NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, tests publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

An Electrophysiological Search for Candidate Pathways Responsible for the Rewarding Effect of Medial Forebrain Bundle Stimulation

Ivan Kiss

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy at
Concordia University
Montréal, Québec, Canada

November, 1987

ISBN 0-315-41585-1

ACKNOWLEDGEMENTS

Peter Shizgal has been a major influence upon my academic life. He has provided material, intellectual and emotional support for my efforts. His guidance has been invaluable throughout this work and he seemed to me to strike a nearly perfect balance between his interests and my own. Thanks to him, I have begun to learn how a scientist takes vague and intriguing questions and derives related questions that retain some of this intrigue while being amenable to scientific scrutiny. It is my great fortune that he continues to affect my thoughts and actions.

Without Ellen, I'm not sure I could have survived the process that culminated in this thesis. She supported all my efforts and contributed directly to the final product. Far more important, she shares my life and allows me to share hers and provides a qualitative dimension that is my deepest source of pleasure.

My parents, Andrew and Eva, have aided me throughout my life. Their advice and support have been invaluable and continue to be so. Kathy, my sister, has been a willing participant in many discussions and has furnished valuable insights.

I am grateful to Dwayne Schindler for his direct assistance on several aspects of this work. Sue Schenk was always willing to provide me with guidance, unique insights and a shoulder to cry on. I thank Beverly Murray, Pierre-

Paul Rompre and Sandra Boye for their help and support.

This thesis is dedicated to the memory of my grandfather, Ivan Kiss. Opa, kész vagyok.

ABSTRACT

An Electrophysiological Search for Candidate Pathways Responsible for the Rewarding Effect of Medial Forebrain Bundle Stimulation

Ivan Kiss, Ph. D.
Concordia University, 1987

Psychophysically based estimates of the refractory periods of directly fired neurons responsible for the rewarding effect of electrical stimulation of the medial forebrain bundle were collected. The stimulation electrodes, and parameters used to gather the psychophysical data were also used to elicit compound, axonal action potentials from regions caudal to the hypothalamic level of the MFB. Methods were devised to isolate and quantify the compound potentials and to estimate the refractory periods of the neurons whose firings produced them.

By scaling the psychophysically-based and electrophysiologically-based refractory period data in analagous ways, valid comparisons between the two types of data were made possible. These comparisons lend support to previous findings regarding the trajectories of medial forebrain bundle reward neurons through the ventral tegmental region and to a recent demonstration that such reward neurons also connect to the ventral portion of the central grey.

TABLE OF CONTENTS

LIST OF FIGURES

GENERAL INTRODUCTION

In his heirarchical formulation of the organisation of behaviour, Gallistel (1974, 1980) sees motivational states as variable strength permissive or preclusive influences upon behaviour. These motivational processes are placed at the top of his sensori-motor heirarchy so that motivational influences might, through selective potentiation and inhibition of lower levels (e.g. locomotion, ingestion, reflexes), provide direction and aptness to behaviour. His scheme gives reinforcement the role of a tonic organising principle, determining the relative influences of various motivational states upon the lower levels of the heirarchy, and the action that ultimately results. There is a long-standing and distinguished tradition of research on the mechanisms of action that comprise the lower levels of such a heirarchy, mechanisms that include reflexes, oscillators and taxes (e.g. Sherrington, 1904; Von Holst, 1937; Fraenkel, 1927). However, it has only more recently seemed feasible to study the physiological properties of the neurons responsible for motivation and reinforcement in mammals.

The startling demonstration that animals will repeatedly and vigorously self - administer brief electrical stimuli to intracranial sites (Olds and Milner, 1954) has been instrumental in promoting this shift of focus. As a necessary first step toward the location of these neurons and the eventual identification of the circuits responsible for

reward and motivation, a series of psychophysical methods were developed whereby electrophysiological properties of the neurons directly activated by the stimulating probe could be inferred (Bielajew and Shizgal, 1986; Deutch, 1964; Fouriezos and Wise, 1984; Matthews, 1977; Shizgal et al., 1980; Yeomans, 1975; Yeomans, 1979; Yeomans et al., 1979;).

Brain Stimulation Reward

Self-stimulation (SS), the self administration of electrical stimuli to central sites, is a strongly goal directed set of actions that putatively initiate a transient signal resulting in brain stimulation reward (BSR). It has been argued that the study of SS may reveal the properties of the neural circuits that play a role in motivation and learning (Gallistel, 1974). An understanding of the properties of such circuits could aid in the eventual development of a more complete neurophysiologically-based model of human behaviour. A better understanding of the physiological bases of a number of psychopathologies involving learning and motivational deficits such as depression, bipolar disorders, schizophrenia and autism could also result.

There is evidence that a record of the magnitude of rewarding stimuli is retained so that subjects' responding reflects their previous exposure to brain stimulation (Gallistel, Stellar and Bubis, 1974). One can also summon evidence for the contention that BSR and conventional rewards

share a neural substrate. BSR is modulated by a gastric load (Hoebel, 1968), cells activated during BSR may also be activated by the sight and/or taste of food in food deprived animals (Rolls, Burton and Mora, 1979) and most hypothalamic neurons whose firing is inhibited during feeding behave similarly during self-stimulation (Sasaki et al., 1984).

The BSR phenomenon may be elicited via electrodes aimed at widely dispersed central sites (German and Bowden, 1974). Particularly vigorous and persistent responding is directed toward obtaining stimulation of the diencephalic portion of the medial forebrain bundle (MFB). Self-stimulation of this MFB region is also resistant to satiation (Olds, 1958) and typically shows sharp, stable threshold discrimination (Gallistel, 1974). Thus, in a practical sense, the MFB is especially suitable for psychophysical studies of BSR.

Linking Brain Circuits and Behaviour

Even if a detailed and complete map of BSR sites were available, one would still be faced with the daunting task of precisely locating neurons whose direct activation is needed for BSR, since these neurons are only a subset of the population fired by the stimulation. Attempts at localization of BSR neurons within the MFB are further impeded by the structural complexity of the region.

The MFB courses between the olfactory tubercle and the ventromedial regions of the mesencephalic tegmentum and is composed of fibres of many calibres with widely distributed

origins and extensive projection fields. In the rat, up to fifty MFB fibre compartments confined to a cross sectional area of about 4 square mm have been identified at the hypothalamic level (Nieuwenhuys, Geeraedts and Veening, 1982; Veening et al., 1982; Nauta and Haymaker, 1969).

This structural complexity is manifest in the wide range of effects that may be produced by electrical stimulation of the region. Predation (Flynn et al., 1970), escape (Bower and Miller, 1958), changes in nociception (Rose, 1974), motor twitches (Mathews, 1977), exploration (Rompre and Miliaresis, 1980) and alterations in endocrine function (Harris, 1948), blood pressure and heart rate (Perez-Cruet, McIntyre and Pliskoff, 1965) have been electrically elicited and several actions may result from stimulation via a single electrode (e.g. Bower and Miller, 1958; Matthews, 1977; Roberts, 1958; Rolls, 1975; Rompre and Miliaresis, 1980; Valenstein, Cox and Kakolewski, 1968). A means is required to link a particular stimulation elicited effect to a specific subset of the activated neurons.

Combined Methods

In dealing with the problem of linkage, psychophysical techniques (i.e. techniques that map the physical aspects of stimuli onto psychological variables) pioneered by Deutch (1964) have been essential. These methods have provided data that guide the electrophysiological search for neurons that may subserve BSR. For example, if the psychophysically

estimated recovery from refractoriness for a particular BSR placement ranges between 0.4 and 1.5 msec, neural elements activated during SS whose physiologically determined recovery begins at 3.0 msec are ruled out as candidates for inclusion in the directly activated neural population responsible for BSR whereas those whose recovery ranges from 0.4 to 1.5 msec should be considered viable candidates. The term "candidate" is deemed appropriate here since a precise match between the behavioural and electrophysiological RP estimates does not necessarily mean that the neuron in question is part of the directly activated stage of the BSR circuit; elements not participating in BSR may have the same RP's as reward neurons. Neurons deemed to be candidates on the basis of a match between their properties and psychophysically-derived properties of the reward substrate must be subjected to further tests. For example, if destruction of candidate neurons had no effect upon SS, the correspondence of their properties to the psychophysically-derived characteristics of the reward substrate could be deemed coincidental and they would not be considered as parts of the BSR circuit.

The psychophysical results may be seen as placing quantitative constraints upon the choice of candidate neurons from electrophysiological data. If the inferences drawn from the psychophysical results are correct, they dictate the properties that neurons must possess to be considered as candidates for inclusion in the directly stimulated stage of

the BSR circuit. An increase in the number of psychophysically-derived characteristics employed would tighten the constraints. For example, comparisons between psychophysically-based and physiologically-based estimates of current integration properties, conduction velocities, and super- and subnormal excitability characteristics are possible (Gallistel, Shizgal and Yeomans, 1981).

Psychophysical Experiments

The studies described in this section employ trade-offs between independant stimulation variables to hold the behavioural effect of the stimulation at a constant level. It has been shown that if the relationship between stimulus input and performance is monotonic, the outputs of all intervening stages remain constant and trade-off experiments may be used to reveal the properties of the directly activated elements by determining the combinations of stimulus parameters that result in constant behavioural output (Gallistel, Shizgal and Yeomans, 1981). Since this relationship has been shown to be monotonic over a wide range of input values (Edmonds, Stellar and Gallistel, 1974), the subjects' performance can be used to provide data from which the physiological properties of the directly activated elements may be inferred, regardless of the number of synapses between the directly activated neurons and the motor neurons needed for behavioural output. Psychophysical experiments have provided a basis for inferring the current

integration properties, refractory periods, trajectories and direction of orthodromic conduction of reward neurons. The techniques that led to these results have been reviewed elsewhere (Shizgal et al., 1980; Gallistel, Shizgal and Yeomans, 1981; Bielajew and Shizgal, 1986) but the collision and refractory period methods will be described here since they are most relevant to the present work.

The psychophysical methods for refractory period estimation employ Yeomans' (1975) modification of the trade-off or equivalent stimulus paradigm introduced by Deutch (1964). Lever presses are rewarded by constant duration trains of electrical pulse pairs and the number of pulse pairs per train is traded off against the intra-pair (CT) interval so that the SS rate remains constant. As the CT interval is reduced, the second or test (T) pulse has a progressively diminished effect, presumably because of the refractoriness of the directly fired neurons following their firing by the first or conditioning (C) pulse. To hold behavioural output constant, the action potentials lost due to refractoriness must be replaced by increasing the number of pulse pairs per train. The decrease in the rewarding effectiveness of the stimulation due to the refractory state of the directly stimulated neurons is indicated by the size of the required increase in the number of pulse pairs.

The collision procedures can provide a basis for inferring direct axonal linkage between BSR sites. In

contrast to the RP methods, collision experiments require that each member of the stimulus pair be applied to a separate BSR site via separate electrodes. At short CT intervals, an increase in the number of pulse pairs is again required in order for constant behavioural output to be maintained. This reduction in paired pulse effectiveness has been attributed to collision between antidromic and orthodromic action potentials in axons that link both BSR sites (Shizgal et al., 1980). As the CT interval is increased, an often step-like rise in effectiveness is encountered and is taken to indicate that the orthodromic action potentials generated at both stimulation sites successfully propagated to the synaptic terminals. The time at which this abrupt rise occurs is presumed to be the sum of the conduction time between sites and the refractory period of the neurons undergoing collision. Dividing the conduction time by the inter-electrode distance provides an estimate of the conduction velocity of the axons undergoing collision.

The implications of these paired pulse tradeoff experiments include the following :

1) The recovery from refractoriness of directly stimulated MFB elements subserving BSR occurs primarily over an interval of about 0.4 to 1.5 msec.

2) SS sites at the levels of the lateral hypothalamus (LH) and ventral tegmentum (VT) separated by a distance of about 3 mm are directly linked by axons of neurons

responsible for BSR. Orthodromic conduction in at least some of these axons proceeds in the rostro - caudal direction (Bielajew and Shizgal, 1986).

3) MFB reward neurons have estimated conduction velocities of between 1.0 and 8.0 m/sec.

4). These results are not compatible with the notion that the directly fired stage of the BSR circuit consists largely of catecholamine-containing neurons. While there is substantial evidence for the participation of these neurons in SS (Fouriezos and Wise, 1976; Franklin, 1978; Franklin and McCoy, 1979; Gallistel et al., 1982), the paired pulse experiments have indicated that, by and large, CA neurons have longer refractory periods and lower conduction velocities than the behaviourally characterised elements in the MFB (Yim and Mogenson, 1980; Maeda and Mogenson, 1980; Deniau, Thierry and Feger, 1980; German, Dalsass and Kiser, 1980).

Electrophysiological Recording : Rationale

The psychophysical collision experiments have established that axonal elements of MFB reward neurons extend between the LH and VT and probably beyond (Boye, Rompre and Shizgal, 1987). Although further behavioural studies could contribute to finding the origins and terminations of MFB reward fibres, electrophysiological methods guided by psychophysically based inferences will be employed in the present work because of the relative rapidity

with which physiological studies may be performed. Recording data can reveal the locations of elements directly fired by rewarding stimulation whose properties match the behaviourally determined profile for reward neurons. These data could then be used to guide subsequent behavioural experiments focused upon regions known, on electrophysiological grounds, to contain candidates for inclusion in the directly activated stage of the BSR circuit.

In previous electrophysiological experiments, extracellular recordings have been obtained from neurons directly driven by rewarding MFB stimulation (Gallistel, Rolls and Greene, 1969; Rolls, 1971; Rompre and Shizgal, 1986; Kiss, 1982). In the first three studies, microelectrodes were used to record single unit activity in the vicinity of somata. In these cases, the tip of the recording electrode and thus the region over which current was integrated was very small so that, ideally, a single neuron was recorded at a time. Somata generally produce considerably greater local currents than single axons within their vicinity. Thus the activity of cell bodies would be preferentially recorded, probably even in areas containing mostly fibres. The morphology of at least some of the unit responses recorded by Rompre and Shizgal (1986) confirm their somatic origin (Tasaki, Polley and Orrego; 1958).

In contrast, macroelectrodes may be profitably used to record from populations of MFB axons activated by rewarding

stimulation. Since stimulation at reward sites involves synchronous activation of populations of fibres, a larger brain/electrode interface permits simultaneous recording of current flow from multiple fibres yielding records that consist of compound action potentials (CAP's). In previous experiments employing macroelectrodes to measure neural activity at one MFB site elicited by stimulating at another MFB site (Kiss, 1982; Shizgal, Kiss and Bielajew, 1982), the potentials recorded met the major criteria for axonal compound responses (Tasaki, Polley and Orrego, 1958).

Field Potential Recording : Improved Methods

Previous physiological studies provided support for psychophysically-based inferences of the properties of neurons that convey the rewarding effect of electrical stimulation of the MFB (Kiss, 1982; Shizgal, Kiss and Bielajew, 1982). These CAP recording studies provided evidence for the existence of MFB neurons with RP's well within the range of the behaviourally-inferred values and provided support for the interpretation of psychophysical collision experiments by demonstrating that MFB fibres with RP's within this range were continuous between the LH and VT. Nonetheless, these records were deficient in several respects. Since the distance between stimulating and recording electrodes was small, the resultant short latency responses were often partially obscured by the stimulus artifact. In addition, during recording experiments involving

paired stimulus pulses, the artifact and responses produced by the C and T pulses were often at least partially superimposed, making it difficult to assess the degree of recovery. These problems were exacerbated at times by random electrical noise that partially obscured the responses.

These early attempts to provide physiological estimates of refractory periods were based upon direct measurement of the amplitudes of what were judged, on the basis of visual inspection of photographic records of single traces, to be CAP's. The beginning of recovery was taken as the CT interval at which a stable response to the second pulse could just be observed. Recovery from refractoriness was considered complete at an interval equal to the shortest CT at which no difference could be seen between the T pulse response in the paired record and the response in a single pulse record. The need for more precise quantification methods as well as for more objective means of establishing the beginning and end of recovery from refractoriness was clear.

The use, in the experiments reported here, of a digital storage oscilloscope linked to a microcomputer made it possible to improve signal-to-noise ratios, reduce stimulus artifacts and develop more "hands off", quantitative estimates of the range of recovery from refractoriness. Photographs of oscilloscope traces were replaced by sets of single traces stored on magnetic tape. These records could be read back into the digital oscilloscope, averaged to reduce

random noise and stored on diskette. Software techniques that involved subtraction of single pulse records from paired pulse records were developed to reduce artifacts and more rigorously estimate the post-stimulation recovery of excitability. Refractory period estimation was also aided by software capable of calculating response magnitude and by curve fitting procedures.

Mapping Candidate Reward Pathways

An additional improvement over the previous CAP studies (Kiss, 1982; Shizgal, Kiss and Bielajew, 1982) was the behavioural testing of stimulation sites prior to recording. Only those behaviourally-tested subjects that demonstrated vigorous operant responding free of obvious motoric disruption were included in the CAP recording phase. The same electrodes, pulse durations and currents that served to reward lever pressing during behavioural testing were used to drive neural activity during the acute recording phase, thus ensuring that reward neurons were among those activated. In addition, this permitted within-subject comparisons between electrophysiological and psychophysical estimates of refractory periods.

Anatomical studies and psychophysical experiments influenced the selection of recording sites. Nauta and Domesick (1982) have indicated that the descending MFB splits at the level of the VT into medial and lateral components. The medial subdivision is described as continuous between the

VT and the median and dorsal raphe nuclei as well as nearby ventral portions of the central grey. The lateral limb of the MFB's descent is said to be further divisible into three subcomponents. These are 1) fibres that pass through dorsal regions of the sustantia nigra; i.e. virtually the entirety of the pars compacta along with the dorsal third of the pars reticulata 2) fibres travelling to the peripeduncular nucleus (a region bounded by the dorsolateral margin of the substantia nigra, the medial geniculate nucleus and the medial lemniscus) and 3) a branch that, after curving dorsomedially around the lateral extents of the medial lemniscus and red nucleus, terminates mostly in the midbrain tegmentum with some longer fibres extending to the ventral portions of the central grey. The lateral limb is also invaded by the medial subdivision of the MFB at the level of the central grey. These findings suggest that, if they extend beyond the VT, MFB reward fibres might pass through the substantia nigra and/or the raphe nuclei and central grey substance.

Rompre, Boye and Shizgal (1987) have obtained psychophysical evidence for collision between action potentials arising from BSR sites at the VT and the dorsal raphe. Although one cannot be certain that the fibres involved in this collision effect are continuous with reward neurons connecting the LH and VT, psychophysically-derived RP estimates obtained from raphe collision sites overlap with

those for the LH. The most direct approach for showing that reward neurons connect the LH and raphe is to conduct the psychophysical version of the collision test. There is a single case where this has been done with positive results (Boye, personal communication, 1987). CAP recordings of directly driven raphe responses to rewarding LH stimulation would provide a means of testing the interpretation that this effect was indeed due to collision, and might serve to indicate which raphe regions are most likely to permit replication of this finding.

Although they did not attempt to demonstrate collision between the LH and the substantia nigra (SN), Macmillan, Simantarakis and Shizgal (1982) suggested that the observed overlap between LH and SN psychophysical refractory period curves could reflect the involvement of a common population of reward neurons. A CAP study designed to record nigral activity elicited by stimulation of MFB reward sites could provide a useful indication of the likelihood that reward fibres link the LH and SN. This would aid in deciding whether the definitive psychophysically-based collision experiments should be performed.

On the basis of these anatomical and behavioural indications, regions along the trajectories of fibres projecting from MFB regions that contain reward neurons were chosen to search, using electrophysiological recording methods, for the caudal extension(s) of the MFB reward

pathway known to course between the LH and VT. Recording electrode tracks were confined to regions within 3 mm of the midline and no more than 5 mm caudal to the VT.

Regions outside those selected on the aforementioned anatomical and behavioural grounds were also examined. It was hoped that directly driven CAP's with RP's compatible with those of MFB reward neurons could be elicited from novel sites, sites that could then be tested for direct connectivity with MFB reward neurons using psychophysical methods. In addition, regions that were found to be poor recording electrode targets based upon apparent lack of substantial CAP contributions to records and regions that provided CAP's whose RP properties were not similar to those of neurons responsible for BSR could be considered lower priorities for psychophysical study.

TECHNICAL EXPERIMENTS

INTRODUCTION

This series of experiments was designed to test several hypotheses concerning the nature of the electrophysiological records that comprise the larger part of the data presented in this thesis. Two of the experiments test the view that the initial record feature, a brief rapidly rising potential, is a stimulus artifact whereas the later, longer lasting and often multiphasic potential is a neural response. The remaining two experiments were designed to assess the hypothesis that the potential attributed to a neural response reflects the synchronous activation of axons linking the stimulation and recording sites, i.e., that the neural response is a CAP.

METHODS

Subjects

Twenty - two male hooded (Long-Evans) rats obtained from Canadian Breeding Farms and weighing between 330 and 500 g at the time of the initial surgery served as subjects. Standard composition rat chow and water were available ad libitum. Reverse cycle lighting in the animal housing facility was programmed to provide a 12 hour light/dark cycle.

Electrode Construction

Each stimulation electrode consisted of a rigid 254 micron diameter stainless steel wire to which was soldered a

twisted pair of flexible, pre-insulated wires terminating in a single male Amphenol pin. The steel wire was coated with enamel (Formvar) along approximately 11 mm of its length between one end and the solder junction with the twisted pair. The tip of the steel wire was honed to a roughly hemispheric shape and was devoid of insulation.

The bipolar recording electrodes were constructed of pairs of 127 micron diameter, enamel - insulated stainless steel wires. The exposed tips used for recording were approximately square cut and uninsulated either only at their tips or for about 0.25 mm from their tips. In either case, pairs were fixed in parallel using glue or lacquer so that the tips were separated by 0.5 mm in the direction of the long axis of their shafts. The other ends of the apposed pair of wires terminated in male Amphenol pins.

Surgery

Subjects received intraperitoneal injections of atropine methylsulphate (0.6 mg/kg) to reduce mucus secretion and thus improve ventilation during surgery. Approximately 15 min. later, Somnotol (sodium pentobarbital) was injected intraperitoneally (65 mg/kg). Once a sufficient level of anaesthesia was attained, indicated by a lack of overt responding to tail pinch and corneal contact, the subject was placed in a stereotaxic instrument (model 1704, David Kopf) and an incision was made to expose the cranial surface including portions of the frontal and parietal bones. The

skull was levelled by adjusting the incisor bar so that the stereotaxic coordinates for bregma and lambda were identical in the dorsal/ventral plane. In the 9 subjects not tested psychophysically, the surgical procedures were identical except that Urethane (ethyl carbamate, 1200 mg/kg) was used as the anaesthetic.

To provide a return path for current flow, an uninsulated stainless steel wire terminating in a male amphenol pin was wrapped around several jeweller's screws embedded in the frontal and occipital bones. Two small burr holes were drilled over the bilateral stimulation targets at the LH level of the MFB. The tips of the stimulation electrodes were aimed at a target 2.8 mm posterior to bregma, 1.7 mm lateral to the midsagittal suture and 7.7 to 7.8 mm below dura.

The electrodes were fixed to the skull using dental acrylic. The male amphenol pins at the ends of the two stimulating wires and the uninsulated wire leading to the skull screws were inserted into a connector (Molino and McIntyre, 1972) which was cemented to the skull. The remaining exposed region of skull was covered with removable "sticky wax" to facilitate access to the skull for subsequent electrophysiological recording procedures.

Stimulation

Monopolar stimulation was controlled by means of custom-built pulse generators that permitted independent

manipulation of the duration, intensity and frequency of up to four stimulation pulses and a trigger signal (the S pulse) that permitted each electrophysiological record to precede stimulation onset by a known interval. Interpulse intervals could be set independently and train duration was variable. Stimulation was produced via the custom-built pulse generators which were connected to Grass CCU1 constant-current units through stimulation isolation units (Grass SIU5). Stimulation was monitored on a Tektronix 502A oscilloscope by reading the voltage drop across a precision 10 kohm resistor in series with the preparation.

Recording

The two poles of the recording electrodes were led by short wires to separate operational amplifiers (AD515J or AD545J) located on the electrode carrier within 10 cm of the rat's skull. Since neither the spatial locations nor the tip surfaces of the two poles were identical, artifacts recorded from the two poles were of different shapes and sizes. In order to reduce these dissimilarities and thus to facilitate artifact rejection, the signal from each pole was led through a variable gain amplifier and one channel of a graphic equaliser before being combined by a Tektronix 3A9 differential amplifier. The subject was grounded through the stereotaxic instrument.

The resultant record was displayed on a Gould OS4020 digital storage oscilloscope and then transferred in analog

form to FM tape. Records could also be transferred in digital form from the OS2040 oscilloscope to an Apple II+ microcomputer that averaged records and wrote them to disk.

