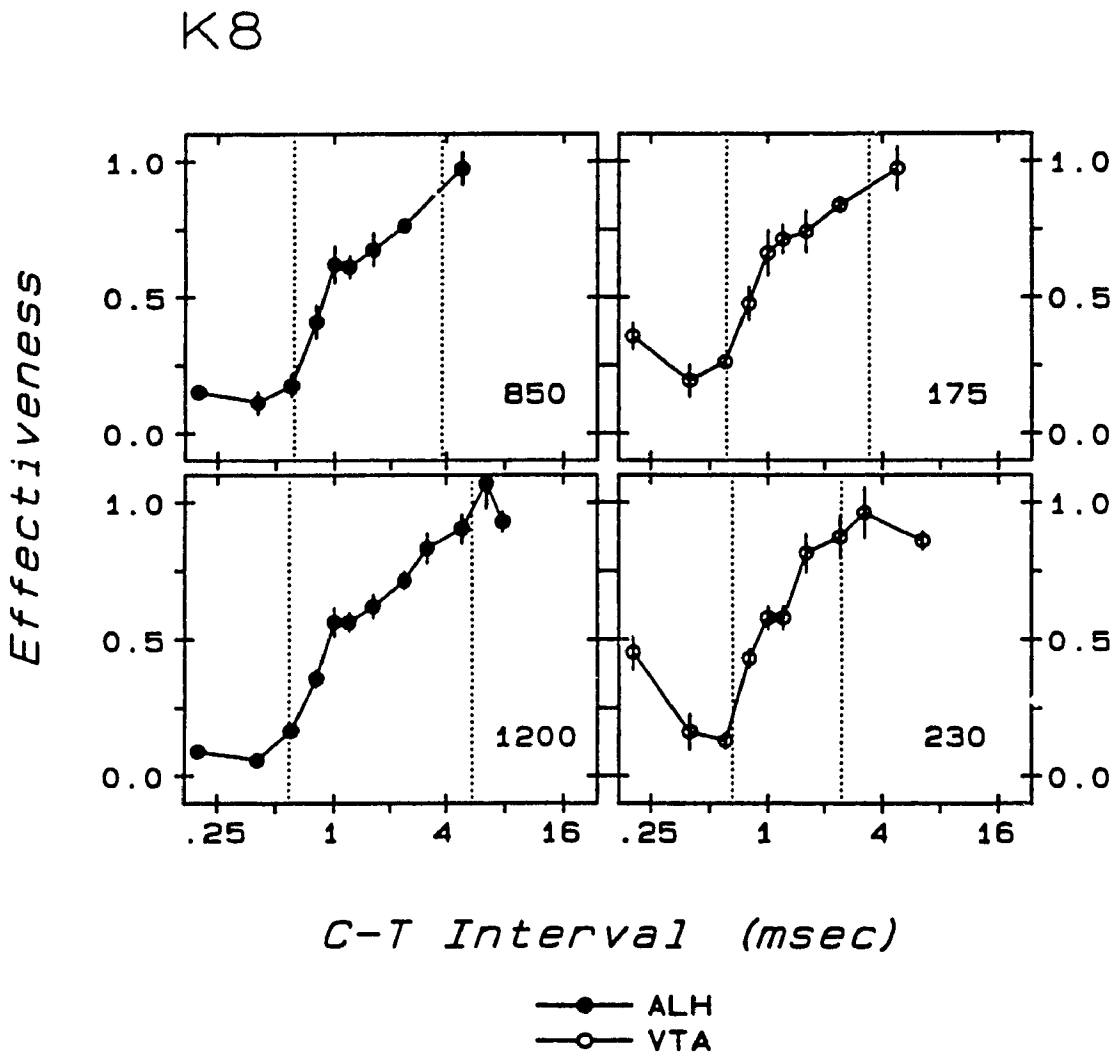
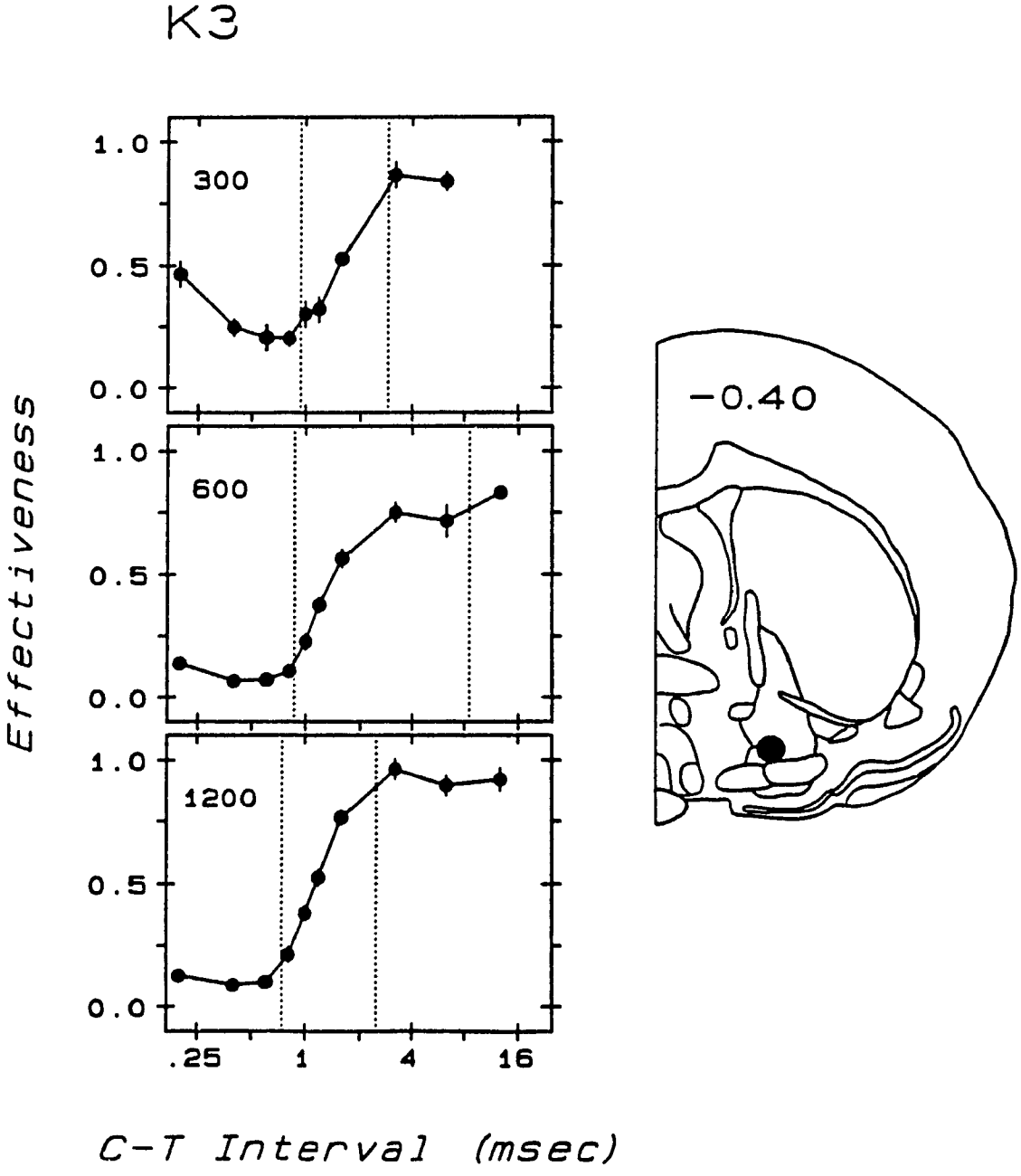

K8
Effectiveness
1.0
0.5
0.0
850
175
1200
230
.25
1
4
16
C-T Interval (msec)
ALH
VTA

Fig. 26. Refractory period data obtained for stimulation of the anterior stimulation site in subject K3. The current (uA) is given in the upper left-hand corner. Data points without error bars correspond to cases where the s.e.m. was less than the radius of the symbol. The electrode tip location for this subject is shown on a tracing of a coronal plate (-0.4 mm behind bregma) from the Paxinos and Watson (1986) atlas.


K3
1.0
0.5
0.0
300
600
1200
.25
1
4
16
Effectiveness
C-T Interval (msec)
-0.40

In the majority of the refractory period curves (24/31), the beginning of recovery was estimated to occur between C-T intervals of 0.6 and 0.8 msec. Due to the fact that C-T intervals between 0.6 and 0.8 msec were not tested, more precise estimates of the beginning of recovery cannot be made with any confidence. Therefore, the beginning of recovery might have been as early as 0.6 msec. In the case of subject K3, recovery did not begin until C-T intervals ranging from 0.8-1.0 msec for 2 of the 3 currents tested. The electrode for this subject was more anterior than the other subjects and was near the ventral border of the VP. Five of the refractory period curves began to rise between C-T intervals of 0.40 and 0.60 msec; 3 of these were collected from stimulation of the ALH (K6 and K8) and 2 from stimulation of the VTA (K4 and K5).

The end of recovery occurred between C-T intervals of 1.6 and 3.2 msec in 17 of the 31 curves. More precise estimates cannot be made because intermediate values were not tested in these subjects. In 4 additional curves, recovery was 90% complete between C-T intervals of 2.4 and 3.2 msec or between 2.4 and 4.8 msec. Recovery was 90% complete in 6 of the curves between C-T intervals of 1.2 and 1.6 msec; 4 of these curves were obtained from stimulation delivered to the more posterior of the ALH sites (K6 and K7). A more gradual recovery was obtained in 4 of the curves (2 ALH, 1 VP, 1 VTA) with 90% recovery occurring between 6.4 and 12.8 msec or between 3.2 and 6.4 msec.

In the case of several of the refractory period curves, effectiveness values stopped rising temporarily at a C-T interval of around 1.0 msec. This plateau frequently occurred between 1.0 and 1.2 msec, sometimes occurred at

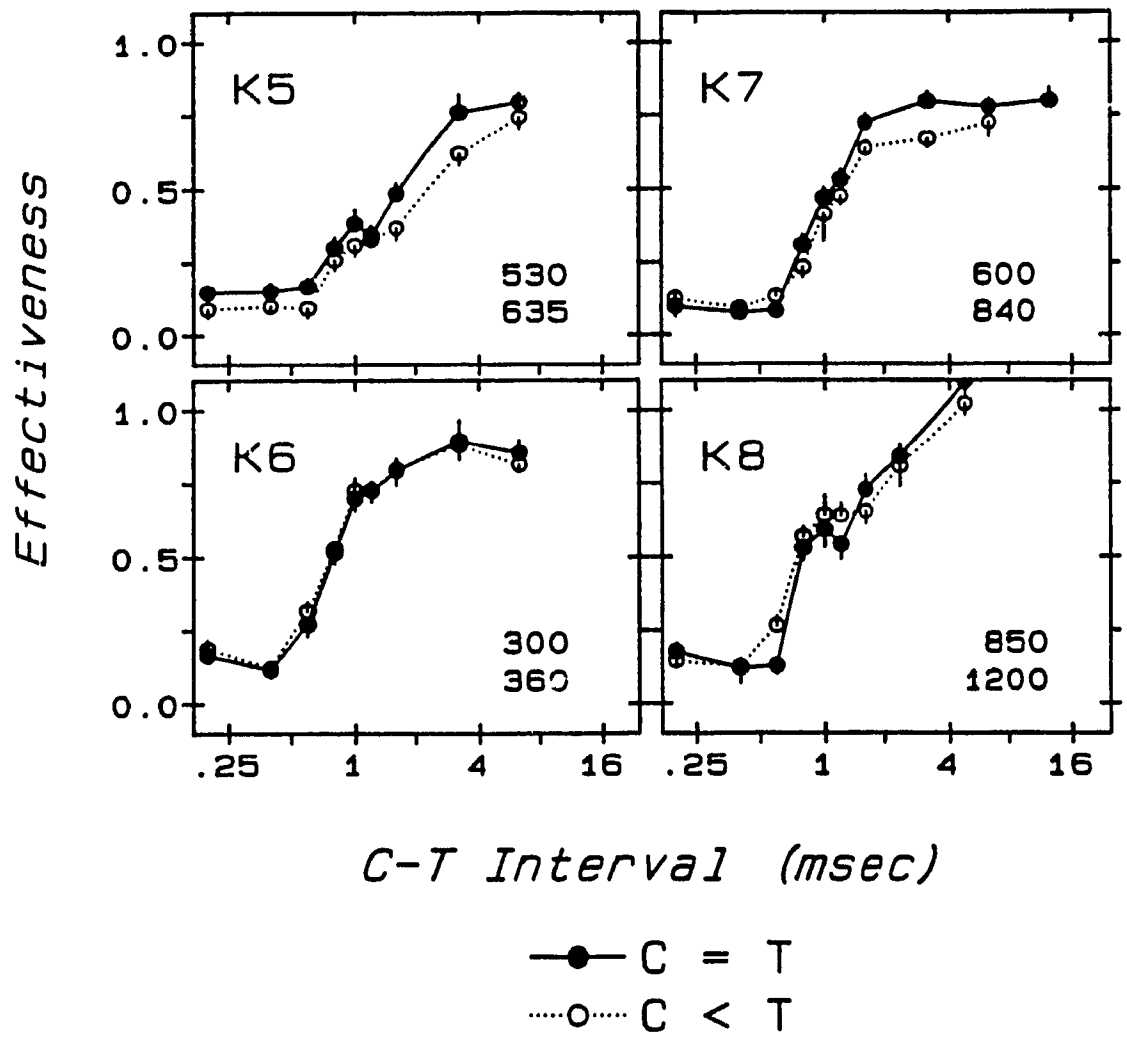
more than one current, and was seen at both ALH and VTA stimulation sites (ALH: K4, K5 and K8, all currents, K3 and K7, low current; VTA: K4, low current, K8, all currents). In 3 of the subjects in which this plateau was observed for the ALH stimulation site, increasing the amplitude of the T-pulse did not eliminate the plateau (see K5, K7 and K8, Fig. 27).

Effect of Current.

Increasing the current of both the C- and T-pulses did not produce any consistent effects on the range of C-T intervals over which recovery occurred. In some cases, increasing the current delayed the end of recovery (K4, VTA site; K6, VTA site), in some cases it hastened the end of recovery (K2, ALH site; K8, VTA site), and in the majority of cases it had little or no effect on the range of C-T intervals over which recovery occurred.

Similarly, increasing the current of the T-pulses had little or no effect on the range over which recovery occurred for the ALH sites tested in this condition. Fig. 27 shows the equal- (filled symbols) and unequal- (open symbols) pulse refractory period curves for the 4 subjects tested in both conditions. The current (in uA) of the C- (top number) and T- (bottom number) pulses is given in the lower right-hand corner of each graph. Increasing the amplitude of the T-pulse by 1.2-1.4 times that of the C-pulse did not hasten the end of recovery in any of the curves. The beginning of recovery was slightly earlier in the unequal-pulse condition for subject K8.

Fig. 27. Equal-pulse (filled symbols) and unequal-pulse (open symbols) refractory period data obtained for the four subjects tested in this condition. In the case of the unequal-pulse condition, the lower of the two numbers shown on each panel gives the C-pulse current (in uA) whereas the higher number gives the T-pulse current. The lower current was used for both the C- and T-pulses in the equal-pulse condition. Data points without error bars correspond to cases where the s.e.m. was less than the radius of the symbol.

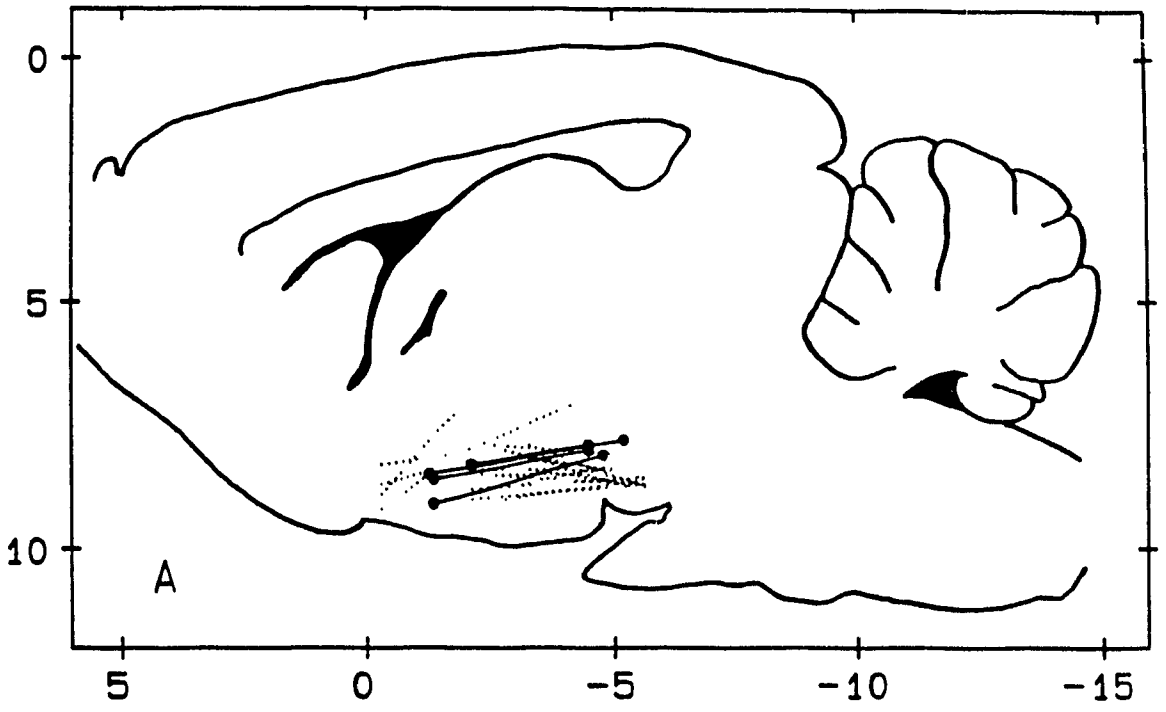

K5
530
635
K7
600
840
K6
300
360
K8
850
1200
1.0
0.5
0.0
.25
1
4
16
Effectiveness
C-T Interval (msec)
C = T
C < T

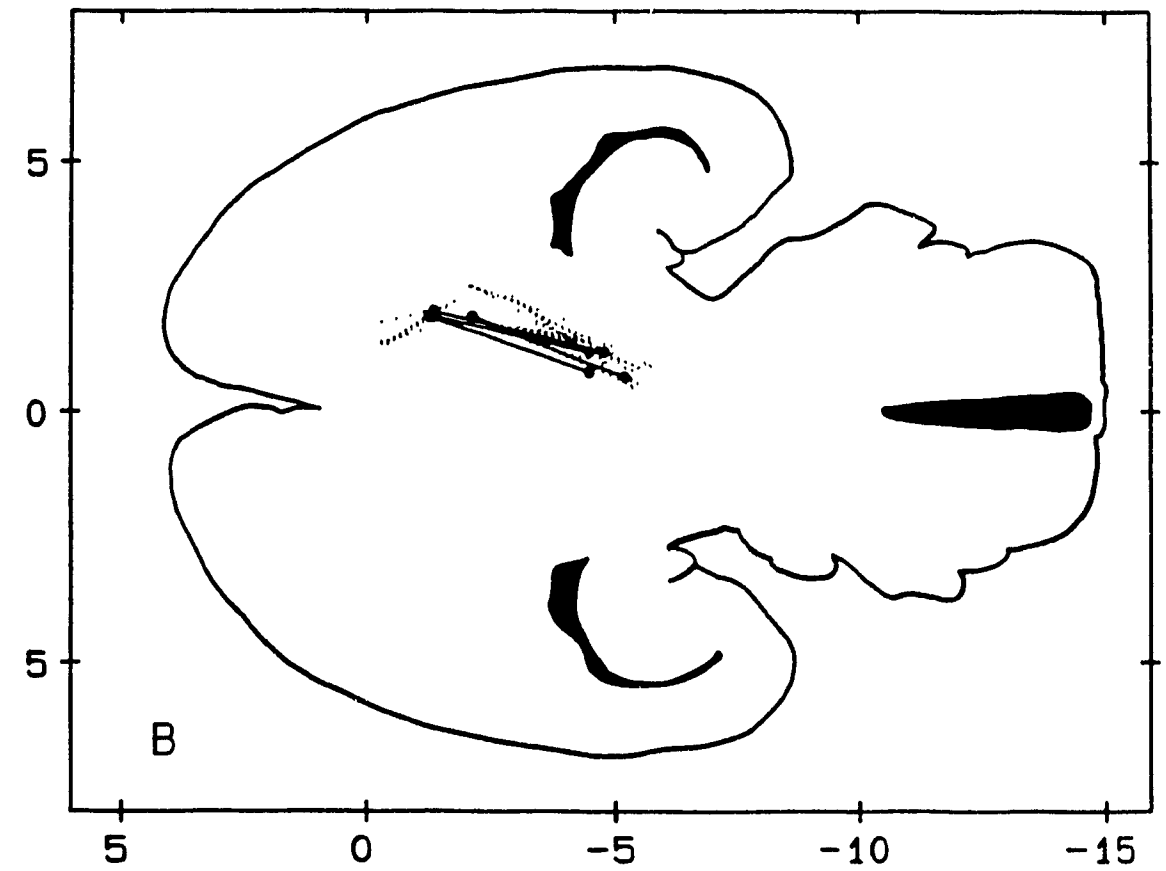
Discussion

In 4 of the 6 subjects tested in the collision experiment, stimulation effectiveness increased with C-T interval in a manner independent of the electrode delivering the C-pulses. These data are consistent with recovery from collision block in reward-relevant neurons directly linking the ALH and VTA. The location of the pairs of self-stimulation sites yielding collision-like effects in this study are summarized in a schematic fashion in Fig. 28. Pairs of stimulation sites that have yielded collision-like effects from previous studies are joined by dotted lines whereas the pairs of stimulation sites from the present study have been joined by solid lines. What is apparent from this figure is that the collision sites from the present study bridge the small gap that had existed between the anterior and posterior cluster of collision sites. These data support the notion that the axons of some reward-relevant neurons directly link the anterior and posterior MFB.

