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A psychophysical and electrophysiological study of forebrain neurons antidromically activated by rewarding stimulation of the medial forebrain bundle in the rat.

Dwayne C. Schindler

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the degree of Doctor of Philosophy at
Concordia University
Montreal, Quebec, Canada

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ABSTRACT

A psychophysical and electrophysiological study of forebrain neurons antidromically activated by rewarding stimulation of the medial forebrain bundle in the rat.

Dwayne C. Schindler

It has been proposed that fibers subserving the rewarding effects of medial forebrain bundle stimulation originate in the septal and/or neighbouring forebrain basal nuclei (Bielajew & Shizgal, 1986; Gallistel, Gomita, Yadin, and Campbell, 1985; Shizgal, Bielajew, Corbett, Skelton, & Yeomans, 1980). To investigate this hypothesis, the paradigm proposed by Gallistel, Shizgal, and Yeomans (1981) was employed. The first step is to behaviourally estimate electrophysiological and anatomical characteristics of the substrate subserving medial forebrain bundle self-stimulation. The behavioural estimates are then used as a filter to select candidate neurons in recording studies. Nine male hooded rats were implanted with stimulation electrodes aimed at the lateral hypothalamus and the ventral tegmental area. In the behavioural phase, it was found that recovery from refractoriness began between 0.4 - 0.6 msec. and ended about 1.0 - 2.0 msec. following stimulation. These results are consistent with those from previous studies. In the recording phase, the activity of single-units was monitored in the

same subjects previously tested in the behavioural phase. Using standard criteria (Humphrey, 1979; Lemon, 1984), it was found that 81 cells were antidromically driven by the stimulation. Using the current that had been employed during the behavioural phase, refractory period estimates were obtained for 28 cells. Of these, 12 cells had refractory periods that overlapped with the behavioural estimates obtained from the same subject. These 12 cells were considered to be candidate reward neurons whereas the other 16 cells were considered noncandidates. About 58% of the candidate neurons were localized to the septal complex (lateral and medial septal nuclei, diagonal band complex, and the bed nucleus of the stria terminalis) whereas 84% of the noncandidate neurons were found there. The limitations of the scheme for distinguishing between candidate and noncandidate neurons are discussed. In addition, the hypothesis that forebrain nuclei give rise to the MFB reward fibers is discussed in view of both the results of this study and of recent knife cut and lesion experiments.

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Dedication

This thesis is dedicated to Chantal Létourneau. Without her emotional support over the years I might never have finished the thesis.

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Introduction

It has been argued that the development of treatment strategies for various problems such as the self-administration of drugs of abuse (Esposito & Kornetsky, 1978; Fibiger, 1978; Wise, 1978; 1980) will arise from an understanding of the neurobiological basis of appetitive behaviours such as eating, drinking, nest building, copulating, or exploration. This argument rests on the assumptions that all appetitively motivated behaviours lead to reward and reinforcement and that these behaviours depend on common circuitry in the limbic system of the brain (Stellar & Stellar, 1985). While noting that the specific details of each behaviour differ, Stellar and Stellar (1985) argue that the underlying mechanisms of reward, reinforcement, and affect are all organized according to the same physiological principles. If so, then studying any one appetitive behaviour will shed light on other appetitive behaviours.

The discovery by Olds and Milner (1954) that rats would perform an operant behaviour to obtain electrical stimulation of discrete brain regions (self-stimulation) gave researchers an appetitive behaviour that could be easily elicited. The neural system underlying self-stimulation has been labelled the "reward" system since many vertebrates will acquire new behaviours to obtain electrical brain stimulation. The study of the rewarding effects of brain stimulation, or brain stimulation reward (BSR), appears to

be a particularly useful tool for investigating the neural basis of appetitive behaviours for several reasons. First, BSR is a very strong reward (Olds & Milner, 1954; Falk, 1961; Routtenberg & Lindy, 1965) and self-stimulation of certain brain sites does not lead to satiation (Olds, 1958). Second, the rewarding effect arises from focal stimulation of the brain, thus providing an opportunity to link the rewarding effect to the activity of identified cells. In addition, the stimulation parameters may be manipulated with a high degree of precision, thus making it feasible to employ powerful scaling methods derived from sensory psychophysics.

The present study is part of a research programme aimed at identifying the various neural elements of the system mediating BSR. Once components of this system have been found, it should be possible to develop a "circuit diagram" by tracing their projections and by characterizing their interconnections. This circuit diagram might lead to an understanding of how the rewarding effect of electrical stimulation is produced. Mechanistic approaches of this type have been applied successfully in the area of invertebrate neurobiology (Kandel, 1970; Pearson, 1976; Roeder, 1976) to investigate the neurobiological basis of behaviour. Given the proposals of Stellar and Stellar (1985), it follows that knowledge about the processes involved in BSR will also be applicable to other appetitive behaviours.

The specific aim of this study is to locate the somata of neurons likely to subserve the rewarding effects of MFB self-stimulation. To accomplish this aim, behaviourally derived refractory period estimates of MFB reward neurons are to be used as a filter for sorting cells observed by means of single-unit recording. Neurons with refractory periods beyond the range of the behaviourally derived estimate would be regarded as poor candidates. The initial section of the introduction deals with the application of the psychophysical approach to the characterization of the neurons subserving BSR. In addition, this section outlines the techniques that have evolved and summarizes what has been learned using these techniques. The second section contains a summary of the anatomical data given the findings from the psychophysical approach. The third section presents issues relevant when comparing recordings of cellular activity to behaviourally derived estimates of physiological characteristics. In addition, studies that have attempted such comparisons are reviewed. The fourth section outlines the specific objectives of the present thesis.

I. Psychophysical approach

Psychophysics has been defined as an area of study aimed at understanding the relationship between the physical properties of various stimuli and the psychological sensations that result (Lahey, 1989). Thus, the psychophysical

approach to understanding BSR can be defined as the attempt to determine the relationship between the physical properties of the electrical stimulation and the rewarding effect inferred from the behaviour of the animal. In this section, the results derived from applying a psychophysical approach to the investigation of BSR are reviewed. However before discussing the research findings themselves, three aspects related to the psychophysical approach are discussed. First, the underlying rationale for applying this approach to BSR research is discussed. Second, a four stage paradigm that has been proposed for research in this area is presented. Finally, the problem of scaling the results of the psychophysical approach is discussed.