Experiment 1: Distinguishing Stimulus Artifact from Neural Response

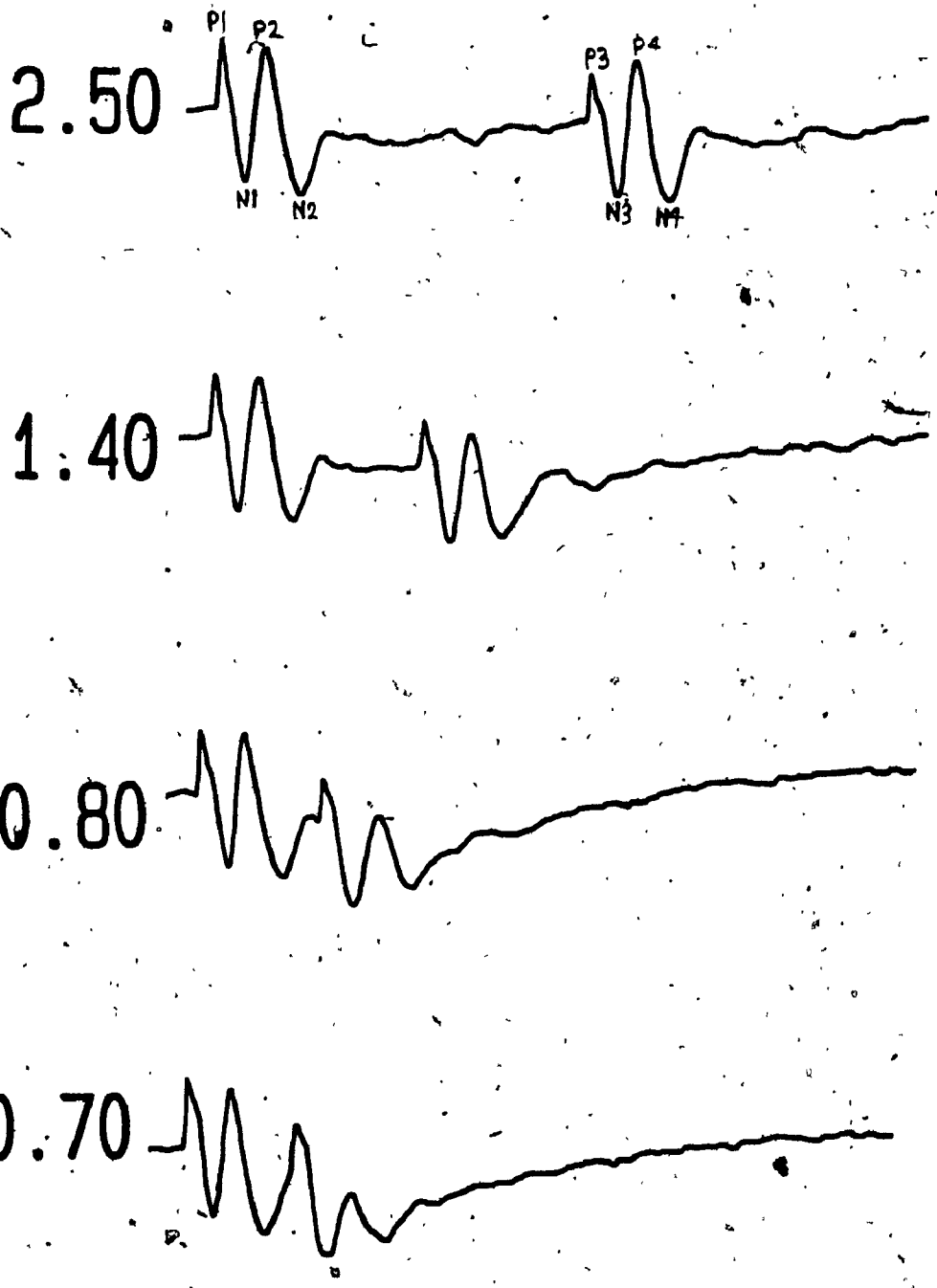
Experiment 1a: Responses to Pulse Pairs

Regardless of the position of the recording electrode, all records share a common feature : a rapidly rising, short duration potential whose onset is synchronised with the stimulation pulse. Some records include a second feature or set thereof : a longer latency, longer duration potential that is often multiphasic. In an earlier study of potentials elicited by MFB stimulation (Kiss, 1982; Shizgal, Kiss and Bielajew, 1982), it was argued that the feature with the same onset as the stimulation pulse is a stimulation artifact whereas the longer-latency, longer-duration feature is a neural response. If so, the second feature should vary with the CT interval as a result of recovery from refractoriness in the stimulated neurons, whereas the first feature should remain constant as the CT interval is varied.

This test consisted of recording potential changes during and after delivery of pairs of stimulation pulses; the CT interval was systematically varied. In Figure 1, the top trace, taken at a relatively long CT interval of 2.5 msec, contains two clusters of features with two positive-going and two negative-going features per cluster; the first cluster is

Figure 1: RESPONSES TO PAIRED STIMULI

The numerals to the left of traces indicate the interval (msec) between stimulation pulses. Trace durations are 5 msec.


2.50
P1
P2
P3
P4
N1
N2
N3
N4
1.40
0.80
0.70

elicited by the C pulse and the second by the T. In the next trace, taken at a CT of 1.4 msec, the feature labelled P4-N4 in the second cluster is of reduced amplitude. This reduction in P4-N4 amplitude continues in the last two traces in this series and is directly related to the CT interval. It is reasonable to suggest that the marked systematic attenuation of P4-N4 with decreased CT interval reflects a progressive increase in the proportion of the stimulated fibres that are refractory to excitation by the T pulse. None of the other features undergo such marked and systematic attenuation, indicating that they are due to either artifacts of stimulation (P1-N1, P3-N3) or responses to the first stimulus of each pair (P2-N2).

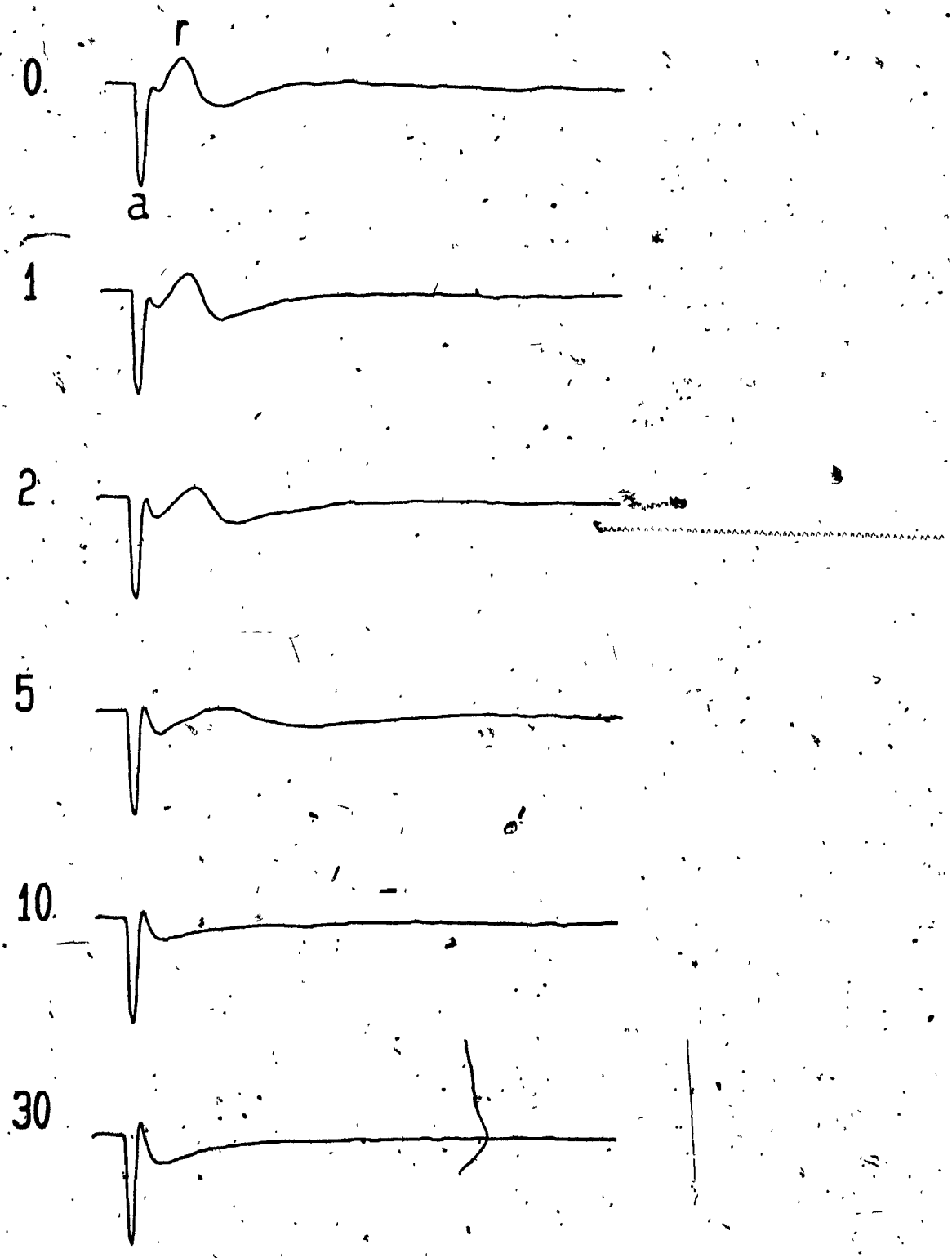
During the course of this study approximately 500 series of traces were collected in which the CT interval was systematically varied. In all records in which the signal/noise is good and there is little overlap of clusters of features in time, this same pattern was seen clearly. In the others, the more elaborate methods described in Section III are required to see systematic changes as a function of CT interval.

Experiment 1b : Response to Single Pulses During Progressive Hypoxia

Figure 2 further supports the notion that the longer latency feature is due to neural activity whereas the short latency event is a stimulation artifact. The subject that

Figure 2: RESPONSES TO SINGLE STIMULI AFTER LETHAL INJECTION

The numerals to the left of traces refer to approximate time (min) after lethal injection of Urethane. Trace durations are 5 msec.


r
0
a
1
2
5
10
30

provided the data for this figure received a lethal overdose of Urethane. Recall that the artifact is thought to propagate by interstitial volume conduction and not to be dependant on neural metabolic activity (Nicholson, 1979). Thus, cerebral anoxia caused by circulatory system failure would lead to the progressive decline and eventual elimination of neural response with little or no change in the stimulus artifact.

The recordings that comprise Figure 2 were made in response to single pulses starting at the time of injection and continuing until about 30 min after cardiac arrest. This figure portrays the gradual decline of neural activity as anoxia progresses. Note that the short latency component (labelled A), referred to from now on as the stimulus artifact, is virtually unchanged over time whereas the longer more extended feature (labelled R), referred to hereafter as the neural response, gradually declines and is completely absent 10 min post-injection and beyond.

Experiment 2: Determining the Composition of the Neural Response

Experiment 2a : Effects of Varying the Stimulaton Frequency

Experiment 1 shows that the records include neural responses. The goal of experiment 2 is to specify the source of this neural activity. Some possible sources include graded post-synaptic potentials, trans-synaptically driven compound action potentials (CAP's) and CAP's arising from fibres directly linking the stimulation and recording sites. The

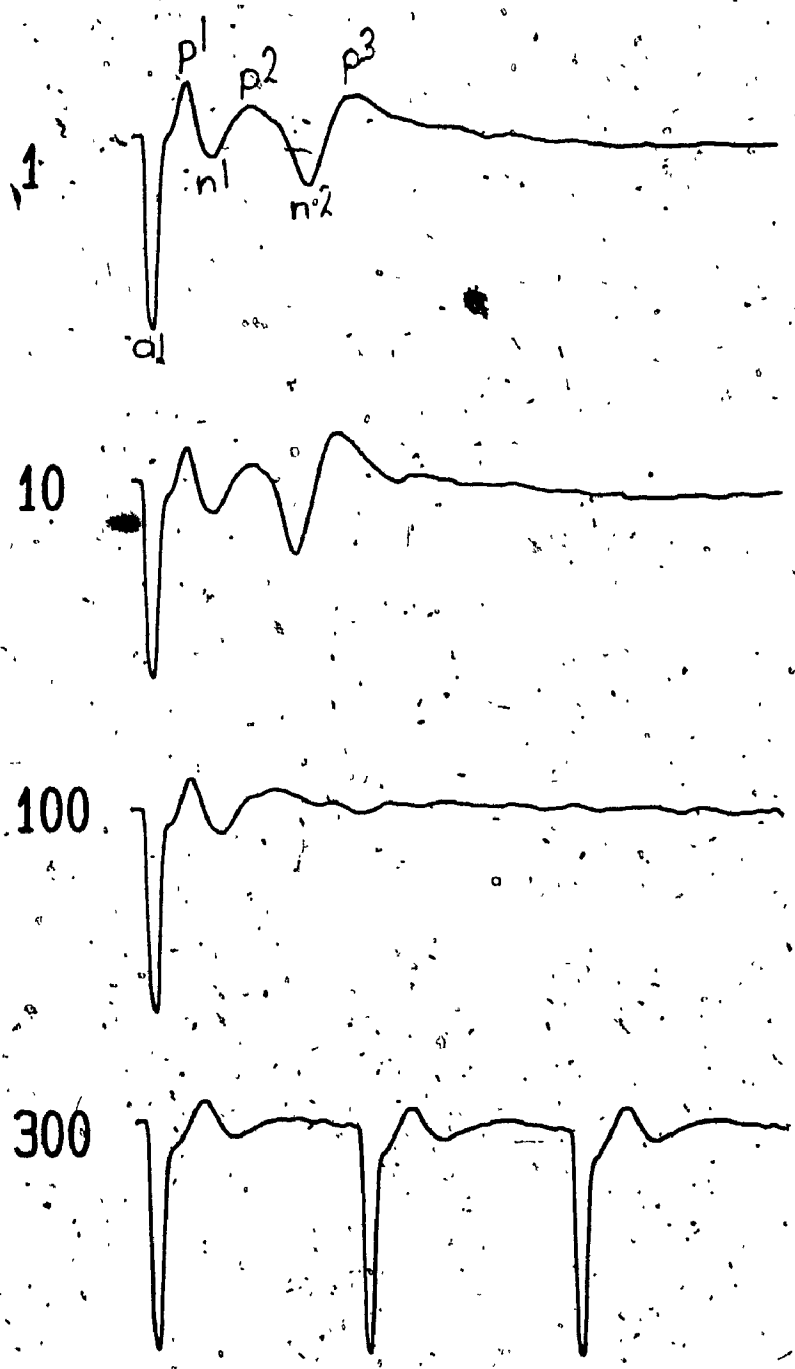
response to high frequency stimulation might aid in differentiating between these possible signal sources.

Figure 3 details the neural response to increasing frequencies of stimulation. The uppermost trace taken at a stimulation frequency of 1 Hz contains the artifact (A) followed by a series of positive (P1, P2, and P3) and negative - going (N1 and N2) features that, on the basis of Experiment 1, are assumed to be of neural origin. At 10 Hz all components remain but the latencies of N2 and P3 are shortened while the latencies of the other features are virtually unchanged. Note that the amplitude of N2 is increased. At 100 Hz, N2 and P3 are absent and the remaining record displays the triphasic form of an axonal CAP. Apart from the feature labelled "A", the record obtained at 300 Hz is, relative to that derived at 100 Hz, somewhat attenuated in amplitude and its phases display slightly longer latencies.

The continuous wave P1-N1-P2 follows high frequency and is of nearly invariant latency until the frequency exceeds 100 Hz, consistent with what is known about axonal responding Erlanger and Gasser, 1937). In contrast, the N2-P3 complex is absent at higher frequencies and undergoes considerable latency reduction and increase in amplitude as the frequency is raised from 1 to 10 Hz. The inability to follow frequencies of 100 Hz and beyond is consistent with the presence of synapses between the stimulation and recording

Figure 3: RESPONSES TO SINGLE STIMULI OF INCREASING FREQUENCIES

The numerals to the left of traces refer to the stimulation frequency (number of single pulses per sec.). Traces are 5 msec in duration.


p1
p2
p3
n1
n°2
a1
1
10
100
300

sites. The increase in N2-P3 amplitude from 1 to 10 Hz may be due to potentiation, a process thought to involve increased transmitter release (Milner, 1970). This apparent potentiation also supports the suggestion that the N2-P3 complex is a record of summed post-synaptic activation.

The first, tripartite complex is similar in form and latency to many of the responses that comprise the recording data for this thesis and suggests that the CAP records may be comprised mostly of the results of the synchronous activation of axons linking the stimulation and recording sites. Features similar to N2-P3 were seen only twice during this study perhaps because longer latency events of synaptic origin would likely not often appear on the relatively short duration records intended to capture shorter latency directly driven CAP's. The duration of traces rarely exceeded 5.0 msec and, had it been longer, it is likely that this putative postsynaptic activity would have been recorded with greater frequency. Due to temporal delays introduced by the interposition of synapses between the stimulation and recording sites, trans-synaptic responses would exhibit longer response latencies than direct axonal connections between these sites and would require longer traces for their registration. Nevertheless, more frequent registration of transynaptic activity was expected and its lack may reflect the fact that sufficient numbers of transynaptically activated fast conducting fibres did not connect the

stimulation and recording fields.

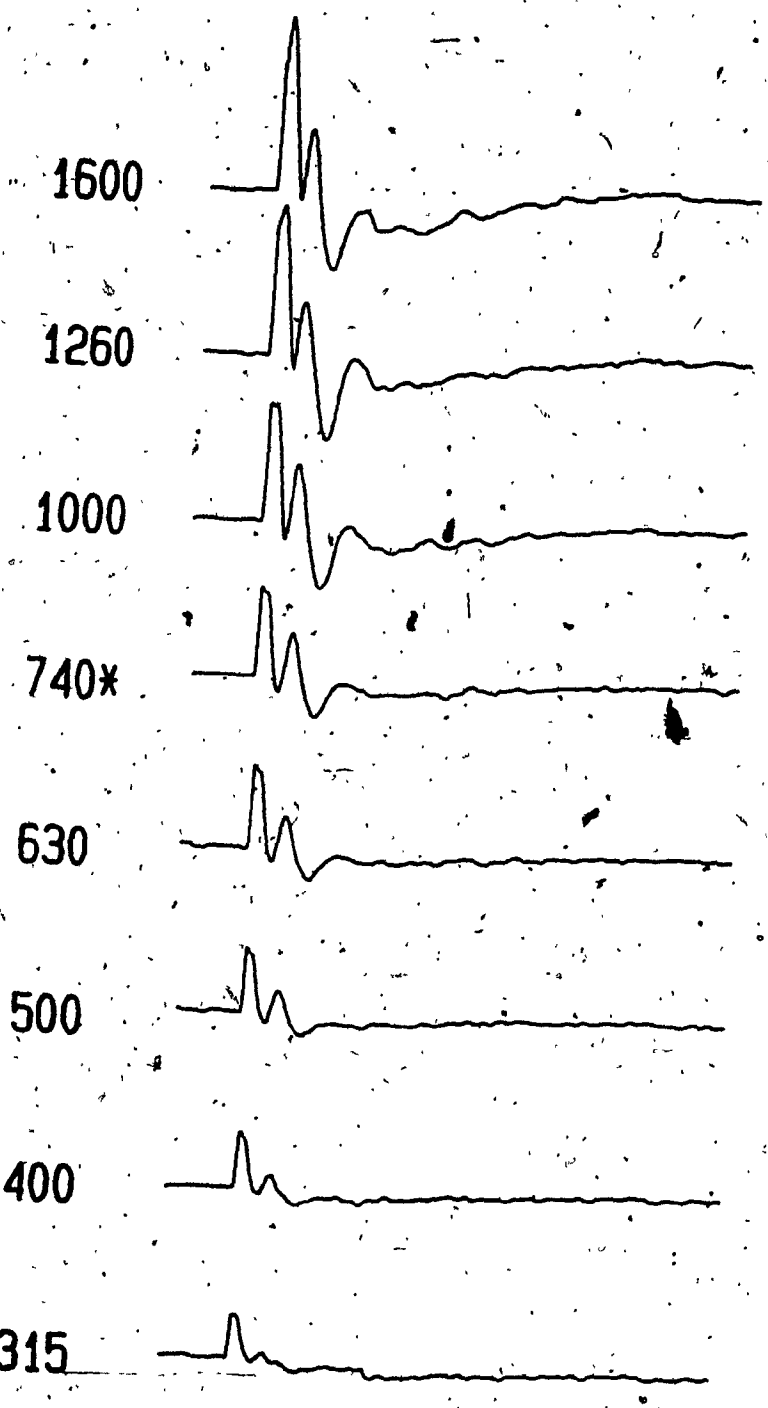
The features attributed to CAP's were seen to follow frequencies of 100 Hz in all instances that allowed physiological RP estimation.

Experiment 2b : Effects of Varying the Stimulation Current

In Figures 1,2 and 3 we see gradual decreases in response amplitude as a result of decreasing CT interval, increasing hypoxia or increasing frequency. This supports the notion that the response is compound, that is, due to the activity of a population of cells. Nonetheless, all three manipulations could also affect the amplitude of single unit responses. Varing stimulation current permits one to assess whether single units or CAP's comprise the neural responses. Units should behave in "all or none" fashion as intensity is varied, that is, their responses should appear full sized above some threshold current and be completely absent below threshold.

Figure 4 is a series of records taken in response to MFB stimulation of decreasing intensities. In this and in nearly all of the intensity series recorded in the present study there was a gradual decline in the amplitude of both the artifact and the CAP, with a preservation of the latencies of the main response features. This supports the hypothesis that the response is a CAP. As intensity declines the size of the stimulation field decreases and the number of cells contributing to the records should decline as well.

Figure 4: RESPONSES TO SINGLE STIMULI OF DECREASING INTENSITY
The numerals to the left of traces refer to stimulation current (uamp). Trace durations are 5 msec. The asterisk indicates the current at which psychophysical studies were carried out at the stimulation site.


1600
1260
1000
740*
630
500
400
315

Abrupt changes in response size and/or shape were occasionally observed in intensity series. These cases may result from loss of contributions from neurons concentrated at the fringes of the stimulation field.

The findings that the features attributed to neural activity 1) exhibit good frequency following up to 100 Hz and 2) decline in a gradual fashion as stimulus intensity is graually reduced; lend support to the notion that these features are CAPS arising from fibres directly fired by the stimulation electrode.

METHODS FOR DESCRIBING COMPOUND ACTION POTENTIALS EVOKED BY SINGLE- AND PAIRED-PULSE STIMULATION

INTRODUCTION

The main goal of this section is to illustrate the methods developed to reduce random noise, improve isolation of responses and allow quantification of responses to single- and paired-pulse stimulation. Attempts to reduce random noise consisted of averaging successive responses produced under identical conditions. In order to improve isolation of responses to single and paired stimuli, several multiple subtraction procedures were devised. Quantification of response size was accomplished using calculations of response area.

The previous section demonstrated that records elicited by single stimulation pulses contain potential changes due both to the artifacts of stimulation and to evoked neural events likely to consist of directly driven axonal CAP's. A subtraction method was developed in an attempt to remove the artifact, leaving only a neural response. In paired records, the response to the C pulse often overlaps the T pulse artifact and response, particularly at shorter CT intervals. In order to isolate the T pulse response from a paired-pulse record, subtraction was applied to remove the contribution of the C pulse response as well as the two stimulus artifacts.

Responses were quantified by means of an area measure. It will be demonstrated that, although this quantification

scheme is not perfect, it has advantages over measurement of peak amplitude.