If at least some reward-relevant neurons directly link the ALH and VTA, one would predict that refractory period estimates for the two sites would also show some overlap. Refractory period estimates for the two stimulation sites were substantially the same, supporting the view that some of the reward-relevant neurons at the anterior MFB sites were common to the posterior sites. Refractory period estimates were obtained in order to calculate conduction velocity estimates for the reward-relevant neurons undergoing collision and for comparison with the electrophysiological estimates obtained in Experiment 3.

Fig. 28. Schematic representation of the pairs of self-stimulation sites that yielded collision-like effects in Experiment 2 (solid lines) and in previous collision studies (dotted lines). The tips of the two stimulating electrodes for each pair have been joined by straight lines. The stimulation sites are shown in the sagittal (panel A) and the horizontal (panel B) plane. Tracings of the brain were taken from the Paxinos and Watson (1986) atlas. The numbers along the bottom of each panel give the anterior-posterior distance (mm) from bregma; numbers along the side of each panel give the dorsal-ventral distance from bregma (panel A) or the medial-lateral distance from the midline (panel B).


0
5
10
A
5
0
-5
-10
-15


5
0
5
B
5
0
-5
-10
-15

Refractory Period Data

The majority of the refractory period curves obtained from stimulation of ALH and VTA sites began to rise over C-T intervals of 0.6-0.8 msec and finished their rise over C-T intervals of 1.6-3.2 msec. In a few cases, recovery from refractoriness began earlier or finished later, however there was no tendency for these cases to be associated with one of the two sites. There was a tendency for the curves obtained from stimulation of the more posterior ALH sites to finish their recovery at earlier C-T intervals (1.2-1.6 msec). This is consistent with previous refractory period estimates obtained from stimulation of middle to posterior LH; these curves have generally been found to asymptote at C-T intervals between 1.2 and 2.0 msec (Bielajew & Shizgal, 1982, 1986; Bielajew et al., 1982; Bielajew et al., 1981; Macmillan et al., 1985; Rompré & Miliaressis, 1980; Schenk & Shizgal, 1982; Yeomans, 1975, 1979), earlier than the end of recovery observed for most of the ALH and VTA sites tested here. Bielajew and Shizgal (1986) also found that recovery from refractoriness at their VTA stimulation sites was in some cases more prolonged than recovery at their mid-LH stimulation sites. Thus, it would appear that stimulation of sites in the ALH and VTA recruits more slowly recovering reward-relevant neurons than stimulation of the middle LH.

It is unlikely that the gradual rise in the ALH refractory period curves is due to the recruitment of a homogeneous population of neurons with a pronounced relative refractory period given the similarity of the data obtained in the equal and unequal pulse conditions. Relative refractory period contributions should be reduced or eliminated by larger amplitude T-pulses since the greater current density should fire more neurons in this

hypoexcitable phase of their excitability cycle (Yeomans, 1979). Increasing the amplitude of the T-pulses by 1.2-1.4 times that of the C-pulses did not hasten the end of recovery at any of the 4 ALH sites tested in this condition. Bielajew et al. (1982) found that larger amplitude T-pulses hastened recovery by up to 2.5 msec at 5 out of 6 of their stimulation sites which were located in more posterior MFB and surrounding regions. In one of their subjects, a T-pulse intensity equal to 1.73 times that of the C-pulse was required in order to shorten the time course of recovery. Thus, although these data support the idea that relative refractory period contributions are not pronounced for ALH sites, it is possible that had larger intensity T-pulses been used in this study, a more substantial effect of the relative refractory period would have been detected. Nonetheless, the data obtained at this time support the hypothesis that the gradual recovery in the refractory period curves obtained at ALH sites is due primarily to the recruitment of several subpopulations of neurons with a range of absolute refractory periods.

The plateau in effectiveness values that was occasionally observed at C-T intervals of around 1.0-1.2 msec may reflect a discontinuity in the distribution of refractory periods at some ALH and VTA self-stimulation sites. Similar explanations have been offered to explain plateaus observed in refractory period curves obtained for self-stimulation sites on or near the midline in the metencephalon (Rompré & Miliaressis, 1987) and for more posterior LH sites (Gratton & Wise, 1985), however in these cases the plateau occurred at different C-T intervals. Other MFB refractory period studies have obtained plateaus in effectiveness values between 1.0-1.2 msec

for at least one of their subjects (Gratton & Wise, 1985; Rompré & Miliaressis, 1980) so this finding does not appear to be entirely peculiar to this study. The fact that the plateau occurred around C-T intervals of 1.0-1.2 msec may be significant. Refractory period curves obtained from some LH and VTA sites have been found to reach their maximum effectiveness values by C-T intervals as short as 1.2 msec (see references above). In contrast, some self-stimulation sites in the anterior MFB and nearby basal forebrain regions have been found to begin their recovery from refractoriness at C-T intervals as long as 1.0 msec (Fouriezos et al., 1987). One subject in the present study (K3) with an electrode near the anterior MFB in the ventral aspect of the VP also exhibited a delay in the beginning of recovery. Thus, the plateau in effectiveness values that was found to occur at C-T intervals of around 1.0 msec may reflect these two populations of reward-relevant neurons, one more slowly recovering population that is recruited at the anterior MFB sites and a faster population that is evident at posterior MFB sites.

Collision Data

Previous collision studies (Bielajew & Shizgal, 1986; Shizgal et al., 1980) have found that the rise in effectiveness evident at high currents was eliminated when the current was decreased. This finding is consistent with the idea that the alignment of the stimulation fields within the MFB determines whether or not a collision-like effect is observed. At low currents, the small cross-sectional area of the stimulation fields is less likely to recruit the same reward-relevant neurons at the two stimulation sites. At

higher currents, the partial nature of the collision-like effects is due to imperfect alignment of the electrodes within the bundle of reward-relevant axons so that only some of the reward-related neurons project through the cross-sectional area of the two stimulation fields.

In the case of subject K2, the effect of current on the collision curves was similar to that reported by Bielajew and Shizgal. At the lowest pair of currents tested, the effect of C-T interval on effectiveness values was not significant. At higher currents, effectiveness values increased with increasing C-T interval. These data are consistent with the notion that the reward-relevant neurons undergoing collision were located at some distance from the electrode tips and therefore only recruited at the higher currents. Nonetheless, a more trivial explanation can be proposed to account for these effects. At the beginning of the testing period for subject K2, a current of 350 uA delivered to the VTA resulted in a required number of 11.9 pulses. At a later point in time when the low current collision test was run, 350 uA delivered to the VTA resulted in a required number of 16.2 pulses, which is an increase of 36%. Thus, the lack of a significant collision-like effect at the end of the testing period for this subject could be due to a shift in the position of the VTA electrode or to an inadvertent lesion made through the VTA stimulating electrode.

Current-dependent effects that could not be attributed to a sudden shift in thresholds were also obtained in the remaining subjects. The magnitude of the collision effect increased with current for subjects K6 and K5 suggesting that the higher currents recruited proportionately more reward-relevant neurons undergoing collision than the lower currents for these subjects. It is

interesting to note that effectiveness values decreased with current for subject K5. These two findings appear to be contradictory; increasing the current recruited proportionately more reward-relevant neurons common to the two sites and yet the stimulation was less effective at the longer C-T intervals. Similarly, it appears contradictory that the subject with the highest overall effectiveness values (K2) was the subject with the smallest collision effect.

These two features of the collision curve, maximum effectiveness values at long C-T intervals and the percent change in effectiveness values, are thought to reflect the properties of different stages of the reward-relevant substrate. Recall that the percent change in effectiveness values is presumed to reflect the proportion of reward-relevant neurons undergoing collision (p. 9). For instance, E-values that go from 0.5 to 1.0 would occur if half of the neurons stimulated at each site undergo collision. On the other hand, maximum effectiveness values at long C-T intervals are presumed to reflect the characteristics of postsynaptic elements that integrate activity in the first stage neurons. In practice, the integration of the activity from the two sites is rarely perfect, thus E-values rarely reach 1.0 at the long C-T intervals. For instance, if the required number of single pulses at each stimulation site is found to be 20, then imperfect summation would imply that the required number of pulses pairs when stimulating <u>both</u> sites (at long C-T intervals) is greater than 10 (10 pulse pairs = 20 pulses). Thus, a total of 24 pulses might be needed to produce half maximal responding when stimulating both sites (12 per site) as compared to only 20 pulses when stimulating one site. As long as the influence of this imperfect summation

is proportionally the same for all C-T intervals, then the percent change in collision will still reflect the percentage of neurons undergoing collision. For example, if 50% of the neurons are undergoing collision and summation at all C-T intervals is only 80% of maximum, then E-values will range from 0.4 (0.5 x 80%) to 0.8 (1.0 x 80%) which is still a 50% collision effect.

According to these assumptions, it is theoretically possible for these two features of the collision curves, maximum effectiveness values at long C-T intervals and the percent change in effectiveness values, to vary independently. In the case of subject K5, increasing the current may have recruited proportionately more reward-relevant neurons undergoing collision and in addition recruited a population of neurons that somehow influenced the postsynaptic processes responsible for integration of the activity in the directly activated neurons. This could result in an increase in the magnitude of the collision effect and also a decrease in the effectiveness of the stimulation at long C-T intervals.

In the case of subject K4, increasing the current decreased the size of the collision effect, an effect that could be due to the alignment of the two electrodes within a bundle of reward-relevant neurons. In this particular example, the neurons undergoing collision would have to pass close to the tips of the two electrodes so that the effect of increasing the current was to recruit proportionately fewer reward-relevant neurons linking the two sites. The data from this subject also demonstrate the relative independence of the maximum overall effectiveness values and the percent collision. Maximum effectiveness values increased and then decreased with current whereas the percent collision consistently decreased with current.

Conduction Velocity Estimates.

Recall that recovery from collision block can only occur after the volley of action potentials initiated at the C-pulse electrode (and their trailing refractory period zone) has had time to pass by the T-pulse electrode. The collision interval is therefore theoretically equal to the sum of the conduction time between the stimulation sites and the refractory period of the neurons stimulated by the T-pulses. Estimates of the conduction velocity for the reward-relevant neurons undergoing collision were obtained by subtracting the refractory period estimate from the collision interval (giving the inter-electrode conduction time) and dividing by the inter-electrode distance computed on the basis of the histological coordinates. The overlap in the refractory period range obtained at the ALH and VTA sites was used in the calculation of conduction time based on the assumption that the neurons undergoing collision would be more likely to have the same refractory period at each stimulation site. The end of recovery in the refractory period curve was subtracted from the end of the collision interval based on the assumption that the neurons with the longest refractory periods would also tend to have the slowest conduction velocities. According to these assumptions, the slowest conduction velocity estimates for the 4 subjects ranged from 0.4 to 1.65 m/sec, which is less than the slowest estimates of 1 m/sec that have been obtained for LH and VTA collision sites.

A difficulty arises when trying to calculate the fastest conduction times. The beginning of the collision interval was estimated to occur at roughly the same C-T interval as the beginning of recovery in the refractory period

curve, thus leading to conduction times that either approached zero or in two cases were negative. It is likely that this occurred because the spacing between C-T intervals was too large. The beginning of the collision interval occurred between C-T intervals of 0.4 and 0.8 msec and the beginning of recovery from refractoriness occurred between C-T intervals of 0.6 and 0.8 msec in each of the 4 subjects. It is possible that the beginning of the collision interval was as late as 0.79 msec and that the beginning of recovery in the refractory period curve was as early as 0.61 msec. The most conservative estimates of the fastest conduction velocities will therefore be obtained by assuming that the longest possible conduction time was 0.18 msec. Assuming a conduction time of 0.18 msec, the fastest estimates of the conduction velocity ranged from 18-20 m/sec, which is considerably greater than the fastest estimates of 8 m/sec that have been obtained for LH and VTA collision sites.

The estimates of conduction velocity for the reward-relevant neurons linking the ALH and VTA includes both faster and slower estimates than those previously obtained for reward-relevant neurons linking the LH and VTA. This finding is readily apparent from the striking difference between the steepness of the LH-VTA collision curves compared to the ALH-VTA collision curves. The range of the collision interval in the present study varied from 4.4 to 10.3 msec, consistent with recovery from collision block in a heterogeneous population with a wide range of conduction velocities and refractory periods. Earlier collision studies using LH and VTA stimulation sites had generally found that the rise in effectiveness occurred over a very narrow range of C-T intervals, as small as 0.5 msec, consistent with

recovery from collision block in a homogeneous population of neurons with a narrow range of conduction velocities and refractory periods. What is puzzling about these data is why the heterogeneous population that is evident in the collision curves obtained for ALH and VTA sites is not evident in the collision curves obtained for sites in the LH and VTA since presumably the neurons linking the ALH and VTA would have to project past the LH on their way to or from the VTA.

Comparison with Previous LH-VTA Collision Studies.

Most of the collision curves reported in earlier studies with LH and VTA electrodes rose over a very short range of C-T intervals, less than 0.5 msec. Indeed the range may have been shorter in many cases since the increase in effectiveness occurred between two adjacent C-T intervals. In the three studies published by Shizgal and Bielajew in which they report LH-VTA collision effects, collision curves obtained from 12 of the 15 subjects rose within 2 adjacent C-T intervals (Bielajew & Shizgal, 1982; 1986; Shizgal et al., 1980).

In addition to the collision data obtained by Bielajew and Shizgal, Gratton and Wise (1988) have also reported a collision-like effect for LH and VTA self-stimulation sites that rose sharply between two adjacent C-T intervals separated by only 0.5 msec. The collision curve obtained from the second subject in their study was more gradual; it rose over C-T intervals ranging from 1.0 to 3.5 msec. The LH-VTA collision data reported by Durivage and Miliaressis (1987) were more consistent with the gradually rising collision

curves found in the present study. Their collision curves rose over a 1.2 to 8.5 msec range of C-T intervals.

One possible explanation for the differences in the slope of the collision curves across studies is the location of the MFB stimulation sites. It could be the case that some MFB stimulation sites recruit a more heterogeneous population of reward-relevant neurons than other MFB sites. Murray and Shizgal (1992) have recently obtained LH-VTA collision effects that more closely resemble the collision effects obtained in this study for stimulation of the ALH-VTA. The range of the collision interval varied from 2.2-7.7 msec across the 5 subjects and effectiveness values were lower than many of the previously reported LH-VTA collision effects. Thus, it appears that at least for some mid LH sites, the collision effects can be as gradual as those reported here.

Given the known topographic organization of the constituent pathways of the MFB, small differences in the location of the stimulation sites could substantially alter the composition of neurons recruited by the stimulation (Nieuwenhuys et al., 1982; Veening et al., 1982; Geeraedts et al., 1990a; Geeraedts, Nieuwenhuys, & Veening, 1990b). Comparison of the stimulation sites across studies reveals that there are consistent differences between studies, particularly in the location of the posterior electrodes. The VTA sites used in the Bielajew and Shizgal studies are more posterior than the VTA sites used in the present study and the study by Murray and Shizgal (1992). However, not all of Bielajew and Shizgal's abruptly rising collision curves are from stimulation of the caudal portion of the VTA (e.g. B-1 and D-29) and their only gradually rising collision curve was obtained from their

most posterior VTA stimulation site (CB-1, although this placement was also slightly dorsal to the VTA). In addition, Gratton and Wise (1988) obtained an abrupt collision effect from stimulation of an anterior VTA site (AG813). Thus, it seems unlikely that the stimulation of anterior versus posterior VTA sites can completely explain the differences between the abrupt and gradual collision effects although it is possible that the effect depends upon a more subtle pattern of alignment. For instance, the more posterior VTA sites used in the Durivage and Miliaressis study tended to be more medial than those used by Bielajew and Shizgal and the anterior VTA site in the Gratton and Wise study (AG813) was more lateral than most of the VTA sites in the present study.

Although there is no clear anatomical explanation for the differences between the self-stimulation sites yielding abrupt versus gradual collision effects, we suspect that there are some relevant differences between our stimulation sites and those used by Bielajew and Shizgal as well as Gratton and Wise since our effectiveness values at long C-T intervals were substantially lower than the maximum effectiveness obtained in these studies. Direct comparison of the E-values obtained here and in the study by Durivage and Miliaressis (1987) is not possible since they used a different scaling formula that tended to push their E-values to 1.0 at long C-T intervals.

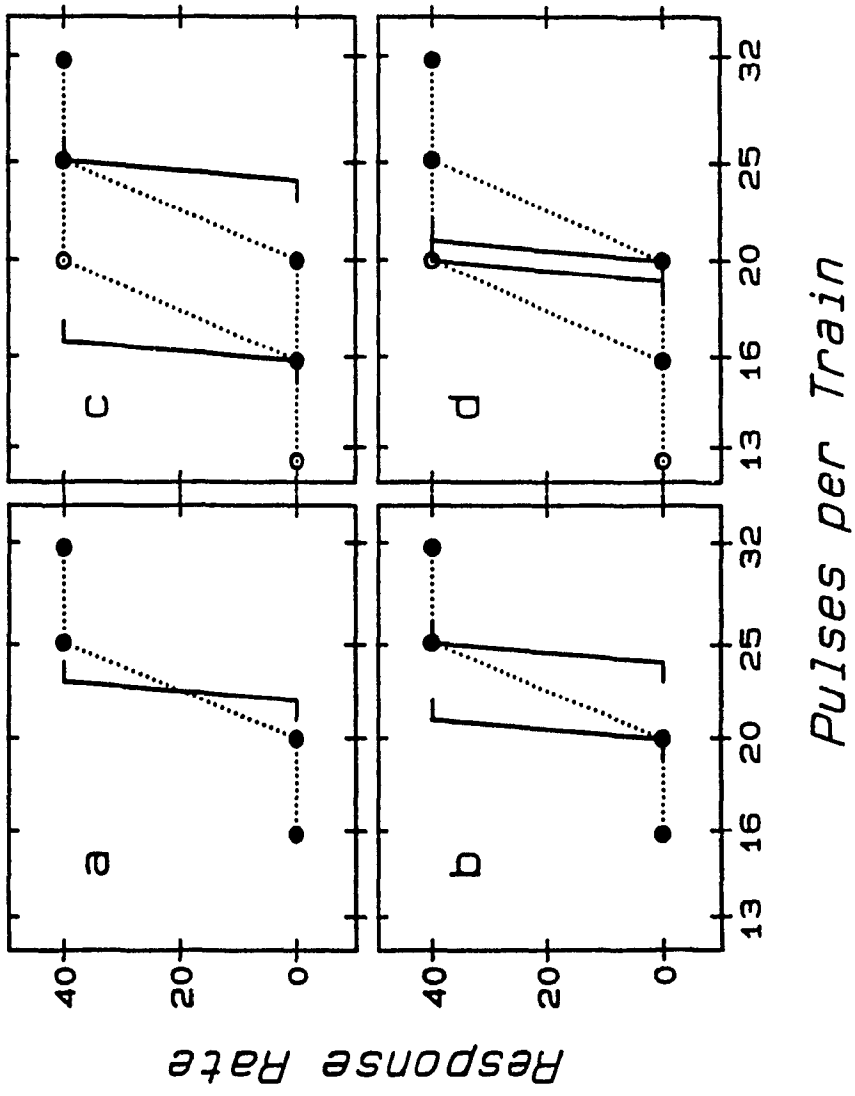
In order to more directly assess the effect of stimulation site on the collision effect, it would be preferable to test multiple MFB sites within the same subject, thus keeping constant any differences across subjects or across studies. Using a moveable electrode design, Boye and Rompré (1992) have

made such a within-subject comparison of collision curves obtained for self-stimulation sites in the VTA and central grey. They have found that relatively small movements of the electrode (around 0.16 mm) can result in substantial alterations in the collision curve, lending support to the notion that differences in the location of the stimulation sites within the MFB could be responsible for the differences in the steepness of the collision curves. However, it should be pointed out that Boye and Rompré did not obtain any abruptly rising collision curves in the more than 46 sites they tested across their 6 subjects.