Rationale

The ability to derive a circuit diagram of the reward-relevant fibres depends on the degree to which the researcher can distinguish between fibres that subserve the rewarding effect of the stimulation and those that subserve other behaviours. Many of the structures that support self-stimulation such as the medial forebrain bundle (MFB) are both structurally and functionally complex. Structurally, the MFB is composed of at least 50 neural sub-populations differing in origin and calibre (Nieuwenhuys, Geeraedts, & Veening, 1982). Functionally, stimulation of the MFB can elicit a variety of behaviours such as feeding (Hoebel,

1975), drinking (Mogenson & Stevenson, 1966), copulatory behaviour (Caggiula & Hoebel, 1966); it can also affect endocrine secretion (Harris, 1948) and nociception (Rose, 1974).

Four-stage paradigm

Gallistel, Shizgal, and Yeomans (1981) have proposed a four-stage approach aimed at distinguishing the reward-relevant fibres from others activated by the electrical stimulation. In stage one, the relationship between two of the stimulation parameters is characterized by selecting values of one parameter and adjusting another parameter so as to maintain a constant behavioural output on the part of the subject. For example, Yeomans (1975) trained rats to lever press in order to obtain electrical stimulation consisting of pulse pairs delivered to the posterior hypothalamus. The first pulse of each pair is designated the C-pulse (conditioning pulse) while the second pulse is designated the T-pulse (test pulse). Yeomans (1975) derived a trade-off function by relating T-pulse effectiveness to the C-T interval.

Stage two involves interpreting the results of the behavioural experiments in terms of the electrophysiological and anatomical characteristics of the neurons responsible for the rewarding effect. Yeomans (1975) interpreted the trade-off function as reflecting the excitability cycle (Erlanger & Gasser, 1937) of the directly stimulated (first-

stage) neurons. The excitability cycle is composed of four separate phases: the absolute refractory period, the relative refractory period, the supernormal period, and the subnormal period.

Stage three involves recording the electrophysiological activity of neurons driven by the same stimulation parameters used in the behavioural experiments. In order for the estimates of the electrophysiological characteristics derived at this stage to be comparable to those derived in stage one, the tests performed during the recording session must be analogous to the psychophysical tests performed. Thus, if a refractory period estimate is behaviourally derived using an electrode aimed at the MFB and a current of 500 microamperes (μA), then a similar procedure using the same subject ought to be employed during the recording session (Rolls, 1974).

Stage four involves screening the neurons recorded in stage three through the "filter" that has been established in stages one and two. Only those cells that exhibit the physiological and anatomical characteristics consistent with the behavioural trade-off functions pass through the filter and are considered as candidate reward neurons. Therefore, a researcher who had recorded a neuron with a refractory period longer than the range of values estimated in stage one would consider it as having a low probability of being a reward neuron. Thus, the results of the trade-off (or equi-

valent-stimuli) functions can place quantitative constraints on the directly stimulated substrate for BSR.

To more fully appreciate how the psychophysical approach may be used to investigate the neural basis of BSR, it will be necessary to consider, in more detail, one of the key elements of the psychophysical approach: the trade-off function.

The trade-off function permits the researcher to investigate the characteristics of each stage of a monotonic system up to and including the stage that combines the effects of the two input variables under study (Gallistel, 1978; Gallistel et al., 1981). The underlying logic assumes that if the behavioural output of a system of monotonic stages is constant then the output of the preceding stage must have been constant. Using the same logic and backing through the system to the point where the two input variables are combined, it follows that the outputs of each of the preceding stages must also be constant in order for the final output to be constant. Thus by maintaining a constant behavioural output, the intervening stages become "invisible" and the trade-off function describes the relationship between the effects of the two stimulation parameters at the point at which they combine within the system.

The reward system would be monotonic if a monotonic relationship were found between the final output and one of

the stimulation parameters, such as the pulse duration, the train duration, the current intensity, and the number of stimulation pulses in a train. In fact, the monotonicity of the reward system has been substantiated over a wide range of all four of these stimulation parameters using the runway paradigm (Edmonds, Stellar, & Gallistel, 1974; Gallistel, 1978; Matthews, 1977). Thus, the psychophysical approach enables the researcher to characterize the early stages of the circuit mediating BSR even though the researcher is observing behaviour many steps removed from the actual point of stimulation.

Using the psychophysical approach, the researcher can establish a list of behaviourally derived electrophysiological characteristics of the reward neurons. Armed with the list of characteristics, the researcher in search of a reward neuron is in much the same position as a police officer armed with a composite drawing in search of a criminal. Both have some information concerning some of the distinguishing features of the desired suspect but not a full picture. Similarly, the more information that is known about the suspect the more readily the identification will be made. To impose a tight constraint and thereby reduce the number of suspects, a police officer will interview many individuals to improve the composite drawing so as to obtain as detailed a drawing as possible. Similarly, the number of possible candidate reward neurons may be reduced, if the

researcher has a list of electrophysiological and anatomical characteristics derived from several different trade-off functions.

Scaling

Deutsch (1964) was the first to attempt to characterize reward neurons by behaviourally estimating an electrophysiological property. To estimate the refractory periods of the first-stage neurons, he delivered trains of pulse pairs (C- and T-pulses) through a single electrode and varied the C-T interval. Unfortunately, some of the refractory period estimates derived by Deutsch (1964) and other researchers (see Yeomans, 1975) were based on the false assumption that there was a linear relationship between the level of excitation and the level of performance. Yeomans (1975) demonstrated that measures of recovery from refractoriness based on this assumption are dependent on the arbitrary choice of stimulation parameters.

Although the relationship between the rate of responding and the level of excitation is monotonic over an appreciable range as noted above, it is ogival rather than linear (Edmonds et al., 1974; Gallistel, 1978; Matthews, 1977). Using this less stringent assumption, Yeomans (1975) developed a new method of scaling the results of refractory period experiments. The new method was based on the counter

model of spatio-temporal integration in the reward substrate (Hawkins, 1976; Yeomans, 1975).