METHODS

Averaging

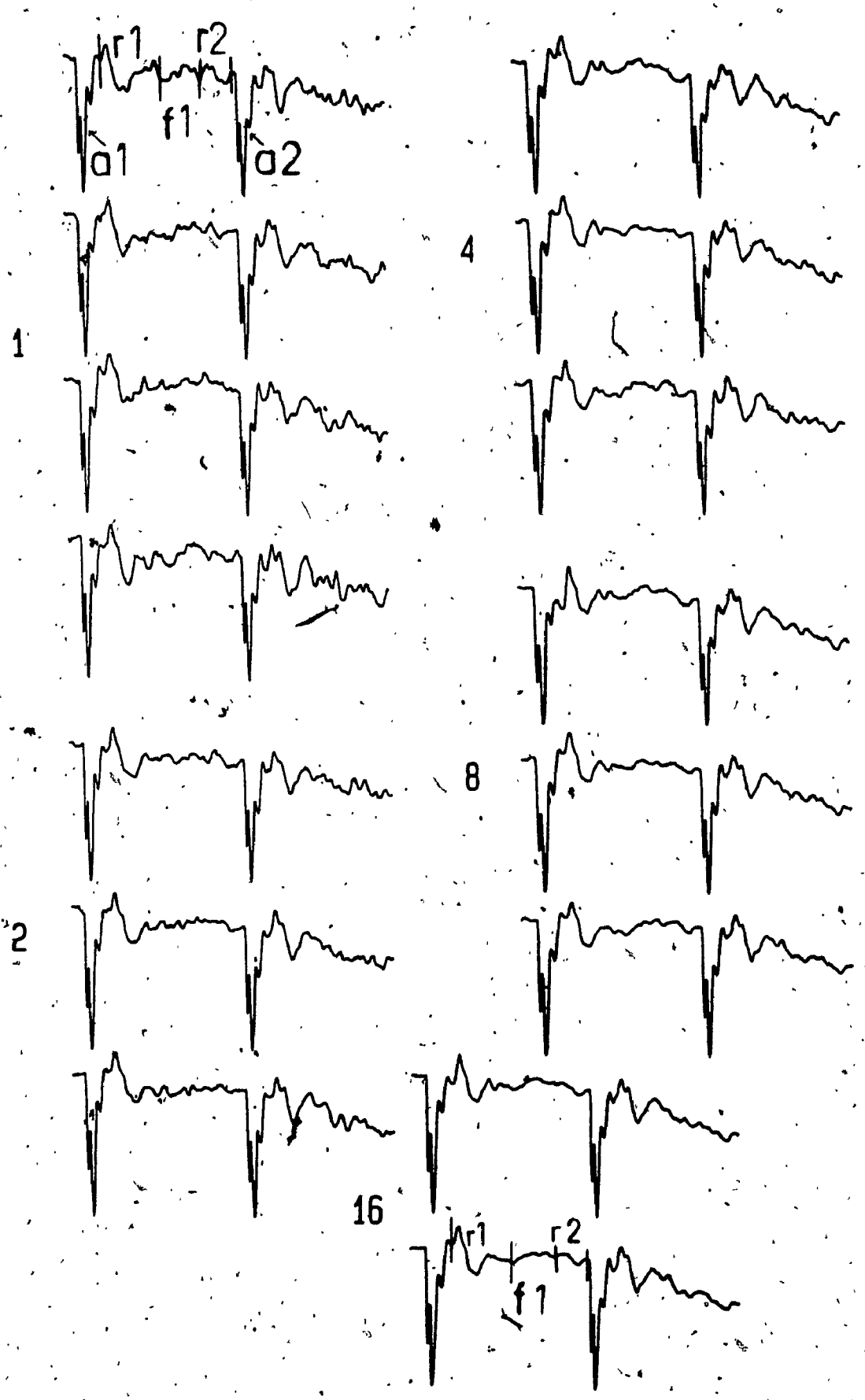
Signal averaging is widely used to reduce random noise in electrophysiological records. The reduction in noise should be a function of the square root of the number of traces averaged whereas fixed latency neural events such as CAP's should remain unattenuated (Ferris, 1974).

In the upper left trace of Figure 5, three regions have been labelled (r1, f1, and r2). The stimulus artifacts are designated a1 and a2. The region designated f1 may be attributed mostly to random variation (noise) since it is frequently absent in the other raw traces (the second, third and fourth traces from the upper left) and thus tends to nearly disappear as more and more traces are averaged (groups of traces labelled 2, 4, 8 and 16 to reflect the number of traces averaged in producing the members of each group). Contrast this pattern with the changes in the feature labelled r2. It is claimed that this feature, which is less prominent on the unaveraged record on the upper left than the random variation region labelled f1, contains a considerable neural contribution. Although like f1, it appears inconsistently in raw traces; unlike f1, r2 becomes more consistently evident as more and more responses are averaged.

Larger responses such as the feature labelled r1 are

Figure 5: RESPONSES TO PAIRED STIMULI WITH INCREASED NUMBERS OF TRACES PER AVERAGED RECORD

The numerals to the left of trace groupings refer to the number of averages per group member. Trace durations remain 5 msec.


r1
r2
f1
à1
à2
1
2
4
8
16
r1
r2
f1

made more evident and consistent in form when random variation is reduced by averaging traces. The form of this response varies considerably in the raw records but becomes progressively more consistent as the number of traces per average is increased. This response looks very similar in each average of 8 or 16 traces.

Averages of 8 traces seem considerably less variable than the raw records. Although averaging greater numbers of traces might have further reduced random variations, it would have been prohibitively time-consuming. Thus, averages of 8 were used in the great majority of the records that appear in this study.

Subtraction

Subtraction techniques were used extensively in the present work to isolate neural responses. In some instances, (see Figure 2), the response and artifact are largely separate. In others, perhaps due to the combined filtering properties of the recording apparatus and the brain itself, the artifact is extended in time and may overlap the response.

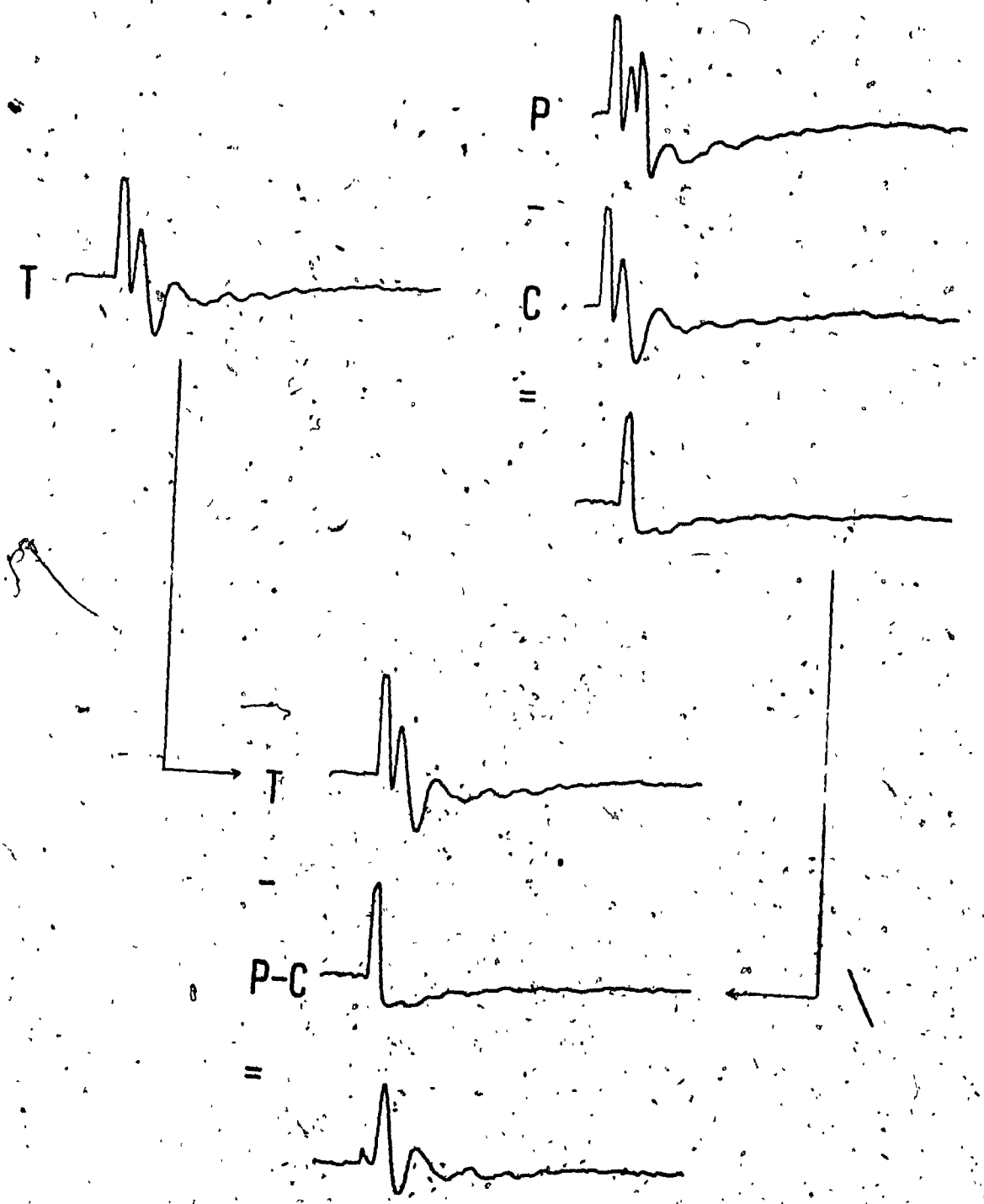
To remove artifacts from averaged single-pulse records, subtraction techniques were applied. The single pulse subtraction technique involves subtraction of a record containing only artifact from a record that includes artifact and response. To obtain a record that contains only artifact, a paired pulse trace is recorded using a CT

interval sufficiently short so that no response to the second pulse occurs (i.e. so that the second pulse arrives during the absolute refractory period of the neurons activated by the C pulse) and sufficiently long so that latent addition is minimized. Thus, little or no T pulse response should result. If a record produced by a single (C) pulse is subtracted from this paired record, a record containing little apart from the T pulse artifact should be obtained. If this T pulse artifact record is subtracted from a record containing T pulse artifact and response to produce a record of the isolated T pulse response.

Figure 6 illustrates the single pulse subtraction technique. The three traces required for subtraction-based artifact removal, are : 1) a paired record with a CT interval of 0.4 msec (upper right), 2) a C only record (second from the top, right side) temporally aligned with the C pulse of the paired record and 3) a T only record synchronized with the second pulse of the paired record (upper left). To remove the artifact from the single (T) pulse record, the C pulse record is subtracted from the paired record. Since the CT interval is sufficiently short to preclude any significant responding to the second of the paired stimuli but not short enough to produce much latent addition, this first subtraction produces a difference record (third trace, right side) consisting of little other than an isolated T pulse artifact. Subtracting this difference

Figure 6: AN ILLUSTRATION OF METHODS TO ISOLATE NEURAL RESPONSES TO SINGLE STIMULI

Traces labelled "T" are averaged responses to test pulses, the one labelled "C" is an averaged response to a conditioning pulse, the one labelled "P" is an averaged response to paired (C and T) pulse stimuli and the trace labelled "P-C" is the result of subtraction of the trace labelled "C" from the one labelled "P". The traces without labels are the algebraic difference of the two traces immediately above them. All trace durations are 5 msec.

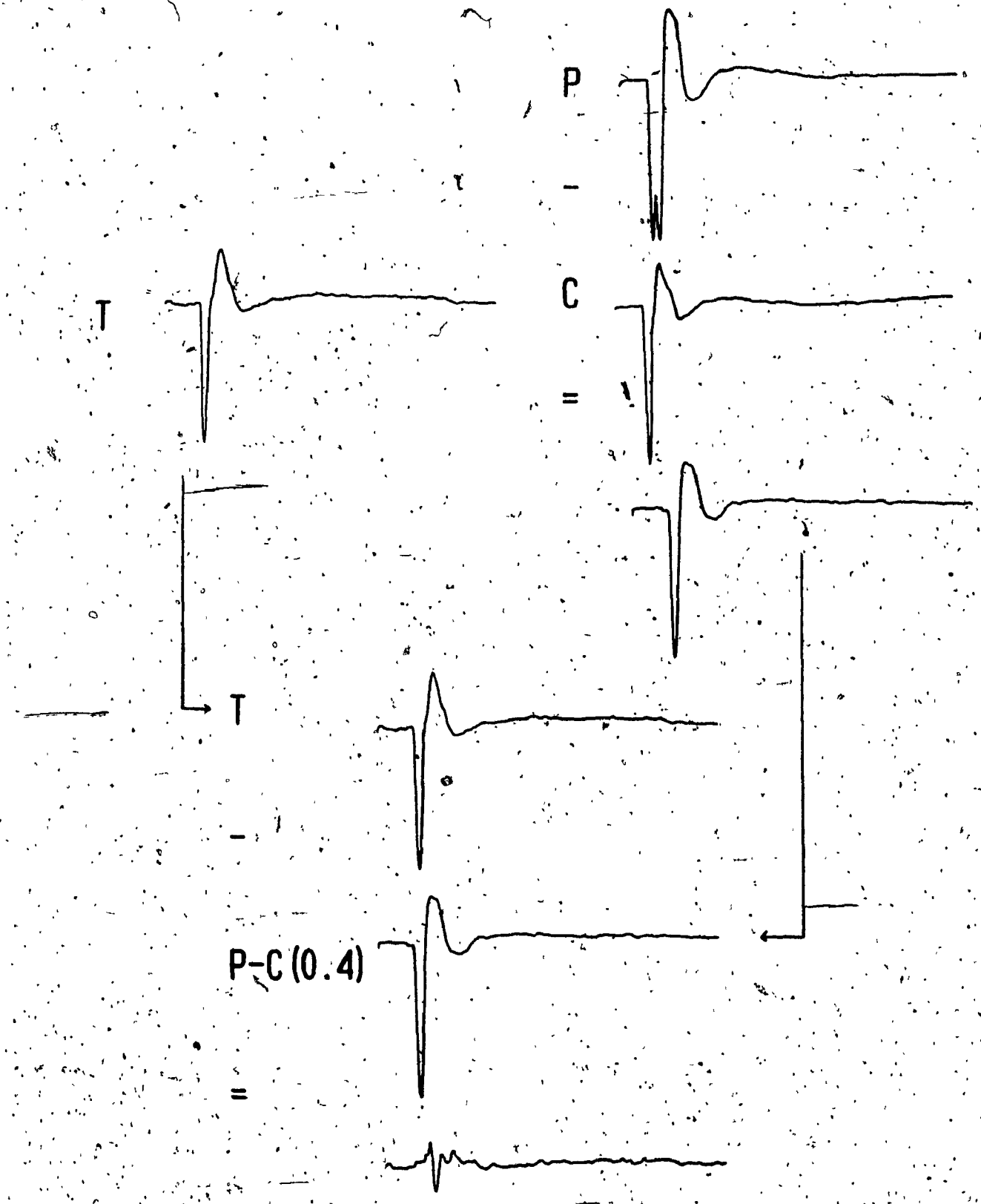

P
T
C
=
T
-
P-C
=

(labelled P-C) from the T pulse record, a record which includes artifact and neural response, removes much of the artifact and reveals a nearly artifact-free response to single pulse stimulation (bottom-most trace). Examination of this final record in Figure 6 indicates that the isolated CAP is triphasic in form and that a small artifact remnant, temporally synchronised with the artifact in the isolated artifact record that appears directly above, persists after subtraction.

Figure 7 illustrates the same single-pulse subtraction technique and the analagous traces appear in the same order on both figures. Observe that the final post-subtraction record (bottom-most trace) in Figure 7 appears to contain little other than artifact remnant, despite the apparent response-like form contained in the unsubtracted, T only record. Unaided visual examination of the single (T) pulse record in figure 7 may have been misleading and, without subtraction, it would have been exceedingly difficult to determine whether the record contained a response. This might have resulted in erroneous conclusions regarding response size and morphology. Thus subtraction may be fruitfully applied to single pulse data to aid in response isolation and, perhaps in some cases, to indicate instances where apparent responses consist largely of artifact. The response isolation capabilities of this method are apparent upon comparison of the pre- and post- subtraction intensity

Figure 7: AN ILLUSTRATION OF METHODS TO ISOLATE NEURAL RESPONSES TO SINGLE STIMULI

Traces are labelled in the same way as in Figure 6. That is "T", "C" and "P" indicate responses to test, conditioning and paired pulses respectively, "P-C" refers to the difference between "P" and "C" records and unlabelled traces are the differences of the two traces immediately above them. Traces are again 5 msec in duration.


P
–
T
C
=
T
–
P-C(0.4)
=

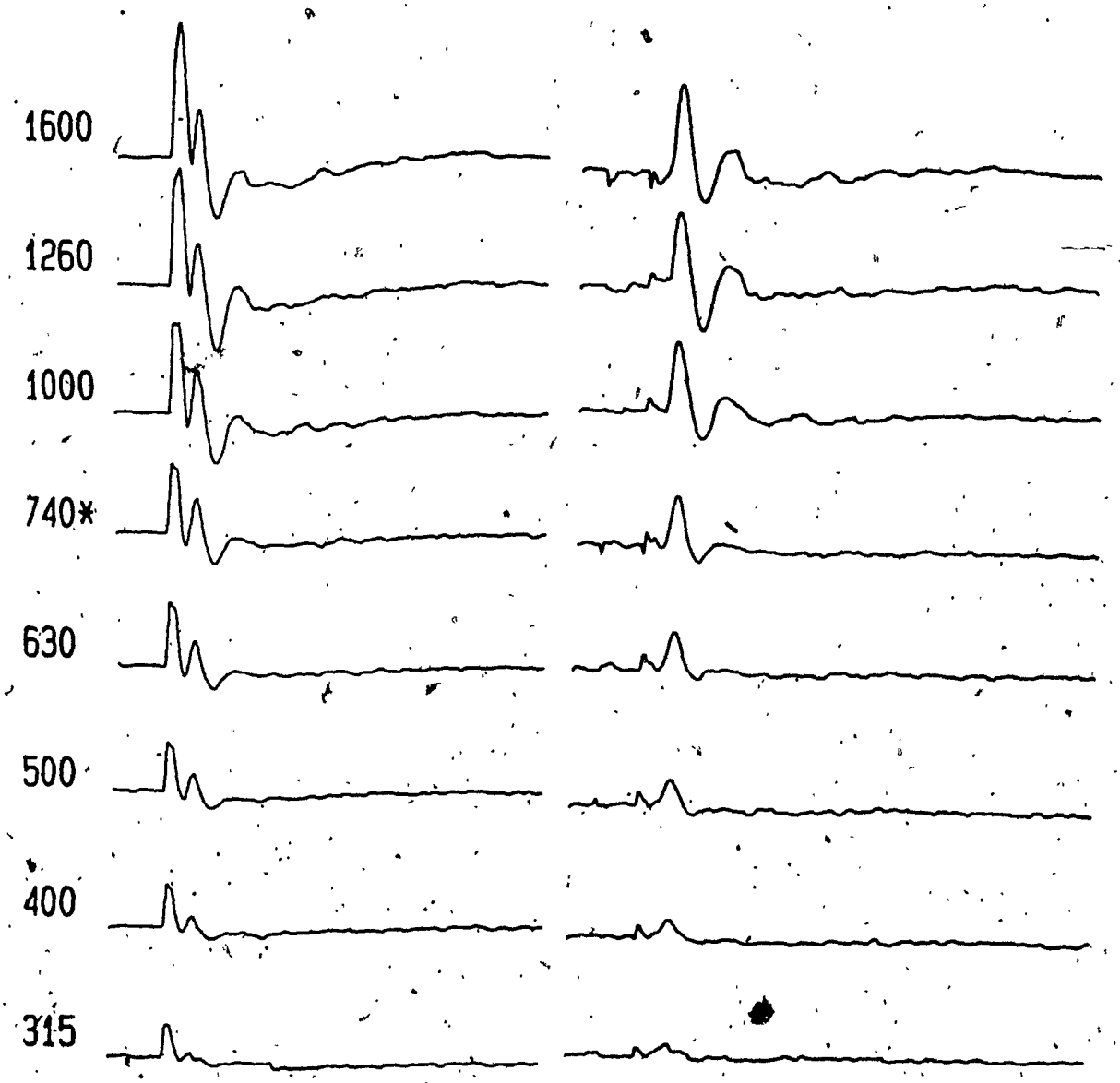
series that appear in Figure 8. The first feature of each member of the unsubtracted series in the left column is a stimulus artifact. This initial event overlaps the beginning of the first phase of the CAP. Examination of the post-subtraction records in the column on the right reveals that, at least between 1600 and 630 uA, a triphasic set of features is present. Additional experiments support the hypothesis that this set of features is an axonal CAP. A relatively small artifact remnant is seen in the post-subtraction records but the response is certainly far more isolated than in the pre-subtraction series.

However, it should be recalled that a key assumption underlying this procedure is that there is no response to the second of a pair of pulses with a CT interval of 0.4 msec due to absolute refractoriness. If, in fact, there is some slight recovery or some latent addition at this CT interval, the resultant small responses would be subtracted from single pulse records during the response isolation process. This would serve to reduce the apparent size of the final post-subtraction record but could not result in augmentation of response feature. That is, this method could only bias the results against the experimenter; i.e. towards response reduction.

Subtraction methods are perhaps even more useful in isolating responses due to the second stimulus in records

Figure 8: A COMPARISON OF RESPONSES TO STIMULI OF VARIOUS INTENSITIES BEFORE AND AFTER SUBTRACTION

Traces on the left are unsubtracted averages of responses to single pulse stimuli of decreasing intensities, those on the right are the same traces after subtraction methods for neural response isolation had been applied. Numerals refer to stimulation currents (uamp). The asterisk indicates the current at which psychophysical studies had been carried out at this stimulation site. Traces are 5 msec in duration.

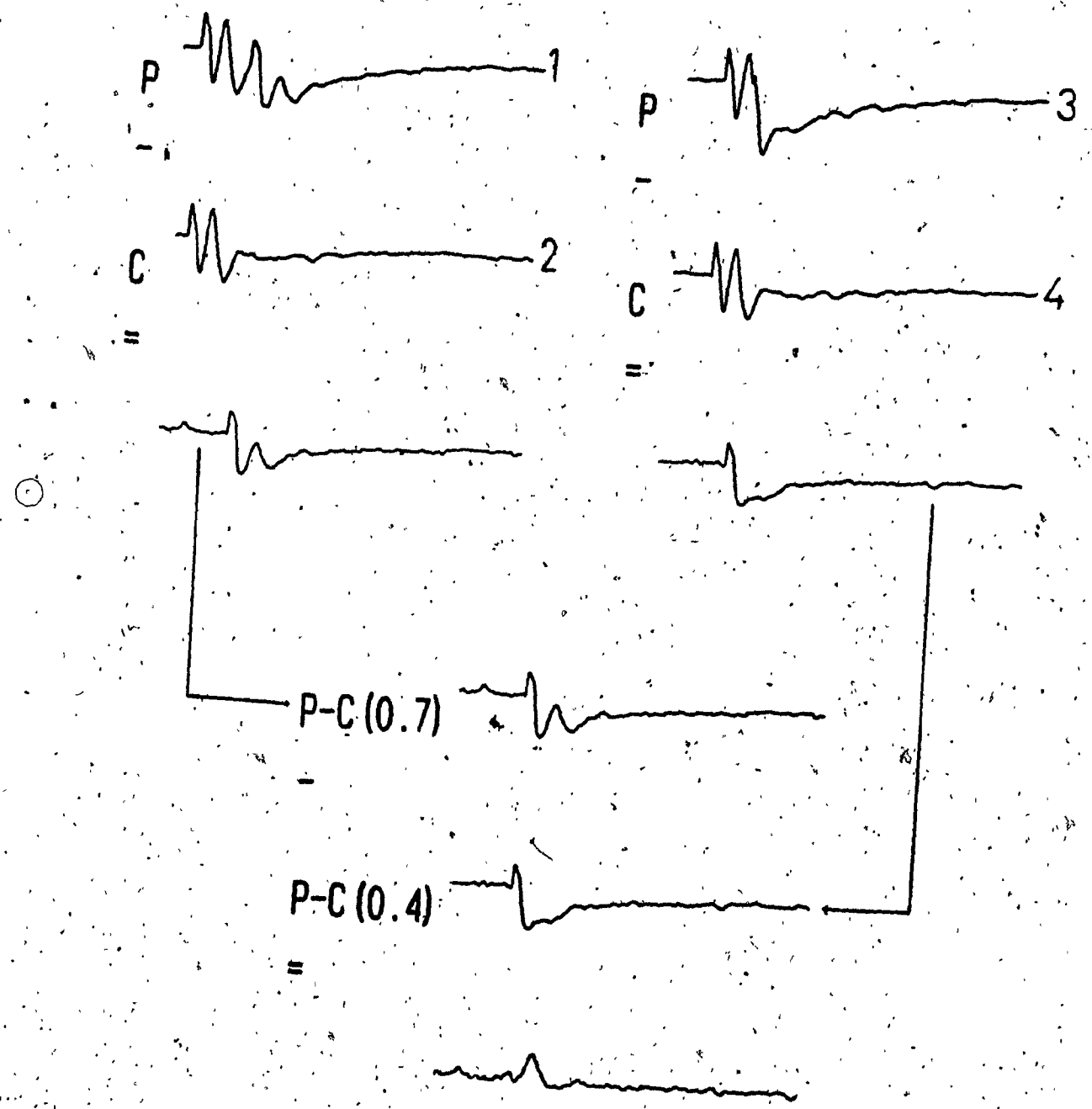

1600
1260
1000
740*
630
500
400
315

produced using pulse pairs. In these traces, not only might artifacts and responses overlap, but responses to the C pulse may overlap with T pulse responses, especially if the duration of the C pulse response exceeds the CT interval.