Effects of Undersampling the Rate-frequency Function

In addition to anatomical differences between the location of the stimulation sites, there also exist methodological differences between the studies that may account for some or all of the differences in the steepness of the collision curves. One difference between the present study and the earlier collision studies by Bielajew and Shizgal is the manner in which the rate-frequency (or rate-number) curves were obtained. In the Bielajew and Shizgal collision studies, the number of pulses per train was decreased in 0.1 $\log_{10}$ unit steps over successive trials whereas in the present study the number of pulses was decreased in 0.05 $\log_{10}$ unit steps. We have found that the rate-frequency curve frequently rises over the 0.05 $\log_{10}$ unit frequency steps that we have used. This suggests that previous collision studies may have undersampled the underlying rate-frequency function and that this could have potentially steepened the rising portion of the collision curves.

Fig. 29. The effects of undersampling. The rate-number function (solid line) in panel 'a' is much steeper than the rate-number curve (dotted line) obtained by sampling in 0.1 $\log_{10}$ unit steps. In panel 'b', two rate-number functions (solid lines) separated by 0.08 $\log_{10}$ units result in the same sampled rate-number curve (dotted line). A 0.18 $\log_{10}$ unit (panel 'c') and a 0.02 $\log_{10}$ unit (panel 'd') shift in the rate-frequency function (solid lines) results in the same 0.10 $\log_{10}$ unit shift in the rate-frequency curve (dotted lines, open and filled circles).


a
b
c
d
40
20
0
13
16
20
25
32
Response Rate
Pulses per Train

The effects of undersampling are illustrated in Fig. 29. Hypothetical rate-frequency functions (solid lines) rise over an arbitrary 0.02 $\log_{10}$ unit range in frequency. The rate-frequency function is continuous. However, we can only test a finite number of frequencies, and thus the rate-frequency curve (dotted lines) is a digitized approximation of the function and is obtained by sampling the rate-frequency function at discrete values of frequency. If the sampling is done using steps that are larger than the range over which the function rises, (in this case using 0.1 $\log_{10}$ unit steps), then the rate-frequency curve will not accurately represent the rate-frequency function. Panel 'a' of Fig. 29 illustrates such a case of undersampling, the rate-frequency curve is much shallower than the rate-frequency function it is supposed to represent.

In addition to altering the shape of the rate-frequency curve, undersampling also introduces uncertainty about the exact position of the rate-frequency function along the abscissa. Panel 'b' of Fig. 29 shows two rate-frequency functions shifted by 0.08 $\log_{10}$ units along the frequency axis. Sampling these functions at the same 0.10 $\log_{10}$ unit steps can result in the same values in the rate-frequency curve (dotted line). If we perform some manipulation which produces a shift in the rate-frequency curve (e.g. change the C-T interval or make a brain lesion), the uncertainty has now doubled; the corresponding shift in the rate-frequency function could be between 0.18 $\log_{10}$ units (panel 'c') and 0.02 $\log_{10}$ units (panel 'd').

The effects of undersampling the rate-frequency function in the collision experiment will depend upon the nature of the collision effect. A gradually rising collision curve would be obtained if increasing the C-T interval over

some range (defined as the collision interval) resulted in small shifts in the corresponding rate-frequency functions. These intermediate rate-frequency functions would be bracketed by two clusters of rate-frequency functions; a high frequency cluster corresponding to C-T intervals that are shorter than the collision interval, and a low frequency cluster corresponding to C-T intervals that are longer than the collision interval.

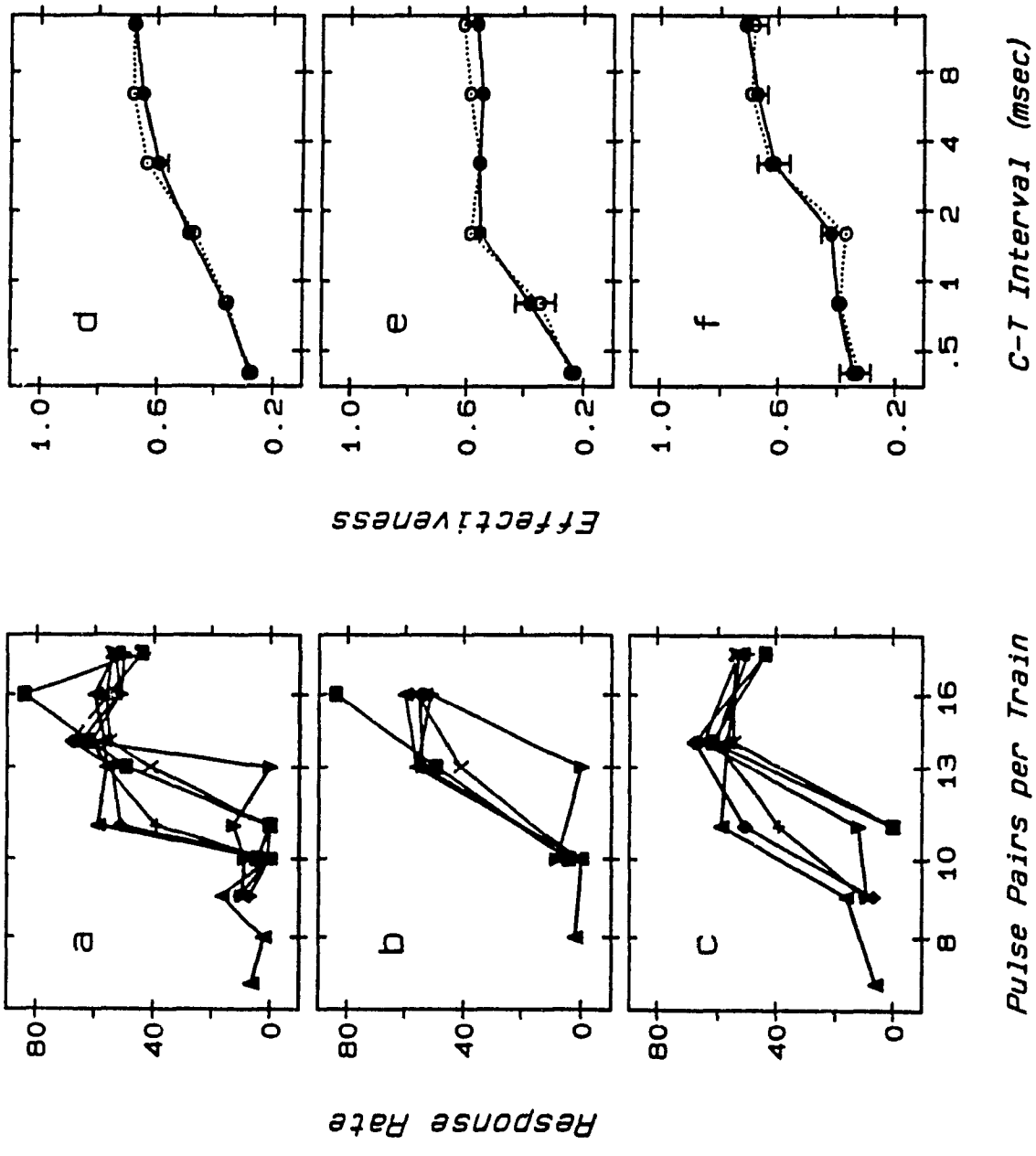
If the separation between the high and low frequency cluster is on the order of 0.10 $\log_{10}$ units, then undersampling will produce rate-frequency curves that tend to fall into one of two clusters. For example, all the functions that rise between 16 and 20 pulses will result in one cluster and all of the functions that rise between 20 and 25 pulses will result in the second cluster. The rate-frequency curve obtained at each of the C-T intervals tested is assigned an effectiveness value which is inversely related to the position of the curve along the frequency axis. One of two E-values is therefore assigned to the two clusters of rate-frequency curves and the result is a collision curve with an abrupt rise in effectiveness values.

Undersampling can also explain why the refractory period curves for posterior MFB sites appeared to rise more gradually than the collision curves for these same sites. Recall (p. 9) that an E-value of 0 is assigned to a particular C-T interval if the required number of pulse pairs for that interval is equal to the required number of single pulses. An E-value of 1 is assigned to a particular C-T interval if the required number of pulse pairs for that interval is equal to one half the required number of single pulses. Thus, an increase in E-values ranging from 0 to 1.0 corresponds to a 50% decrease in the required number of pulse pairs or a shift of 0.3 $\log_{10}$ units.

Thus, the maximum shift in the underlying rate-frequency curve that can occur in either the refractory period or collision experiment is 0.3 $\log_{10}$ units. In general, the magnitude of the LH-VTA collision effects has ranged from 40% to 60% which corresponds to a shift in the underlying rate-frequency curves on the order of 0.10 $\log_{10}$ units. If the frequencies are sampled in 0.10 $\log_{10}$ unit steps, then only 2 clusters of rate-frequency curves will be obtained for a collision effect of this magnitude. E-values in the refractory period experiment have been found to span a wider range than in the collision experiment, often close to the maximum of 0 to 1.0. Thus, the underlying rate-frequency curves in the refractory period experiment span close to a 0.30 $\log_{10}$ unit range of frequencies. This would result in 3 clusters of rate-frequency curves if sampling in 0.1 $\log_{10}$ unit steps. Effectiveness values in the refractory period experiment would therefore tend to take on one of 3 values (low, medium, high) whereas effectiveness values in the collision experiment would tend to take on one of two values (low or high), leading to the false impression that effectiveness values in the refractory period curve increased over a wider range of C-T intervals than in the collision curve.

In order to determine whether sampling at a coarser grain could produce artifactually steepened collision curves, LH-VTA collision data obtained from a subject in the Murray and Shizgal (1992) study were re-analyzed using a 0.1 $\log_{10}$ unit sampling grain. Since the rate-frequency curves were originally collected by sampling at a 0.05 $\log_{10}$ unit grain, two new sets of rate-frequency curves, sampled at a 0.1 $\log_{10}$ unit grain, were created by selecting alternate points along the original curve. For instance, if the

Fig. 30. Re-analysis of collision data collected in a previous study (Murray & Shizgal, 1992). Rate-number curves from a single test session (panel 'a') sampled in 0.05 $\log_{10}$ unit steps. Each curve was collected at one of the six C-T intervals tested in this subject. Alternate points along each curve were selected to create two new sets of curves (panel 'b' and 'c') with a 0.1 $\log_{10}$ unit grain. Right-hand panels ('d','e','f') show the average effectiveness values corresponding to the sampling grain shown to the left. Undersampling the rate-number curves either advanced the end of recovery ('e') or delayed the start of recovery (f') depending upon the frequencies sampled. Data obtained in the LH-VTA condition (filled circles, solid line) are shown separately from data obtained in the VTA-LH condition (open circles, dotted line). Data points without error bars correspond to cases where the s.e.m. was less than the radius of the symbol.


Response Rate
Effectiveness
Pulse Pairs per Train
C-T Interval (msec)
a
b
c
d
e
f
80
40
0
1.0
0.6
0.2
8
10
13
16
.5
1
2
4
8

original curve consisted of points taken at 28, 25, 22, 20, 18 and 16 pulse pairs (or single pulses) per train, then one new curve would consist of the data obtained at 25, 20, and 16 pulses and the other new curve would consist of the data obtained at 28, 22 and 18 pulses. The number of pulse pairs (or single pulses) required for half-maximal responding was again interpolated from each new curve and scaled using the same effectiveness formula.

The result of the re-analysis is shown in Fig. 30. The actual rate-frequency curves (paired pulse condition) that were collected during one testing session are shown in panel 'a'. Each of the six rate-frequency curves were collected using a different C-T interval ranging from 0.4 to 12.8 msec. Alternate data points were selected from each curve and are shown in panels 'b' and 'c'. Sampling at the 0.1 $\log_{10}$ unit grain (panels b and c) tends to place intermediate rate-frequency curves into one of two clusters. The corresponding effectiveness values (averaged across several sessions) are shown on the right in panels 'd', 'e', and 'f'. Both sets of collision curves obtained with the 0.1 $\log_{10}$ unit grain (panels e and f) rise more abruptly than the original curves collected using a 0.05 $\log_{10}$ unit grain (panel d). The collision interval obtained for the original curve sampled at a 0.05 $\log_{10}$ unit grain was 0.8 to 3.0 msec (panel d). Using the same criteria for the beginning and end of recovery (20% and 80%), the two "undersampled" collision curves rise between C-T intervals of 0.6 to 1.4 msec (panel e) and 1.7 to 3.5 msec (panel f). The end of recovery from collision occurs earlier in panel 'e' whereas the beginning of recovery occurs later in panel 'f'.

The re-analysis of the data from this subject clearly demonstrates that sampling at a 0.10 $\log_{10}$ unit grain <u>can</u> produce artifactually steepened

collision curves due to undersampling. Whether the artifact consists of a delay in the beginning of the rise or an advance in the end of the rise depends upon the frequencies at which the underlying functions are sampled. These data, in addition to the observation that the rate-frequency curve can rise over a 0.05 $\log_{10}$ unit range of frequencies (e.g. panel a of Fig. 30), suggests that a sampling grain of 0.1 $\log_{10}$ units is inadequate and may have produced or contributed to the abrupt rise found in earlier LH-VTA collision studies.

It is not known whether undersampling accounts for all of the abruptly rising collision curves collected in previous studies, nor whether a smaller sampling grain can account for all of the gradually rising collision curves collected in the present study. For instance, Gratton and Wise (1988) obtained an abrupt collision effect despite the fact that they tested, when possible, at a finer sampling grain than that used in the present study (0.02 $\log_{10}$ units). However, if their rate-frequency curves rose over low values of pulse number, then a 0.02 $\log_{10}$ unit sampling grain would not have been possible. When using fewer than 19 pulses (or paired pulses) per train, the sampling grain is necessarily larger than 0.02 $\log_{10}$ units since the steps between pulse numbers below 19 are larger than 0.02 $\log_{10}$ units (only integer values of pulse number can be tested). Similarly, when using fewer than seven pulses (or paired pulses) per train, the sampling grain is necessarily larger than 0.05 $\log_{10}$ units. Thus, a 0.05 $\log_{10}$ unit sampling grain may not have been achieved in the case of the collision curves collected in this study at the higher currents and low pulse numbers. It would be interesting to ascertain whether the sampling grain used in the

collision studies by Durivage and Miliaresis (1987) and Bielajew et al. (1987), in which more gradual collision curves were obtained, was finer than 0.10 $\log_{10}$ units.

An adequate sampling grain for the collection of the rate-frequency curve would depend upon the range of frequencies over which the underlying functions rise. To my knowledge, there have not been any studies aimed at determining the precise range of the rate-frequency curve. Even if an adequate sampling grain could be determined, it may be impossible to test at this grain when using higher currents. High currents are frequently employed in collision tests in order to increase the size of the stimulation field and therefore the probability of activating the same reward-relevant neurons. Since high currents push the rate-frequency curve towards lower pulse numbers, testing at an adequate grain may limit the values of current that can be used and still avoid undersampling. Using longer train durations or manipulating the schedule of reinforcement (e.g. the fixed delay after each train) may allow future studies to test at adequately high currents while still maintaining the rate-frequency curve at pulse numbers that permit an adequate sampling grain. There is also the possibility that there may be site-related differences in the range of the rate-frequency function, so that some electrode placements may require a finer sampling grain than other placements.

Implications of Undersampling

The results of the collision experiment have been interpreted in terms of the properties of the directly activated reward-relevant neurons. What are

the implications of undersampling for the conclusions that have been drawn from these earlier studies? The most important conclusion that was drawn from the results of previous collision studies was that reward-relevant neurons directly linked the LH and VTA. Undersampling cannot create a shift in the rate-frequency curve that does not exist in the underlying rate-frequency function and therefore does not change the validity of this conclusion. Undersampling may have created or contributed to the steepness of the collision curves reported in earlier studies. Thus, the earlier collision studies may have underestimated the range of conduction velocities in the reward-relevant neurons undergoing collision.

The abrupt nature of many of these earlier collision curves was in fact a puzzling finding that could never be adequately explained. Recall that the collision interval is theoretically equal to the sum of the conduction time between the stimulation sites and the refractory period of the neurons stimulated by the T-pulses. If it is assumed that the neurons undergoing collision have the same range of refractory periods as in the refractory period experiment, then the range of collision intervals should be at least as large as the range of refractory periods since the collision interval is equal to the conduction time plus the refractory period. Of course, neurons with the same refractory period could have different conduction velocities (and thus different conduction times) which would lead to an even wider range of collision intervals. Thus, it would be expected that the range of the collision interval would be greater than the range of recovery in the refractory period curve. In order to explain the abrupt nature of the collision curves in relation to the more gradual refractory period curves, it

was necessary o postulate that of the reward-relevant neurons recruited at each site, only a homogeneous subpopulation linked the LH and VTA. This explanation was never entirely satisfactory. The estimates of conduction velocity varied substantially between subjects with similar stimulation sites and therefore required the added assumption that there were different homogeneous subpopulations with different conduction velocities and only one such subpopulation was recruited at each pair of stimulation sites yielding collision-like effects. Given the high currents (and therefore large stimulation fields) used for most of the subjects, it was not clear why only a single subpopulation would be recruited for each pair of stimulation sites. If the abrupt nature of many of these collision curves was due to undersampling, then the population of neurons undergoing collision may be less homogeneous than previously assumed. The gradual nature of the collision curves obtained by Murray and Shizgal (1992), although they represent a small sample of subjects, suggest that the reward-relevant neurons linking the LH and VTA may consist of a more heterogeneous population similar to the population implicated by the ALH-VTA collision curves obtained in this study.

Role of Dopaminergic Neurons.

One of the most important questions addressed by the refractory period and collision experiments was whether catecholamine neurons were part of the directly activated substrate for MFB self-stimulation. The behavioural refractory period estimates of 0.5-1.5 msec and the conduction velocity estimates of 1-8 m/sec obtained from MFB self-stimulation sites did not

overlap with the longer refractory periods and slower conduction times that had been obtained for catecholamine neurons. It was therefore concluded that catecholamine neurons were not part of the directly activated substrate but were perhaps involved at some later stage in the reward-relevant system.

How does the problem of undersampling influence these conclusions? Undersampling may have delayed the start of recovery and advanced the end of recovery so that both faster and slower conducting neurons may play a larger role than suggested by previous collision studies. Clearly, shorter conduction times would be even less compatible with a role for dopamine neurons so it is still possible to conclude that dopamine neurons cannot account for the fastest estimates of conduction velocity. The potential contribution of a slower population of neurons now raises the possibility that dopaminergic neurons may play a role in this later phase of recovery. Yeomans (1989) has argued that dopamine neurons may contribute to the later recovery observed in the refractory period curves for MFB self-stimulation sites, especially if the electrode tips are small and the currents large. Absolute refractory period estimates of 1.2-2.5 msec for putative dopaminergic neurons places them within the tail end of the estimates that have been obtained for MFB reward neurons (Yeomans et al., 1988).