A key assumption of the counter model is that the magnitude of the rewarding effect is proportional to the total number of action potentials that arrive at the integrator within a specific time interval. Yeomans (1975) reasoned that the degree to which the T-pulse was effective in eliciting action potentials ought to be proportional to the number of neurons that had recovered from refractoriness following their firing in response to the C-pulse. The scaling procedure that he developed to estimate T-pulse effectiveness compared the effectiveness of a series of pulse pairs (paired-pulse condition) to the effectiveness of evenly spaced, single pulses (single-pulse condition). At one extreme, if the T-pulse fires all the reward-relevant fibres fired by the C-pulse then half as many pulse pairs would be required as compared to the number of single pulses required to maintain a constant behavioural output. In this case, the scaling procedure would yield a T-pulse effectiveness of one. At the other extreme, if the T-pulse is unable to fire any of the reward-relevant fibres fired by the C-pulse due to their refractory state, then the same number of pulse pairs would be required as single pulses. In this case, the scaling procedure would yield a T-pulse effectiveness of zero. Whereas previous refractory period estimates tended to vary, both across and within subjects,

Yeomans (1975) obtained consistent estimates using his new scaling technique. He found that increases in excitability of the neurons mediating BSR could be observed between all C-T intervals ranging from 0.4 to 1.2 msec. These results have been subsequently replicated (Yeomans, 1979; Bielajew, Jordan, Ferme-Enright, & Shizgal, 1981; Bielajew, Lapointe, Kiss, & Shizgal, 1982; Bielajew & Shizgal, 1982).

Literature review

In reviewing the available psychophysical data, Gallistel et al. (1981) sketched a portrait of the electrophysiological characteristics of the neural substrate subserving BSR. This substrate appears to be primarily comprised of small, myelinated axons with absolute refractory periods in the range of 0.5 - 1.2 msec. and conduction velocities in the range of 2 - 8 meters per second. Additionally, the psychophysical approach has been used to investigate more anatomical questions such as determining whether two self-stimulation sites excite common reward-relevant fibres and inferring the direction of conduction. It is these anatomical findings that will now be discussed because of their relevance in determining potential sites for the somata of the reward neurons.

Shizgal, Bielajew, Corbett, Skelton, and Yeomans (1980) modified the paired-pulse experiment. Rather than sending the C- and T-pulse through the same electrode, the stimula-

tion pulses were sent through two different electrodes. In the following discussion, it must be remembered that axonal stimulation elicits two action potentials. One action potential propagates towards the synaptic terminals (orthodromic) whereas the other propagates towards the cell body (antidromic). In this model, it is assumed that only the orthodromic action potentials are responsible for the transmission of the neural signal across the synapse. Shizgal et al. (1980) reasoned that if reward neurons linked the two sites then at short C-T intervals the antidromic action potentials elicited by the electrode distal to the cell bodies (downstream) would collide with and cancel orthodromic action potentials elicited by the electrode proximal to the cell bodies (upstream). The number of action potentials reaching the terminal buttons would be reduced because of these collisions.

Using a variant of the scaling technique developed by Yeomans (1975), Shizgal et al. (1980) related the effectiveness of the paired-pulse stimulation to the C-T interval. They found that, when stimulating the lateral hypothalamic and ventral tegmental levels of the MFB, there was an abrupt change (usually within a 0.4 msec. interval or less) in the effectiveness of the stimulation as the C-T interval increased in the range from 1.0 to 2.0 msec. They attributed this step-like change to collision rather than to refractoriness for two reasons. First, the recovery occurred at intervals longer than the refractory period estimates

(Yeomans, 1975; 1979) previously obtained at MFB sites. The longer interval was assumed to reflect the sum of the refractory period and the conduction time rather than the refractory period alone. Second, the almost flat portion of the curve prior to recovery suggested that there was no local potential summation (LPS). LPS results when neurons on the fringe of the effective region of excitation produced by the C-pulse undergo sub-threshold depolarization and are then brought to threshold by additional depolarization caused by the T-pulse. In many refractory period curves, an LPS effect is observed at short C-T intervals.

Additional support for the hypothesis that the abrupt change in effectiveness is due to the collision of the antidromic and orthodromic action potentials in reward-related neurons has come from recording data. Shizgal et al. (1980) calculated conduction velocity estimates by dividing the interelectrode distance by the difference between the collision interval and the refractory period. These estimates ranged from 2.6 - 7.8 meters per second. Shizgal, Kiss, and Bielajew (1982) and Rompré and Shizgal (1986) have shown that there are fibres linking the LH and the VTA with conduction velocities within the behavioural range. Thus, it appears that some of the fibres linking the LH and VTA have conduction velocities within the range of the psychophysiological estimates and could account for the abrupt change in effectiveness.

In addition to determining whether two self-stimulation sites share common reward-relevant fibres, the psychophysical approach has also been used to infer the direction of conduction. Bielajew and Shizgal (1986) proposed that if anodal hyperpolarization were applied between the site of stimulation and the axon terminals then the orthodromic action potentials propagating to the terminals could be blocked. On the other hand, if anodal hyperpolarization were applied between the site of stimulation and the soma, then only antidromic action potentials could be blocked. Since it is the orthodromic action potentials and not the antidromic action potentials that are believed to be involved in the rewarding effect of the stimulation, the location of the hyperpolarization block would indicate the direction of conduction. That is, an effective block in the LH would indicate a direction of conduction from the VTA to the LH whereas an effective block in the VTA would indicate the opposite.

The predicted result was only seen when the anodal hyperpolarization block was applied to the VTA. According to Bielajew and Shizgal (1986), the simplest anatomical arrangement that could account for the obtained results would be one in which at least some of the somata of the reward neurons are located in the forebrain.

Thus, the results of the psychophysical research suggest that the MFB includes reward-relevant fibres that

descend from forebrain nuclei and course through the LH towards the VTA. In addition, it has been determined that, for the most part, these neurons have absolute refractory periods in the range of 0.5 - 1.2 msec. and conduction velocities in the range of 2 - 8 meters per second. By taking into account the relationship between conduction velocity, diameter, and myelination (Hursh, 1939; Waxman, 1978; Waxman & Bennett, 1972), it has been inferred that these fibres are small, myelinated axons (Shizgal et al., 1980).

Summary

The scaling method developed by Yeomans (1975) to estimate the refractory period of the first-stage neurons yields consistent and replicable results. This is essential since behaviourally derived refractory period estimates of first-stage neurons provide one criterion that candidate reward neurons must meet. That is, any neuron activated by MFB stimulation having a refractory period within the behaviourally derived range has a greater chance of being a reward neuron than one having a refractory period greater than that range. In addition, the behavioural data suggest that the same fibres link the LH and the VTA and that at least some of these fibres have somata rostral to the LH or in the LH.