To apply the subtraction procedures to the paired pulse records, four traces are needed. As may be seen in Figure 9, these include 1: a paired record at the CT interval at which one wishes to isolate the T pulse response, 2: a single pulse record synchronised in time with the C pulse in record 1, a paired record with a CT interval of 0.4 msec whose T pulse is temporally aligned with the T pulse in record 1 and 4: a single pulse record temporally aligned with the C pulse in record 3. Subtraction of record 2 from record 1 should result in a record devoid of response and artifact resulting from the C pulse, leaving the T pulse artifact and any neural response due to the T pulse (third from top, left side). Subtraction of 4 from 3 should, as in the single pulse subtraction methods, result in a difference record consisting of little other that the artifact attributable to the T pulse (third from top, right side). Subtraction of this difference from the difference of traces 2 and 1 (third from top, left side) should reveal any response due to the T pulse stimulus in trace 1 virtually devoid of stimulus artifacts and C pulse responses (bottom-most trace). In fact, the relatively small response to the second pluse of the pair of stimulation pulses applied in the production of 1 is well isolated from

Figure 9: AN ILLUSTRATION OF METHODS USED TO ISOLATE THE SECOND NEURAL RESPONSE TO PAIRED STIMULI

"P" and "C" refer to responses to paired and conditioning pulse stimuli respectively. Unlabelled traces are the differences of the two traces immediately above them and are each identical to one of two traces labelled "P-C" as indicated on the figure. The values in the brackets refer to CT intervals used in the paired records from which the difference traces adjacent to these values were derived.


P
1
C
2
P
3
C
4
P-C (0.7)
P-C (0.4)

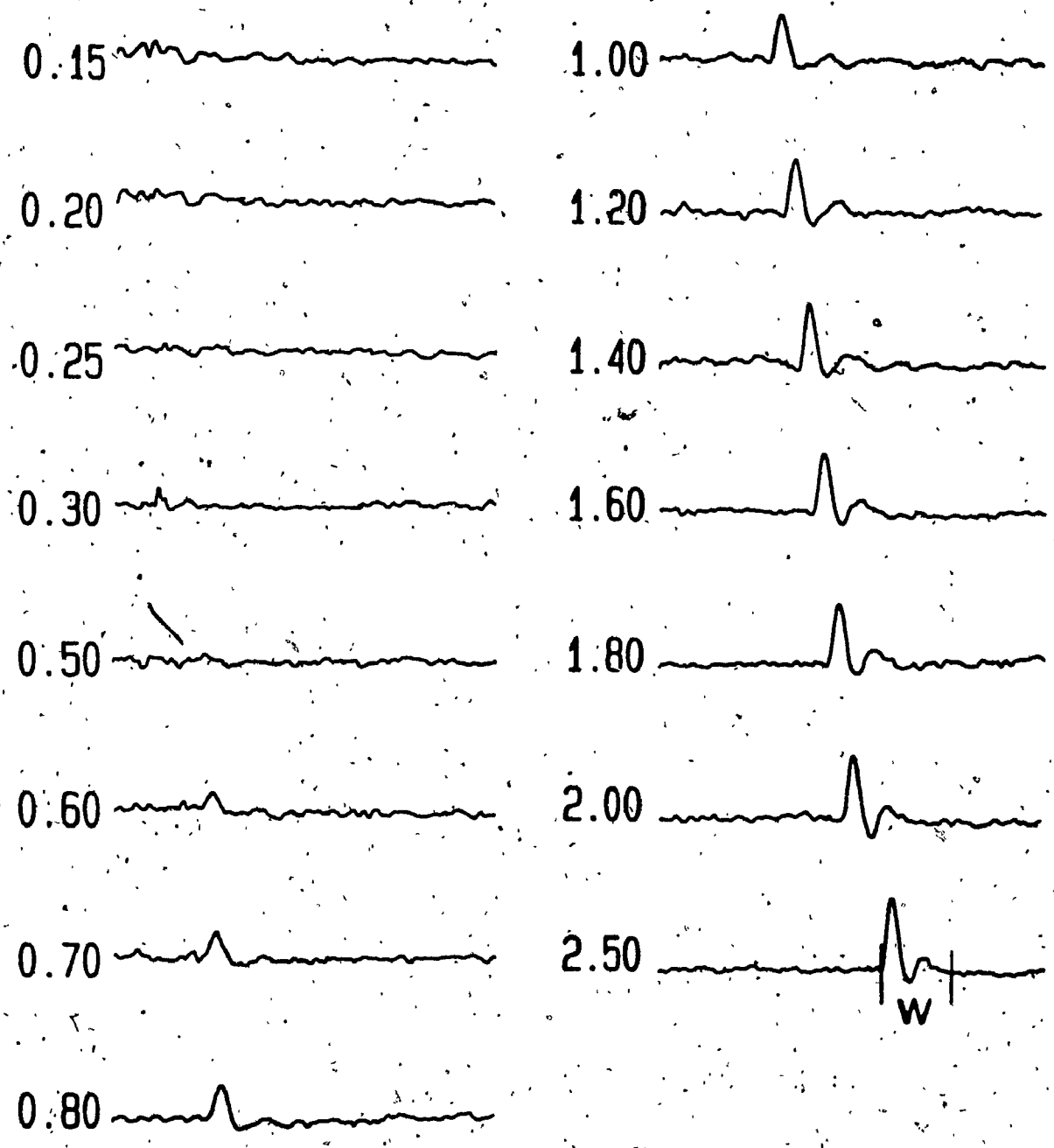
both artifacts and from the response to the C pulse in this final record. There is a very small artifact remnant, exhibiting the same latency as the positive peak in the isolated artifact record that appears above this final trace, preceding the response.

Subtraction of an appropriately synchronized "artifact only" record from each of a series of paired records covering a range of CT intervals that have been stripped of the contributions of the C pulse artifact and response by subtraction, should provide a picture of the isolated CAP responses to the T pulse and may be used to record the recovery from refractoriness in CAP's. Figure 10 is such a post-subtraction RP series. Comparison of this figure with Figure 1, which includes pre-subtraction paired pulse data used to prepare some of the records in Figure 10, illustrates the improvement in response isolation that may be achieved using the paired-pulse subtraction process.

Despite its ability to isolate the T pulse response in single and paired pulse records and thus provide improved response visualisation, some imperfections in the subtraction methods have been noted. In Figure 10 and others, a small artifact remnant precedes the isolated response. The subtraction procedures are based upon the assumption that the T pulse artifact produced at a CT of 0.4 msec is identical to the T pulse artifact produced at other CT intervals as well

Figure 10: AN ILLUSTRATION OF THE RESULTS OF METHODS TO ISOLATE RESPONSES TO THE SECOND OF A PAIR OF PULSES

The numerals to the left of traces refer to CT intervals (msec) used in the paired records from which these post-subtraction records were derived. "w" refers to a time window whose width is kept constant for a given set of traces and is the region over which response area is calculated. For a given trace the window begins at a point that is the sum of the SC and CT intervals and a constant. Traces are of 5 msec duration.


0.15
0.20
0.25
0.30
0.50
0.60
0.70
0.80
1.00
1.20
1.40
1.60
1.80
2.00
2.50
W

as in single pulse records. If this assumption were true, no artifact remnant would be produced. Violation of this assumption may result from post-stimulation alterations at the brain/electrode interface and/or in the recording components that are time-dependant. Perhaps some time is required for the brain/electrode and/or recording systems to return to their pre-stimulation state. If so, T pulse artifacts produced at different CT intervals might differ and the subtraction of T pulse artifacts produced using a CT of 0.4 msec from those produced at different CT's may yield artifact remnants whose shape and size vary with CT interval. This problem cannot simply be attributed to the presence of a small neural response to the T pulse at a CT of 0.4 msec since this would merely reduce the size of T pulse responses at all CT intervals by a constant amount and no feature, either artifactual or neural response, would be affected in a way related to CT interval. Such artifact remnant changes with changes in CT interval are frequently observed in post-subtraction records however, even when they are of considerable amplitude, they rarely obscure neural responses (e.g. see figure 19).

Calculation of Response Area

Since the size of the neural responses changes as a function of current (see Figure 8) and CT interval (Figure 10), a method was required to quantify CAP amplitude. A response area measure was used toward this end. Since the

digital records consisted of samples taken every 25 usec, response area measures consisted of the algebraic sums of the absolute values of deviations from the mean value in an experimenter selected, post T pulse time window. All such calculations were done on the final results of the subtraction procedures. Mean deviations were used because unlike the simple sums of absolute values they are not affected by possible baseline changes across records. The width of the window was held constant within each single or paired pulse series.

An area-based measure was chosen as opposed to the perhaps more standard peak measurement approach since in relation to unit responses, CAP's can be morphologically complicated. This complexity may be due to the fact that CAP's reflect the activities of multiple and probably heterogeneous subpopulations of neurons. During electrical stimulation used to elicit CAP's, neurons near the tip of the stimulation electrode are subjected to higher currents than those more distant. Thus, neurons more proximal to the stimulation electrode are likely earlier in their relative refractory periods than more distal neurons. This would cause the peaks of action potentials to arrive at the recording site at different times and the peak of the CAP would not fully reflect the summed amplitude of the peaks of the neural firings that comprise it. Therefore, a measure of CAP size based upon peak height would be misleading. It may also

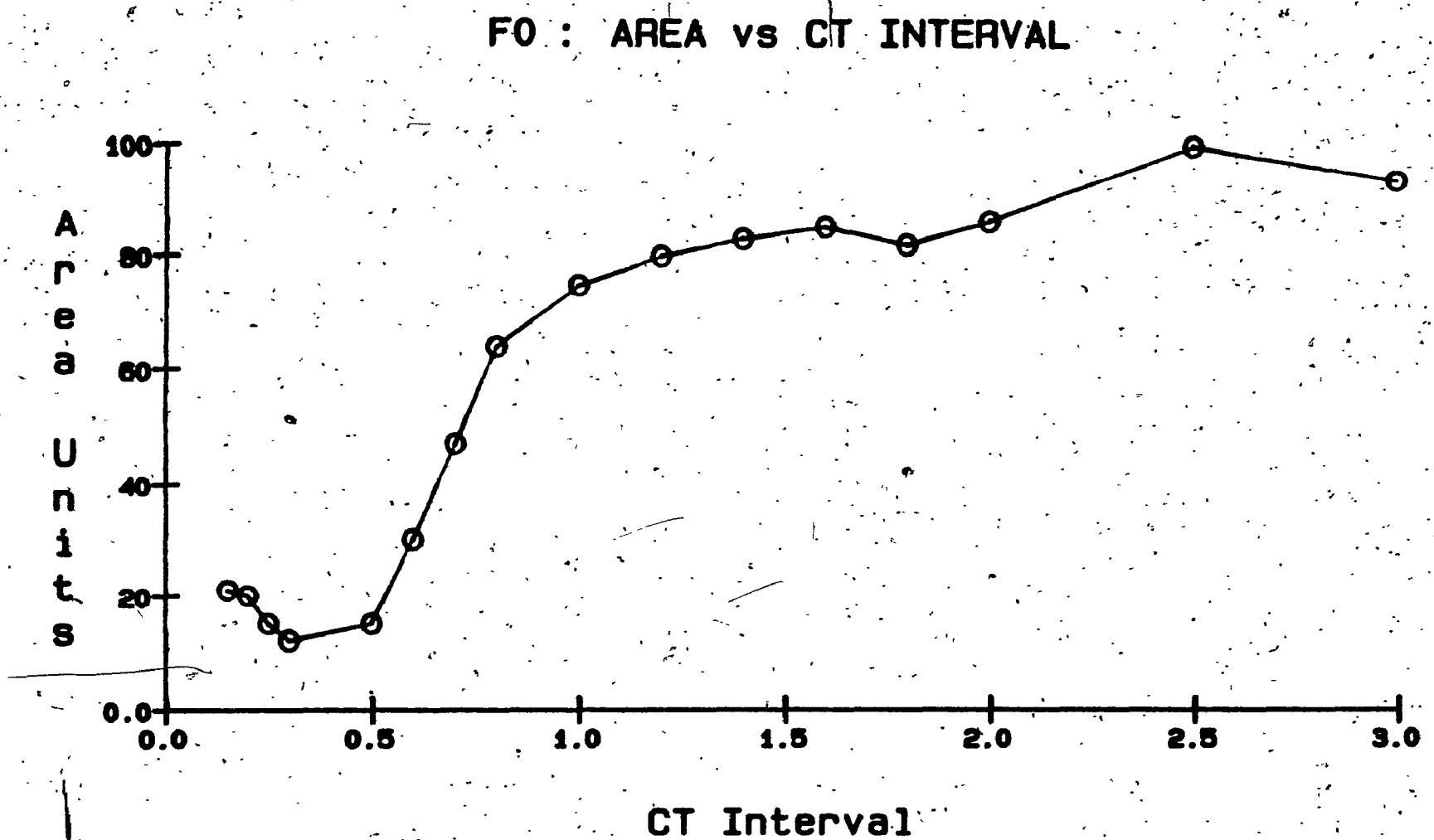
be that, due to multiple subpopulation, multiple peaks would be evident in CAP records.

An area measure is not dependant on CAP height for response quantification. An area measure circumvents difficulties in deciding which (and perhaps how many) peaks should be subject to measurement within a given set of traces and how one is to maintain comparability across sets whose response components may vary markedly due to differences in the relative orientations of stimulated fibres and recording electrodes and differences in the relative contributions of axonal subpopulations with different action potential durations. Furthermore, area measures are equally capable of quantifying peak height as peak measures and, unlike peak height calculations, can quantify changes in CAP duration.

Figure 11 is a curve of area units vs CT interval derived from Figure 10 and employing the time window displayed in the latter (indicated on the lower right trace by "w"). Upon examination of these figures, it would appear that the area measure tracks the apparent change in response size with CT interval. Nevertheless, the measure has its flaws. As suggested above, CAP's are likely the result of firings in multiple neural subpopulations which may differ in RP. These subpopulations may recover from refractoriness over different time ranges. Since conduction velocity varies during the relative refractory period (Koscis et al., 1979), a changing pattern of overlap between the recorded responses

Figure 11: VARIATION OF THE AREA OF THE ISOLATED RESPONSE TO THE SECOND OF A PAIR OF PULSES AREA WITH CT INTERVAL

The data that comprises this figure were derived from the post-subtraction records displayed in figure 10. The window's width and its starting point are as described in the caption for that figure. Units for the x-axis are in msec, and units for the y-axis are percentages of the maximum area response. As indicated in the figure title, these data are derived from subject F0.


F0 : AREA vs CT INTERVAL
Area Units
100
80
60
40
20
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
CT Interval

that contribute to a CAP could result as CT interval is varied. The overlap of peaks and troughs could cause mutual cancellation when the peak of one subpopulation is superimposed upon the trough of another and a reduced area measure would result. Alterations in the pattern of overlap would occur as CT interval was changed thus changing the effects of overlap on peak amplitudes and CAP response durations. Therefore, some inaccuracies in measurement of true CAP size using response area are likely, particularly when a fixed duration area window is used. Note that this difficulty is not circumvented using peak measures.

Thus this area measure, though imperfect, is seen as preferable over peak measurement for quantification of CAP size since it is better suited to dealing with the often complicated multiphasic morphology of CAP records and because of its increased sensitivity to changes in response area which are not reflected in peak amplitude.

COMPARISON OF PSYCHOPHYSICALLY-DERIVED AND ELECTROPHYSIOLOGICALLY-DERIVED MEASURES OF RECOVERY FROM REFRACTORINESS

INTRODUCTION

The main goal of this project was to use CAP recording methods to search for regions through which MFB reward neurons are likely to project. In such regions, CAP's elicited by rewarding MFB stimulation would be recorded and the psychophysically-derived range of RP's of MFB reward neurons would overlap with the RP's of the neurons giving rise to the CAP's. The larger the portion of the psychophysically-derived range of RP's that overlaps with the electrophysiologically-derived range, the greater the likelihood that reward neurons contribute to the CAP's.

The comparison of electrophysiologically- and psychophysically-derived measures of RP's is most meaningfully carried out when the same subject, stimulation electrode and stimulation field are used to collect both sets of data. In that instance, one knows that reward neurons are fired by the stimulation driving the CAP's and that neurons linking the recording and stimulation sites are fired by the rewarding stimulation.

The psychophysical evidence for collision between antidromic and orthodromic action potentials elicited by rewarding stimulation of the LH and VT implies that these two

sites are directly linked by axons of reward neurons. In the present experiment, projections of neurons that link these two sites and extend beyond the VT were examined by comparing measures of their RP's derived from CAP data to psychophysically-derived measures of recovery from refractoriness in MFB reward neurons activated by the same electrodes and stimulation fields that elicited the CAP's.

In the preceding section, methods for isolating and measuring CAP's elicited by T pulses were described. These methods were applied in the present experiment to describe the RP's of the neurons responsible for the CAP's elicited by rewarding MFB stimulation. The measures of CAP magnitude were then rescaled in a manner analagous to the scaling of the psychophysical data.

PSYCHOPHYSICAL METHODS

Screening

1) Apparatus

The screening chamber was a wooden enclosure with a 25 X 25 cm grid floor. The walls were 70 cm high and the front wall was clear Plexiglas. A Lehigh Valley rodent lever protruded from the center of the right wall 6 cm above the floor. A cable attached to the subject's electrode connector was routed to the stimulator outputs via a seven channel, slip ring commutator (model CAY-652, Airflyte Electronics) fixed to the center of the ceiling.

Stimulus parameters were determined by integrated circuit

pulse generators and dual channel constant - current amplifiers (Mundl, 1980). When neither channel was active, their outputs were shorted to ground through a 1 Kohm resistor to reduce the buildup of charge at the brain/electrode interface. Stimulus current was monitored on a Telequipment (model D61A) oscilloscope by reading the potential drop across a precision 1 Kohm resistor in series with the subject.

2) Screening and Shaping

Surgical methods for implanting stimulation electrodes have been fully described in the Technical Experiments. After a post-surgical recovery period of at least 24 hours, subjects were tested to acertain whether the implanted electrodes, aimed bilaterally at the LH level of the MFB, would support self-stimulation. Screening was carried out using 500 msec trains of rectangular, cathodal 100 usec constant current pulses. Once a combination of current and frequency was found that produced increased locomotion, sniffing and apparent searching for the source of stimulation, subjects were shaped to approach and eventually press the lever that triggered the stimulation. Current and frequency were optimized to yield vigorous self-stimulation (SS), (> 40 presses per minute) without severe motoric disruption. A series of pulse frequencies descending in 0.1 log unit steps was applied until responding ceased. This was repeated until the rate of lever pressing varied in an

orderly and reliable manner with the frequency; that is, until the frequency at which half-maximal rate of responding ocurred varied by less than 0.2 log10 units across 5 consecutive series. Subjects that failed to exhibit vigorous responding devoid of motoric disruption were eliminated from the study.

Automated Refractory Period Determination

1) Apparatus

The operant chambers and electrode - to - stimulator connections used for the psychophysical determinations of refractory period closely resembled those used for screening and shaping, with the following exceptions:

i) the lever was offset from the center of the wall by 6cm,

ii) parallel 1.25 cm diameter Plexiglas rods separated by 0.6 cm replaced the grid floor,

iii) a yellow, 1 cm diameter, hemispherical light was placed about 5 cm above the lever and

iv) all four walls were composed of Plexiglas.

The entire chamber was enclosed in a wooden, sound attenuation chamber lined with 1 inch thick Styrofoam insulation. The external enclosure included a 60 W ceiling light and a ventilation fan which provided a level of background noise that served to reduce the likelihood of the subjects' distraction by extraneous noise. The enclosures were housed in a room separate from the experimenter; the

subjects' performance could be observed through a 11 by 15 cm Plexiglas window in the front of the sound attenuation chamber by means of a video camera system.

2) Parametric control

In the screening and shaping apparatus, all stimulus parameters were set by hand. In the automated apparatus, parameters were under microprocessor control. Temporal parameters and electrode selection were controlled by external logic and a dedicated microprocessor equipped with a custom constructed interface. Pulse amplitude was determined by the output of a digital to analog converter fed to a voltage-controlled constant-current amplifier. Pulse and train duration, pulse polarity and output gating circuitry were the same as for the screening sessions.

3) Stabilization

The initial stimulation parameters employed were identical to those in the shaping procedure, with each trial lasting 30 sec. The number of pulses required to produce half-maximal rate of responding was repeatedly determined as follows: The initial, maximum and minimum number of pulses per train were preset by the experimenter. If the subject did not press on the initial trial, the number of pulses was increased by 0.3 log10 units until responding was elicited or until the preset maximum value for the number of pulses was reached. If the animal responded on the initial trial, the number was sucessively increased by 0.1 log10 units until the

preset maximum number was attained or until the rate of responding failed to increase by more than 10% of the rate on the previous trial. The number of pulses was then returned to 0.1 log10 units below the value used on the initial trial and was sucessively decreased in 0.1 log10 unit steps until the rate of responding failed to reach 10 per cent of maximum on two consecutive trials or until the preset lower limit was reached.

Daily stabilizatiion sessions consisted of twenty such series of trials. The refractory period determinations were begun after the range of the numbers of pulses required for half-maximal rates of responding, referred to hereafter as the "required number", was less than 0.1 log10 units across an entire stabilization session . Subjects that performed in a particularly vigorous and reliable manner during screening and shaping and reached the stability criterion for sceeening and shaping within the first 10 repetitions of the descending frequency series did not undergo stabilization but proceeded directly to refractory period determination.

4) Refractory period determination

Each session began with at least four determinations of the required number of single pulses. These were followed by determinations of the required number of pulse pairs with CT intervals ranging between 0.15 and 5.0 msec. A single pulse evaluation was carried out after every fifth CT interval and after the last paired pulse condition. The sequence of CT

interval presentation was pseudo-random. The algorithm for determining the required number of pulse pairs was identical to that used in stabilization except that the starting number for each determination was automatically adjusted to prevent excessive numbers of effective pulses from being delivered to the subject. Refractory period data were considered suitable for averaging only if the single pulse required number range within a session did not exceed 0.1 log10 units. The required number values for the first two single pulse determinations were not included in this range because these initial determinations were regarded as a "warm-up" needed for performance to stabilize. At times, subjects were given two sessions within a day with an inter-session rest period of at least one half hour.

The measure of recovery from refractoriness was based upon the change in the required number as a function of CT interval. The computer calculated an estimate of T pulse effectiveness using the following formula :

$$E = \frac{RN(SP)}{RN(CT)} - 1$$

where E = effectiveness of the T pulse,

RN(SP) = required number in the single pulse condition

and RN(CT) = required number of pulse pairs at a given CT interval.

At sufficiently short CT intervals, the T pulse will be ineffective because the neurons stimulated by the C pulse will be in a state of absolute refractoriness. If so, the required number of pulse pairs will be the same as the required number of single pulses and, according to the formula, E equals zero. With a sufficiently long CT interval, the T pulse becomes fully effective in firing neurons, and thus the required number of pulse pairs is half the required number of single pulses. The fact that the T pulses are now as effective as the C pulses is reflected in an E value of 1. Between these extremes there exists a range of CT intervals over which the T pulse becomes progressively more effective as the CT interval is increased and the directly stimulated elements recover from refractoriness.

Scaling of RP Data Derived from CAP Responses to Facilitate Comparison with Behaviourally-Derived RP Data

A previous section describes the area measure used to quantify the growth of the CAP elicited by the second of a pair of pulses as CT interval is increased. This growth is attributed to recovery from refractoriness, as is the increase with CT interval of the T pulse effectiveness (E) statistic used to scale results of the psychophysical pulse pair test carried out with a single electrode. If scaled in a way analagous to the E-value calculation for

psychophysical data, one could compare the growth of a given CAP to the increases in E as a function of CT interval.

To carry out such comparisons, an area ratio measure was used to scale the electrophysiological data. The electrophysiological T pulse effectiveness for a given CT was the T response area at that CT divided by the maximum T response area calculated across all CT intervals tested at a particular recording site. Thus, for both data types the RP range could be defined as that range of tested CT intervals over which the E values went from minimum to maximum.

The similarities between the two measures of T pulse effectiveness can be made more apparent by dealing with a hypothetical neural population with simplified properties. If the behavioural weight of each reward neuron during psychophysical RP estimation is the same, then T pulse effectiveness at a given CT interval is equivalent to the number of neurons fired by the T pulse divided by the number of neurons fired by the C pulse. This relationship is readily derived from the behavioural effectiveness formula given above and the counter model of BSR (Edmonds, Stellar and Gallistel, 1974). The area ratio, the physiological measure of T pulse effectiveness, is equivalent to the number of neurons fired by the T pulse divided by the number fired by the C pulse, provided that the fired elements in the recording field are homogeneous and the contribution of each of them to CAP amplitude is the same. Although it is unlikely

either population possesses these properties, this example illustrates the relationship between the two measures.

Quantification of Refractory Periods

1) Determining the Range of Recovery from Refractoriness

As previously described, E value vs CT interval curves can be derived from both physiological and behavioural data. In order to compare recovery from these two sources one requires a means of quantifying the points at which this recovery begins and ends. Examination of Figure 12, a curve depicting the change in T pulse effectiveness as a function of CT interval derived from CAP recording data, and Figure 13, an E vs CT curve based upon psychophysical results, indicates that the minimum E value need not occur at the shortest CT tested, a finding consistent with evidence for latent addition at very short CT intervals, a phenomenon unrelated to the refractory period (Yeomans et al., 1979).