In spite of this overlap in the refractory period and conduction velocity estimates, other characteristics of dopaminergic neurons make it improbable that they comprise even a small proportion of the directly-activated substrate for MFB reward, at least with the methods used in this study. For instance, dopamine neurons are known to have a prolonged relative refractory period lasting up to 8 msec (Yeomans et al., 1988). Even with equal

amplitude C- and T-pulses, refractory period curves obtained from most MFB self-stimulation sites asymptote between 1.2 and 3.0 msec (Bielajew et al., 1982; Yeomans, 1975, 1979). Yeomans et al. (1988) used stimulation currents 1.5-3 times threshold in order to obtain their estimates of the absolute refractory period for dopaminergic neurons. It seems highly unlikely considering the current intensities (170-1200 uA), pulse duration (0.1 msec), and tip size (250 um) used in this study, that we were stimulating substantial numbers of dopamine neurons at a level sufficient to fire them immediately after their absolute refractory period.

Summary

In 4 of the 6 subjects tested in the collision experiment, stimulation effectiveness increased with C-T interval in a manner independent of the electrode delivering the C-pulses. These data are consistent with recovery from collision block in reward-relevant neurons directly linking the ALH and VTA. The majority of the refractory period curves obtained for the ALH and VTA sites began to rise over C-T intervals of 0.6-0.8 msec and finished their rise over C-T intervals of 1.6-3.2 msec. Conduction velocity estimates for these neurons ranged from 0.4 to at least 20.0 m/sec. These data are consistent with a heterogeneous population of reward-relevant neurons directly linking the ALH and VTA, with a wide range of conduction velocities and refractory periods. Previous collision studies may have underestimated the range of conduction velocities for the reward-relevant neurons directly activated by MFB stimulation due to undersampling of the rate-frequency function.

Experiment 3

Experiment 3 was aimed at assessing the refractory periods and conduction velocities of the descending fibers of rostral MFB somata that were antidromically activated by stimulation of the LH and/or VTA. These estimates could then be compared to the psychophysically derived estimates for the directly activated substrate for MFB self-stimulation. The cells characterized in Experiment 3 would either arise in or project through the regions damaged by the effective lesions made in Experiment 1 and project past sites that were shown to be connected by common reward-relevant neurons in Experiment 2. If some anterior MFB neurons possess characteristics that match the psychophysical profile, then it will be more likely that they comprise at least part of the first stage for MFB self-stimulation.

In order to render the electrophysiological estimates as comparable as possible to the psychophysical estimates, stimulation procedures developed by Swadlow (1982) were used to estimate recovery at or near the site of stimulation. These procedures are necessary because different portions of the neuron, with different physiological properties, are characterized in the behavioural and electrophysiological tests. In the behavioural experiment, orthodromic action potentials initiated at the site of stimulation must successfully reach the terminals in order to impact on subsequent stages of the reward-relevant system and finally influence behaviour. Thus, refractory period estimates obtained in the behavioural experiment characterize the portion of the neuron between the stimulation site and the terminals.

In the electrophysiological experiment, antidromic action potentials initiated at the site of stimulation must successfully reach the region of the cell body in order to be detected by the recording electrode. The relatively large magnitude and spatial extent of the extracellular field produced by the cell body action potential makes this the most likely site for extracellular recording. In addition, a cell body response can be differentiated from an axonal response on the basis of its longer waveform and the presence of the initial segment/somatodendritic break (Humphrey, 1979). Assuming the recording site is in fact near the cell body, refractory period estimates obtained in the electrophysiological experiment characterize the portion of the neuron between the stimulation site and the soma. However, the refractory period of the soma and initial segment may exceed the refractory period of the axon (Swadlow, 1982) and therefore electrophysiological estimates based on the refractory period of the soma could be deceptively longer than psychophysical estimates based on the refractory period of the axon even if both were obtained from the same population of neurons.

The procedures introduced by Swadlow (1982) were designed to estimate recovery from refractoriness at or near the site of stimulation and therefore should result in electrophysiological estimates that are more comparable to the psychophysical estimates. In the standard procedure that is conventionally used, a pair of pulses is delivered and the interval between C- and T-pulses is varied. The shortest C-T interval at which two antidromic responses are always recorded is used as the estimate of the refractory period. In the Swadlow test, the pair of pulses is delivered in the interval following the detection of a spontaneous, orthodromic action potential and

before its arrival at the stimulation site. The antidromic action potential initiated by the C-pulse will never reach the cell body due to collision with the spontaneous orthodromic action potential. Thus, the refractory period is now estimated by the shortest C-T interval at which the antidromic response from the T-pulse is always recorded. In the Swadlow procedure, the cell body is allowed more time to recover from refractoriness (the interval between the spontaneous spike and T-pulse response) than in the standard procedure (interval between the arrival of the action potentials triggered by the C- and T-pulses) and is therefore less likely to dominate the refractory period estimate. Swadlow-type estimates of the refractory period therefore characterize the segment of the axon between the point of collision and the stimulation site.

A similar problem exists in comparing conduction velocity estimates obtained in the behavioural and electrophysiological experiments. In the behavioural version of the collision test, the conduction velocity is based on the time required for the action potential initiated at one stimulation site to arrive at the second stimulation site. Typically, conduction velocity estimates in the electrophysiological experiment are obtained by dividing the estimated distance between a single stimulating site and the recording site by the latency of the antidromic response. If the axonal trajectory was non-linear or if conduction time was altered due to non-uniformities in the axons, then the behavioural and electrophysiological estimates would be based on different physiological measures.

In order to circumvent this problem, a two-electrode version of the collision test (Rompré & Shizgal, 1986) was employed in the

electrophysiological assessment of conduction velocity. In the event that a single unit was directly driven by stimulation of the LH and VTA sites, pulse pairs were delivered to the two stimulation sites, the C-pulse to the LH and the T-pulse to the VTA. The shortest C-T interval at which a C- and T-pulse response were always obtained was used as the estimate of the collision interval. Theoretically, the collision interval is the sum of the time required for the C-pulse response to travel to the VTA stimulation site and then for the axon at the VTA to recovery from refractoriness. If the refractory period for the VTA site is subtracted from the collision interval, an estimate of inter-electrode conduction time will be obtained in a manner analogous to the estimation of the inter-electrode conduction times from the results of the behavioural version of the collision test.

Whenever possible, Swadlow-type estimates of the collision interval were obtained using a procedure analogous to that described above for the refractory period test. The pulse pairs were delivered in the interval following the detection of a spontaneous spike and before its arrival at the LH stimulation site. The antidromic C-pulse response from the LH therefore would collide with the spontaneous spike and not invade the cell body region. This procedure allows the cell body more time to recovery before the arrival of the T-pulse response, thus increasing the likelihood that the conduction velocity estimate is not biased by properties of the soma or initial segment. The shortest C-T interval at which a T-pulse response was always observed was used as the estimate of the collision interval. The estimates of inter-electrode conduction time based on the two-electrode collision test were compared to estimates of conduction time based on the difference between

the latencies of the responses from the LH and VTA stimulation sites as a check of the validity of the assumptions underlying the two-electrode collision test.

Method

Subjects

Eight male rats of the Long-Evans strain (Charles River Breeding Farms) served as subjects. Weight at the time of surgery varied from 530-800 grams. The animals were individually caged with unlimited access to food and water and were maintained on a reverse 12 hour light/12 hour dark cycle. Subjects were food deprived approximately 12 hours before surgery.

Electrodes

Stimulating electrodes (the cathode of the stimulation circuit) were constructed from no. 00 stainless-steel insect pins insulated with Formvar to within 0.5 mm of the pointed tip. The anode of the stimulation circuit consisted of a 0.25 mm stainless-steel wire insulated to within 3 mm of the rounded tip. The ground of the recording circuit consisted of an uninsulated no. 00 stainless-steel insect pin.

Recording electrodes were constructed from two lacquer-insulated, tungsten microelectrodes (Frederick Haer) with shank diameters of 0.125 mm and tip impedances of 9-12 Mohm at 1000 Hz. The two tungsten

microelectrodes were glued together so that their tips were separated by approximately 0.5 mm in the vertical plane. A stainless-steel microelectrode (Frederick Haer) was then glued to the assembly so that its tip was approximately 0.5 mm above the shorter of the tungsten electrodes. Prior to the recording session, the distances between the tips of the three microelectrodes were measured under a microscope in all three planes. The stainless steel microelectrode was used to pass a lesioning current in order to mark the position of the recording electrode.

Surgery

Atropine methyl nitrate (0.40 mg/kg, i.p.) was administered 20 minutes prior to anesthesia. Surgery and electrophysiological recordings were carried out under urethane anesthesia (ethyl carbamate, 1.2 g/kg, i.p.) with supplements administered as required. Body temperature was maintained at 37 degrees Celsius by means of a feedback controlled heating pad equipped with a rectal temperature probe. Heart rate was monitored throughout the surgery and recording session.

Subjects were mounted in a stereotaxic instrument (Kopf 1700) so that lambda and bregma lay in the same horizontal plane. The bone and dura mater overlying the stimulating and recording sites were removed and the surface of the exposed cortex covered with Gelfoam soaked in 0.9% saline. Several jewelers screws were driven into the skull to serve as anchors for the stimulation electrodes. A stainless-steel wire wrapped around one of the screws served as the cathode during lesioning.

Stimulation electrodes were aimed at the ipsilateral LH and VTA using the following level-skull coordinates: -2.8 to -3.3 mm from bregma, 1.6 to 1.7 mm lateral to the mid-sagittal sinus, and 7.8 to 8.3 mm below the dura mater for the LH and -4.8 mm from bregma, 1.0 mm lateral, and 7.5 to 8.1 mm below dura for the VTA. The anode was positioned in the ipsilateral hemisphere such that the recording sites would lie as close as possible to the zero potential surfaces located mid-way between each stimulation electrode (cathode) and the anode. The ground electrode was positioned in the contralateral hemisphere such that it would lie between the two zero potential surfaces. The electrodes were attached to the skull and skull screw anchors with dental 'sticky wax' applied in a molten state.

Electrical Stimulation

Stimulation consisted of cathodal, rectangular pulses produced by two constant-current amplifiers (Grass CCIU1) connected to a pulse generator (A.M.P.I. Master-8) via stimulation isolation units (Grass SIU5). Pulse duration was fixed at 0.1 msec for all testing with the exception of the strength-duration experiments to be described later. Stimulation current was monitored by reading differentially across a 1 kohm, 1% resistor in series with each electrode. In order to minimize the stimulation artifact, Wagner grounds (Ranck, 1981) were connected across the outputs of the constant-current amplifiers. When the Wagner grounds were switched in, the zero potential surfaces passed through the ground electrode and could be rotated about it by adjusting a potentiometer.

Electrophysiological Recording

Precision FET-input operational amplifiers (AD515J or AD545J), configured as unity-gain voltage followers, served as headstage amplifiers and were mounted as close to the recording electrode as possible. The signals recorded by each of the tungsten microelectrodes were passed through separate headstage amplifiers, variable gain amplifiers, and filters before being combined by a differential amplifier (Tektronix 3A9) in order to cancel the stimulation artifact. The output of the differential amplifier was displayed on a digital storage oscilloscope (Gould OS4020) and stored on VCR tape with an audio description of the experimental procedure.

The recording electrodes were aimed at sites in the basal forebrain ipsilateral to the stimulation electrodes. Coordinates for the recording electrode ranged from 0.30 to 1.30 mm posterior to bregma and from 2.6 to 2.2 mm lateral to the mid-sagittal sinus. The recording electrode was initially advanced 6.6 mm below dura using the coarse stereotaxic manipulator and then left in place for at least 20 minutes to allow for dissipation of the compression of brain tissue caused by the penetration of the electrode. The recording electrode was then slowly lowered through the brain using a hydraulic microdrive (Narishige MO-8) while alternate stimulation pulses were delivered to the LH and VTA electrodes at a frequency of 2 Hz and a current of 500 uA. Movement of the recording electrode was stopped in order to assess putative spontaneous or driven responses (see Analysis below). The recording electrode was lowered a total of 2.0-3.3 mm for each penetration. At the end of the penetration, a marking lesion was made by delivering a direct current of 0.15 mA for 15

sec using the skull screw as the cathode and the stainless-steel wire glued to the recording electrodes as the anode. Lesions were made in some cases at the bottom of the penetration and in all cases at the start of the penetration after the recording electrode had been raised to its initial dorsal-ventral position. In some subjects, a second penetration was made after moving the recording electrode to a new coordinate. At the completion of the recording session, lesions were made at the tip of each of the stimulation electrodes by delivering a direct current of 0.15 mA for 15 sec using the skull screw as the cathode and the electrode as the anode.

Analysis of the Electrophysiological Responses

Putative cellular responses were analyzed if their signal-to-noise ratio was at least 3:1. In order to determine which of the two tungsten microelectrodes recorded a given response (spontaneous or evoked), the differential recording was compared to the recordings obtained when one of the microelectrodes was disconnected. Evoked responses were classified as single-unit if their amplitude was invariant over a range of suprathreshold current intensities. The current threshold for the response was obtained by gradually reducing the current until the evoked response was "never" obtained (no more than 1 evoked response for every 10 stimulation pulses) and then gradually increasing the current until a response was "always" obtained (at least 9 evoked responses for every 10 stimulation pulses). The criterion used for the current threshold of the cell was the lowest current at which a response was "always" obtained. If an evoked response varied in amplitude

with suprathreshold currents, then it was classified as a compound action potential and no further tests were performed.

Synaptically evoked responses were distinguished from antidromically evoked responses using two main criteria. If the cell was spontaneously active, then collision between evoked and spontaneous spikes was used to establish whether the cell was antidromically activated. In the collision test, a window discriminator (Bak Electronics DDIS-1) was used to detect the occurrence of a spontaneous action potential which then triggered a C-pulse to follow after a pre-set delay. The delay between the spontaneous spike and the C-pulse (S-C interval) was varied in order to determine the shortest S-C interval at which an evoked response was always obtained (at least 9 responses to 10 C-pulses) and the longest S-C interval at which an evoked response was never obtained (no more than 1 response to 10 C-pulses). The collision interval was defined as the shortest S-C interval at which an evoked response was always obtained. The collision test was performed using currents equal to 1.2 times the threshold current and in some cases repeated using currents equal to twice the threshold current. If spontaneous firing was absent or erratic, then latency stability and the ability to follow high frequency stimulation (up to 100 hz) were used to distinguish antidromic from synaptic responses. In some cases, closely spaced (around 10 msec) pulse pairs were used to assess the ability of the cell to follow the second pulse of each pair at high frequencies.

Refractory Period Test

If a single-unit response was classified as antidromically activated, an estimate of the refractory period of the cell was obtained using one of two procedures depending upon the spontaneous activity of the cell. The standard procedure was used if the cell was not spontaneously active and consisted of delivering pairs of equal amplitude C- and T-pulses at a frequency of 1 Hz to the stimulation electrode driving the response. In the Swadlow test, the same pulse pairs were used but the C-pulse was delivered a short time after the occurrence of a spontaneous spike (detected using a window discriminator) so that collision always occurred between the spontaneous and C-pulse responses. In both the standard and Swadlow tests, the interval between the C- and T-pulses was varied in order to determine the shortest interval at which the T-pulse always evoked a response and the longest interval at which the T-pulse never evoked a response. The shortest C-T interval at which the T-pulse always evoked a response was used as the estimate of the refractory period. The amplitude of the C- and T-pulses was set to either 1.2 times threshold or twice threshold and in several cases the test was performed at both current intensities.

Two-electrode Collision Test

In several cases, both stimulation electrodes produced evoked responses that were similar in amplitude and form but differed in latency. In those cases, a two electrode collision test was performed to determine whether the two electrodes were stimulating the same axon at two different points along its trajectory and to provide an estimate of the interelectrode conduction

time. The test was identical to the refractory period test described above except that instead of delivering the pulse pairs to a single electrode the C-pulses were delivered to the LH electrode and the T-pulses to the VTA electrode. As in the refractory period test, standard and Swadlow versions of the two-electrode collision test were used depending upon the spontaneous activity of the cell. Currents 1.2 times and twice threshold were used in most cases.

Strength-duration Test

In several cases, current thresholds were obtained at pulse durations ranging from 0.1 to 6.4 msec in order to obtain the strength-duration relationship. The chronaxie of the cell, defined as the pulse duration at which the current threshold is equal to twice the rheobase, was estimated by fitting a hyperbolic function of the form $I = I_r(1 + C/D)$ to the strength-duration data where I_r is the current at rheobase and C is equal to the chronaxie. The procedure for estimating the current threshold was the same as that described earlier.

Histology

Subjects were given an overdose of Somnotol and perfused intracardially with 50 mL of 0.9% saline followed by 50 mL of 10% formalin and 50 mL of a Prussian Blue solution consisting of 3% potassium ferricyanide, 3% potassium ferrocyanide, and 0.5% trichloroacetic acid dissolved in 10% formalin. The brains were removed and stored in 10% formalin for approximately 48 hours and then transferred to a 20% sucrose-formalin

solution for an additional 24 hours. The brains were quick-frozen in pulverized dry ice and then sliced in a freezing microtome. Alternate 30-um-thick sections were mounted onto two sets of glass slides coated with gelatin and then stained using a formol-thionin solution.

The locations of the recording sites were estimated in the following manner. First, the coordinates of the lesion produced at the top of each penetration were estimated according to the Paxinos and Watson (1986) atlas. The coordinates for each of the penetration microelectrodes were then calculated based on its spatial relationship to the stainless-steel lesioning electrode as determined prior to the recording session. The site at which a particular response was recorded was then estimated according to the dorsal-ventral distance the recording electrode had moved from the start of the penetration. If a second lesion was made at the bottom of the penetration, its location was used to determine whether the recording electrode descended along a vertical path and also whether any shrinkage of the brain had occurred during histological processing. Shrinkage was estimated to be minimal and therefore was not accounted for in estimating the location of the recording sites.

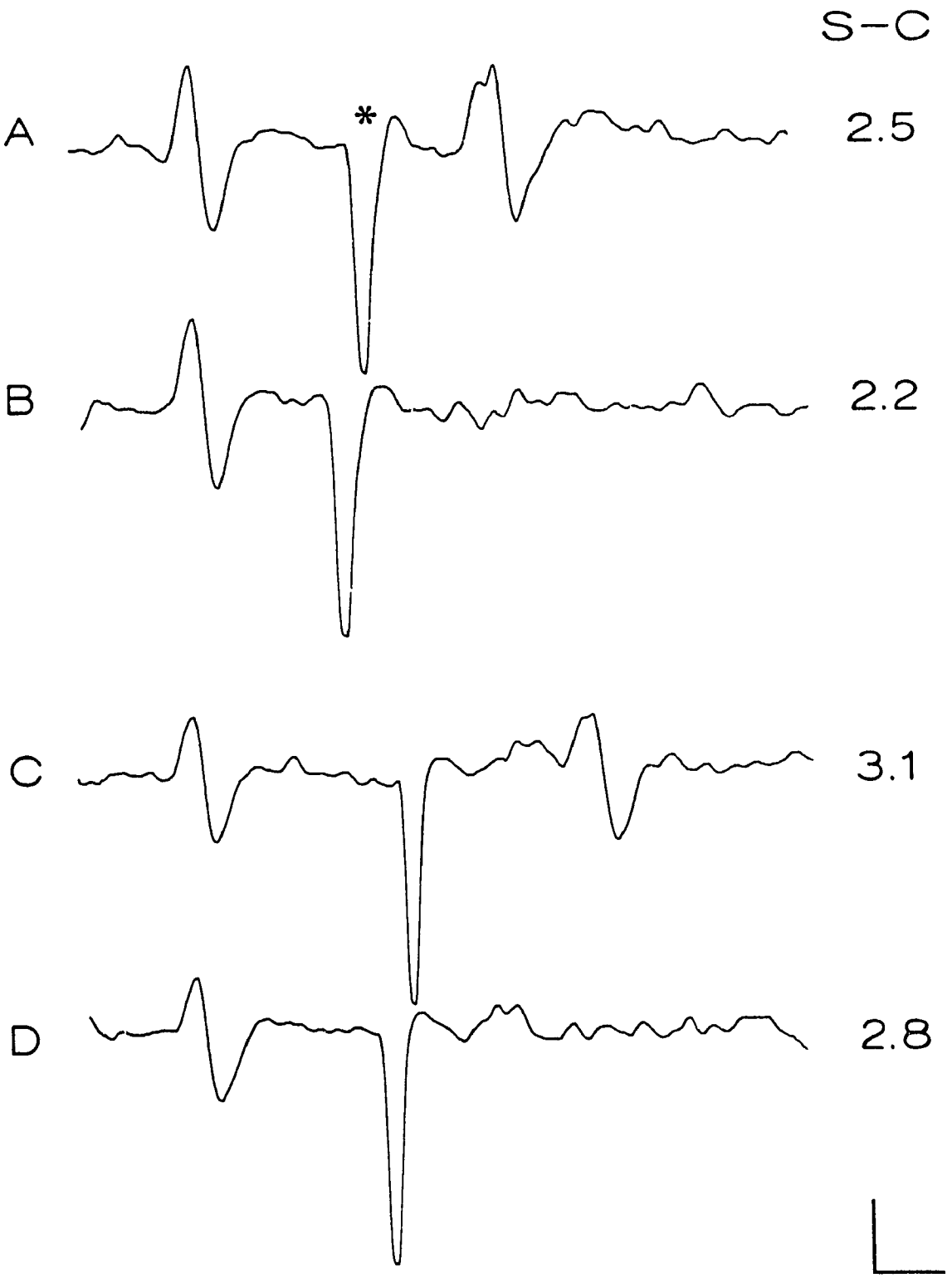
Results

Single Unit Responses

One hundred and forty-two responses met our criterion of a signal-to-noise ratio of 3:1. Of these responses, 17 were classified as antidromic, single-unit action potentials according to the criterion of collision between spontaneous and evoked responses. The results of one such test are shown in Fig. 31 for a cell (#1.104) that was localized to the SI. This particular cell was driven from both of the stimulation sites and had an amplitude of around 400 uV. The results of the collision test are shown in traces labelled A and B for the LH site and traces C and D for the VTA site. The occurrence of the spontaneous action potential triggered the C-pulse (the stimulation artifact is marked by an asterisk) to follow after a pre-set delay (S-C interval, shown to the right of each trace). At an S-C interval of 2.5 msec for the LH site and 3.1 msec for the VTA site, an evoked response was always obtained (traces A and C). When the S-C interval was reduced to 2.2 msec for the LH and 2.8 msec for the VTA, an evoked response was never obtained (traces B and D). Notice that the latency of the evoked response from the LH stimulation site (trace A) is shorter than the latency of the evoked response from the VTA stimulation site (trace B) which is consistent with a longer conduction time from the VTA.