II. Anatomical data

As noted above, some of the psychophysical techniques such as the collision test yield information concerning the structure of the reward substrate. For example, it is possible to determine whether two sites share a common subset of reward relevant neurons. However, given the number of sites that would need to be investigated, the search for the somata of the first stage neurons would take considerable time if only psychophysical techniques were used and no other structural information were available. Fortunately, results from anatomical studies indicate the brain regions that give rise to MFB fibres. Studies that monitor the metabolic activity of the brain indicate the subset of these regions that are active when subjects are responding to MFB stimulation. Each of these research areas will be discussed below.

Anatomical studies

Anatomical studies concerning the structure of the MFB (for a review see Nieuwenhuys et al., 1982) indicate that there are at least 50 different fibre components that comprise this pathway. However, not all of these fibre components fulfill the anatomical criterion of the first stage neurons imposed by the psychophysical approach. That is, not all of these fibre components (a) have cell bodies

rostral to or in the LH and (b) send projections through the LH to the VTA. From among the 50 different fibre components comprising the MFB, only 10 satisfy these criteria. These pathways are: fibres arising from the septal nuclei (SE), the nucleus of the diagonal band of Broca (DBB), the nucleus interstitialis of the commissura anterior (BCA), the nucleus interstitialis of the stria terminalis (BST), the nucleus accumbens septi (ACB), the substantia innominata (SI), the amygdala and the overlying cortex (AMYG and CPA), the regio preoptica (APL and APM), the nucleus paraventricularis (PVA), and the area hypothalamica lateralis (LH). Eventually, it will be necessary to verify the role that each of the 50 pathways discussed by Nieuwenhuys et al. (1982) plays in the rewarding effect of MFB stimulation. However, the 10 pathways noted above appear to warrant initial investigation given the behavioural data.

Metabolic activity

A technique has been developed that allows an investigator to gauge the relative activity of neural tissue subsequent to some experimental manipulation (Sokoloff, 1981). Called [^{14}C]-2-deoxy-D-glucose (2DG) autoradiography, the technique uses 2DG, a radioactively labeled analogue of glucose, to mark metabolic activity. The 2DG is taken up by a cell as readily as glucose but is metabolized through only a part of the glucose pathway and so the meta-

bolic product of 2DG, [^{14}C]-2-deoxy-D-glucose-6-phosphate (2DG-6-phosphate), accumulates within the cell. Quantitative autoradiographic techniques can then be used to determine relative levels of radioactivity and subsequently metabolic activity in tissue slices. The main assumptions germane to work on BSR is that glucose utilization is directly related to the rate of cell firing (Sokoloff, 1981).

Using 2DG autoradiography, Gallistel, Gomita, Yadin, and Campbell (1985) attempted to restrict the number of candidate pathways for self-stimulation in the MFB. To limit the number of systems activated by the stimulation, parameters were chosen so as to produce a just-submaximal rewarding effect. With respect to forebrain regions, the researchers found that rewarding posterior LH stimulation increased metabolic activity in only the DBB and the BST.

Two points need to be considered when interpreting the results of this experiment. The first point concerns the 2DG technique itself. Sokoloff (1981) has proposed that the 2DG technique reflects the increased metabolic activity of axon terminals. If this is correct, then the activity noted by Gallistel et al. (1985) in the DBB and BST might represent the axon terminals of the first-stage neurons. This result appears to be inconsistent with the behaviourally derived finding that at least some of the somata of reward relevant fibres are located in the forebrain (Bielajew & Shizgal,

1986). To reconcile these divergent hypotheses, it will be necessary to understand the 2DG technique in more detail.

Sokoloff (1981) argues that activity of enzyme-catalyzed reactions involving Na^{+}, K^{+}-ATPase place energy demands on the cell. He points out that the axon terminals with a relatively large surface area compared with their volume are likely to undergo large changes in ionic concentration for a given level of activity and therefore involve more enzyme-catalyzed reactions than the soma. From this, it follows that the axon terminal requires more glucose or 2DG (glucose analogue) and accumulates relatively larger concentrations of radioactive 2DG-6-phosphate than the soma (Sokoloff, 1981). To support this hypothesis, Sokoloff (1981) discussed the 2DG work that has been done on layer IVB of the visual system in the monkey. The 2DG technique shows that layer IVB is clearly the most active region in the striate cortex when the subject is receiving visual stimuli with both eyes open. With both eyes covered, this layer shows the greatest decrease in glucose utilization in that it is almost indistinguishable from the other layers in the autoradiographs. Since this layer contains predominately neuropil and axodendritic connections, Sokoloff (1981) concludes that the 2DG technique reflects mainly the metabolic activity of synaptic terminals. The key issue here is the word, "mainly". If the interpretation of the behaviourally derived data is accurate then stimulation of the posterior MFB will result in both

antidromic and orthodromic activity. The antidromic activity might be responsible for the increased activity in the DBB and BST reported by Gallistel et al. (1985). That is, the antidromic activity might increase the metabolic activity of the somata rostral to the stimulation electrodes. In such a case, there ought to be sites caudal to the stimulation electrodes that show increased metabolic activity as a result of the orthodromic activity. In fact, Gallistel et al. (1985) found increased activity in the substantia nigra and in the ventral tegmental area, on the border of the interpenduncular nucleus.

Finally, the results of the 2DG experiments performed by Gallistel et al. (1985) can be interpreted with respect to the work by Nieuwenhuys et al. (1982). In the semidiagrammatic representations of the inputs to and outputs from the MFB in the rat developed by Nieuwenhuys et al. (1982), almost all of the nuclei that send fibres to or through the MFB also receives a synaptic input that originates from or passes through the MFB. Given this maze of interconnections, it is not surprising then that the 2DG experiments found increased glucose utilization both rostral and caudal to the stimulation electrodes (Gallistel et al., 1985). What is both surprising and encouraging is the fact that so few regions showed increased glucose utilization given the complexity of the MFB (Nieuwenhuys et al., 1982). This is encouraging because these findings suggest that the mapping

of the first-stage neurons might not be as complicated as one might have feared on the basis of traditional anatomical data.

Summary

What can those interested in recording from reward-relevant somata conclude at this point? By matching the characteristics inferred from the psychophysical data (Shizgal et al., 1980; Bielajew & Shizgal, 1986), the trajectories of known pathways (Nieuwenhuys et al., 1982), and the 2DG data (Gallistel et al., 1985), the DBB and the BST nuclei appear to merit special attention during any initial search for the candidate nuclei of the reward-relevant MFB fibres.