After C pulse delivery, beyond the region fired by the pulse there is a region in which the stimulation pulse brings neurons close to threshold without firing them. If the T pulse arrives sufficiently soon after the C pulse, temporal summation of the rapidly decaying depolarizations produced by each of the two pulses may result in firings additional to those produced by the C pulse. According to Yeomans et al., (1979) the contribution of local potential summation to E values has declined to a low level before recovery from refractoriness begins in MFB reward neurons. This phenomenon,

Figure 12: EFFECTIVENESS AS A FUNCTION OF C-T INTERVAL BASED UPON DATA RECORDED FROM A NIGRAL SITE

These data are derived from area measurements performed on CAP recordings from the SN. "E" refers to the area of the T pulse response at a given CT interval as a proportion of the maximum area of the T pulse response at all CT intervals (msec).

E5 : SN : Electrophysiological Data

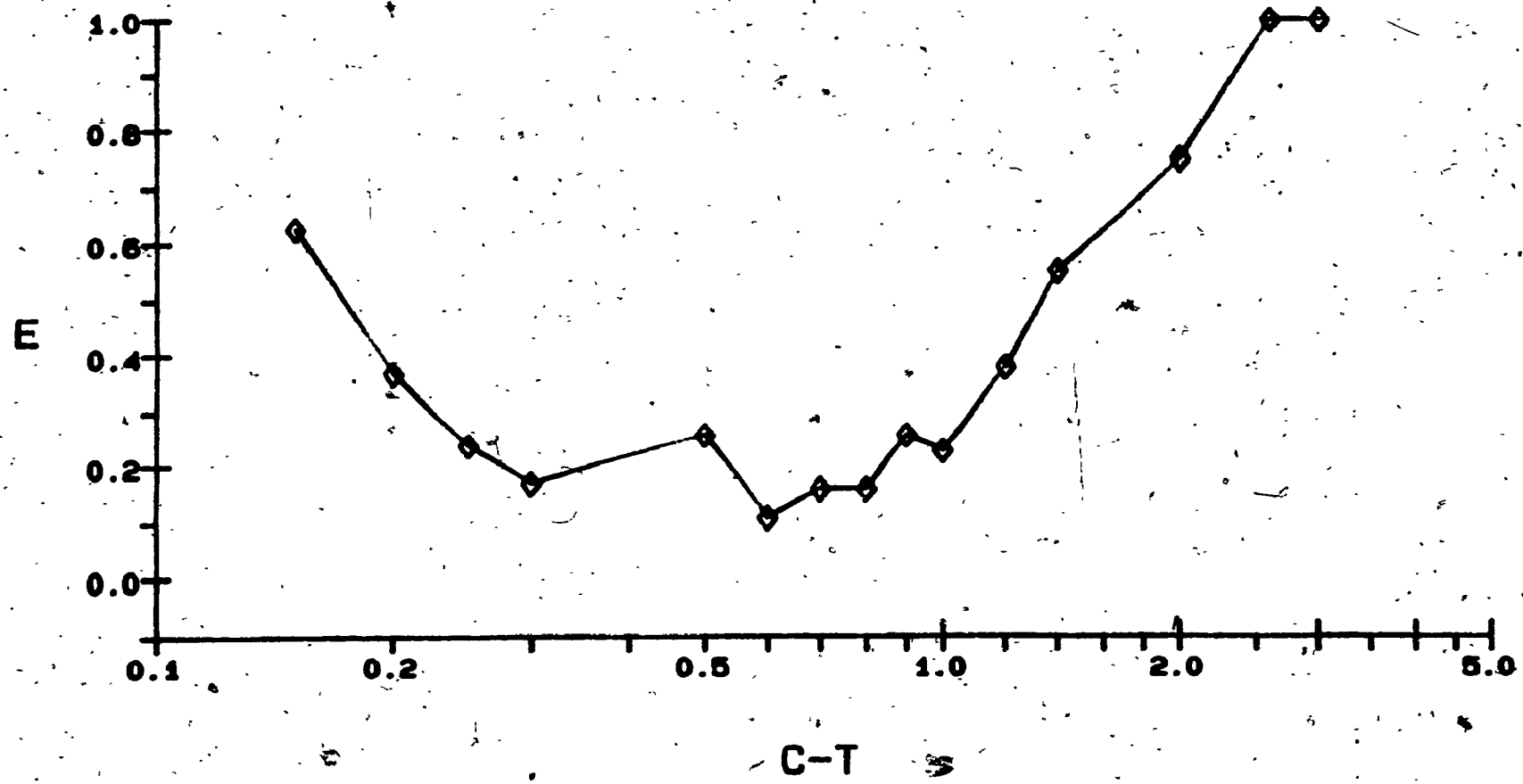
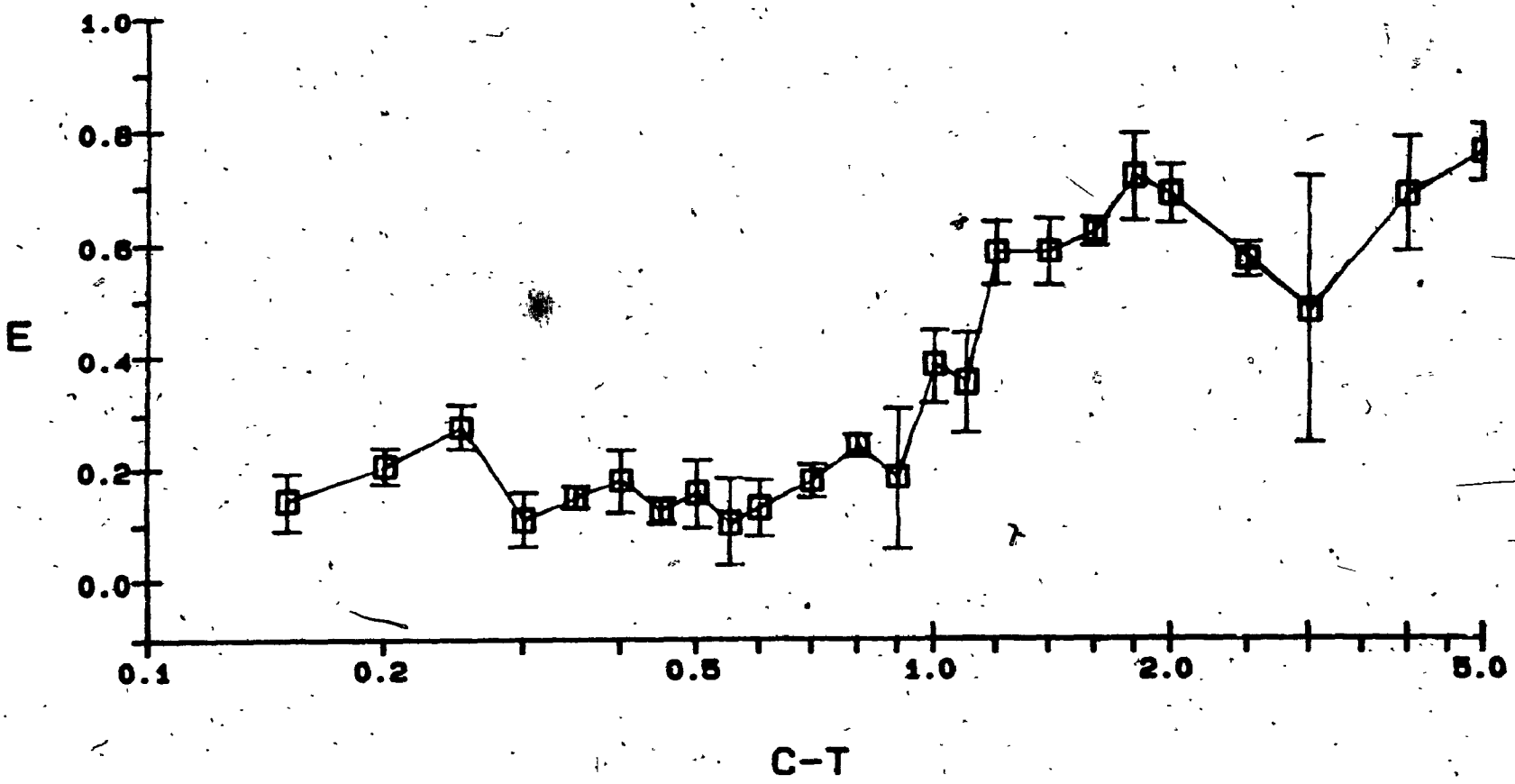


Figure 13: EFFECTIVENESS AS A FUNCTION OF C-T INTERVAL BASED UPON PSYCHOPHYSICAL DATA DERIVED FROM AN MFB REWARD SITE

These data are derived from psychophysical measurements of the effectiveness of the second of pairs of rewarding stimulation pulses directed at the MFB locus used to elicit the CAP data upon which Figure 12 is based. "E" refers to T pulse effectiveness as described in the psychophysical methods section, "C-T" refers to C-T interval (msec) and error bars about data points indicate +/- 1 standard error of the mean.

E5 : LH : Behavioural Data



may mask the initial increase in effectiveness at the beginning of recovery from refractoriness. A precise end of recovery also may not be apparent.

A practical solution to the quantification of the beginning and end of recovery from refractoriness that could be applied to both recording and psychophysical results involves the fitting of a three component line to E vs CT curves from both sources. A decaying exponential is fit between an initial extrapolated Y - intercept (A1) and a CT interval at which an initial estimate of the minimum E value occurs (X2). The time constant of the exponential is designated "T". From this minimum a straight line of positive slope is drawn to an initial point at which recovery appears complete (A3, CT=X3) and a line of zero slope from this point is extended to the maximum CT value tested. Using a combination of in-house and commercial software the values of A1, A3, X2, X3 and T are repeatedly subjected to simultaneous manipulation until the residual sums of squares between the resultant line and the data are minimized. This has provided us with a relatively "hands-off" way of estimating the beginning and end of recovery, which correspond to the final values of X2 and X3 respectively. Although, as indicated above, a straight line connects X2 and X3 on all E vs CT figures, this should not be taken to imply that there are any theoretical grounds for expecting the

recovery to occur linearly over time. A straight line was selected merely for convenience.

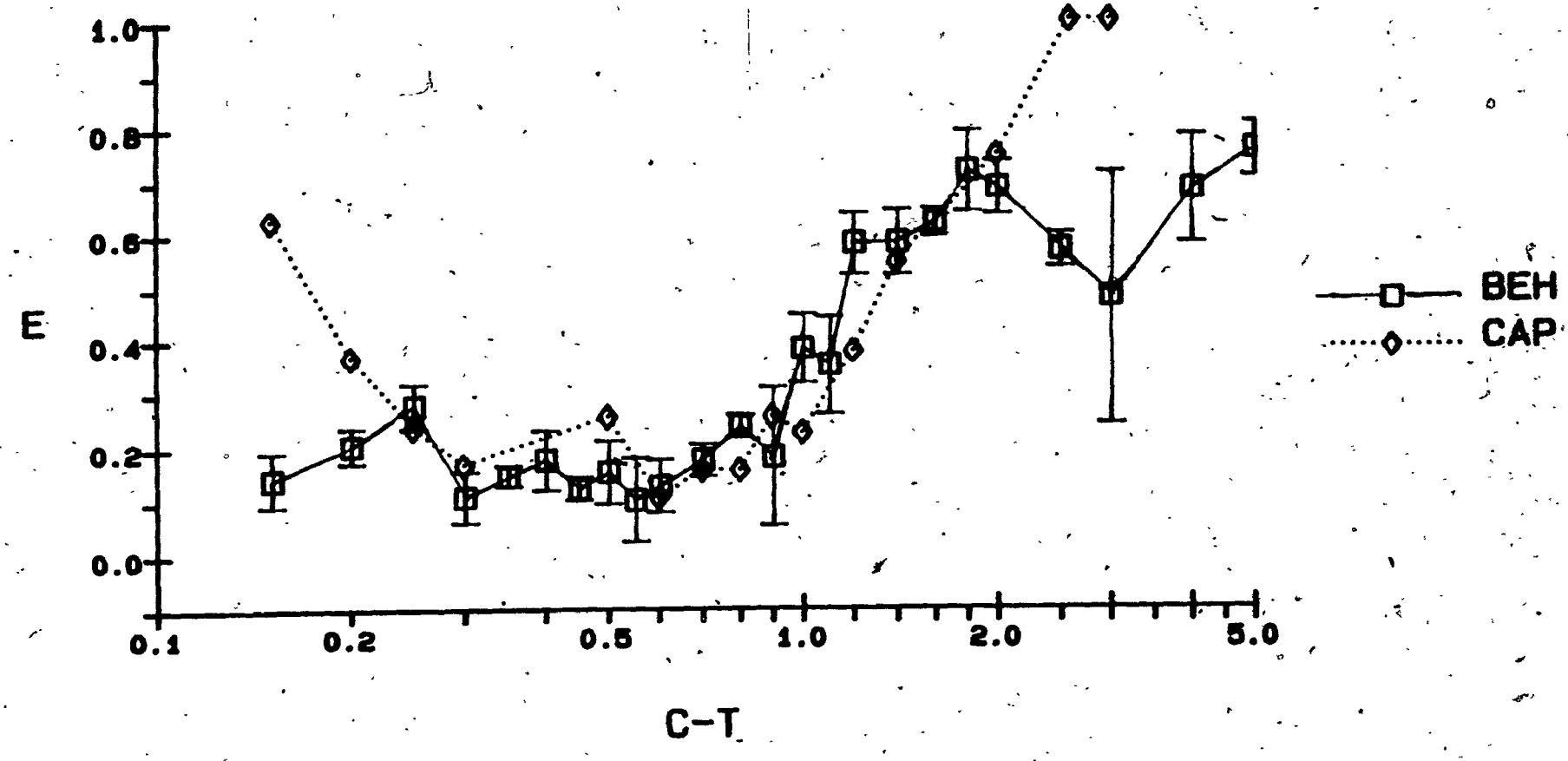
2) Linear Transformation of Data After Determination of the Range of Recovery from Refractoriness

Figure 14 presents applications of the curve-fitting procedure described above applied to both psychophysical and electrophysiological data derived from the same subject. It may be seen that neither set of data includes an E value of 0. Nor does the E value of the behavioural data reach unity. Possible reasons the minimum E value for physiological data does not reach zero are : 1) the minimum physiologically based E value is determined from area calculations and, due to random noise, the area can never be zero and 2) at the shorter CT intervals where the T pulse may unable to activate neurons stimulated by the C pulse, local potential summation may add to the E value. This second reason may also explain why the E values calculated from behavioural data are never 0. The E value calculation for CAP data sets the maximum E value to one; a value not reached by E values based on the behavioural data for reasons that have not been established.

Since the two divergently derived data sets do not span the same range on the Y axis, visual comparison of their ranges on the X axis (represented by the line segments with positive slopes) is made more difficult. Therefore, the behaviourally- and electrophysiologically-based values on E vs CT figures were scaled after the addition of the best fit

Figure 14: COMBINED BEHAVIOURAL AND ELECTROPHYSIOLOGICAL DATA

This figure is a combination of the curves that appear on the previous two figures. The psychophysical data are labelled "BEH" whereas the electrophysiological data are labelled "CAP".


E5 : Combined Data
1.0
0.8
0.6
0.4
0.2
0.0
E
0.1
0.2
0.5
1.0
2.0
5.0
C-T
BEH
CAP

line to each data type. This scaling was accomplished by using the following formula :

$$E(s) = \frac{E - Emin}{Emax - Emin}$$

where Es = the E value after scaling

E = the E value before scaling

Emax = the maximum E value for a particular site

and Emin = the minimum E value for a particular site.

In effect, this linear transformation forces the ranges of Y (or E) values for the best fit lines to be between 0 and 1 and thus visual comparison of the X values, that is, the CT interval ranges, over which the E values rise from 0 to 1, is facilitated (see Figure 15).

HISTOLOGY

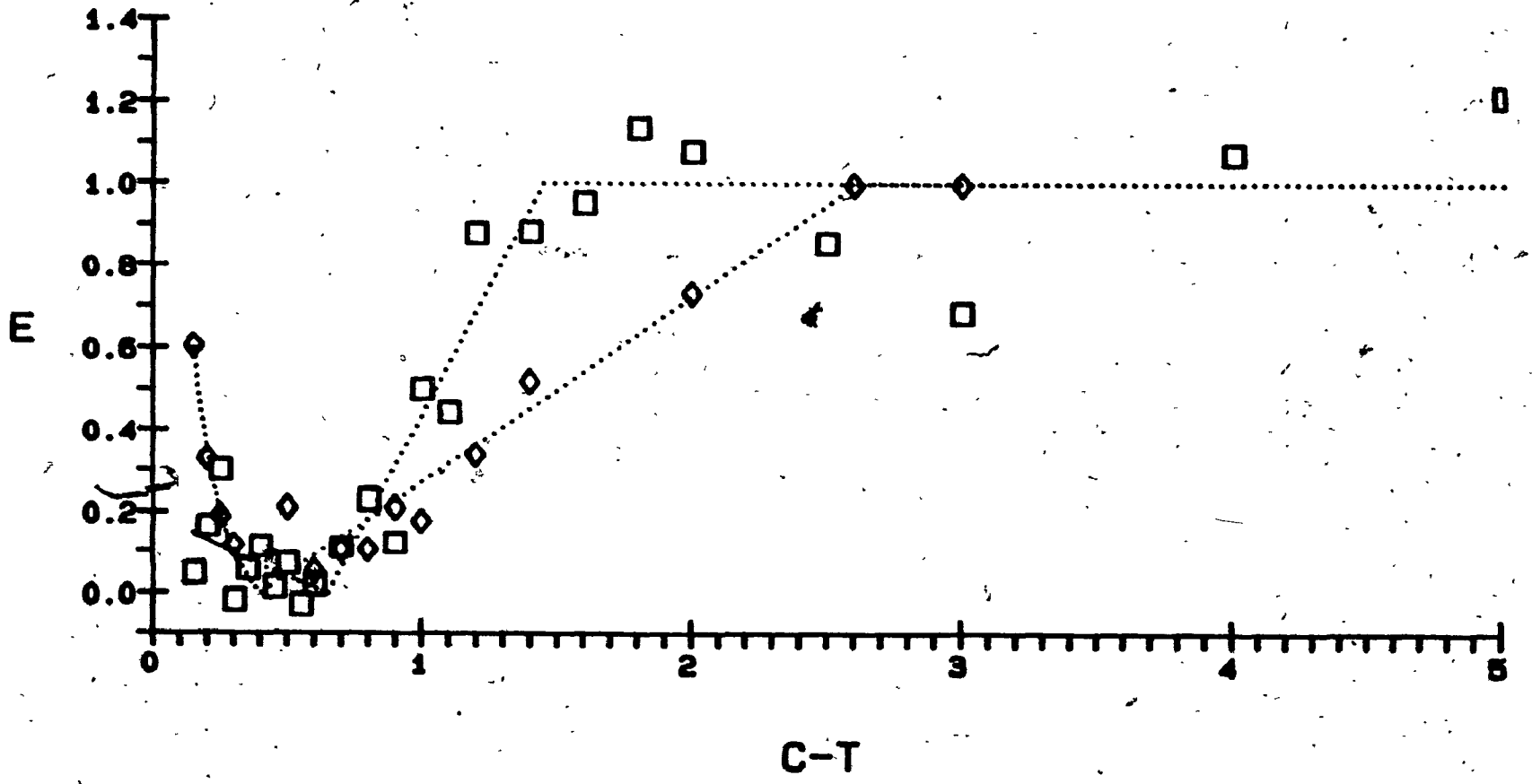
After completion of recording sessions, animals were perfused intracardially with 100 ml of isotonic saline followed by 3 gm potassium ferrocyanide, 3 gm potassium ferricyanate and 0.5 gm trichloacetic acid dissolved in 10 % formalin to make 100 ml solution. The formalin solution was freshly prepared after each recording session and served to form a blue precipitate in reaction with iron ejected by anodal marking lesions made through all electrode tips at the end of recording experiments. This made it easier to find electrode tips in sections of brain tissue.

Immediately after perfusion, subjects were decapitated and the brains were removed and stored in 10 % formalin for

Figure 15: BEST FIT LINES TO TRANSFORMED COMBINED DATA

Figure 15 presents best fit lines for transformed versions of the two curves presented in Figure 14.

E5 : Line Fit to Transformed Combined Data



at least one week. Frozen 20 or 40 micron sections were taken, mounted on gelatin - coated glass slides and stained using the formol - thionin method. The stained, Permount covered sections were microscopically examined to locate stimulation and recording electrode tips.

RESULTS AND DISCUSSION

Subjects

Twenty-two subjects provided electrophysiological data. Six of these do not appear on summary tables and were excluded from statistical consideration due to : 1) lack of histological verification of electrode sites and/or 2) failure of circuitry responsible for recording synchronisation pulses thus precluding averaging and response isolation.

The 16 remaining subjects included three acute subjects (A4, A6, and A9) from which psychophysical data were not collected and thirteen animals that provided both behavioural and physiological information. The acute animals were excluded from histological figures and summary tables since their stimulation sites were not shown to support SS data and therefore could not provide behavioural data. However, these acute experiments aided in the development and refinement of methods applied to behaviourally tested subjects.

Stimulation Sites

Figure 16 provides a diagrammatic description of the stimulation sites for electrophysiological experiments. These

Figure 16: LOCATIONS OF MFB REWARD SITES USED TO PROVIDE PSYCHOPHYSICAL DATA AND ELICIT CAP'S

This figure is based upon tracings from the Paxinos and Watson atlas (1982) and includes all psychophysically characterised MFB reward sites. Some of the indicated loci represent multiple placements that are too close to each other to resolve. Numerals refer to the distances of the coronal sections from bregma.

2.8
3.3

sites also provided psychophysically based estimates of recovery from refractoriness. These electrode loci are concentrated at the anterior-posterior plane located 2.8 mm behind bregma in the Paxinos and Watson (1982) atlas, with three sites located in the plane 3.3 mm behind bregma. The tips of the stimulation electrodes are clustered in the perifornical MFB and are confined to a region extending between 8.3 and 9.6 mm below the skull surface and between 1.2 and 1.9 lateral to the midline.

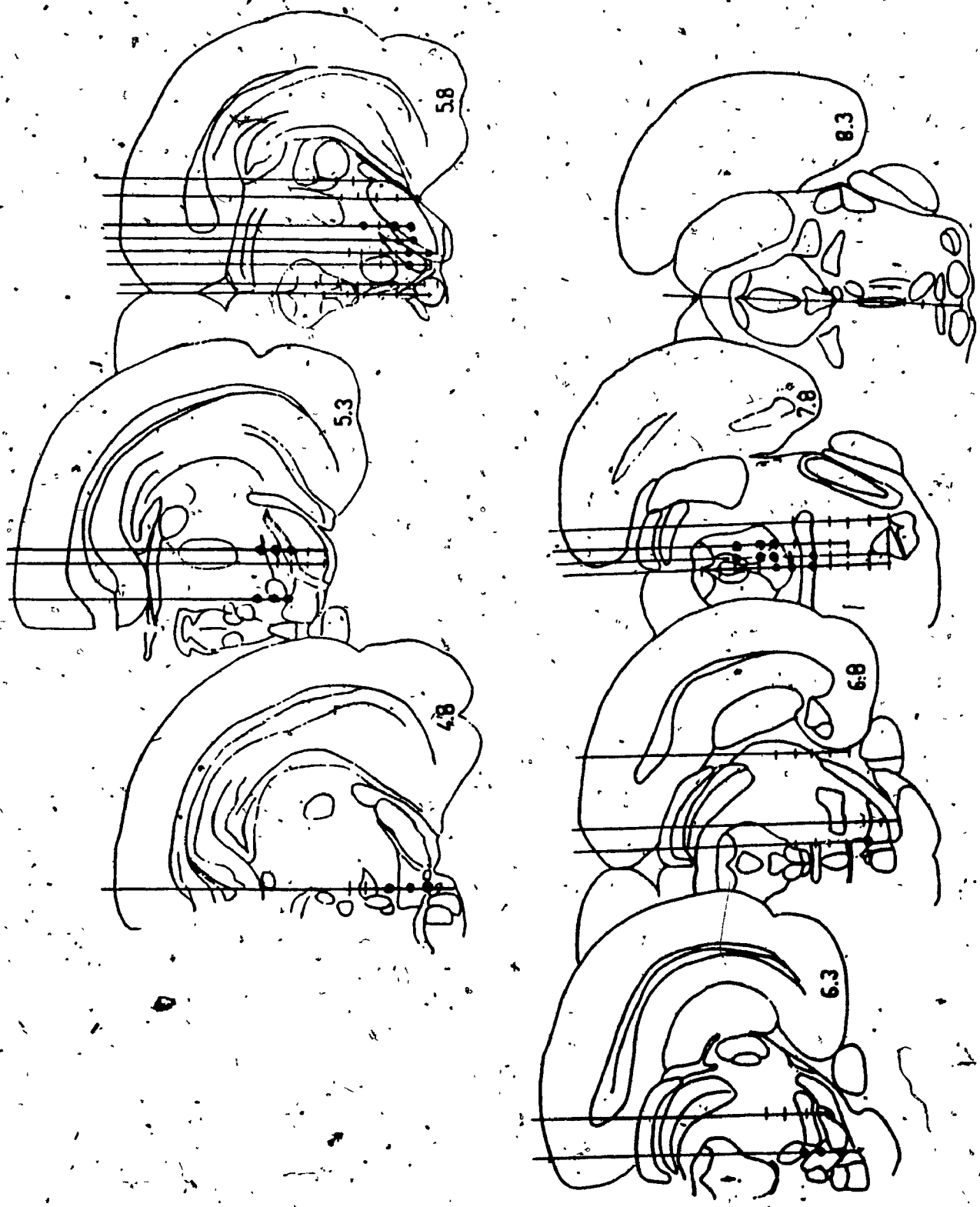
Recording Sites

Recording targets were placed in one of three categories. A recording site was classified as "positive" if a feature distinguishable from stimulus artifact and background noise and stable in time was visible during stimulation at the stimulation current used for behaviourally-based RP estimation. A "negative" classification was given when these requirements were not met. In cases where features were marginally distinct from artifact and noise but met the remaining stated criteria for a "positive" classification, recording sites were labelled as "marginal". Figure 17 diagrammatically represents all recording sites.