An additional 27 responses were classified as antidromic, single-unit action potentials using the criteria of latency stability and frequency following. Fifteen of the antidromic responses were driven only by stimulation of the

Fig. 31. Collision between spontaneous and evoked responses obtained from a cell (#1.104) located in or near the SI that was directly driven from both the LH (traces A and B) and VTA (traces C and D). The interval between the spontaneous response and the C-pulse (S-C interval) is shown to the right of each trace. Stimulation artifacts are labelled with an asterisk. The evoked response from the LH was always present at an S-C interval of 2.5 msec (trace A) but was never present at an S-C interval of 2.2 msec (trace B). The evoked response from the VTA was always present at an S-C interval of 3.1 msec but never present at an S-C interval of 2.8 msec. Note that the latency of the response from the VTA is longer than the latency from the LH. The calibration (1 msec, 200 uV) is given in the lower right-hand corner.


S-C
A
*
2.5
B
2.2
C
3.1
D
2.8

LH site, 18 responses were driven only by stimulation of the VTA site, and 11 responses were driven by stimulation of both sites. Refractory period and/or collision tests were completed on 40 of the 44 responses classified as antidromic and unitary.

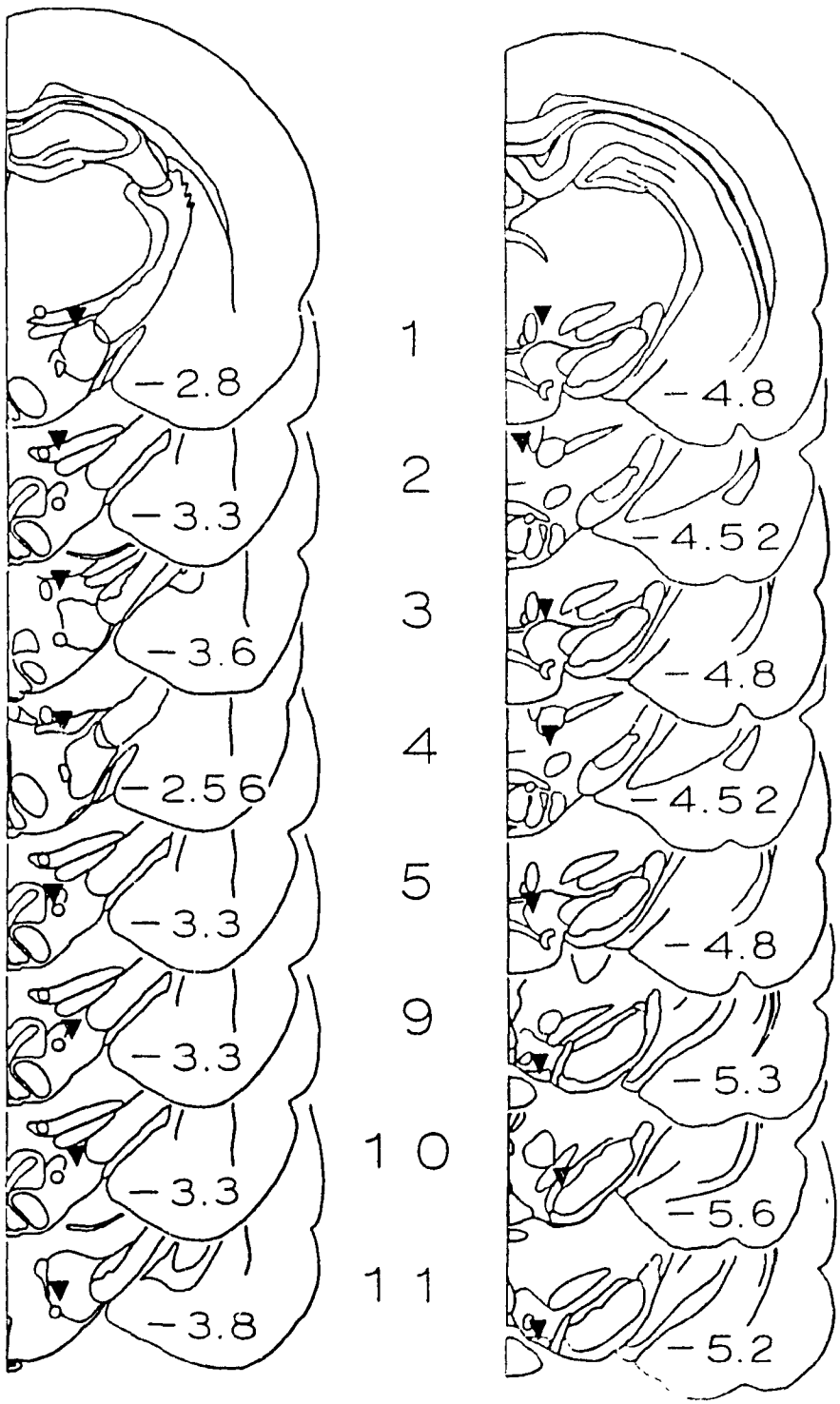
Stimulation and Recording Sites

The location of the tips of the stimulating electrodes for the 8 subjects were reconstructed onto tracings from coronal plates of the Paxinos and Watson (1986) atlas (Fig. 32). The anterior stimulation sites were located between -2.56 and -3.8 mm from bregma. In 5 of the 8 subjects (1, 5, 9, 10, and 11), the anterior stimulation electrode was in or bordering the LH. In the case of subjects 2, 3, and 4, the anterior stimulation sites were dorsal to the LH. In subjects 2 and 4, the tip of the anterior electrode was located in the middle and ventral border of the zona incerta respectively. The anterior electrode for subject 3 was located just lateral to the mammillothalamic tract.

The posterior stimulation sites were located between -4.52 and -5.6 mm from bregma. In 4 of the 8 subjects (3, 5, 9, and 11) the posterior stimulation sites were located in or bordering the VTA. The posterior stimulation site for subject 10 was in the substantia nigra pars compacta. In the case of subjects 1 and 4, the posterior electrodes were located dorsal to the anterior VTA and posterior LH respectively. The posterior electrode for subject 2 was medial and dorsal to the intended target within the rostral interstitial nucleus of the medial longitudinal fasciculus.

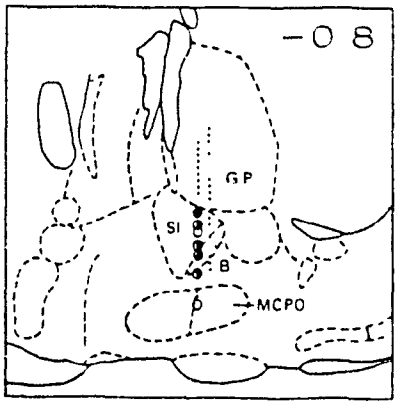
Fig. 32. Electrode tip locations reconstructed onto tracings of coronal plates from the Paxinos and Watson atlas (1986) for the subjects in Experiment 3. The anterior stimulation sites are shown in the left-hand column and the posterior stimulation sites are shown in the right-hand column. The distance (mm) of the plate from bregma is given in the lower right corner of each section.

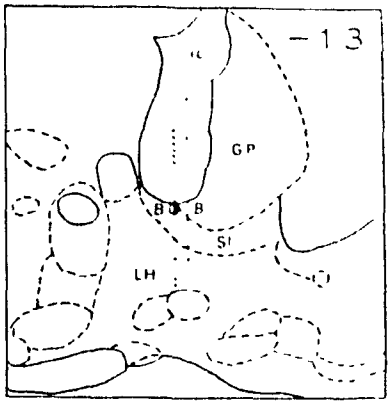
Stimulation Sites



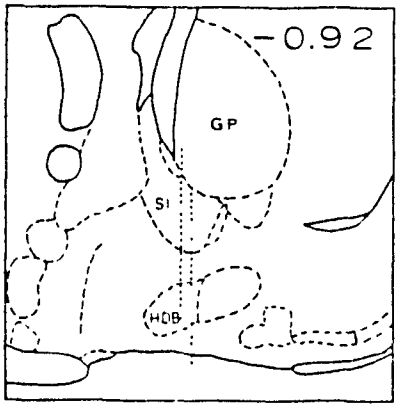
Figs. 33-34. Trajectory of the recording electrodes and location of the antidromically activated cells reconstructed onto tracings of coronal plates from the Paxinos and Watson (1986) atlas. The distance (mm) from bregma is given in the upper right-hand corner of each plate. Dotted lines show the trajectory of each recording electrode whereas the circles denote the cell locations. The location of cells driven from the LH, VTA, or both the LH and VTA are represented by filled, open, and half-filled circles respectively.

1


-0 8
GP
SI
B
MCPO


-1 3
GP
B
B
SI
LH

2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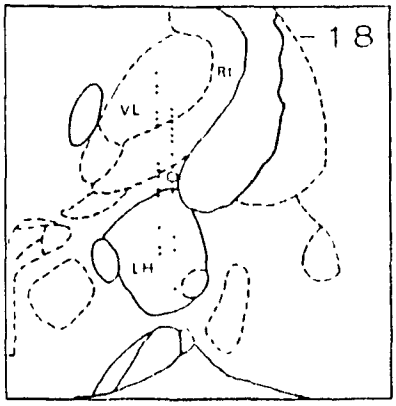
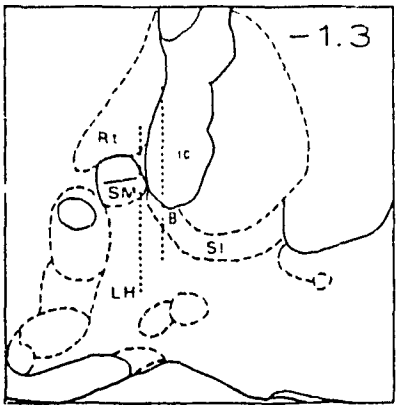
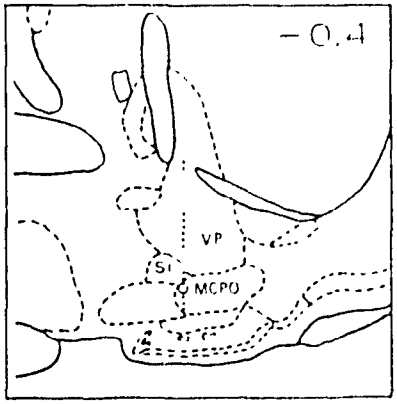
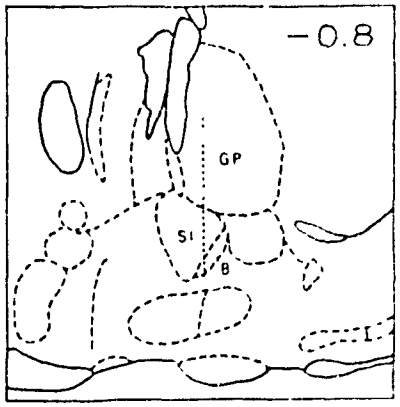
SI
HDB

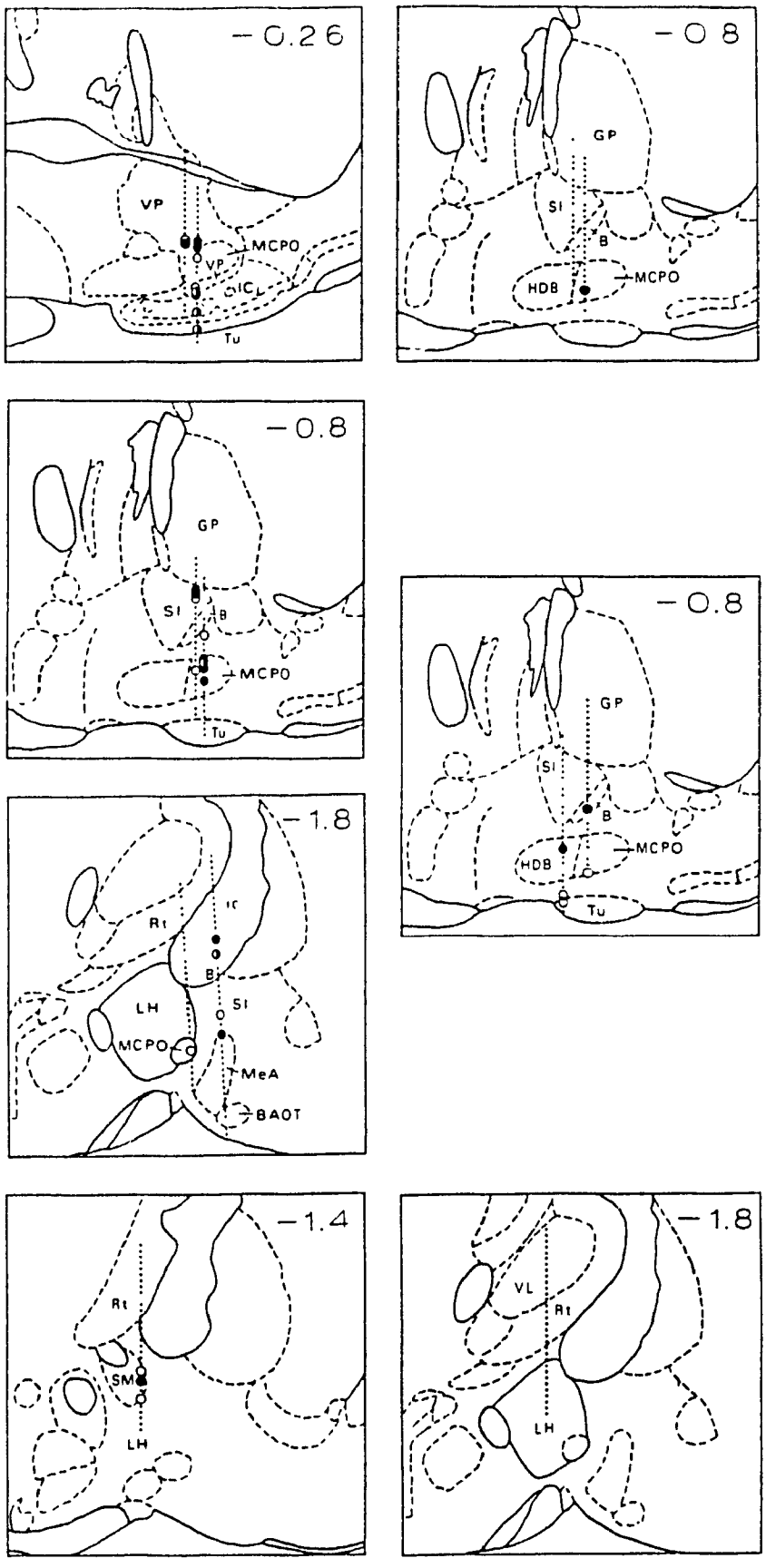

-1 8
Rt
VL
LH

3


-1.3
Rt
ic
SM
B
SI
LH


-0.4
VP
SI
MCPO


-0.8
GP
SI
B


4
−0.26
VP
MCPO
VP
CIC
Tu
−0 8
GP
SI
B
HDB
MCPO
5
9
−0.8
GP
SI
B
MCPO
Tu
−0.8
GP
SI
B
HDB
MCPO
Tu
10
−1.8
Rt
IC
B
SI
LH
MCPO
MeA
BAOT
11
−1.4
Rt
SM
LH
−1.8
VL
Rt
LH

The estimated trajectory of the recording electrodes and the location of the antidromically activated cells were reconstructed onto tracings of coronal plates from the Paxinos and Watson (1986) atlas (Figs. 33 and 34). Dotted lines show the trajectory of each recording electrode. Filled, open, and half-filled circles show the location of cells driven by LH, VTA, or both LH and VTA stimulation respectively. Two penetrations were made in the case of subjects 1, 2, 3, 4, 10, and 11 while only a single penetration was made in the case of subjects 5 and 9. In those subjects with multiple penetrations, the reconstructions for each penetration are shown in side-by-side panels with the second penetration on the right (except for subject 10 in which case the second, more lateral penetration was reconstructed onto the same coronal plate). In the case of subjects 10 and 11, the separation between the two microelectrodes was less than 0.2 mm in the anterior-posterior and medial-lateral planes and therefore the trajectory of the two microelectrodes is denoted by a single dotted line showing the start of the penetration for the shortest electrode and the end of the penetration for the longest electrode. In the case of subject 3, the trajectory of the two microelectrodes for the second penetration (right-hand panels) were reconstructed onto separate coronal plates.

Half of the antidromically-activated cells were localized to the magnocellular preoptic nucleus (MCPO, 12 cells) or the substantia innominata (SI, 10) and an additional 4 cells were recorded in the adjacent basal nucleus of Meynert (B). Other regions that contained antidromically-activated cells include the ventral pallidum (VP, 6 cells) and olfactory tubercle (Tu, 6 cells), the nucleus of the stria medullaris (SM, 3 cells), the internal capsule (ic, 2

cells), and the nucleus of the horizontal limb of the diagonal band (HDB, 1 cell). The number of antidromically-activated cells recorded on a given penetration varied substantially across subjects. Sixty-six percent of all the cells recorded were obtained from 3 of the 8 subjects (1, 4 and 5) compared to only 11% of the total cells recorded from another 3 subjects (2, 3 and 11).

Refractory Period Estimates

A total of 29 refractory period estimates were obtained from 25 cells using the standard procedure. In the case of 4 cells that were driven from both electrodes, refractory period estimates were obtained from stimulation of each site. Nineteen refractory period tests were completed on 14 cells using the Swadlow procedure; 5 of these cells were driven from both sites. Refractory period data obtained using the Swadlow procedure are shown in Fig. 35 for cell 1.104. The Swadlow procedure was used so that the response elicited from the C-pulse delivered to the VTA site always collided with the spontaneous action potential and therefore never reached the recording site. At a C-T interval of 1.5 msec (trace A), the T pulse reliably triggered an evoked response. When the C-T interval was reduced to 1.4 msec (trace B), the response was probabilistic; only about half of the T-pulses triggered an evoked response. At a C-T interval of 1.3 msec (trace C), a T-pulse response was never obtained. When the current was increased from 1.2 times threshold to twice threshold (traces D and E), the refractory period was reduced from 1.5 msec to 1.1 msec.