III. Recording

As noted earlier, the aim of the thesis is to locate the somata of neurons subserving BSR by using behaviourally derived refractory period estimates as a filter while doing single-unit extracellular recordings. From the psychophysical data, it appears that reward neurons link the LH and VTA and have somata rostral to the LH. From the anatomical data, it appears that there are at least 10 fibre pathways that satisfy these criteria. The DBB and the BST appear to be two sites that warrant initial investigation. In this section,

recording studies that have attempted to map the reward substrate will be reviewed.

One means that has been used to analyze reward pathways has been to apply stimulation with parameters similar to those typically used in BSR experiments to sites that usually support self-stimulation and then to record neural activity from different parts of the brain. Tests can then be performed to determine whether the neuron is directly or synaptically driven. The pathways activated by the stimulation may be traced through the central nervous system using this procedure (Rolls, 1974). In addition, behaviourally derived electrophysiological characteristics of the MFB reward neurons may be used to interpret the data from previous recording experiments. Neurons that exhibit characteristics similar to the behaviourally derived characteristics have a greater probability of having participated in the rewarding effect.

Prior to reviewing these studies, some technical issues will be discussed. These issues need to be considered when evaluating the likelihood that a given cell participated in the rewarding effect of the stimulation during the behavioural phase of the experiment. First, is the activity recorded that of a single-unit or a compound action potential? Second, is the activity recorded that of a directly or a synaptically driven action potential? Third,

is the activity recorded that of somatic or axonal origin? Each of these questions will be dealt with below.

Technical issues

Single-unit versus compound action potential

The criterion traditionally employed to distinguish between single-unit responses versus compound action potentials is invariance in the amplitude of the response in the face of suprathreshold increases in the stimulation current. It is assumed that a single-unit action potential is an all-or-none phenomenon. If the response amplitude increases with increases in current, this is interpreted to reflect the recruitment of additional neurons.

Directly versus synaptically driven action potentials

Three criteria have been agreed upon to distinguish directly driven from synaptically evoked action potentials (Humphrey, 1979; Lemon, 1984). They are (1) collision between spontaneous and stimulation-evoked action potentials, (2) near-constant latency at threshold intensity, and (3) ability to follow high stimulation frequencies.

The collision test is probably considered the most crucial test to determine whether a cell is directly driven (Humphrey, 1979). This test takes advantage of the finding that orthodromic and antidromic action potentials arriving

at the same site collide and cancel each other (Lucas, 1913). In the case of a synaptically driven cell, the spontaneous orthodromic action potential can never collide with a stimulation-evoked antidromic action potential since the action potentials are propagating along different axons. This is not the case for a directly driven cell where both the spontaneous orthodromic and stimulation-evoked antidromic action potentials propagate along the same axon. Additionally, the stimulation pulse must be triggered within the time interval required for an action potential to propagate between the stimulation site and the recording site plus the refractory period interval at the site of stimulation. Thus, the absence of a response to a stimulation pulse triggered by a spontaneous action potential is considered proof that the cell is directly driven. Although considered the most crucial test, it has been pointed out that a monosynaptic input can display collision-like effects when the somatic refractory period is greater than the latency (Barillot, Bianchi, Dussardier, and Gauthier, 1980).

With respect to near-constant latency, Humphrey (1979) has pointed out that directly stimulated action potentials show a variation in latency of approximately 20%. Lemon (1984) noted that most workers have selected an upper limit of 0.1 msec. allowable variation in response latency. These variations are the result of several factors because of the 'noise' inherent in biological pro- cesses. Swadlow, Waxman,

& Rosene (1978) have proposed an alternative to use in these cases. In delivering pairs of pulses, they found that there was more variance associated with the response latency to the first pulse (C-pulse) than to the second pulse (T-pulse). They therefore suggested that latency invariability to the second pulse would provide a fruitful criterion to distinguish between directly driven versus synaptic activity.

Humphrey (1979) has noted that most directly driven fibres are able to faithfully respond to each pulse in a stimulation train at 100 - 300 Hertz. In contrast, synaptically driven fibres respond typically to only the first few pulse in such trains. Thus, the ability of a cell to follow high stimulation frequencies is generally indicative of a directly driven response. However, somatic responses known to be antidromically activated due to collision may fail to follow frequencies less than 100 Hertz. Thus, the ability to follow high frequency stimulation is not an absolute criterion.

Of the three criteria used to distinguish directly driven from synaptically evoked action potentials, the collision test is generally considered the most crucial test. However, one difficulty of the collision test is that the neuron must be spontaneously active or it must be possible to stimulate the cell via the recording electrode at least. When the cell is not spontaneously active or can-

not be activated at the recording site, the method proposed by Swadlow et al. (1978) is the method of choice. Near-constant response latency and the ability to follow high frequencies can also be used as criteria but are less reliable.

Somatic versus axonal activity

Somatic response criteria. Generally, the larger electrical fields generated by the soma makes it likely that a recorded response will be due to somatic activity. However, a number of criteria may be used to determine whether the recorded response is somatic or axonal in nature. First, the prominence of an initial segment/somatodendritic (ISSD) break has been considered evidence that the recorded activity originates near the soma of the neuron (Nelson & Frank, 1964; Terzuolo & Araki, 1961). The ISSD break results from differences in the electrophysiological characteristics of the initial segment and somatodendritic portions of the neuron. Typically seen as a notch on the rising portion of the recorded response, the ISSD break becomes more pronounced as the activity of the cell increases. Second, the action potential duration provides a means to distinguish between somatic and axonal responses. Humphrey (1979) noted that cell body action potentials tend to be on the order of 0.7 - 1.0 msec. in duration whereas axon action potentials tend to be on the order of 0.4 - 0.5 msec. in

duration. Lemon (1984) summarized the results from a number of studies where the action potentials' durations were recorded. For the most part, cell body action potential were greater than 0.8 msec. in duration. Two exceptions were cell body action potentials in the motor cortex (0.5 - 0.9 msec. for 'fast' pyramidal tract nuclei) and dorsal column nuclei (0.2 - 0.5 msec.) of the cat. Of particular relevance for the present study, hippocampal, hypothalamic, and reticular formation fibres in the rat had cell body action potentials of 0.8 - 2.2 msec. duration. Of the two criteria, the presence of an ISSD break is generally considered the most crucial.