Recording electrode tracks were found over an anterior-posterior extent of 3.5 mm, from 4.8 to 8.3 mm behind bregma (Paxinos and Watson, 1982). Positive sites were not found

Figure 17: LOCATIONS OF ELECTRODE PENETRATIONS AND RECORDING SITES

This figure is also based upon coronal section tracings from Paxinos and Watson (1982). Numerals again refer to distance (mm) caudal to bregma. The vertical lines represent electrode tracks separated by about 0.5 mm within coronal sections. Within each electrode track, recording attempts were separated by about 0.5 mm. Sites marked with a dash (-) are "negative" those marked with a closed circle are "positive" and those marked with open circles are "marginal" (see text). In some cases, the vertical lines represent multiple tracks (see Table 1).


5.8
5.3
4.8
8.3
7.8
6.8
6.3

beyond 3.0 mm from the midline and above 5.5 mm below the skull. However, the number of recording sites aimed beyond these regions were relatively few.

Within this region, the great majority of positive sites appear to be in close proximity to (i.e. within 0.5 mm of), if not confined by one of the following areas : the VT, the medio-dorsal half of the SN or the medio-ventral part of the central grey along with the medial portion of the decussation of the superior cerebellar peduncle (Paxinos and Watson, 1982). There appears to be a single positive site clearly distal to these regions; a deep mesencephalic locus recorded in subject F9. The distribution of negative sites about these regions supports the suggestion that a fairly concentrated and distinct group of projections may be activated by stimulation of the MFB.

Comparisons Between Psychophysical and Physiological Refractory Period Data

1) Comparative Indices

Two indices were calculated to compare the congruence between psychophysically-based and physiologically-derived RP ranges. The ranges themselves were arrived at by subtracting the beginning of recovery from the end of recovery derived from the line fitting procedure described above. The overlap index is the proportion of the behaviourally-based RP range for a BSR site that falls within the range of the physiologically-derived RP estimate from a recording site

activated by stimulation of that BSR site. The electrophysiological purity index is the overlap range divided by the entire physiological range (see Table 1).

These indices did not differ across regions as indicated in Table 2. Across all sites for which these figures could be calculated, the mean overlap index was 0.836 (+/- 0.214) and the mean purity index was 0.680 (+/- 0.262). In 13 of 23 cases the range of physiologically-based RP data exceeds the corresponding RP range derived from behavioural data.

Ideally, the overlap ratio reflects the degree to which the spectrum of RP's contributing to the psychophysical data is represented in the spectrum of RP's contributing to the CAP data. If the overlap ratio is zero, then there is no evidence that any of the neurons contributing to the CAP were among the directly stimulated cells responsible for the rewarding effect. If this ratio is one, then the fibre types responsible for the rewarding effect are fully represented in the population of fibres linking the SS site and the recording site. However, one cannot determine from these data whether two different groups of fibres with the same RP spectra are responsible for the rewarding effect and for the CAP's or whether the same fibres are responsible for both sets of data.

Ideally, the purity ratio reflects that portion of the RP range of a CAP that overlaps with the behaviourally-

derived RP range attributed to the MFB reward site stimulated to elicit the CAP. This ratio may serve to inform electrophysiologists as to the likelihood of activating neurons with RP's similar to those derived from the psychophysical data. If this ratio is high for a given MFB reward site and a corresponding CAP recording site, there is a high likelihood that stimulation at this recording site will activate neurons that, at the very least, project through MFB regions known to contain reward neurons and that have similar RP's to MFB reward neurons.

Of these two ratios, the overlap ratio is more relevant to future psychophysical experiments. This measure informs the experimenter where neurons that can be directly activated by rewarding MFB stimulation and having RP spectra similar to reward neurons may be found. For example, a CAP recording site contributing to a high overlap ratio and thus indicating the presence of neurons with the appropriate RP characteristics would provide a good site to search, using psychophysical collision techniques, for extensions of the MFB reward substrate despite the fact that a low purity ratio suggests the presence of other fibres with properties incompatible with those of reward neurons.

2) Examples of CAP and Behavioural Data from Subjects with Positive Ventral Tegmental, Nigral and Central Grey Recording Sites

Each of the data sets in this subsection includes a

figure depicting electrophysiologically-derived RP data. Isolated responses to the second members of pairs of pulses with progressively longer CT intervals are presented. This is followed by a figure containing behavioral RP data gathered from the MFB site used to elicit the CAP's providing the data for the initial figure. In each data set, the current used to gather psychophysically-based RP data was the same as the current applied to the MFB reward site to elicit CAP's.

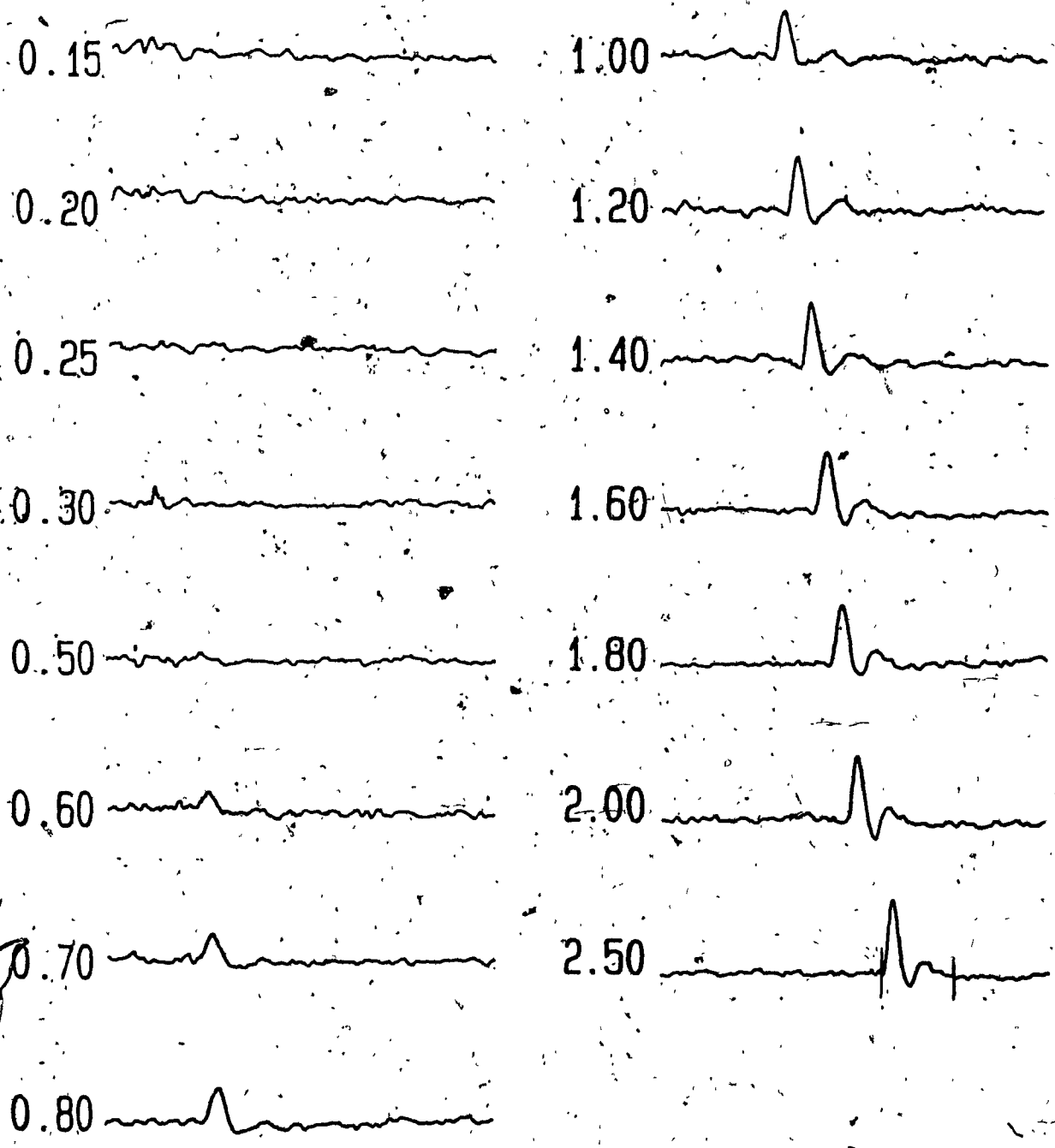
Two additional figures appear in each set. One is a combined E vs CT figure containing two curves: 1) a curve identical to the behaviourally-derived E vs CT curve appearing in the preceding figure and 2) an E vs CT curve obtained by performing the previously described area ratio calculations upon the T pulse response at each CT presented in the initial figure of the set. The second combined figure contains the same data as the first combined figure but the best fit line for each E vs CT curve is included and the linear transformation has been performed on both the data sets and the best fit lines so that the positive slope portions of best fit lines span an E value range from 0 to 1.

a) VT Recording Site

Figure 18 depicts CAP responses recorded at the VT and elicited by rewarding stimulation of the MFB. The CT interval at which each record was produced appears to its left. No response is apparent before 0.60 msec and the CAP

Figure 18: CAP REFRACTORY PERIOD SERIES RECORDED FROM A VENTRAL TEGMENTAL LOCUS

These are averaged isolated records of responses to the second of a pair of rewarding electrical pulses. The interpulse (CT) interval appears to the left of traces. The window region used for area measurement appears on the bottom-most trace on the right.


0.15
0.20
0.25
0.30
0.50
0.60
0.70
0.80
1.00
1.20
1.40
1.60
1.80
2.00
2.50

reaches maximum size by around 2.0 msec. Figure 19 presents the psychophysically-derived E vs CT data from the MFB site used to elicit the VT CAP responses. There is some evidence for latent addition at the earliest CT intervals tested but recovery from refractoriness does not appear to begin before 0.7 msec and is apparently complete by around 1.8 msec. The first combined data figure of this set (Figure 20) seems to indicate fairly good overlap between the recovery from refractoriness portions (regions of positive slope) for the two curves. From the final figure of this set (Figure 21), the CT ranges over which the linearly transformed best fit lines exhibit positive slopes are perhaps more divergent that one might expect from the preceding figure; i.e., 0.42 to 1.51 and 0.41 to 1.05 for the behaviourally- and electrophysiologically-based ranges respectively. In addition, both ranges begin and end earlier than one might have expected from visual examination of the next-to-last figure of this series. For these data the overlap ratio is 0.578 and the purity index is 0.984.

b) SN Recording Site

Figure 22 illustrates CAP responses to MFB stimulation; responses recorded from an SN locus. The CT intervals range from 0.15 to 2.4 msec and a portion of the artifact remains despite subtraction at virtually all CT intervals and may be observed in islolation at 0.60 msec. At the shortest CT

Figure 19: EFFECTIVENESS AS A FUNCTION OF CT INTERVAL FOR AN MFB REWARD SITE USED TO ELICIT CAP'S FROM A VT LOCUS

As in Figure 13, these data depict the effeciveness of the second of a pair of rewarding pulses directed at th MFB. This site was used to elicit the CAP's displayed in the previous figure. The Y axis represents T pulse effectiveness (E) and the X axis represents C-T interval (msec). Bars about points represent +/- 1 standard error of the mean.

FO : LH : Behavioural Data

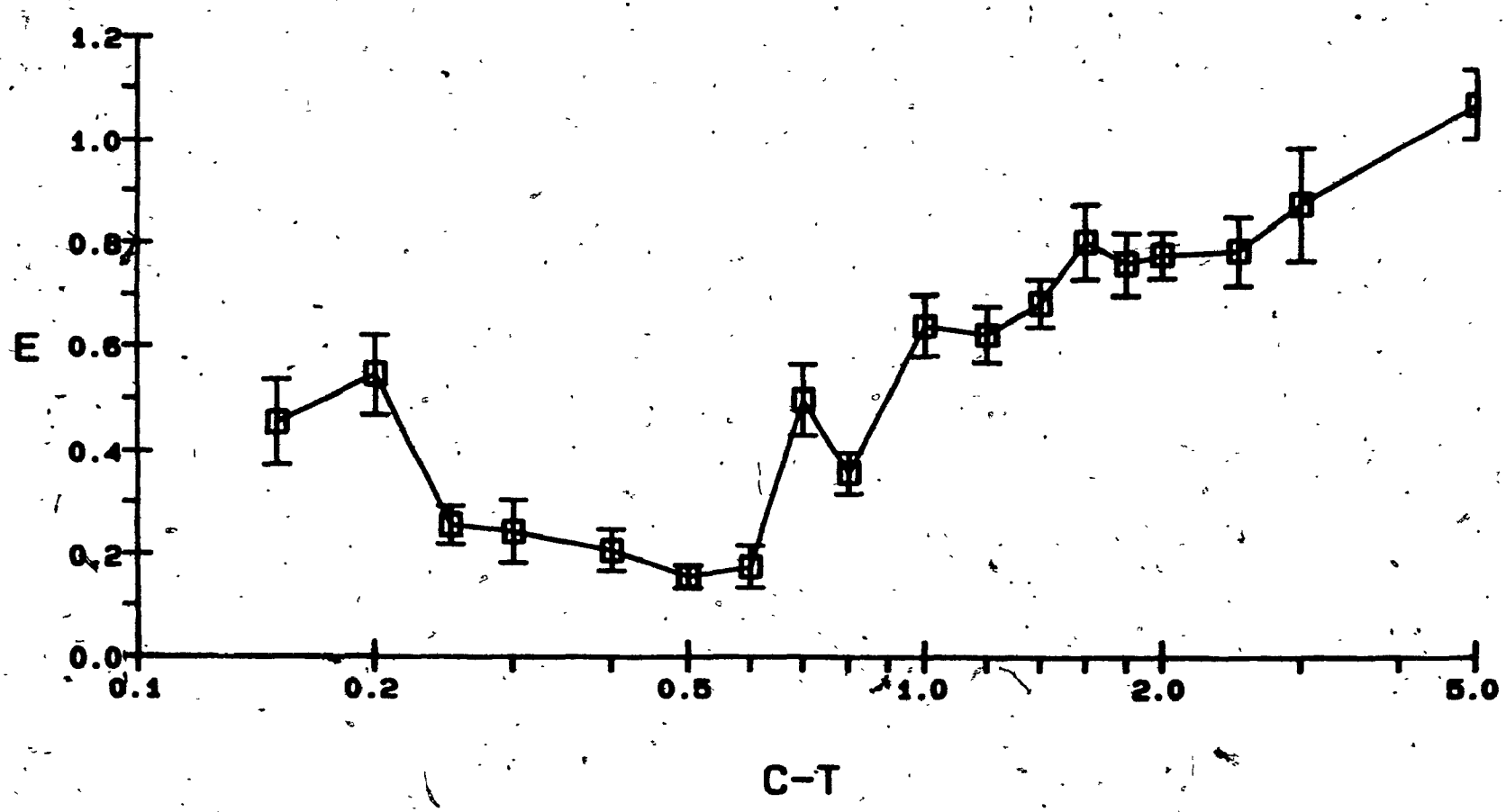


Figure 20: COMBINED BEHAVIOURAL AND ELECTROPHYSIOLOGICAL DATA This figure combines the curve that appears upon the previous figure with a curve based upon area measures performed upon the CAP data in figure 18. "BEH" and "CAP" refer to psychophysically- and electrophysiologically-based curves respectively.

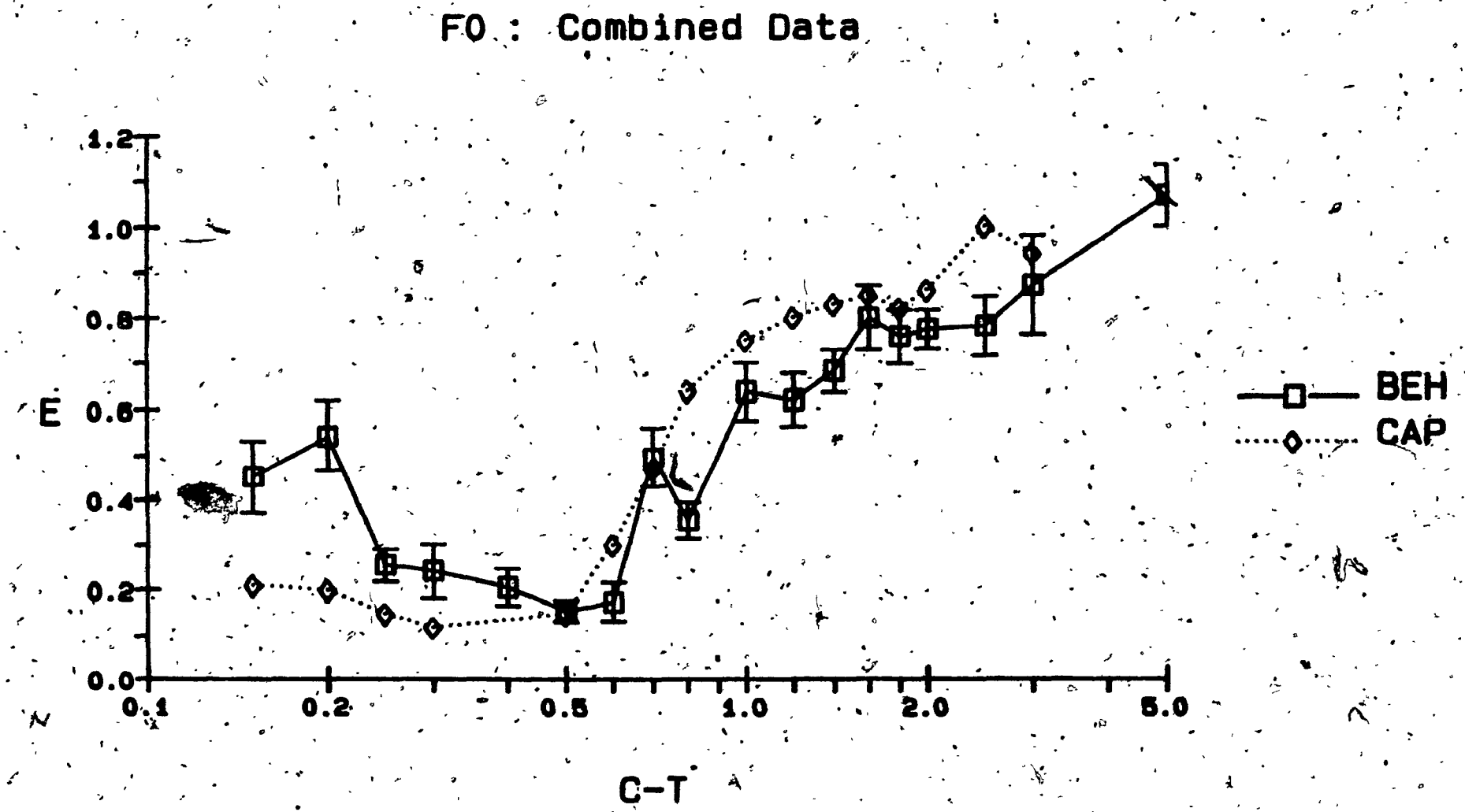

F0 : Combined Data
E
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.1
0.2
0.5
1.0
2.0
5.0
C-T
BEH
CAP

Figure 21: BEST FIT LINES TO TRANSFORMED COMBINED DATA

This figure presents the best fit lines for transformed versions of the two curves in figure 20.

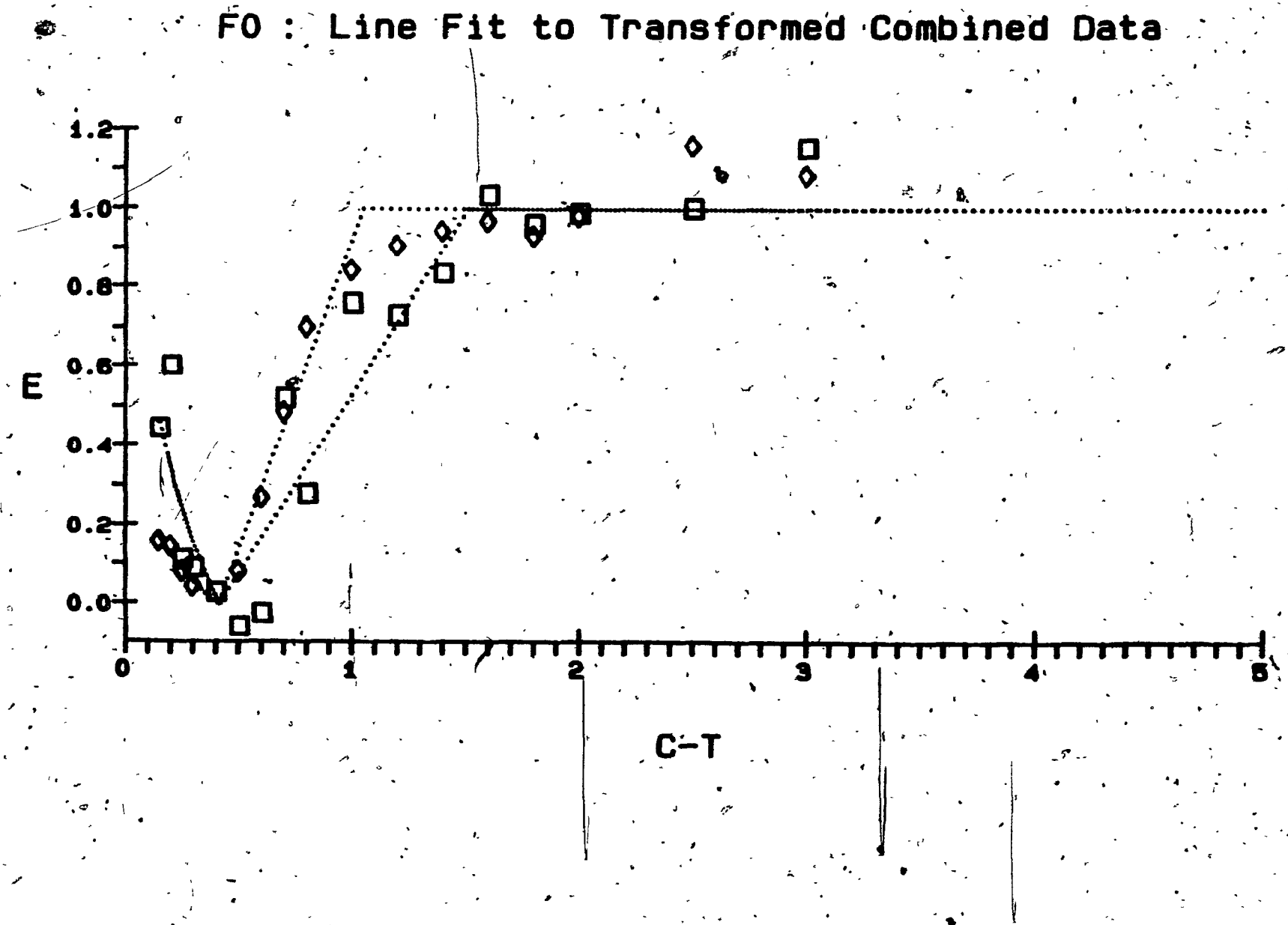
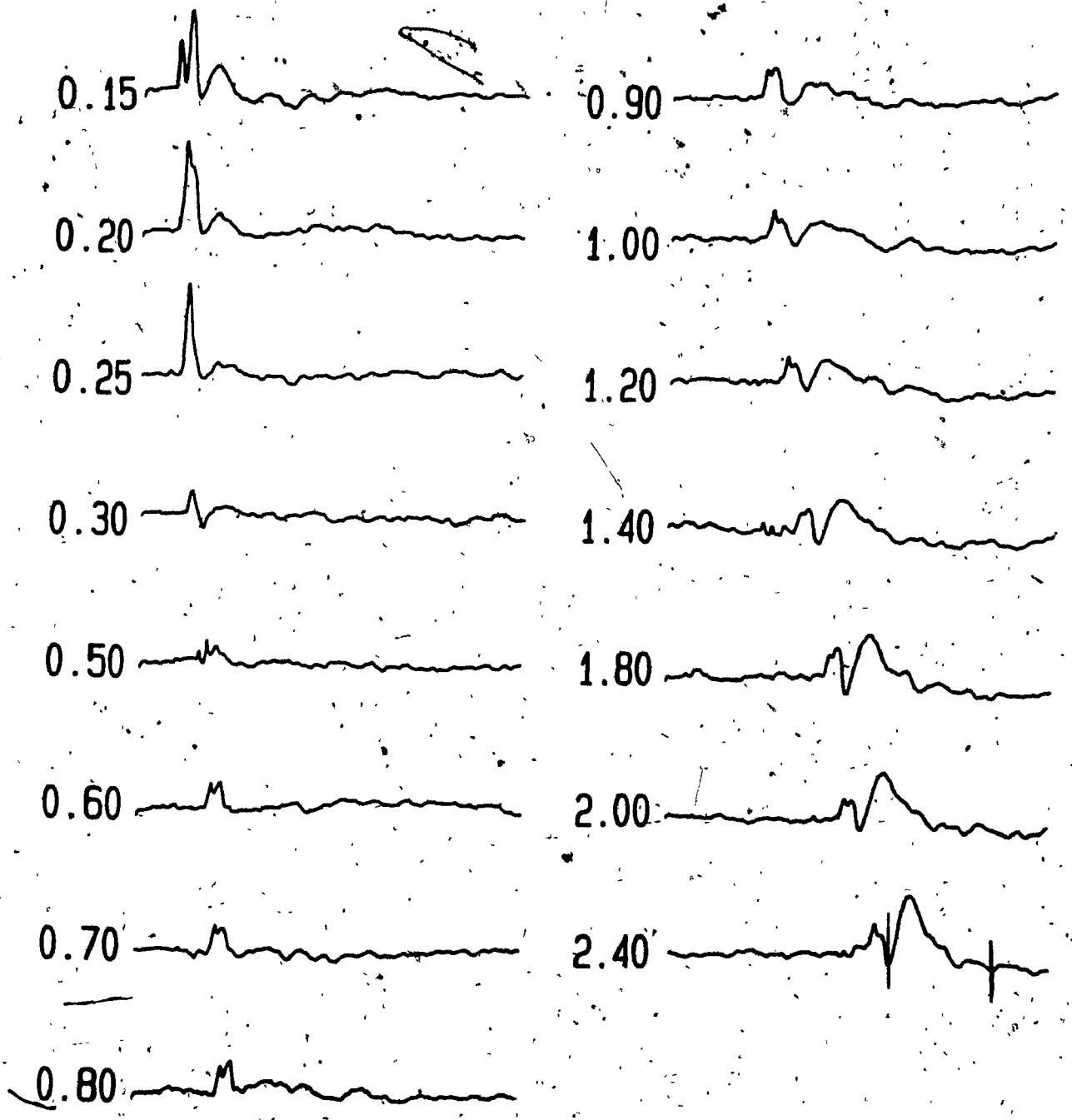

F0 : Line Fit to Transformed Combined Data
E
C-T
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5

Figure 22: CAP REFRACTORY PERIOD SERIES RECORDED FROM NIGRAL A LOCUS

These traces are averaged, isolated CAP responses to the second of a pair of rewarding pulses. The CT interval (msec) appears to the left of traces and the window region used for area measurement appears on the bottom-most trace on the right.