Fig. 35. Swadlow-type refractory period test for cell #1.104 driven by VTA stimulation. The S-C interval was set so that collision always occurred between the spontaneous and C-pulse spike. The stimulation artifacts from the C- and T-pulses are labelled with an asterisk. Traces A - C show the records obtained for a current of 1.2 times threshold. The T-pulse response was always present at a C-T interval of 1.5 msec, probabilistic at a C-T interval of 1.4 msec, and never present at a C-T interval of 1.3 msec. Raising the current to twice threshold (traces D and E) decreased the estimate of the refractory period. At twice threshold, the T-pulse response was always present at a C-T interval of 1.1 msec (D) and never present at a C-T interval of 1.0 msec (E). The calibration (1 msec, 200 uV) is given in the lower right-hand corner.

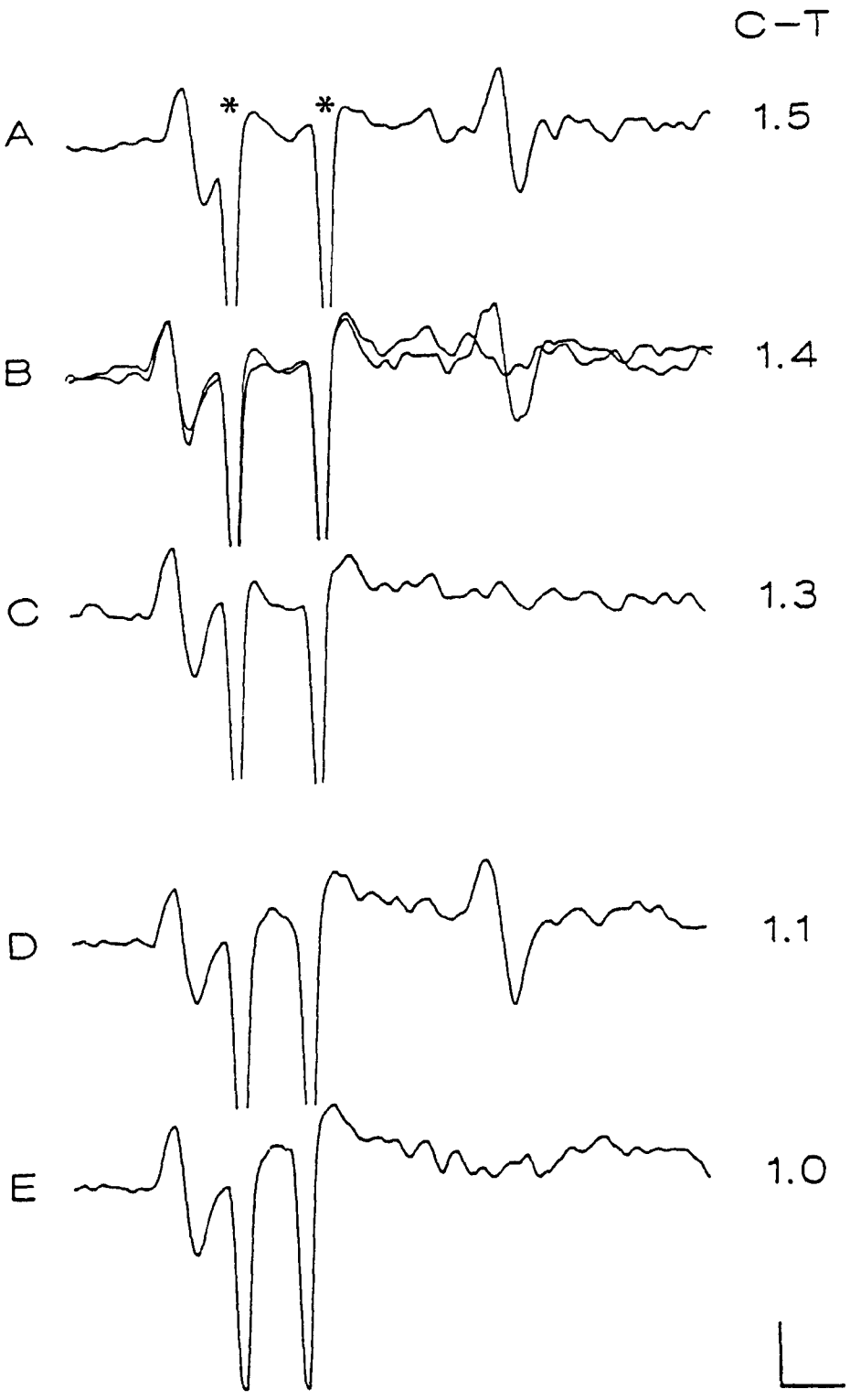
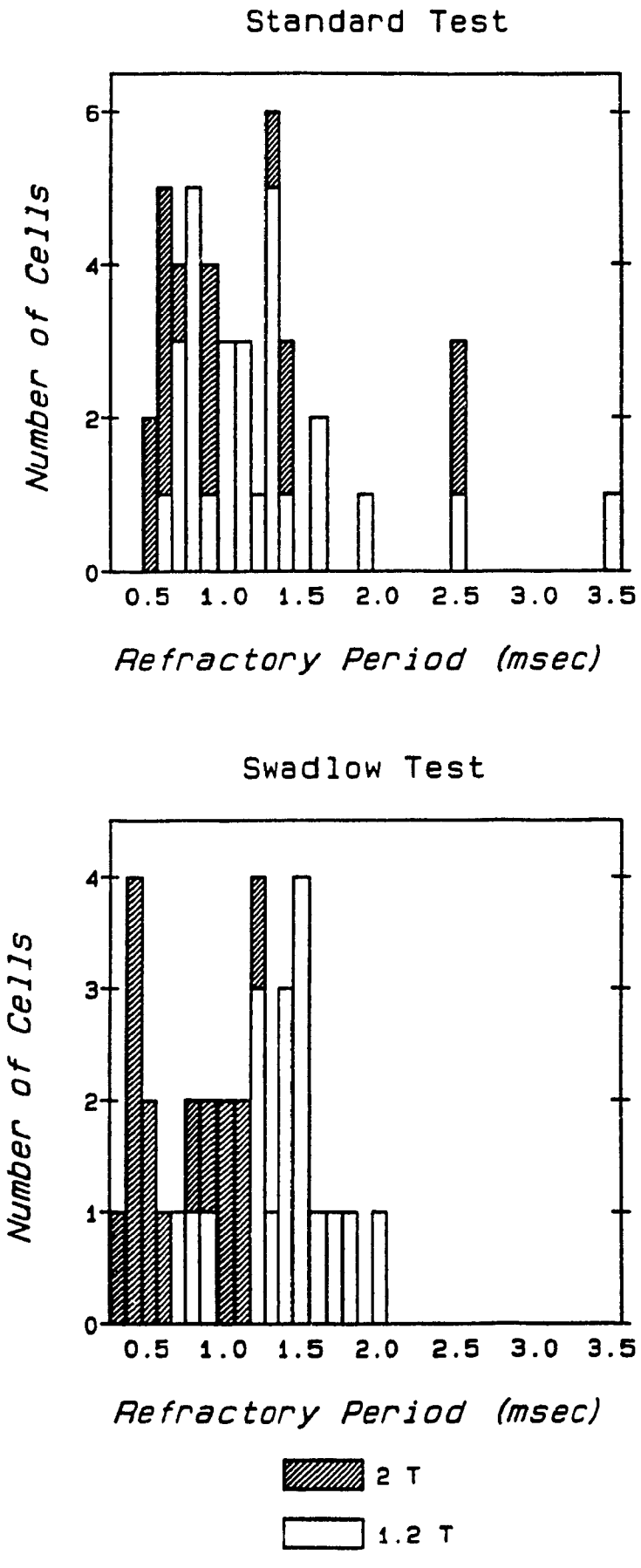

C-T
A
*
*
1.5
B
1.4
C
1.3
D
1.1
E
1.0

Fig. 36. Distribution of refractory periods obtained in the standard (top panel) and Swadlow (bottom panel) tests. Height of the open and hatched bars gives the number of cells at a given refractory period using currents of 1.2 and twice threshold respectively.


Standard Test
Number of Cells
0
2
4
6
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Refractory Period (msec)
Swadlow Test
Number of Cells
0
1
2
3
4
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Refractory Period (msec)
2 T
1.2 T

The distribution of refractory periods obtained in the standard and Swadlow tests are shown in the top and bottom panels respectively of Fig. 36. The distribution of refractory periods obtained with stimulation currents equal to twice the threshold current are shown with hatched bars and the distribution obtained using currents equal to 1.2 times threshold are shown with open bars. The shortest C-T interval at which the cell would always respond to the T-pulse varied from 0.52 to 3.4 msec for the standard test and 0.35 to 2.0 msec for the Swadlow test. In both tests and at both current intensities, the majority of cells had completely recovered from refractoriness by C-T intervals of 1.5 msec.

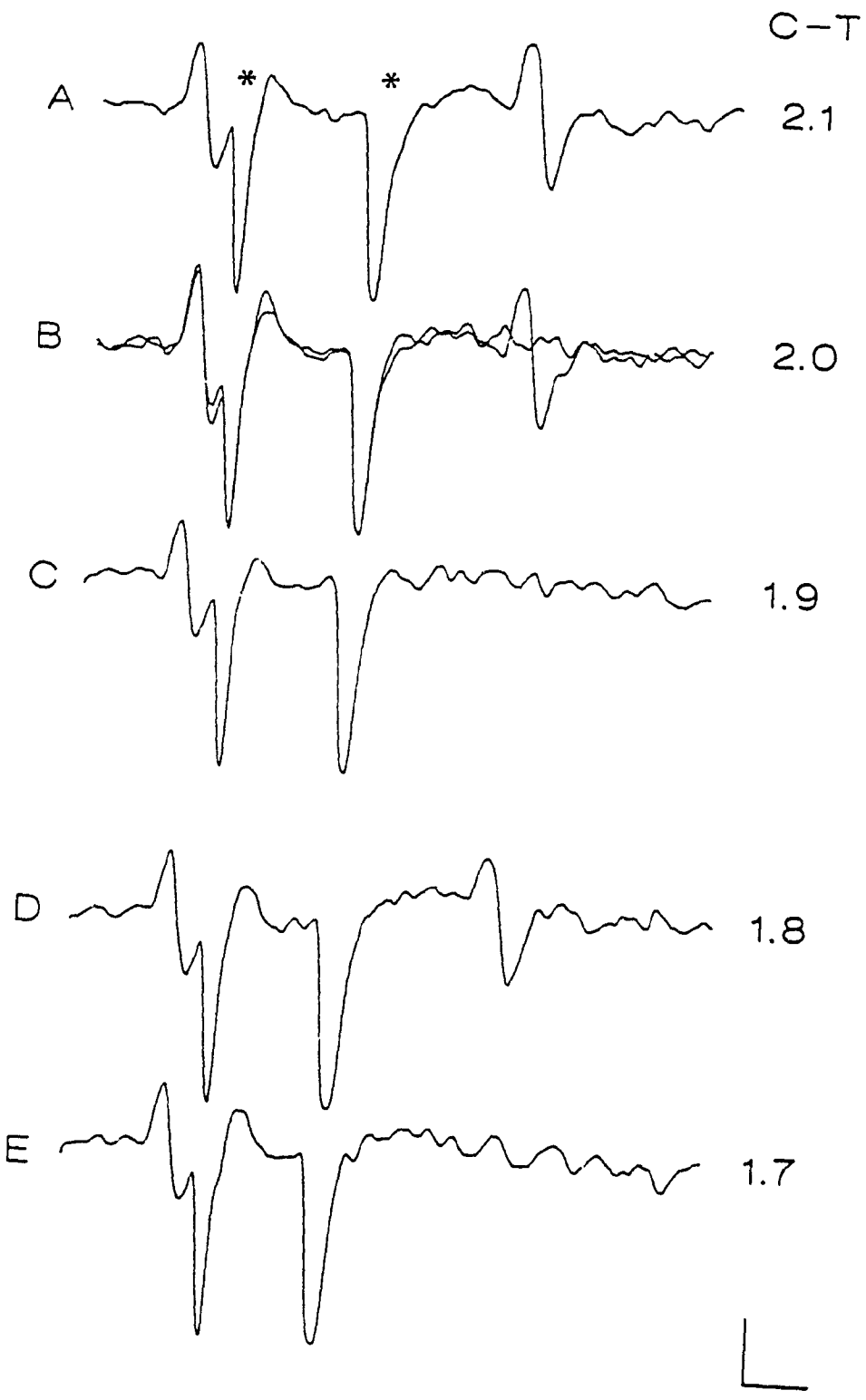
The effect of current on the refractory period estimates was more consistent in the Swadlow test. In all of the 14 Swadlow tests conducted at 1.2 and twice threshold, increasing the current reduced the refractory period estimate by 17-74%. In 6 of the 14 standard tests conducted at both currents, increasing the current had little or no effect on the refractory period estimate (+/- 10%). In the remaining 8 standard tests, increasing the current reduced the refractory period estimate by 20-57%. The more consistent effect of current in the Swadlow test compared to the standard test suggests that this procedure was in fact providing a better estimate of the refractory period at the site of stimulation. Increasing the stimulation current would not be expected to alter the estimate of refractoriness if that estimate was determined by sites remote from the stimulation field (i.e. soma/initial segment).

Collision Between Spontaneous and Evoked Responses

Twenty-nine tests of collision between spontaneous and evoked responses were performed on 16 cells. Five cells were driven from both sites and therefore the collision test was conducted at each. In the 8 cases where the collision test was performed at 1.2 times and twice threshold, increasing the current reduced the collision interval by 3.4%-38.9%.

In theory, the sum of the latency and the refractory period at the site of stimulation should exceed the collision interval by an amount equal to twice the utilization time (Fuller and Schlag, 1976). The utilization time (the time between the onset of the stimulus and the initiation of the response) is generally accepted to be about 0.10 msec in many fast axonal systems (Swadlow, 1982). Swadlow-type estimates of the refractory period were obtained at the same current intensity for 25 of the 29 collision tests. In 6 of the 25 cases there was good agreement between the experimental and predicted collision interval, the sum of the latency and the refractory period exceeded the collision interval by an amount ranging from 0.10-0.30 msec. In more than half the cells, the sum of the latency and the refractory period exceeded the collision interval by an amount ranging from 0.35-0.80 msec. In 4 cases, the sum of the latency and refractory period was approximately equal to the collision interval (within +/- 0.1 msec) whereas in 2 cases it was less than the collision interval by 0.10-0.15 msec.

Fig. 37. Swadlow-type inter-electrode collision test for cell #1.104 driven by LH and VTA stimulation. The C-pulse was delivered to the LH and the T-pulse to the VTA. The S-C interval was set so that collision always occurred between the spontaneous and C-pulse spike. The stimulation artifacts from the C- and T-pulses are labelled with an asterisk. Traces A-C show the records obtained for a current of 1.2 times threshold. The T-pulse response was always present at a C-T interval of 2.1 msec, probabilistic at a C-T interval of 2.0 msec, and never present at a C-T interval of 1.9 msec. Raising the current to twice threshold (traces D and E) decreased the estimate of the collision interval. At twice threshold, the T-pulse response was always present at a C-T interval of 1.8 msec (D) and never present at a C-T interval of 1.7 msec (E). The calibration (1 msec, 200 uV) is given in the lower right-hand corner.


C-T
A
*
*
2.1
B
2.0
C
1.9
D
1.8
E
1.7

Inter-electrode Collision Data

The inter-electrode collision test was performed on 10 of the 11 cells that were antidromically activated by stimulation of both the anterior and posterior sites. The collision interval varied from 0.30-2.40 msec using the Swadlow procedure and from 0.61-2.90 msec using the standard procedure. Inter-electrode collision data obtained for cell 1.104 are shown in Fig. 57. The Swadlow procedure was used so that the response elicited from the C-pulse delivered to the LH always collided with the spontaneous action potential and therefore never reached the recording site. At a C-T interval of 2.1 msec (trace A), the T pulse, which was delivered to the VTA, reliably triggered an evoked response. When the C-T interval was reduced to 2.0 msec (trace B), the response was probabilistic; only about half of the T-pulses triggered an evoked response. At a C-T interval of 1.9 msec (trace C), a T-pulse response was never obtained. When the current was increased from 1.2 times threshold to twice threshold (traces D and E), the collision interval was reduced from 2.1 msec to 1.8 msec.

Six cells were tested for inter-electrode collision at currents of 1.2 and twice threshold for the VTA site. In each of these cases, increasing the current at the VTA site (or both sites) decreased the estimate of the inter-electrode collision interval by an amount ranging from 0.20-0.74 msec (8-55%). Four cells were tested using the standard procedure, 4 were tested with the Swadlow procedure, and 2 were tested using both procedures. For the 2 cells tested using both procedures, the collision interval was reduced by 50% (0.61 msec versus 0.30 msec) and by 36% (1.1 msec versus 0.70 msec) when the Swadlow compared to the standard procedure was used.

Estimates of the inter-electrode conduction time were obtained by subtracting the refractory period obtained for the posterior site from the inter-electrode collision interval. The inter-electrode conduction time was found to vary from -0.10-1.20 msec for data obtained using the Swadlow procedure. In 4 of these cases, the inter-electrode conduction time was estimated to be either zero or negative. In the standard procedure, inter-electrode conduction time varied from 0.0-0.40 msec. These estimates were then compared to the estimate of conduction time based on the difference between the latencies of the responses from each stimulation site. For those cells tested using the Swadlow procedure, the difference between the latencies of the LH and VTA responses was in each case greater than the estimate of inter-electrode conduction time obtained from the collision test by an amount ranging from 0.15-0.70 msec. Using the standard procedure for the inter-electrode collision test, the estimate of inter-electrode conduction time was less than the latency differences in 4 out of 6 cases by an amount ranging from 0.07-0.30 msec. In one case there was good agreement between the two estimates of conduction time and in the remaining cell the estimate obtained from the inter-electrode collision test was 0.20 msec longer than the estimate based on the latency differences.

Estimates of the conduction velocity for these cells can be obtained by dividing the inter-electrode distance (computed from the histological coordinates of the electrode tips) by the difference in the latencies for the two sites. Using these latency differences, conduction velocities of 1.39-20.0 m/sec were obtained. The estimate of inter-electrode conduction time

obtained from the inter-electrode collision test was not used to estimate the conduction velocity since in 8 cases this value was negative or close to zero.

Strength-duration Data

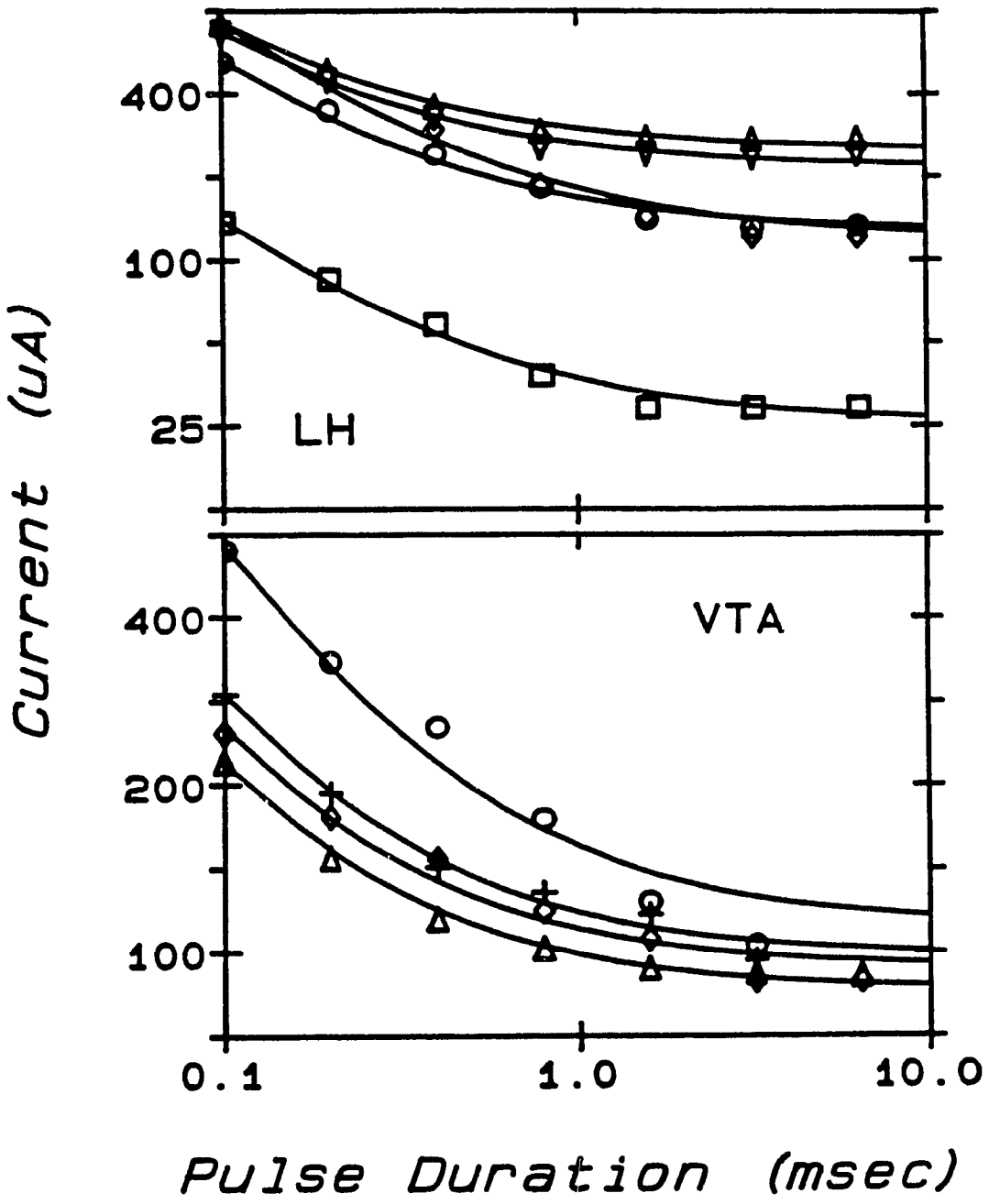
The 9 strength-duration curves obtained in the case of 6 antidromically-activated cells are shown in Fig 38. In the case of 5 cells, strength-duration curves were obtained for stimulation of the LH site (top panel of Fig. 38) and in the case of 4 cells for stimulation of the VTA site (bottom panel). The solid lines in each graph show the hyperbolic function that was fit to the data obtained for each cell. Chronaxie estimates based on the fitted functions ranged from 0.15-0.50 msec.