Axonal refractory period estimation. Swadlow (1982) has raised an important issue for those who record from cell bodies but who are in fact concerned with the refractory period of the axon. This arises when electrophysiological characteristics that have been derived through behavioural experiments are compared to those derived during recording sessions as was the case for Rompré and Shizgal (1986) and as is case for this thesis. Swadlow (1982) pointed out that the refractory period of the soma or axon hillock may be significantly longer than the refractory period of the axonal site where the initial stimulation occurs. If so, electrophysiological estimates derived during the recording session may differ from those derived during behavioural experiments as a result of procedural differences.

The following example will illustrate the situation that Swadlow (1982) described. For the sake of simplicity, changes in conduction velocity due to the relative refractory period and supernormal period (Kocsis, Swadlow, Waxman, & Brill, 1979) will be ignored. Figure 1 shows a simple neuron with a stimulation electrode at site S, a recording electrode located at site A (soma), and another recording electrode located at site B (a point near the terminals). Suppose that in this example the axon has a refractory period of 0.5 msec. (at both sites, B and S) while the soma has a refractory period of 1.0 msec.

Assume that pairs of pulses are delivered and that the interval between the pulses can be varied. With an interpulse interval of 1.2 msec., site S will have had sufficient time to recover from refractoriness by the time that the second pulse arrives. Therefore, a second action potential will be elicited. The second action potential will arrive at site B late enough to find that it has recovered from refractoriness. Therefore, the second action potential will be recorded there as well. Similarly, a second action potential will be recorded at site A. With an interpulse interval of 0.7 msec., site S will again have had sufficient time to recover from refractoriness by the time that the second pulse arrives. Again, in this case, the second action potential will arrive at site B late enough to find that it has recovered from refractoriness. Therefore, a second

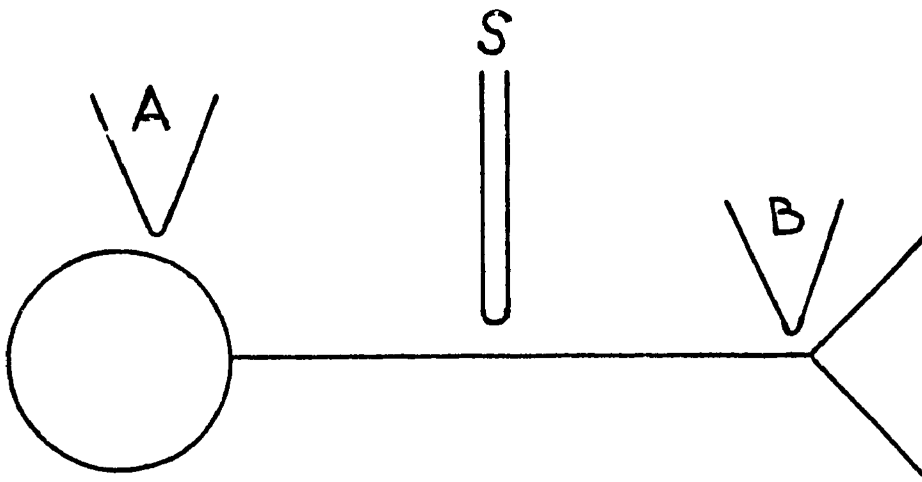


Figure 1. Schematic illustrating the rationale for the Swadlow method. The stimulating electrode, S, elicits action potentials that are recorded at either site A (the soma) or site B (near the terminal region). Suppose that the axon has a refractory period of 0.5 msec. at sites B and S but that the soma has a refractory period of 1.0 msec. If a pair of pulses is applied at site S with an interpulse interval of 0.7 msec., both of the resulting action potentials would be recorded at site B. This would not be the case at site A where the soma would still be recovering from refractoriness when the second action potential arrived. Given this hypothetical illustration, a researcher would incorrectly estimate the refractory period of the stimulation site to be 1.0 msec. when recording at the soma.

action potential will be produced and will be recorded. However, in this case, the events at site A will not be identical. When the second action potential arrives, site A will still be refractory to excitation. Therefore, no action potential will be recorded. The researcher recording at site A would estimate the refractory period to be 1.0 msec. whereas the researcher recording at site B would estimate the refractory period to be 0.5 msec.

When combining behavioural parametric and extracellular recording techniques, this issue is very important. In the parametric experiments, it is as if the investigator is recording at site B since it is the orthodromic activity of the neurons that results in the transmission of the rewarding signal. In contrast, during recording experiments, the activity at site A is recorded because of the ease of recording at the soma. Thus, if there is a discrepancy between the refractory periods of the axon and the soma, a cell may be rejected as being a candidate reward neuron when in fact the cell did participate in the behaviour.

Swadlow (1982) proposed a procedure (hereafter designated as the Swadlow procedure) for obtaining an estimate of the refractory period at or near the point of stimulation. The stimulator is set to deliver a conditioning pulse (C-pulse) following the occurrence of a spontaneous action potential. The interval between the spontaneous action potential and the delivery of the stimulation pulse (S-C inter-

val) is reduced until the antidromic action potential resulting from the stimulation is no longer recorded because of collision between it and the spontaneous orthodromic action potential. The S-C interval is then fixed so that every spontaneously occurring action potential will be cancelled by collision and a pair of pulses is delivered via the stimulation electrode. The interval between the C- and T-pulses is varied to determine the axonal refractory period. When the C-T interval is less than the refractory period at the site of stimulation, the T-pulse will fail to trigger an action potential and thus the T-pulse will not fire the soma. The refractory period of the stimulation site is calculated to be the least C-T interval at which the T-pulse triggers an action potential that propagates to the recording site.

It has been found that the discrepancy between estimates of the refractory period using the standard and Swadlow procedures may depend on the characteristics of the axonal system being studied. Swadlow (1982) found an average discrepancy of 0.02 msec. when recording from 21 callosal axons or axonal branches in the rabbit. Only in one of these cases was the difference greater than 0.1 msec. However, an average discrepancy of 0.09 msec. was found while recording from 25 corticotectal axons of the rabbit. In seven of the 25 cases, the difference was more than 0.1 msec. and in one case the difference was 0.65 msec. Rompré and Shizgal (1986)

compared refractory period estimates based on the Swadlow and standard procedures for forebrain nuclei in the rat that were activated by MFB stimulation. Based on 14 cells, the Swadlow procedure yielded refractory period estimates with a median difference that was 0.2 msec. less than estimates obtained using the standard procedure. In eight of the 14 cells, the Swadlow procedure yielded an estimate less than that obtained using the standard method (two extreme differences were 1.7 and 4.0 msec.). For the other six cells, the refractory period estimates obtained using the standard method were considered similar to those obtained using the Swadlow procedure.