0.15
0.20
0.25
0.30
0.50
0.60
0.70
0.80
0.90
1.00
1.20
1.40
1.80
2.00
2.40

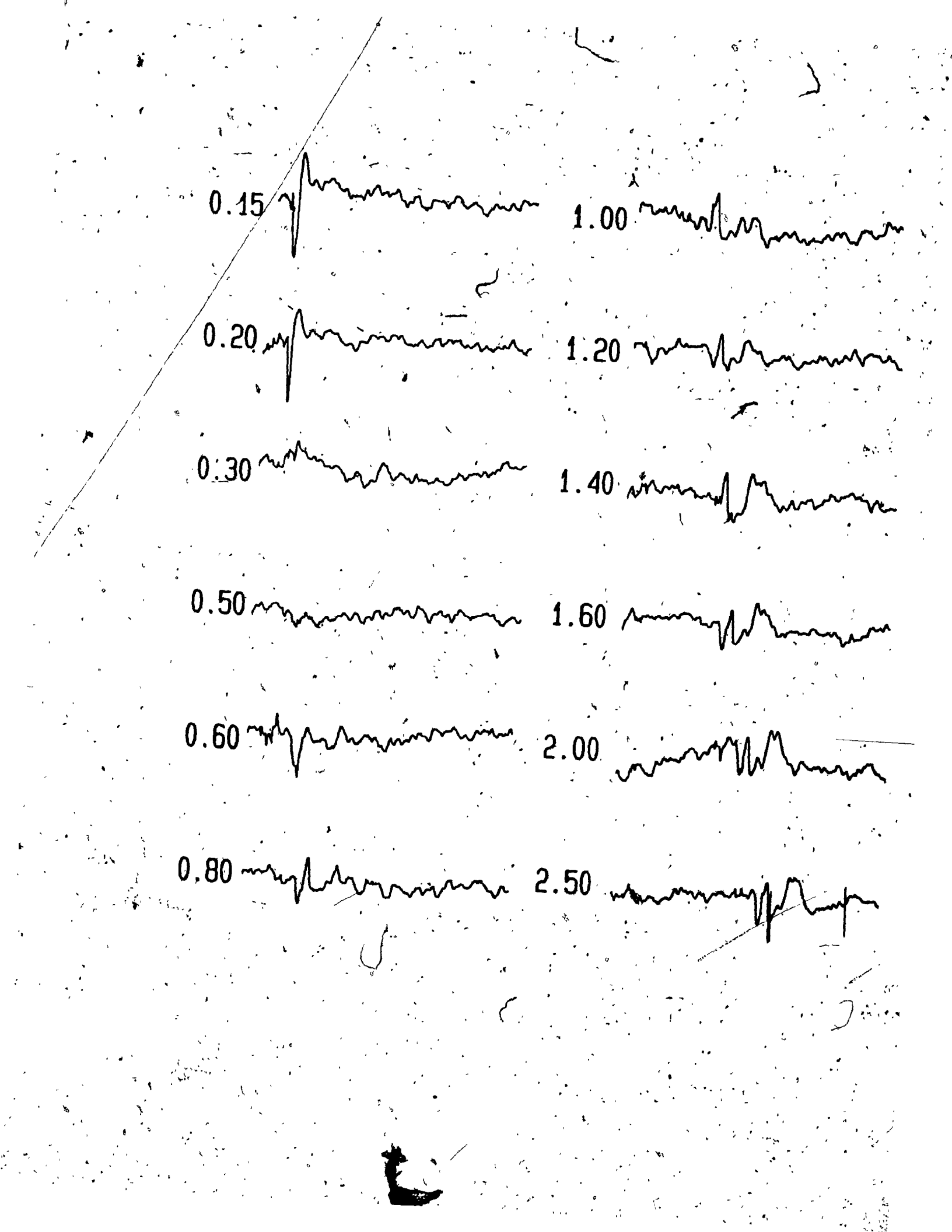
intervals, a response is observed and may be due to latent addition. Recovery from refractoriness does not seem to begin until the CT interval exceeds about 0.7 msec and appears nearly complete by 2.0 msec. The behaviourally-based E vs CT figure for this set (see Figure 13) indicates little or no latent addition, and reward neurons that begin to recover from refractoriness at about 1.0 msec and complete their recovery at around 2.0 msec. The untransformed combined data figure for this set (Figure 14) seems to indicate that the CAP recovery from RP range includes virtually the entire range of the corresponding psychophysically derived RP data. In addition, the CAP RP range appears to extend well beyond its psychophysical counterpart. The transformed version of this combined figure shows that the best fit lines to the data confirm this impression (Figure 15). For these data the overlap ratio is 1.00 and the purity index, 0.36.

c) CG Recording Site

In general, more random noise was seen on CG recording that on records from other regions, since the responses were generally of lower amplitude and higher amplification settings were required. In the CAP RP record presented in Figure 23, the maximum response amplitude was around 25 uV as compared to between 100 and 200 uV for the CAP records presented for the other two sites. The reasons for these apparent amplitude and signal-to-noise ratio differences are not known, but could involve a relatively low concentration

Figure 23: CAP REFRACTORY PERIOD SERIES RECORDED FROM A CENTRAL GREY LOCUS

These traces are averaged isolated CAP's recorded in the central grey region in response to rewarding MFB stimulation. The CT intervals are represented by the numerals to the left of traces and the window region used for area measurements appears on the bottom-most right trace. Traces are 5 msec in duration.


0.15
1.00
0.20
1.20
0.30
1.40
0.50
1.60
0.60
2.00
0.80
2.50

of reward neurons in the CG. Examination of the postsubtraction averaged CAP recordings taken at this CG site suggests an RP range beginning at about 0.80 msec and ending around 1.40 msec. This agrees well with the RP range for the psychophysical data as suggested by the E vs CT curve for the MFB reward site (Figure 24) whose stimulation elicted the CAP's from the CG. The untransformed combined figure (Figure 25) supports this impression of similar RP ranges. The final figure of this series (Figure 26) demonstrates the potential utility of the line fit and linear transformations of these data as visual aids in confirming this impression of congruence between E vs CT curves for the two data types.

For this data set, the overlap index was 0.91 and the purity was calculated as 1.00. If the relatively small CAP amplitude noted for this site does indeed indicate that few fibres connect this portion of the CG and the MFB reward site used to drive these CAP's, the high overlap suggests that these few fibres are, nevertheless, excellent candidates for psychophysical tests aimed at increasing the known extent of reward axons passing through the MFB. The maximal purity figure indicates that stimulation at this CG site would drive neurons that 1) pass through an MFB reward site and 2) these driven neurons are likely to include proportionately few elements whose RP ranges exceed the behaviourally-derived RP range for the MFB reward site.

Figure 24: EFFECTIVENESS AS A FUNCTION OF CT INTERVAL FOR AN MFB REWARD SITE USED TO ELICIT CAPS FROM A CG LOCUS

This figure depicts the the effectiveness of the second members of rewarding pulse pairs directed at the MFB. This site was used to elicit the CAP's that appear in the previous figure. T pulse effectiveness and CT interval (msec) are referred to as "E" and "C-T" respectively.

F9 : LH : Behavioural Data

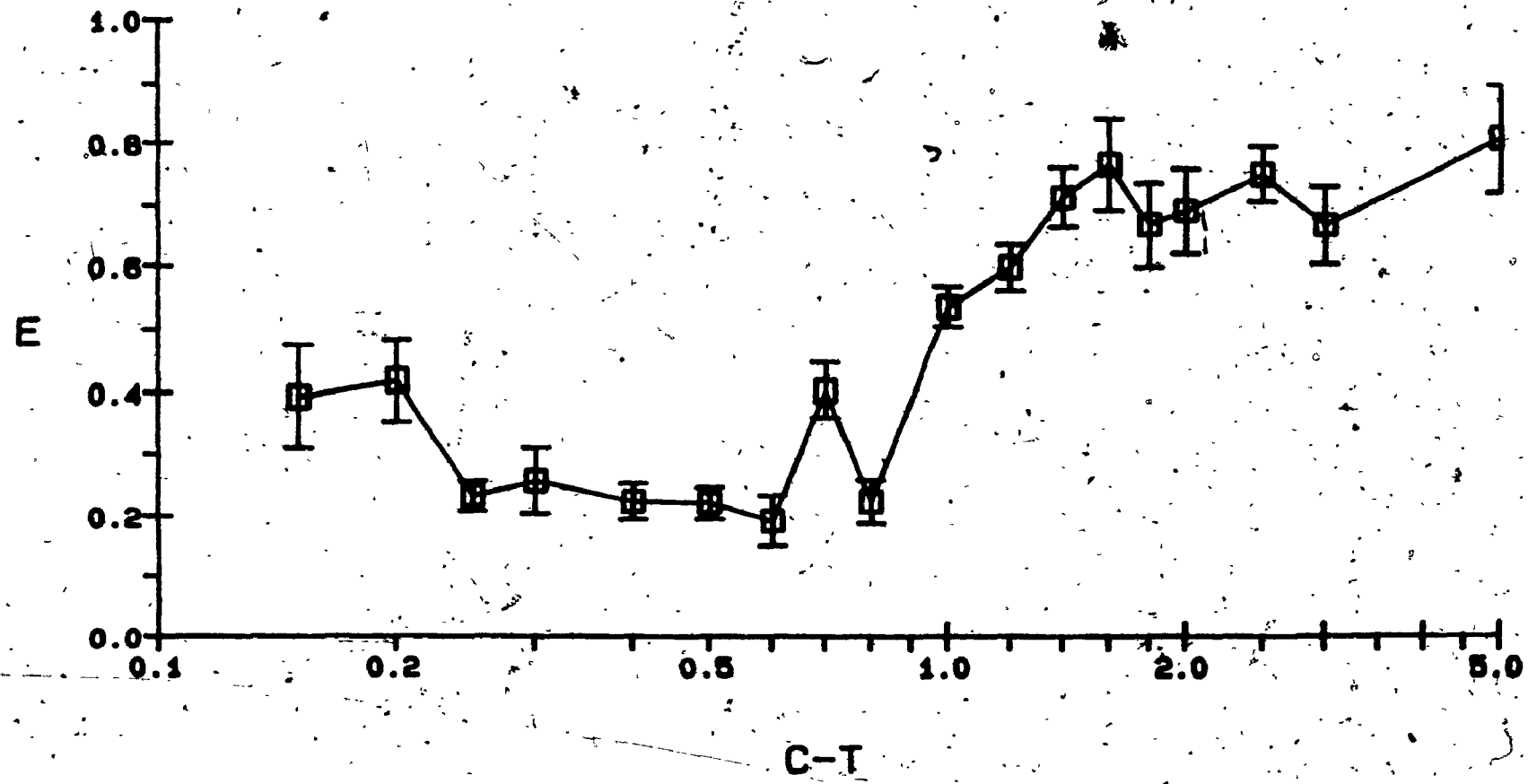


Figure 25: COMBINED BEHAVIOURAL AND ELECTROPHYSIOLOGICAL DATA A combination of the curve from figure 24 and a curve based upon area measuements of the CAP's that appear in figure 23 comprise this figure. "E" refers to T pulse effectiveness and "C-T" refers to CT interval (msec).

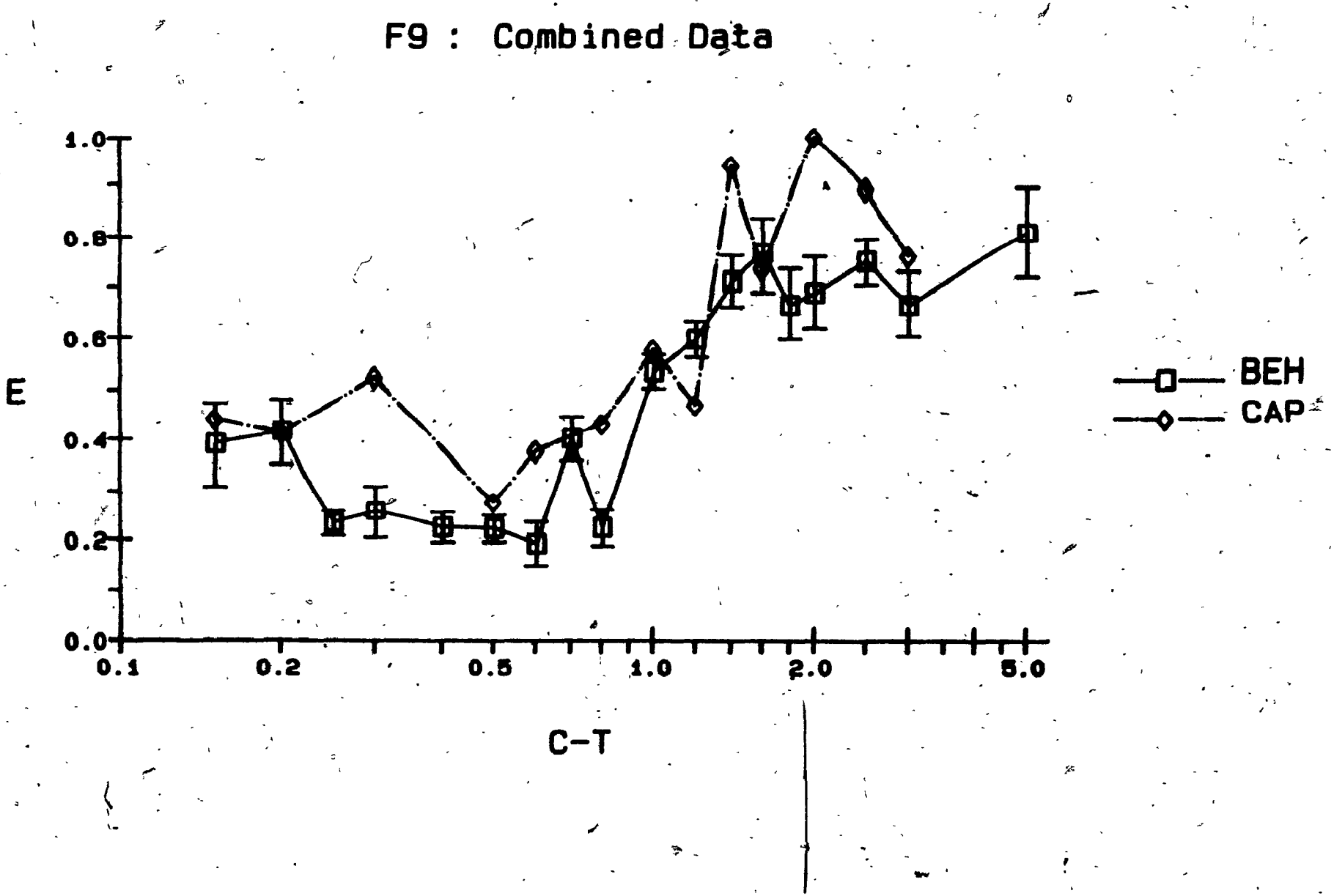
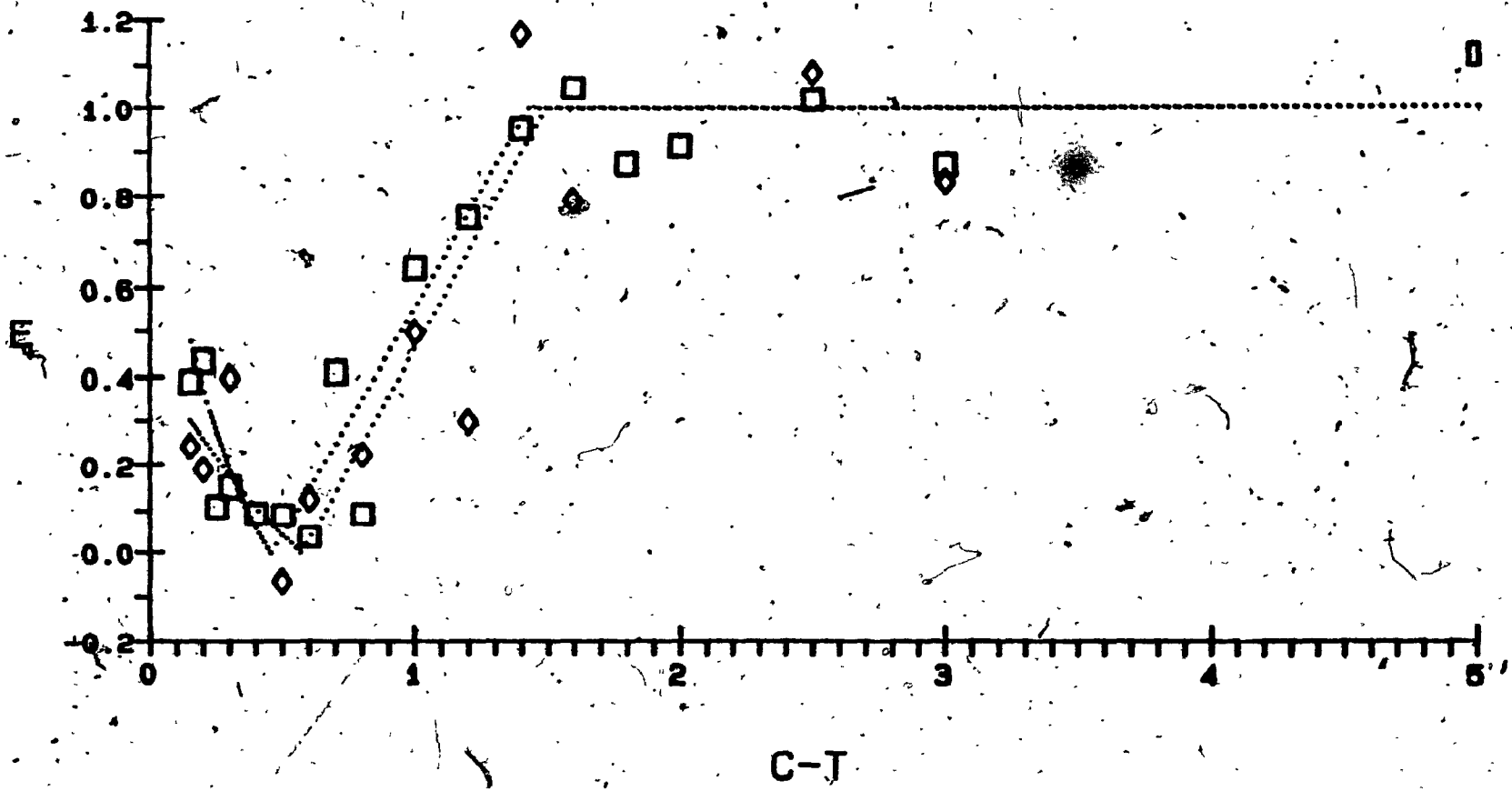

F9 : Combined Data
E
1.0
0.8
0.6
0.4
0.2
0.0
0.1
0.2
0.5
1.0
2.0
5.0
C-T
BEH
CAP

Figure 26: BEST FIT LINES TO TRANSFORMED COMBINED DATA

This figure presents the best fit lines to the transformed versions of the two curves in Figure 25.

F9 : Line Fit to Transformed Combined Data



GENERAL DISCUSSION

Apart from providing evidence for the compound axonal nature of the neural events that contibuted to the electrophysiological data presented here, the technical experiments were intended to illustrate a method for isolation of CAP responses to single pulses and to the second of a pair of pulses. Once isolated, these could be quantified permitting comparisons to be made between electrophysiologically-based and behaviourally-based RP estimates. This chapter includes an evaluation of the CAP response isolation and quantification methods and the techniques for comparing psychophysical and electrophysiological results. The empirical findings are considered next. A consideration of possible future directions for studies aimed at enlarging the known extent of the neural elements responsible for carrying the reward signal during SS of the MFB completes this work.

Technical Experiments

1) Response Isolation

The subtraction techniques appeared successful in isolating responses from artifacts in single pulse experiments (i.e. CAP experiments in which either the current or frequency of single pulses was varied), and in isolating T pulse responses from artifacts and C pulse responses in paired pulse experiments. In virtually all cases, the response of interest was made more distinct from the artifact

since, if not removed entirely, the artifact underwent considerable attenuation. These subtraction techniques were particularly useful in isolating T pulse responses in paired records where there was an overlap between components of the artifacts, the C pulse response and the T pulse response. As has been demonstrated in the Technical Experiments, it would have been exceedingly difficult to discern the T pulse response and to estimate RP's without subtraction.

However, an artifact remnant often remains, after subtraction, in final traces. This remnant may be due to non-linear changes at the brain/electrode interface and/or in the recording circuit components. In other words, the brain/recording circuit system does not immediately return to its prestimulation condition following delivery of the C pulse and therefore there is a difference in the C and T pulse artifacts that results in the post-subtraction remnant. Any attempt at a specific solution to this difficulty must await determination of the precise source of these nonlinearities. In any case, the artifact remnant rarely, if ever, overlapped the response and thus did not cause any practical difficulties for area measurement.

2) Area Measures: Response Quantification and Comparisons Between CAP- and Psychophysically-Based RP Estimates

Area measurements are the bases for the methods used to quantify changes in responses as a function of varying the intensity and CT interval. It was suggested that a main

advantage of this approach over peak measurement methods is that increases in the number of axons fired need not necessarily be accompanied by changes in peak height and, at least in these cases, area measures are more sensitive than peak height measures. However, one difficulty not adequately dealt with by area measures involves the additive cancellation of components of responses so that, in some cases, underestimates of the actual compound response magnitude result. To deal with this problem, means capable of disentangling the subpopulations that comprise responses (e.g. some sort of spectral decomposition) and subsequent computation of the total of their separately determined areas may be required.