Discussion

The goal of this experiment was to determine the physiological characteristics of fibers arising from rostral MFB somata in order to assess the likelihood that they comprise part of the first stage neurons for MFB self-stimulation. These cells would arise in or project through the regions damaged by the effective lesions of Experiment 1 and project near the stimulation sites that were psychophysically characterized in Experiment 2.

Conduction velocity estimates for cells driven by stimulation of both the LH and VTA sites ranged from 1.39-20.0 m/sec. These estimates were based on the differences between the response latencies from the two stimulation sites. Conduction velocity estimates based on the inter-electrode collision test were not used due to the fact that inter-electrode conduction time in

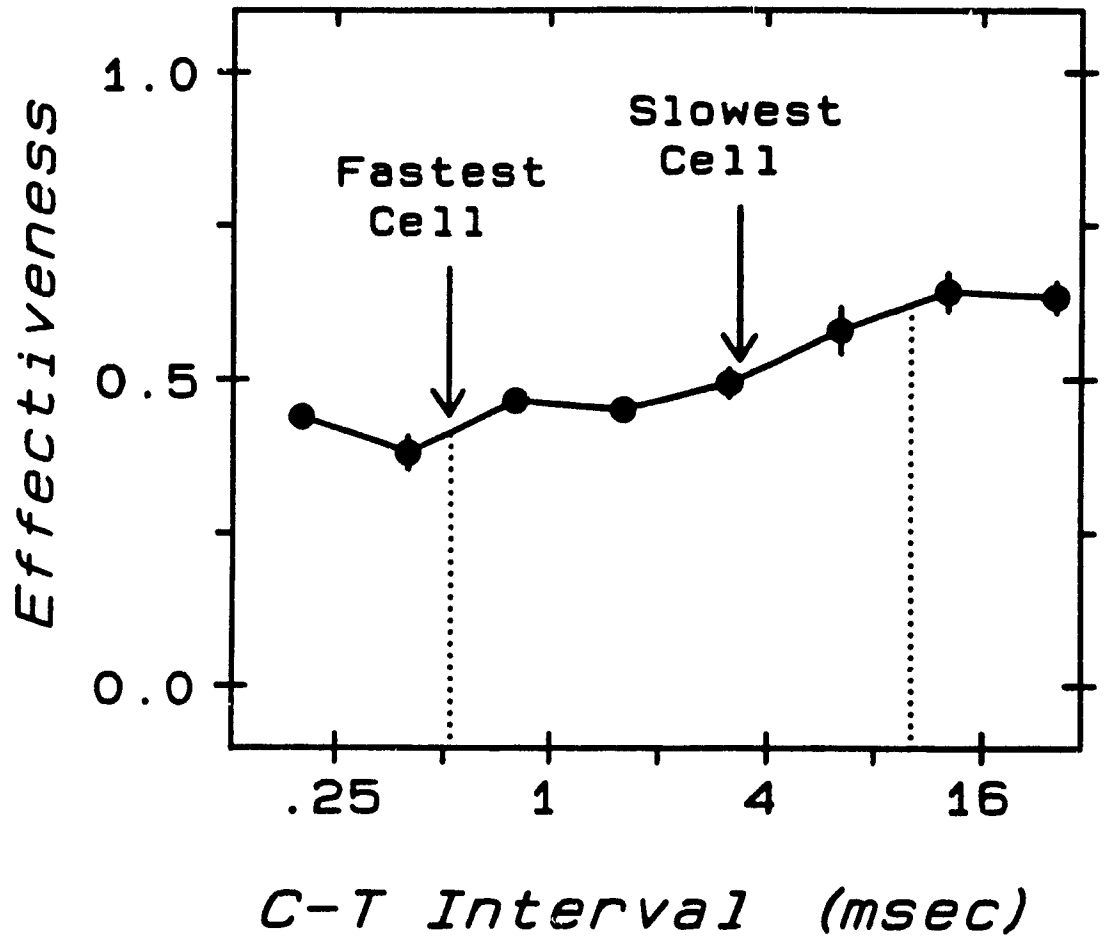
Fig. 38. Relationship between the current threshold and the pulse duration for 6 cells driven by stimulation of the LH (top panel) or VTA (bottom panel). Data obtained from each cell are represented by different symbols. Solid lines show the hyperbolic function fit to the data obtained for each cell.


Current (uA)
400
100
25
LH
400
200
100
VTA
0.1
1.0
10.0
Pulse Duration (msec)

this test was estimated to be zero or negative in several cases (see discussion below). The electrophysiologically derived estimates of 1.39-20.0 m/sec overlap with the estimates of 0.4-20.0 m/sec for the reward-relevant neurons directly linking the ALH and VTA. Estimates of the refractory period at or near the site of stimulation for cells antidromically activated from either the LH or VTA site ranged from 0.35-1.2 msec using currents equal to twice threshold. These estimates of the absolute refractory period overlap with the estimates of 0.6-3.2 msec for the reward-relevant neurons directly activated at the ALH and VTA stimulation sites characterized in Experiment 2.

Fig. 39 illustrates the degree of overlap between the electrophysiological and behavioural data in the case of the collision experiment. The behavioural collision curve shown in Fig. 39, obtained for one of the subjects from Experiment 2 (K6), is the average of the ALH-VTA and VTA-ALH conditions for the currents yielding the highest percent collision. The dotted, vertical lines show the estimates of the beginning and end of the collision interval corresponding to 10% and 90% of the total recovery. The collision interval that would be expected for each of the cells driven from both the LH and VTA sites was calculated as follows: The conduction time for that cell was obtained by dividing the inter-electrode distance for subject K6 (3.3 mm) by the estimate of the conduction velocity for that cell. The collision interval was them calculated by adding the estimate of the refractory period (Swadlow-type at twice threshold) to the conduction time. According to this calculation, the collision intervals that would have been obtained for these cells ranged from 0.52-3.4 msec, which overlaps with the collision interval of

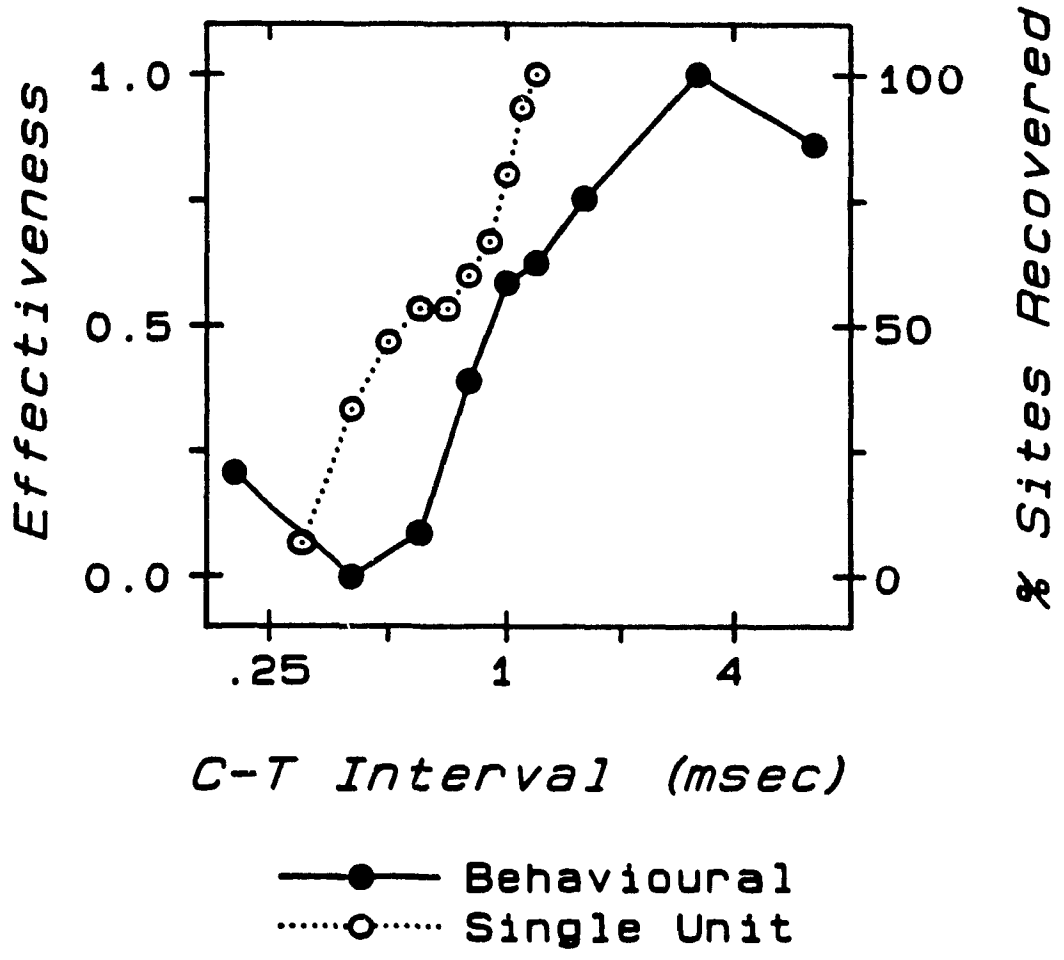
Fig. 39. The overlap between the electrophysiological and behavioural data in the case of the collision experiment. The behavioural collision curve is the average of the ALH-VTA and VTA-ALH conditions for the currents yielding the highest percent collision for subject K6. The dotted, vertical lines show the estimates of the beginning and end of the collision interval corresponding to 10% and 90% of the total recovery. The collision interval that would be expected (assuming the same conduction distance) for the fastest and slowest cell from Experiment 3 is indicated by the arrows. Estimates of the collision interval for these cells overlap with the collision interval obtained from the behavioural curve.


1.0
0.5
0.0
Effectiveness
Fastest Cell
Slowest Cell
.25
1
4
16
C-T Interval (msec)

0.52-10.1 msec that was obtained for subject K6. Thus, recovery from collision block in fibers arising from the present sample of anterior MFB cells could have contributed to the early recovery in the behavioural collision curve for ALH and VTA self-stimulation sites.

Fig. 40 illustrates the degree of overlap between the electrophysiological and behavioural data for the refractory period experiment. Behavioural refractory period data obtained for stimulation delivered to the ALH (filled symbols) are shown for one of the subjects (K4) from Experiment 2. The E-values have been re-scaled so that they range from 0 to 1.0 in order to aid in the comparison with the electrophysiological data. Superimposed on this curve is the percentage of stimulation sites in the electrophysiological experiment (open symbols) that had recovered by the C-T interval shown on the x-axis. The electrophysiological data were obtained at currents equal to twice threshold using the Swadlow procedure and therefore give an estimate of the absolute refractory period at or near the site of stimulation. The single-unit curve in Fig. 40 illustrates how a behavioural curve would look if the reward-relevant neurons were comprised entirely of the sample of cells from this study. What is apparent from this figure is that the single-unit curve overlaps with the early part of the rise in the behavioural curve. Thus, recovery from refractoriness in fibers arising from the present sample of anterior MFB cells could have contributed to the early recovery in the behavioural refractory period curve for ALH and VTA self-stimulation sites.

Fig. 40. The overlap between the electrophysiological and behavioural data in the case of the refractory period experiment. Behavioural refractory period data obtained for stimulation delivered to the ALH (filled symbols) are shown for one of the subjects (K4) from Experiment 2. The E-values from this curve have been re-scaled so that they range from 0 to 1.0. The percentage of stimulation sites in the electrophysiological experiment (Swadlow-type at twice threshold) that had recovered by the C-T interval shown on the x-axis is represented by the open symbols. Estimates of the absolute refractory period for these cells overlap with the fastest estimates obtained from the behavioural curve.


Effectiveness
1.0
0.5
0.0
100
50
0
% Sites Recovered
.25
1
4
C-T Interval (msec)
Behavioural
Single Unit

Determination of Antidromic Activation

In order for the electrophysiological estimates to be meaningfully compared to the behavioural data obtained in Experiment 2, the assumption that these estimates were obtained from single cells that were antidromically activated by our stimulation electrodes must be a valid one. The most reliable demonstration of antidromic activation is obtained using the collision test. In the collision test, the interval between the detection of a spontaneous action potential and the delivery of a stimulation pulse (S-C interval) is varied. The collision interval is defined as the minimum S-C interval at which an evoked response is always detected. In the case of a spontaneous neuron that is antidromically activated from stimulation at a site along its axon, once the spontaneous action potential and its trailing refractory period zone has passed by the stimulation site, then the antidromic response initiated at the stimulation site will be able to propagate successfully to the soma. The collision interval will therefore be equal to the latency of the evoked response plus the refractory period at the site of stimulation. Fuller and Schlag (1976) have argued that the utilization time, the time between the onset of the stimulus and the initiation of the response, must also be taken into consideration in this calculation. Thus, antidromic activation should result in a collision interval equal to the sum of the latency, refractory period at the site of stimulation, and twice the utilization time. In the case of a spontaneous neuron receiving a strong, monosynaptic input from stimulation of an afferent pathway (Barillot, Bianchi, Dussardier, and Gauthier, 1980), the minimum S-C interval will be equal to the refractory period of the soma minus the response latency. The

cell cannot be activated if the synaptic input arrives during the refractory period following the spontaneous spike. Cases of antidromic activation can therefore be differentiated from cases of monosynaptic activation on the basis of the value of the collision interval.

In the majority of cells tested for collision, the sum of the refractory period and the response latency exceeded the estimate of the collision interval by an amount ranging from 0.04-0.80 msec, a finding not consistent with a monosynaptic input. In only 3 of the cells was the collision interval less than the sum of the refractory period and response latency. In none of these cases was the value of the collision interval less than the estimate of the refractory period as would be expected by a monosynaptic input. The collision intervals obtained are therefore more consistent with the notion that the cells characterized in this study were antidromically activated by the stimulation.

In those cases in which the collision test could not be used (because the cell was not spontaneously active), latency stability and frequency following were used to distinguish synaptic from antidromic responses. Although the determination of antidromic activation cannot be made unambiguously in these cases (Humphrey, 1979; Swadlow, Waxman, & Rosene, 1978), given the short refractory periods found in the majority of the driven cells (see below), it is unlikely that many were synaptically driven cells.

Location and Possible Trajectory of the Antidromically Activated Cells

The antidromically activated cells characterized in this study were located in several basal forebrain nuclei that have collectively been called the rostral bed nucleus of the MFB (Geeraedts et al., 1990a). With the exception of the LPO, an area traversed by none of the recording electrodes, directly activated cells were recorded from all of the constituent cellular groups of the rostral MFB as described by Geeraedts et al. (1990a).

Several neuroanatomical papers have described descending MFB projections arising from the cellular regions characterized in this study. Using the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L), Grove (1988) found that descending fibers from the SI terminated in caudal regions that included the VTA, SN, peripeduncular area, as well as pontine and medullary structures. Although interspersed with the cholinergic neurons that are prominent in this area (see Saper, 1984), Grove determined that the descending neurons from the SI were primarily, if not exclusively, non-cholinergic. Swanson et al. (1984) also described a descending projection from SI and LPO that terminated in the pedunculopontine nucleus (PPN). Single axons from this projection were found to give rise to terminal boutons in the PPN and nearby periaqueductal grey. In addition, terminal boutons were observed along the course of the MFB, implying that this projection innervates regions along the length of the bundle.

In an autoradiographic study of the preoptic area of the rat, Swanson (1976) described descending projections arising from the MCPO that travelled through the MFB to sites including the VTA and central gray. Also using

autoradiographic methods, Veening et al. (1982) concluded that the descending fibers from the MCPO and olfactory tubercle were found primarily in the large-fibered ventral part of the MFB (area a). The efferents of the olfactory tubercle have been found to split into a dorsal and caudal branch with the dorsal branch leaving the MFB via the stria medullaris and the caudal branch continuing in the ventrolateral MFB to terminate in the caudal part of the LH (Scott & Chafin, 1975). Injections of the retrograde tracer horseradish peroxidase (HRP) into the VTA have been found to label cells in regions that include the LH, HDB, MCPO, SI and VP (Phillipson, 1979). The descending projection from the VP to the VTA appears to arise primarily from the medial aspect of the VP whereas fibers destined for the substantia nigra originate in lateral parts of the VP (Zahm, 1989). In agreement with the study by Phillipson (1979), Zahm (1989) also found that injections of HRP into the VTA labelled cells in the VP, HDB, LPO, and LH. Thus, these anatomical studies confirm the finding of the present study using electrophysiological techniques that anterior MFB somata give rise to descending MFB projections. Some of the possible regions in which these cells may terminate include the caudal LH, VTA, SN, periaqueductal grey, and pedunculopontine nucleus.

The Importance of Alignment

The ability to antidromically activate cells in these regions appeared to depend upon the alignment of the stimulation electrodes within the MFB. Only a single driven cell was obtained in the case of subject 2 despite the fact that the recording electrode traversed a region (the SI) that contained

many driven cells in other subjects with stimulation electrodes closer to the MFB (e.g. subjects 1 and 5). This finding is consistent with the idea that the antidromically activated neurons located in this region projected through, and not dorsal, to the MFB. Even with stimulation sites that were bordering or within the MFB, stimulation of some sites was able to directly activate substantially more neurons in particular regions than stimulation of other sites. For example, no driven cells were recorded from the SI in subjects 3, 4 or 9 whereas many SI cells were antidromically activated in subjects 1 and 5. Given the complex topography of the fiber components of the MFB (Veening et al., 1982) it is not surprising that different stimulation sites would activate different distributions of cells in the rostral MFB.

Refractory Period Estimates

Rompré and Shizgal (1986) found that approximately one third of their sample of 76 antidromically activated cells had refractory period estimates within the behavioural range typically found for MFB self-stimulation sites. They classified a cell as a good candidate if its refractory period estimate was less than or equal to 1.2 msec in the Swadlow test or 1.4 msec in the standard test when using currents equal to twice threshold. In contrast, 24 of the 26 cells tested in Experiment 3 at twice threshold had refractory period estimates less than or equal to the same criterion set by Rompré and Shizgal. Both of the cells that exceeded the criterion were tested using the standard procedure and had refractory period estimates of 2.5 msec. One of the cells was unusual in that the estimate obtained at the anterior stimulation site was considerably shorter than the estimate obtained at the

posterior site (0.9 compared to 2.5 msec), a result consistent with the interposition of a constricted branch point in the inter-electrode segment of the axon.