It is important to make the conditions under which the electrophysiological estimates are obtained during the recording session as similar as possible to the conditions during the behavioural experiments. Otherwise, discrepancies may reflect procedural differences rather than real differences. By substituting the Swadlow procedure for the standard method for estimating refractory periods, one source of differences is removed.

Combined behavioural and recording studies

In this section, recording studies aimed at mapping the reward substrate will be reviewed. Two approaches have been employed. One approach involves applying stimulation parameters similar to that employed in BSR to sites that

usually support self-stimulation and then recording neural activity from different parts of the brain (Ito, 1972; Ito & Olds, 1971; Routtenberg & Huang, 1968). The other approach involves application of the four-stage paradigm (Gallistel, Rolls, & Greene, 1969; Matthews, 1977; 1978; Rompré & Shizgal, 1986). Each of these approaches will be discussed below.

To illustrate the first approach, the experiment by Routtenberg and Huang (1968) will be discussed. They attempted to determine whether the reticular formation (RF) is a site of convergence for telencephalic and diencephalic reward neurons. They implanted 20 rats with two stimulation electrodes, one aimed at the posterior hypothalamic region and the other at the septal region. Single-unit activity was recorded from microelectrodes in the midbrain and the brain stem. Of the 150 single-units studied, 73 were in the reticular formation (RF) of the midbrain, pons, and medulla while 77 were in other brainstem areas. Following the recording sessions, 16 posterior hypothalamic placements and 15 septal placements were found to support self-stimulation. It was found that stimulation in the posterior hypothalamus activated more brainstem cells than did septal stimulation, and rewarding sites tended to activate more cells than non-rewarding sites. In fact, less than 5% of the cells were activated by septal stimulation sites.

Although the experiment by Routtenberg and Huang (1968) is one of the first aimed at tracing the neural pathway that the "reward signal" follows in the brain, they noted that it would be necessary, in future experiments, to differentiate between activity due to the stimulation itself and activity due to motor patterns involved in obtaining the stimulation. The assumption here is that the stimulation elicits either a rewarding effect or a motor effect. However, this is an oversimplification since MFB stimulation can elicit many different behavioural, physiological, and endocrine effects (Caggiula & Hoebel, 1966; Harris, 1948; Hoebel, 1975; Mogenson & Stevenson, 1966; Rose, 1974). As Routtenberg and Huang (1968) point out, it is important to be able to make a functional distinction between neurons. One difficulty of initial BSR experiments was the inability to make a functional discrimination. In this case, the only definite conclusion is that posterior hypothalamic neurons activated more cells in the brainstem than did septal neurons. Whether these neurons are responsible for the rewarding effects of the stimulation or some other behaviour is unknown. A similar criticism of work done by Ito and Olds (1971) and Ito (1972) may be made.

Gallistel et al. (1981) suggest that a functional link may be established by behaviourally determining the electrophysiological and anatomical characteristics of the neurons subserving the rewarding effects of the stimulation.

These characteristics then provide a means of differentiating between the neurons activated by the stimulation electrodes. The ability to differentiate neurons on the basis of their function is essential to deriving a circuit diagram of BSR. It has been argued that this approach ought to increase the likelihood that a selected neuron is a reward candidate (Gallistel et al., 1981).

Gallistel et al. (1969) attempted to distinguish between sub-populations of neurons on the basis of a behaviourally derived electrophysiological characteristic. They hypothesized that the running speed of a rat for BSR is a function of (a) the reward received for a specific operant and (b) the amount and recency of stimulation just prior to each operant ("priming" stimulation). Further, they hypothesized that the neurons subserving these two functions might be distinguished electrophysiologically on the basis of differences in refractory periods. In the behavioural phase, refractory period estimates of neurons in the MFB were obtained under two conditions: first, when the rewarding stimulation was varied and second, when the priming stimulation was varied. The behaviourally derived refractory period estimates ranged from 0.53 to 0.64 msec. for the rewarding effect and from 0.9 to 1.1 msec. for the priming effect. In the recording phase, stimulation electrodes aimed at the MFB were implanted in a second group of subjects and the current required to yield self-stimulation was determined. The

electrophysiological activity of single-units rostral to the stimulation electrode was recorded. The behaviourally derived refractory period estimates were used as a filter when monitoring single-unit activity. It was found that neurons having refractory period estimates similar to those that were behaviourally determined for the rewarding effect were located in the lateral thalamic nucleus while neurons having refractory period estimates similar to those for the priming effect tended to be located in the nucleus parafascicularis.

A number of criticisms of this experiment can be raised. First, the scaling procedure used to determine the behavioural refractory period estimates has been shown to be inadequate (Yeomans, 1975). Therefore, the behaviourally derived refractory period estimates may not be correct. Second, the electrophysiologically derived refractory period estimates may not reflect the refractory periods at the point of stimulation (Swadlow, 1982). Finally, the fact that different subjects were used in the behavioural and electrophysiological phases reduces the power of this experiment. That is, variability in refractory period estimates resulting from the use of different subjects in the two phases adds another source of error. Notwithstanding these criticisms, this experiment represents the first attempt to distinguish reward-relevant and non-reward-relevant neurons on the basis of a behaviourally derived electrophysiological

characteristic. As such, this experiment laid the groundwork for the four-stage approach advocated by Gallistel et al. (1981).

To determine whether strength-duration characteristics can discriminate neural elements subserving reward and those subserving stimulation-induced movement, Matthews (1977) collected anodal and cathodal strength-duration curves. Using a runway paradigm, he obtained cathodal strength-duration curves that yielded chronaxies between 0.8 and 3.0 msec. for MFB reward neurons. Cathodal strength-duration functions for neurons subserving brief twitches of somatic musculature had chronaxies ranging from 0.15 to 0.48 msec. Given the difference in chronaxie estimates for neurons subserving reward and muscle twitches, Matthews (1977) concluded that the use of anodal and cathodal strength-duration characteristics could aid researchers in discriminating reward neurons from among the various elements activated during MFB stimulation.