Another difficulty, particularly vexing during area measurement of the response to the second of a pair of pulses, involves the use of a fixed width time window that starts at a fixed time after stimulus presentation. Since conduction velocity, which affects response latency, and CAP response duration both vary during recovery from refractoriness (Kocsis et al., 1979; author's personal observations) a combination of window width and latency that selectively includes the CAP response at one CT interval is not likely to do so at all CT intervals. A solution to this problem may involve use of a variable width and latency time window.

The CAP area ratio calculations, despite the flaws of

the area measures itself, permit a reasonable basis for comparing RP recovery ranges derived from the behavioural and physiological experiments. As previously detailed, this basis for comparison rests upon the fact that, for both data types, the E value calculations upon which both RP estimates are based reflect the number of neural firings attributable to the T pulse.

Empirical Contributions

In summary, it may be said that, using MFB reward sites as stimulation sites, elicited CAP's may be found at loci clustered about the VT, and medial portions of the SN, CG and decussation of the superior cerebellar peduncle. These findings are consistent with several anatomical studies of direct projections of MFB neurons (Arbthnott et al., 1976, Nauta and Domesick, 1979). The overlap index described previously suggests that neurons projecting through these sites may be good candidates for inclusion in the set of descending axonal extensions of MFB reward neurons. This index is not different across the various clusters of sites, one of which (the VT) has already been shown to include such extensions (Shizgal et al., 1980).

These empirical findings lend additional support for behavioural evidence regarding not only MFB reward neurons traversing to the VT, but also for the recent findings concerning the course of such fibres between the VT and the CG. The present studies argue for continued intensive

psychophysically-based RP and collision studies of the region between the LH and CG region and strongly suggest that such studies should be directed toward the region between the LH and SN as well.

Future Directions

It is reasonable to view this work as an attempt at the development of tools for the isolation and quantification of CAP responses elicited by rewarding MFB stimulation and the application of these tools to the location of likely candidates for the axonal extensions of the MFB reward substrate. The most obvious extension of the work is to fill in those recording sites within the stated cerebral boundaries that were not investigated here. An investigation of CAP's driven from MFB reward sites and with less separation between recording targets and more extensive boundaries would, despite the considerable time required, likely be extremely useful as a guide for subsequent psychophysical experiments and might possibly provide data that would aid in locating the terminal regions of MFB reward neurons.

As has been mentioned, all sites for which CAP-based RP data are reported were also used to collect intensity series data. It was hoped that these data would provide a means of classifying recording sites in a more graded fashion than the essentially dichotomous (i.e. "positive" or "negative") scheme that was eventually used. In other words,

we hoped to see a gradual growth in CAP's at a particular stimulus intensity as one approached a maximally responsive site. Since this graded growth was not readily observed, the intensity series served merely to add evidence to our contention that compound neural activity comprised our neural responses.

The reasons for this inability to see graded CAP amplitudes as one proceeded toward and passed beyond our "positive " sites are not clear but may perhaps be attributed to the following combination of factors. It may be that, at the regions investigated and the amount of recording electrode travel between recordings (i.e. 0.5 mm), the organisation of the brain was not conducive to observing graded response size changes with successive electrode movements. Certainly, laminar organisation like that of the hippocampus, where such "depth profile" data is more easily obtainable, is not to be found in the regions where the CAP's in this study were recorded. In addition, pairs of wires with a fixed interelectrode distance were used for differential recording in this study. The interelectrode distance selected may have resulted in differential rejection of not only much of the random noise, but also some of the neural responses that might have provided the finer resolution that successful depth profile data collection probably requires. Experimentation with the separation between the poles of the recording electrodes and their exposed tip areas may result

in electrodes better suited to gathering depth profile data.

This thesis is an example of combining research methodologies to gather complementary and convergent data aimed toward a single goal: the location of neural circuitry responsible for the rewarding properties of MFB stimulation. In that spirit, it seems reasonable to suggest that the addition of a third method could only serve to increase the plausibility of any results on which these combined methods agree. It is suggested that anatomical methods may be fruitfully conjoined to the present behavioural/electrophysiological approach.

In order to achieve this three-pronged approach, the main requirements are 1) a concentric stimulation electrode/cannula combination and 2) a tracer substance that is taken up selectively and transported in the orthograde direction by axons but not capable of crossing synapses. This novel electrode would serve to provide psychophysical RP data, elicit axonal CAP's that could be recorded elsewhere and allow tracer injection in the vicinity of the stimulation site. The specificity of such an approach could be further enhanced if the uptake of such a tracer substance was a function of the transmembrane voltage, so that maximal uptake occurred during conduction of action potentials !

This triple method approach is perhaps not as far-fetched as it might appear. There would be no great obstacle to the construction of a suitable electrode/cannula

combination, if one is not commercially available. Although the author is not aware of any tracers taken up specifically by axons, there is ongoing research in dyes whose uptake is proportional to transmembrane voltage (R. Dasheiff, personal communication) and such dyes are being investigated as potential tools for the study of animal models of electrographic seizures. If the required electrodes and tracers were available, there would likely be more rapid progress toward the elusive goal of locating the terminals of MFB reward neurons and the initiation of studies of the next neural link in the neural circuit responsible for MFB brain stimulation reward.

REFERENCES

Arbuthnott, G.W., Mitchell, M.J., Tulloch, I.F. & Wright, A.K. Efferent pathways from lateral hypothalamic neurones. Journal of Physiology, 1976, 263, 131-132.

Bielajew, C. & Shizgal, P. Evidence implicating descending fibers in self-stimulation of the medial forebrain bundle. The Journal of Neuroscience, 1986, 6, 919-929.

Bower, G.H. & Miller, N.E. Rewarding and punishing effects from stimulating the same place in the rat's brain. Journal of Comparative and Physiological Psychology, 1958, 51, 669-674.

Deniau, J.M., Thierry, A.M. & Feger, J. Electrophysiological identification of mesencephalic ventromedial tegmental (VMT) neurons projecting to the frontal cortex, septum and nucleus accumbens. Brain Research, 1980, 189, 315-326.

Deutch, J.A. Behavioral measurement of the neural refractory period and its application to intracranial self-stimulation. Journal of Comparative and Physiological Psychology, 1964, 1, 1-9.

Edmonds, D.E., Stellar, J.R. & Gallistel, C.R. Parametric analysis of brain stimulation reward in the rat. II. Temporal summation in the reward system. Journal of Comparative and Physiological Psychology, 1974, 87, 860-870.

Erlanger, J. and Gasser, H.S. Electrical signs of nervous

activity. University of Pennsylvania Press, 1937, 1-242.

Ferris, C.D. Introduction to Bioelectrodes. New York: Plenum Press, 1974.

Flynn, J., Vanegas, H., Foote, W. & Edwards, S. Neural mechanisms involved in a cat's attack on a rat. In R.F. Whalen, M. Thompson, M. Verzeano & N. Weinberger (Eds.), Neural Control of Behavior. New York: Academic Press, 1970.

Fouriezos, G. & Wise, R.A. Pimozide-induced extinction of intracranial self-stimulation: response patterns rule out motor or performance deficits. Brain Research, 1976, 103, 377-380.

Fouriezos, G. & Wise, R.A. Current-distance relation for rewarding brain stimulation. Behavioral Brain Research, 1984, 14, 85-89.

Fraenkel, G. On geotaxis and phototaxis in littorina. In C.R. Gallistel, The Organization of Action: A New Synthesis. Hillsdale, New Jersey: Lawrence Erlbaum, 1980.

Franklin, K.B.J. Catecholamines and self-stimulation: Reward and performance deficits dissociated. Pharmacology, Biochemistry and Behavior, 1978, 9, 813-820.

Franklin, K.B.J. & McCoy, S.N. Pimozide-induced extinction in rats: Stimulus control of responding rules out

motor deficit. Pharmacology, Biochemistry, and Behavior, 1979, 11, 71-75.

Gallistel, C.R. Motivation as central organizing process: the psychophysical approach to its functional and neurophysiological analysis. In J. Cole and T. Sonderegger (Eds.), The Nebraska Symposium on Motivation, 1974, 22, 183-250.

Gallistel, C.R., Boytim, M., Gomita, Y. & Klebanoff, L. Does pimozide block the reinforcing effect of brain stimulation? Pharmacology Biochemistry & Behavior, 1982, 17, 769-781.

Gallistel, C.R., Rolls, E. & Greene, D. Neuron functions inferred from behavioral and electrophysiological estimates of refractory period. Science, 1969, 166, 1028-1030.

Gallistel, C.R., Shizgal, P. & Yeomans, J.S. A portrait of the substrate for self-stimulation. Psychological Review, 1981, 88, 228-273.

Gallistel, C.R., Stellar, J.R. & Bubis, E. Parametric analysis of brain stimulation reward in the rat. Journal of Comparative and Physiological Psychology, 1974, 87, 848-860.

German, D.C. & Bowden, D.M. Catecholamine systems as the neural substrate for intercranial self-stimulation: a hypothesis. Brain Research, 1974, 73, 381-419.

German, D.C., Dalsass, M. & Kiser, R.S. Electro-

physiological examination of the ventral tegmental (A10) area in the rat. Brain Research, 1980, 181, 191-197.

Harris, G.W. Electrical stimulation of the hypothalamus: A mechanism of neural control of the adeno-hypophysis. Journal of Physiology, 1948, 107, 418-429.

Hoebel, B.G. Inhibition and disinhibition of self-stimulation and feeding: hypothalamic control and post-ingestional factors. Journal of Comparative and Physiological Psychology, 1968, 66, 89-100.

Kiss, I. Electrophysiological properties of neurons at self-stimulation sites in the medial forebrain bundle of the rat. Unpublished Masters Thesis, 1982.

Kocsis, J.D., Swadlow, H.A., Waxman, S.G. & Brill, M.H. Variation in conduction velocity during the relative refractory and supernormal periods: a mechanism for impulse entrainment in central axons. Experimental Neurology, 1979, 65, 230-236.

Macmillan, C.J., Sigantirakis, P. & Shizgal, P. Self-stimulation of the substantia nigra and lateral hypothalamus: recovery from refractoriness in the directly stimulated substrates. Physiology and Behavior, 1982.

Maeda, H. & Mogenson, G.J. An electrophysiological study of inputs to inputs to neurons of the ventral tegmental area from the nucleus accumbens and medial preoptic

anterior hypothalamic areas. Brain Research, 1980, 197, 365-377.

Matthews, G. Neural substrate for brain stimulation reward in the rat: Cathodal and anodal strength-duration properties. Journal of Comparative and Physiological Psychology, 1977, 91, 858-874.

Milner, P.M. Physiological Psychology. New York, New York: Holt, Rinehart and Winston, Inc., 1970.

Molino, A. & McIntyre, D.C. Another inexpensive headplug for the electrical recording and / or stimulation of rats. Physiology and Behavior, 1972, 9, 273-275.

Mundl, W.J. A constant-current stimulator. Physiology and Behavior, 1980, 24, 991-993.

Nauta, W.J.H. & Domesick, V.B. Neural associations of the limbic system. In A. Beckman (Ed.), Neural Substrates of Behavior. New York, New York: Spectrum, 1982.

Nauta, W.J.H. & Haymaker, W. Hypothalamic nuclei and fiber connections. In W. Haymaker, E. Anderson & W.J.H. Nauta (Eds.), The Hypothalamus. Springfield, Illinois, Charles H. Thomas, 1969.

Nieuwenhuys, R., Geeraedts L. & Veening, J. The medial forebrain bundle of the rat. Journal of Comparative Neurology, 1982, 206, 49-81.

Nicholson, C. Generation and analysis of extracellular field potentials. In Electrophysiological Techniques, Bethesda: Society for Neuroscience, 1979, 93-147.

Olds, J. & Milner, P. Positive reinforcement produced by electrical stimulation of septal and other regions of the rat brain. Journal of Comparative and Physiological Psychology, 1954, 47, 419-427.

Olds, J. Satiation effects in self-stimulation of the brain. Journal of Comparative and Physiological Psychology, 1958, 51, 320-324.

Paxinos, G. & Watson, C. The rat brain in stereotaxic coordinates. Sydney, Australia: Academic Press, 1982.

Perez-Cruet, J., McIntyre, R.W. & Pliskoff, S.S. Blood pressure and heart rate changes in dogs during hypothalmic self-stimulation. Journal of Comparative and Physiological Psychology, 1965, 60, 373-381.

Roberts, W.W. Rapid escape learning without avoidance learning motivated by hypothalamic stimulation in cats. Journal of Comparative and Physiological Psychology, 1958, 51, 669-674.

Rolls, E.T. Involvement of brainstem units in medial forebrain bundle self-stimulation. Physiology and Behavior, 1971, 7, 297-310.

Rolls, E.T. The neural basis of brain-stimulation reward. Progress in Neurobiology, 1975, 3, 71-160.

Rolls, E.T., Burton, M.J. & Mora, F. Neurophysiological Analysis of Brain-Stimulation Reward in the Monkey. Brain Research, 1980, 194, 339-357.

Rompre, P.-P., Boye, S. & Shizgal, P. Society for

Neuroscience Abstracts, 1987.

Rompre, P.-P. & Miliaressis, E. A comparison of the excitability cycles of the hypothalamic fibres involved in self-stimulation and exploration. Physiology and Behavior, 1980, 24, 995-998.

Rompre, P.-P. & Shizgal, P. Electrophysiological characteristics of neurons in forebrain regions implicated in self-stimulation of the medial forebrain bundle in the rat. Brain Research, 1986.

Rose, M.D. Pain reducing properties of rewarding electrical brain stimulation in the rat. Journal of Comparative and Physiological Psychology, 1974, 87, 607-617.

Sasaki, K., Ono, T., Muramoto, K.-I., Nishino, H. & Fukuda, M. The effects of feeding and rewarding brain stimulation on lateral hypothalamic unit activity in freely moving rats. Brain Research, 1984, 322, 201-211.

Sherrington, C.S. Correlation of reflexes and the principle of the common path. British Association Reports, 1904, 728-741.

Shizgal, P., Bielajew, C., Corbett, D., Skelton, R.W. & Yeomans, J.S. Behavioral methods for inferring anatomical linkage between rewarding brain stimulation sites. Journal of Comparative and Physiological Psychology, 1980, 94, 227-237.

Shizgal, P., Kiss, I. & Bielajew, C. Psychophysical and

electrophysiological studies of the substrate for brain stimulation reward. In B.G. Hoebel and D. Novin (Eds.), The Neural Basis of Feeding and Reward, 1982, 419-429.

Tasaki, I., Polley, E.H. & Orrego. Action potentials from individual elements in catgeniculate and striate cortex. Journal of Neurophysiology, 1958, 17, 454-474.

Valenstein, E.S., Cox, V.C. & Kakolewski, J.W. The hypothalamus and motivated behavior. In J. Trapp (Ed.), Reinforcement. New York: Academic Press, 1969.

Veening, J.G., Swanson, L.W., Cowan, W.M., Nieuwenhuys, R. & Geeraedts, L.M.G. The medial forebrain bundle of the rat. II. An autoradiographic study of the topography of the major descending and ascending components. The Journal Comparative Neurology, 1982, 206, 82-108.

Von Hoist, E. The reafference principle. In: C.R. Gallistel, The Organization of Action: A New Synthesis. Hillsdale, New Jersey: Lawrence Erlbaum, 1980.

Yeomans, J.S. Quantitative measurement of neural post-stimulation excitability with behavioral methods. Physiology and Behavior, 1975, 15, 593-602.

Yeomans, J.S. The absolute refractory periods of self-stimulation neurons. Physiology & Behavior, 1979, 22, 911-919.

Yeomans, J.S. Stimulation excitability with behavioral methods. Physiology & Behavior, 1975, 15, 593-602.

Yeomans, J.S. Quantitative measurement of neural post-stimulation excitability with behavioral methods. Physiology & Behavior, 1975, 15, 593-602.

Yeomans, J.S., Matthews, G.G., Hawkins, R.D., Bellman, K. & Doppelt, H. Characterization of self-stimulation neurons by their local potential summation properties. Physiology & Behavior, 1979, 22, 921-929.

Yim, C.Y. & Mogenson, G.J. Electrophysiological studies of neurons in the ventral tegmental area of Tsai. Brain Research, 1980, 181, 301-313.

APPENDIX

Table 1: REFRACTORY PERIOD SUMMARY

subject	Site	Beg-End	Ovlap	Purity
D0	stim	0.44-1.13		
""(VT)	63/07/73	0.40-1.44	1.00	0.66
""(VT)	63/07/78	0.37-1.53	1.00	0.59
""(VT)	63/07/83	0.41-1.52	1.00	0.62
D5	stim	0.33-2.00		
""(SN)	53/18/75-90			
""(VT)	48/10/68-73			
""(VT)	48/10/78	0.50-1.40	0.54	1.00
""(VT)	48/10/83			
""(VT)	48/10/88	1.02-2.22	0.58	0.82
E2	stim	0.41-1.28		
""(SN)	63/16/67-77			
""(SN)	63/16/82	0.50-1.22	0.83	1.00
E5	stim	0.65-1.45		
""(SN)	58/19/70-75			
""(SN)	58/19/80	0.40-2.61	1.00	0.36
""(DR)	78/05/70-85*			
""(SN)	58/15/76-81			
""(SN)	58/15/86	0.45-1.12	0.59	0.70
F0	stim	0.42-1.51		
""	68/10/71-91			
""(SN)	53/20/63-83			
""(VT)	58/08/78-78			
""(VT)	58/08/83	0.41-1.05	0.58	0.98
F1	stim	0.63-1.35		
""(SN)	63/15/55-90*			
""(CG)	83/00/70-105			
""(CG)	58/02/55-90			
F2	stim	0.93-1.26		
""(CG/VT)	68/05/56-76			
""(VT)	68/05/81	0.20-1.37	1.00	0.28
F3	stim	0.38-1.57		
""	78/15/75-95			
""	58/26/61-86			
""	stim	0.47-1.02		
F4	stim	0.52-1.14		
""(CG)	78/10/41-46			
""(CG)	78/10/51	0.41-1.83	1.00	0.44
""(CG)	78/10/56-61n			
""(CG)	78/10/66-81			
""(SN)	58/18/65-75n			
""(SN)	58/18/80	0.39-0.90	0.61	0.75
F5	stim*	0.39-1.41		
F7	stim	0.48-1.23		
""(CG)	78/03/45-50			
""(CG)	78/03/55	0.26-1.05	0.76	0.72
""(CG)	78/03/55	1.06-5.47	0.23	0.04

""(CG)	78/03/60-70n			
""(CG)	78/03/75	0.25-1.58	1.00	0.86
""(CG)	78/03/80-95n			
""(SN)	53/20/46-76n			

Table 1: REFRACTORY PERIOD SUMMARY (continued)

Subject	Site	Beg-End	Ovlap	Purity
F7(VT)	53/10/65-75n			
F8	stim	0.39-1.41		
""(SN)	53/30/40-70			
""(CG)	78/0/55-80*			
""	stim	0.47-1.89		
""(CG)	78/0/55-50*			
F9	stim	0.45-1.43		
""(CG)	78/0/45-60			
""(CG)	78/0/65	0.50-1.40	0.92	1.00
""(CG)	78/0/70	0.57-1.50	0.88	0.92
""(CG)	78/0/75	0.20-1.67	1.00	0.67
""(CG)	78/0/80-100			
""(SN)	60/10/55-60			
""(SN)**	60/10/65	0.20-1.50	1.00	0.75
""(SN)	60/10/70			
""(SN)	60/10/75	0.46-1.41	0.97	1.00
""(SN)	60/10/80			
""	stim	0.49-1.50		
""(CG)	78/0/45-70n			
""(CG)	78/0/75	0.38-1.35	0.85	0.88
""(CG)	78/0/80-100			
""(SN)	60/10/55-80			
H0	stim	0.43-1.58		
""(CG)	58/0/40-90n			
""(SN)	58/10/62-67			
""(SN)	58/10/72s			
""(SN)	58/10/77	0.54-2.34	0.90	0.42
""(SN)	58/10/82	0.20-2.72	1.00	0.46

* The electrode track a/o marking lesion was not located.

** This "SN" site was actually located in the deep mesencephalon about 0.8 mm above the SN. However it is suspected that in fact the shorter of the two recording poles was located placing the actual recording locus associated with the longer pole within 0.3mm of the SN and qualifying the actual site for inclusion in the "SN region" according to the present criteria (i.e. within 0.5 mm from the SN)

n no CAP data recorded despite evidence for response since:
1) no response at behaviourally relevant current (most frequent reason) and/or
2) technical problems (e.g. low signal to noise ratio,

highly variable response, equipment problems, etc.)

CG central grey region including dorsal raphe and decussation of superior cerebellar peduncle (medial portion)

Table 1: REFRACTORY PERIOD SUMMARY (continued)

SN subtantia nigra region

stim lateral hypothalamic stimulation site, RP based upon behavioural tests

VT ventral tegmental region

Table 2: Statistical Consideration of Overlap and Purity Indices

Overlap

	VT	SN	CG	
	1.000	0.828	0.588	
	1.000	1.000	1.000	
	1.000	0.612	0.760	
	0.589	0.969	1.000	
	0.578	0.904	0.878	
	1.000	1.000	1.000	
	0.581		0.851	
			0.918	
				Total
sum(x)	5.698	5.313	6.995	18.006
n	7	6	8	21
mean	0.814	0.885	0.874	
sum(x2)	4.962	4.816	6.261	16.039
(sum(x)2)/n	4.638	4.704	6.116	15.458
(Tsum)2/N				15.438

sums of squares: between 15.458-15.438 = 0.020
within 16.039-15.458 = 0.581
total 16.039-15.438 = 0.601

source	sum of squares	dF	Variance
between	0.020	2	0.010(SSb2)
within	0.581	18	0.291(SSw2)
total	0.601	20	

F = SSb2/SSw2 = 0.035, non-sigificant at alpha=0.05 (df 2,18)

Purity

				Mean
mean	0.709	0.670	0.655	0.680
s.d.	0.251	0.267	0.313	0.262
n	7	6	8	21

For the purity values only means and standard deviations are presented since the proportionate difference between means was less and the standard deviations were larger than for overlap values and since the analysis of variance results for the overlap values were non-significant. Thus, a statistically significant F-ratio for the purity values is ruled out.

Data from one positive recording site are not included in the above. This set of records, taken at relatively slow sweep speed (1/4 of the standard sweep), was not included since 1) at standard sweep speed (5msec/cm) another set of data was recorded and included here and 2) the data set recorded at the slower sweep was not visible at the standard sweep speed. Therefore, since the longer sweep was used only rarely, this same situation (i.e. the presence of slower responses visible only at slow sweep speeds but not included in the calculations of overlap and purity) might have occurred at other positive sites.

Note also that the assumption of independance between groups (i.e. regional divisions) required for this ANOVA is not beyond criticism since, in a number of cases, the same stimulation electrode elicited positive recordings from several recording sites. However, if one claims that all stimulation sites may be taken as equivalent since all produced vigorous SS and psychophysical estimates of recovery from refractoriness that were not correlated with stimulation electrode location (see Table 2B below), it is not unreasonable to treat recording sites as independant.

Table 2B: Relationships Between BSR Site and RP

Subject	D/V	M/L	Beg	End	Range
D0	83	12	0.44	1.13	0.69
D5	90	17	0.33	2.00	1.67
E2	92	18	0.41	1.28	0.87
E5	93	19	0.65	1.45	0.80
F1	93	11	0.63	1.35	0.72
F2	82	17	0.93	1.26	0.33
F3	92	18	0.38	1.57	1.19
F4	97	17	0.52	1.14	0.62
F7	83	17	0.48	1.23	0.75
F8rt	96	17	0.39	1.41	1.02
F8lf	90	17	0.47	1.89	1.42
F9rt	85	17	0.45	1.43	0.98
F9lf	87	17	0.49	1.50	1.01
Mean	89.9	16.7	0.491	1.475	0.954
s.d.	4.94	2.23	0.144	0.275	0.333

Correlation Coefficients (Pearson)

D/V position and	Beg :	-0.31066
" "	End :	0.27462
" "	Range :	0.33792
M/L position and	Beg :	-0.12508
" "	End :	0.37383
" "	Range :	0.33720

To test whether correlations are significantly different from 0 (alpha = 0.05), a T-test was performed on the largest R value. With the degrees of freedom equal to 11 a t-value greater than +/- 2.201 required for statistical significance was not achieved.

D/V : BSR electrode location (tenths of mm) in dorsal/ventral plane
M/L : BSR electrode location (tenths of mm) in medial/lateral plane
Beg : beginning of recovery from refractoriness (based upon best-fit line)
End : end of recovery from refractoriness
Range : End - Beg
lf : left MFB
rt : right MFB