In the present study, recordings were obtained from areas caudal or lateral to the sites tested in either the Rompré and Shizgal (1986) study or the Shizgal et al. (1989) study. The majority of cells in both of these earlier studies were located in the septal complex which includes the lateral septal nuclei, the medial septum/diagonal band complex, the posterior complex formed by the septofimbrial and triangular nuclei, and the bed nucleus of the stria terminalis. In both of these studies the authors found that the majority of cells classified as non-candidates were localized to the septal complex (75%-87%) whereas only about half of the candidate cells were recorded in these same regions (46%-51%). This distinction was sharper in the study by Shizgal et al. (1989), perhaps due to the fact that their stimulation sites had been found to support self-stimulation and because the refractory periods in the behavioural and electrophysiological phases of their study were obtained with the same stimulation sites and parameters.

In contrast to the relatively high numbers of non-candidate cells found in the septal complex, almost all of the driven cells characterized in the present report would be classified as candidate cells according to the same criterion used by Rompré and Shizgal (1986). There is no reason to suppose that nuclei giving rise to reward-relevant neurons would have to contain a higher proportion of short-refractory-period neurons than nuclei that do not give rise to reward-relevant neurons. Therefore, the finding that a higher proportion of short-refractory-period neurons arise from the rostral bed

nucleus of the MFB does not by itself point to a greater role for these neurons. For instance, reward-relevant neurons arising from the septal complex could be interspersed with neurons that happen to project to the same regions but play no role in the rewarding effect of the stimulation. Given the relative homogeneous distribution of refractory period estimates obtained from the cells of the rostral MFB, there is the possibility that a majority of the neurons arising here contribute to the rewarding effect of stimulating the MFB. For this reason, it may be easier to establish that these neurons in fact play a role in the rewarding effect of MFB stimulation compared to regions such as the septal complex in which there would have to be a mixture of reward-relevant neurons and neurons with longer refractory periods that are not likely to contribute to the rewarding effect.

Conduction Velocity Estimates

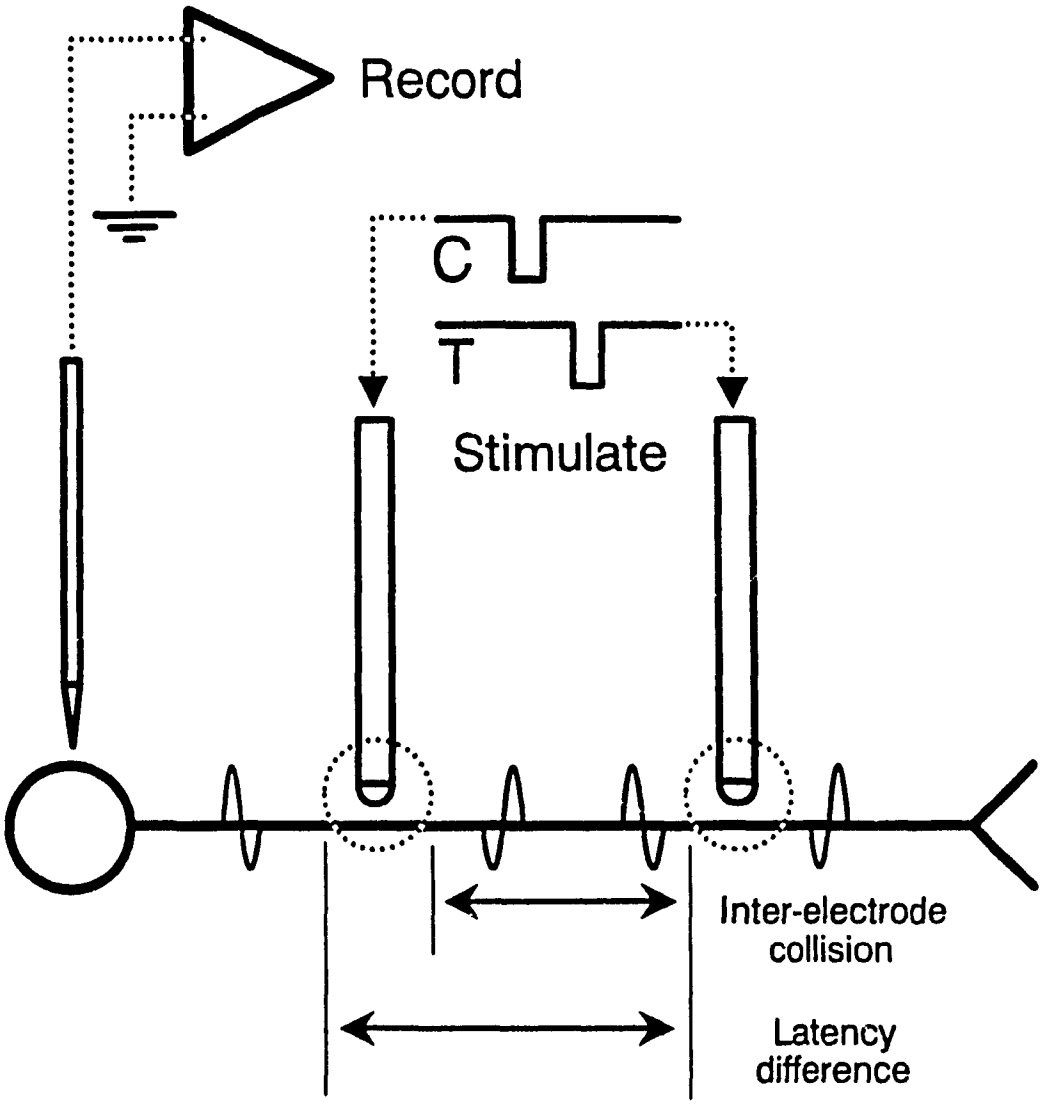
In all of the 11 two-electrode tests that were conducted using the Swadlow procedure, the estimate that was thereby obtained for the inter-electrode conduction time was shorter than the estimate based on the differences between the response latencies by an amount ranging from 0.15-0.70 msec. Part of this discrepancy may be due to an overestimation of the refractory period which would tend to underestimate the conduction time obtained from the two-electrode collision test. Although the Swadlow procedure provides a better estimate of the refractory period at the stimulation site than the standard procedure, recovery from refractoriness will still be limited by any branch points or discontinuities along the portion of the axon between the stimulation site and the site at which collision

occurs. The fact that the estimate of refractoriness obtained from the Swadlow procedure was sometimes longer than the two-electrode collision interval is consistent with the idea that this estimate might have been inflated.

Figure 41 illustrates another possible explanation for the consistent difference in the two estimates of the conduction time. The relevant conduction distance in the case of the latency differences is the distance between the points at which the antidromic action potentials begin propagating toward the cell body. In the case of the two-electrode collision test, the relevant conduction distance is the distance between the point at which the orthodromic action potential is initiated at the C-electrode and the point at which the antidromic action potential is initiated at the T-electrode. If we assume that the action potentials begin propagating from the edge of the stimulation fields, it is apparent from Fig. 41 that the conduction distance in the two-electrode collision test would be less than the conduction distance in the measure of the differences of the response latencies. This could lead to the finding that conduction time in the two-electrode collision test is consistently shorter than conduction time in the latency differences.

Finally, a third possible explanation for the finding that conduction time estimates were consistently shorter in the two-electrode collision test is that branch points may occur between the downstream VTA electrode and the upstream LH electrode. Thus, the segment of the axon stimulated near the VTA electrode may be thinner than the segment stimulated near the LH electrode. The conduction velocity of the action potential increases when

Fig. 41. Illustration of the difference in the conduction distances for the two measures of interelectrode conduction time. The conduction distance in the case of the latency differences is the distance between the points at which the antidromic action potentials from each stimulation site begin propagating toward the cell body. In the case of the two-electrode collision test, the conduction distance is the distance between the point at which the orthodromic action potential is initiated at the C-electrode and the point at which the antidromic action potential is initiated at the T-electrode. Assuming that the action potential is initiated at the edge of the stimulation field, the conduction distance in the two-electrode collision test is therefore less than the conduction distance in the measure of the latency differences.


Record
C
T
Stimulate
Inter-electrode collision
Latency difference

approaching the junction between a thick segment of axon and a thin segment and decreases when travelling in the opposite direction (Swadlow, Kocsis, and Waxman, 1980). Given that the latency differences are measured using antidromic conduction times and the two-electrode collision interval depends upon orthodromic conduction, this asymmetry in the conduction velocity could produce shorter estimates of conduction time in the two-electrode collision test.

Whatever the explanation for the discrepancy between the two measures of conduction time, the fact remains that in 8 of the 11 two-electrode collision tests conducted using the Swadlow procedure, the estimate of the collision interval was approximately equal to (and in some cases less than) the estimate of the refractory period at or near the site of stimulation. Regardless of the degree to which each of the explanations presented above is able to account for this finding, the same factors that determine the value of the collision interval in the electrophysiological test will also influence the value of the collision interval in the behavioural test. Thus, it may not be valid to directly compare conduction velocity estimates obtained using latency measures in the electrophysiological test to conduction velocity estimates based on the value of the collision interval in the behavioural test. The best way to circumvent this problem in future studies would be to conduct the electrophysiological two-electrode test using the same subjects and stimulation parameters as in the behavioural version of the collision test. The likelihood that a particular cell contributed to the behavioural data could then be determined by directly comparing the collision intervals obtained in both tests without having to estimate the conduction velocity.

Strength-duration Data

Chronaxie estimates were obtained for 6 cells based on the hyperbolic function fit to the strength-duration curves shown in Fig 38. Exponential functions were also fitted to the data but it was found in each case that the hyperbolic function provided a better fit. The chronaxie estimates of 0.15-0.50 msec cannot be taken as representative of the entire sample since relatively few cells were tested in the strength-duration phase of the experiment. In addition, rheobase was not accurately determined for all of the cells, especially in the case of cells directly driven from the VTA site. As a result, the data for these cells deviated systematically from the fitted curve at the long pulse durations. Chronaxie estimates may have been underestimated in these cases. Nonetheless, these short chronaxies are consistent with fast conduction times and short refractory periods (West & Wolstencroft, 1983). Chronaxie estimates of 0.6-4.0 msec have been obtained for reward-relevant MFB neurons using cathodal stimulation pulses (Matthews, 1977; Bielajew and Shizgal, 1986). It is therefore possible that these cells contributed to the behavioural data obtained at short pulse durations although they cannot account for the continued decline of the behavioural strength-duration curve at longer pulse durations.

Summary

The results of the present study suggest that cells arising in the anterior MFB could contribute to the early recovery evident in both the refractory period and collision curves obtained for self-stimulation sites in the ALH and VTA. The refractory periods and conduction velocities of these cells overlap

with the refractory periods and conduction velocities of the fastest first stage neurons responsible for MFB self-stimulation. A discrepancy was noted in the electrophysiological collision test: In 8 out of 11 tests the estimate for the collision interval was approximately the same or less than the estimate of the refractory period at or near the site of stimulation. Based on the assumption that the collision interval should be equal to approximately the sum of the conduction time plus the refractory period, this result is consistent with a zero or negative conduction time. The factors responsible for this discrepancy in the value of the collision interval will be present in both the electrophysiological and psychophysical tests. Thus, future studies can validly compare electrophysiologically and psychophysically derived collision data by directly comparing the estimates of the collision interval obtained in the two tests using the same subjects and stimulation parameters.

General Discussion

The findings presented in this thesis support the hypothesis that neurons arising in the rostral MFB comprise at least part of the directly activated substrate for MFB self-stimulation. In Experiment 1, it was shown that some electrolytic lesions to the anterior MFB, centered at the level of the ALH, reduce the rewarding impact of stimulating more posterior MFB sites. Anterior MFB neurons would arise in, or project through, the regions damaged by these effective lesions. In Experiment 2, collision-like effects were obtained for self-stimulation sites in the anterior and posterior MFB, consistent with the notion that reward-relevant neurons directly link the ALH and VTA. Anterior MFB neurons that send descending projections through the MFB would link sites in the ALH and VTA. In experiment 3, it was shown that the refractory periods and conduction velocities of descending MFB fibers arising in the anterior MFB overlap with the estimates for the first stage neurons responsible for MFB self-stimulation. Thus, these neurons could have contributed to the early recovery in both the refractory period and collision curves obtained for ALH and VTA self-stimulation sites.

The psychophysical approach adopted by this thesis has provided converging evidence from lesion, psychophysical, and electrophysiological data all supporting the same conclusion: that fibers arising from anterior MFB somata are part of the directly activated substrate responsible for MFB self-stimulation. In order to further test this hypothesis, several key questions remain to be addressed. The relevant experiments will be discussed below,

in addition to related methodological issues raised by the present thesis that need to be considered by future studies.

Lesion Studies

One critical question that extends naturally from the results of this thesis is whether selective damage to cell bodies in the rostral MFB reduces the rewarding impact of MFB stimulation. Areas of particular interest are the MCPO and SI, regions from which many antidromically activated cells were recorded in Experiment 3. The MCPO is also a region which shows high uptake of the metabolic marker 2DG during self-stimulation of some posterior MFB sites (Gallistel et al., 1985).

The data obtained in Experiment 1 will provide a basis for interpreting the results of future studies aimed at assessing the effects of excitotoxic lesions to the rostral MFB. For instance, we should not necessarily expect selective damage to cell bodies to produce effects that are more consistent or of a larger magnitude than those obtained with electrolytic lesions. It is clear from the electrolytic lesions that many subjects must be tested at multiple stimulation currents before conclusions can be drawn regarding the role of a particular region in the rewarding effect of MFB stimulation.

It had been hoped that the results of Experiment 1 would guide future studies to the specific compartments of the MFB in which excitotoxin lesions should be aimed. Using the histological techniques employed in this thesis, it was not possible to distinguish the anatomical basis of the effective versus ineffective lesions. It is possible that the efficacy of the lesions depended

not only on the location of the lesions but also on their alignment with the stimulation sites. Improving upon the histological techniques used in Experiment 1 may aid future studies in determining the anatomical basis for the effective anterior MFB lesions. Differences between the pathways damaged by effective versus ineffective lesions could be compared using silver impregnation of degenerating neurons thus allowing the behavioural and neuroanatomical effects of a lesion to be assessed within each subject. Additional information regarding the alignment of the lesion and stimulation sites might be obtained by using stimulating electrodes coated with the fluorescent dye, DiI (J.R. Stellar, personal communication), which labels axons in both the anterograde and retrograde directions. In this way, the trajectory of neurons passing near the electrode tip could be charted to determine whether they projected through areas damaged by the lesion. By combining behavioural and neuroanatomical techniques in the same subject, light may be shed on the subtle topographic relationships that apparently exist between reward-relevant neurons in the anterior and posterior MFB.

Combining psychophysical techniques with lesions may also aid in determining the basis for the effective versus ineffective lesions. Prior to the lesion, reward-relevant neurons near the tip of the lesioning electrode could be characterized using psychophysical techniques. One important piece of information that could be obtained prior to the lesion is whether there is a direct connection between reward-relevant neurons at the two sites. If evidence for collision-like effects could not be obtained prior to the lesion, then it would not be surprising if the lesion did not reduce the rewarding impact of the stimulation. Of course, the lesion could damage downstream

efferents of the first stage neurons so that lesions might reduce the rewarding impact of the stimulation even if there was no evidence for a direct connection between the two sites. A more serious challenge would arise if collision-like effects were obtained but the lesion was nonetheless ineffective at reducing the rewarding effectiveness of the stimulation. This would either require that the anatomical interpretation of the tradeoff function obtained in the collision test be re-examined or that the impact of reward-inhibitory or independent reward systems (as discussed previously in Experiment 1) be taken into consideration.

Collision Studies

In addition to assessing the effects of neurotoxic lesions to the rostral MFB, future studies should also determine whether the collision-like effects obtained between self-stimulation sites in the ALH and VTA extend into the more rostral regions that were characterized in Experiment 3. The collision-like effects obtained in Experiment 2 suggest that a heterogeneous population of reward-relevant neurons directly link the ALH and VTA. The collision curves obtained for these sites were gradual, in no case did we obtain the step-like curves that had been obtained for stimulation of more posterior MFB sites. It is likely that the step-like rise evident in many if not all of the collision curves obtained from earlier studies was due to undersampling of the rate-number function and not to collision in a homogeneous population of reward-relevant neurons as was suggested at the time.

In order to avoid undersampling in future collision studies, care should be taken to avoid testing at low pulse numbers or frequencies that do not

permit an adequate sampling grain. What constitutes an adequate sampling grain is still not known and may have to be determined on a site-by-site basis. It can be concluded based on the re-analysis shown in Fig. 30 that a sampling grain of 0.1 $\log_{10}$ units is not sufficient to avoid artifactually steepening the rise of the collision curve. Future studies may need to vary parameters such as the fixed delay after each stimulation train or train duration in order to be able to use high stimulation currents that maximize the probability of recruiting common neurons at the two sites and yet still maintain the high pulse numbers required for an adequate sampling grain.

Electrophysiological Studies

An important improvement to the design of future electrophysiological studies aimed at investigating descending neurons arising in the rostral MFB is the behavioural testing of subjects either prior to or during the recording session. The advantage of prior behavioural testing of stimulation sites is that psychophysical and electrophysiological estimates can be obtained using the same stimulation sites and currents (Shizgal et al., 1989). This ensures that neurons characterized during the electrophysiological phase of the experiment were also recruited during the behavioural phase. In addition, any site-related differences in the psychophysical estimates can be taken into consideration, further reducing the possibility that a neuron will be erroneously classified. The prior behavioural testing of stimulation sites may be particularly important for future studies aimed at comparing the results of the behavioural and electrophysiological collision experiment. The value of the collision interval in the electrophysiological test was often

substantially shorter than what would be predicted on the basis of the refractory period at or near the site of stimulation. Fortunately, the factors responsible for this discrepancy in the value of the collision interval will be present in both the electrophysiological and psychophysical tests. Thus, future studies can validly compare electrophysiologically and psychophysically derived collision data by directly comparing the estimates of the collision interval obtained in the two tests using the same subjects and stimulation parameters.

Undoubtedly, the most informative electrophysiological studies will involve recording from awake subjects during behavioural testing. These experiments will determine whether correlations exist between the behaviour of the subject and the activity of cells in the rostral MFB. Many studies using primates have already found that cells in regions such as the diagonal band, SI, and the primate basal nucleus of Meynert (an area closely related to the SI) are responsive to rewarding stimuli such as food or cues that signal the availability of food (e.g. Delong, 1971; Richardson & Delong, 1986; Richardson, Mitchell, Baker, & Delong, 1988; Wilson & Rolls, 1990). The changes in activity appear to be related to the rewarding properties of the stimuli, or to their ability to predict the occurrence of rewarding stimuli, and not simply to movements used to obtain the reward or to any particular sensory property of the reward (Richardson et al., 1988; Wilson & Rolls, 1990). Many of these studies have assumed that the cells being characterized are ascending cholinergic neurons that are prominant in these regions (Saper, 1984). However, the results of this thesis suggest another possibility: that in some cases these studies were recording from cells that

send descending projections through the MFB. It will be the challenge of future studies to determine whether the same cells that are responsive to naturally-occurring rewarding stimuli are also antidromically activated by rewarding stimulation of the MFB and have refractory periods and conduction velocities that match the first stage neurons. This work could provide strong evidence that neurons arising in the rostral MFB play an important role in both the rewarding effects of MFB stimulation and in directing animals towards naturally-occurring goal objects that are essential for their survival.

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Appendix A

Low, middle, and high current intensities (uA) used in Experiment 1.

Subject	Electrode	Low	Middle	High
J1	LH	315	500	795
J1	VTA	200	400	800
J2	LH	200	400	800
J2	VTA	100	200	400
J13	LH	300	450	675
J13	VTA	200	400	800
F3	LH	300	450	675
F3	VTA	200	300	450
F8	LH	300	510	870
F8	VTA	300	480	770
J9	LH	200	400	800
J9	VTA	125	250	500
J10	LH	300	525	920
J10	VTA	200	400	800
J3	LH	160	315	650
J3	VTA	200	400	800
J4	LH	160	315	630
J4	VTA	125	250	500
J5	LH	200	320	500
J5	VTA	200	320	500
J6	LH	125	250	500
J6	VTA	125	250	500
J7	VTA	315	500	800
J8	LH	300	480	770
J8	VTA	200	300	450
J11	LH	200	400	800
J11	VTA	150	300	600
J12	VTA	125	250	500
K9	VTA	200	400	800