In a later study, Matthews (1978) attempted to ascertain whether single neurons within the MFB had strength-duration characteristics similar to those obtained behaviourally (Matthews, 1977). Stimulation electrodes were aimed at the lateral hypothalamus, and recording sites were located within the posterior midbrain. Anodal and cathodal strength-duration data were collected from 27 different cells. Directly driven cells were characterized by having a

short, unvarying latency, by following a 100 Hertz stimulation frequency, and by a notch on the rising portion of the action potential. The chronaxies of the cathodal strength-duration functions ranged from 0.1 to 0.5 msec. Given the behaviourally determined chronaxie range of 0.8 to 3.0 msec. that Matthews (1977) had found, this would suggest that these single-units could not fully account for the behaviourally derived values. However, Matthews (1978) found that two directly driven cells fired up to three action potentials for pulse durations of more than 2 msec. From this, Matthews (1978) concluded that the difference between the behaviourally derived cathodal chronaxies and the electrophysiologically derived values could be accounted for by the multiple firing at the long pulse durations. That is, the additional action potentials obtained at the longer pulse durations due to multiple firing would permit further decrements in the current than would be possible if the neurons fired only once.

While the studies by Matthews (1977, 1978) yielded considerable information concerning the anodal and cathodal strength-duration characteristics of reward relevant neurons, the ability to apply that knowledge to discriminate functionally between neurons may be limited. The finding that some subcortical cells fire repetitively at long pulse durations means that there may be little concordance between behaviourally and electrophysiologically derived strength-

duration characteristics. An additional weakness arises in that the electrode placements used during the recording experiment (Matthews, 1978) were not behaviourally screened. Thus, it is not possible to determined whether neurons within the stimulation field would support self-stimulation.

Following the 2DG work by Gallistel et al. (1985), Rompré and Shizgal (1986) wanted to determine whether septal and basal forebrain neurons driven by stimulation of the LH and VTA had electrophysiological characteristics similar to those that have been obtained behaviourally.

Rompré and Shizgal (1986) recorded 76 directly driven cells using extracellular electrodes in anesthetized rats. Fifty-two cells were driven from electrodes aimed at the LH only; 14 were driven from the VTA only; 10 were driven from both the LH and VTA. Since no behavioural data had been gathered for the subjects prior to recording, all responses were driven at currents ranging from 1.2 to 2 times threshold.

Eighty-three refractory period estimates, ranging from 0.6 to 6.0 msec., were obtained. These estimates overlap the range of values that have been derived behaviourally (Yeomans 1975, 1979; Bielajew et al., 1981) indicating that some of the cells might be reward neurons. For the purposes of selecting candidate reward neurons, Rompré and Shizgal (1986) used an estimate derived from the behaviourally determined refractory period curves (Yeomans 1975, 1979;

Bielajew et al., 1981). This estimate, 1.2 msec., reflected a median C-T value at which the behavioural curves had attained asymptote. Since the Swadlow procedure yields a refractory period estimate that is comparable to estimates obtained using the psychophysical procedure, the 1.2 msec. value was applied to refractory period estimates obtained from spontaneously active cells using the Swadlow procedure. Rompré and Shizgal (1986) obtained a median difference of 0.2 msec. between refractory period estimates when both the standard and Swadlow procedures were applied to the same cells. Since the standard procedure often yields higher estimates, a criterion value of 1.4 msec. was applied to refractory period estimates obtained using the standard procedure. Using these criteria, thirty-four percent of the sample (28 cells) were designated as likely candidates. Rompré and Shizgal (1986) concluded that there was little tendency for either the better or worse candidates to be concentrated in a particular forebrain structure.

The inability to find a particular forebrain region containing the somata of candidate reward fibres is not that surprising when it is considered that the only major criterion imposed was the refractory period estimates derived from other subjects. Additional constraints could have been imposed if the stimulation electrodes had been behaviourally screened prior to recording. First, it would have been known whether the stimulation site supported self-stimulation.

Second, behaviourally derived refractory period estimates obtained from the same subjects to be used in the recording experiment would have improved classificatior of likely and unlikely candidate reward neurons. For example, recorded cells exhibiting long refractory periods might be designated unlikely candidates using a median estimate of 1.2 msec. However, these same cells might be designated likely candidates if the behaviourally derived curve of the subject under question continued to rise up to 2.0 - 3.5 msec. as has been observed at certain MFB sites (Bielajew et al., 1981). Incorrect labeling of cells could be reduced if behaviourally and electrophysiologically derived refractory estimates for the same rat were employed. In such a case, the stimulation field would be identical in both conditions since the same rat, the same stimulation electrode, and the same stimulation parameters would be used.

Even though no specific forebrain regions were more likely than others to contain somata of candidate reward fibres, Rompré and Shizgal (1986) demonstrated that there are cells with forebrain nuclei that send projections down through the LH to the VTA and share an electrophysiological characteristic with MFB reward neurons. Ideally, the use of multiple constraints such as those imposed by collision data increases the likelihood that a cell deemed as a likely candidate mediates the rewarding effect of the stimulation (Rompré & Shizgal, 1986).

Summary

Although several experiments have been conducted in an attempt to map the reward substrate, some were unable to distinguish between cells likely to participate in the rewarding effect, and other stimulated neurons. The four-stage paradigm permits a functional link to be made since candidate neuron are required to exhibit a number of behaviourally and anatomically derived characteristics. Cells that match all known characteristics or constraints are the most likely to participate in the rewarding effect. Rompré and Shizgal (1986) found that some forebrain somata activated by LH or VTA stimulation had refractory period estimates within the range of values established in behavioural experiments (Yeomans 1975, 1979; Bielajew et al., 1981). However, there was little tendency for either the better or worse candidates to be concentrated in a particular forebrain structure (Rompré & Shizgal, 1986). According to the four-stage paradigm, the ability to correctly identify candidate neurons depends on the number of constraints that are imposed. If behavioural data had been obtained from the subjects prior to recording, more constraints could have been imposed and Rompré and Shizgal (1986) might have obtained different results.

IV. The present investigation

In this project, I sought to identify candidate reward neurons by employing the four-stage paradigm advocated by Gallistel et al. (1981). The objective of the behavioural phase of the experiment was to derive refractory period estimates of the first-stage neurons using the psychophysical approach. The behavioural estimates were to serve as a filter to select candidate reward neurons during the recording phase of the thesis.

To behaviourally derive refractory period estimates, pairs of stimulation pulses (C- and T-pulses) were delivered contingent on a lever press. The C-T interval was varied while the number of pulse pairs required to maintain a half-maximal lever pressing response rate was determined. The effectiveness of the T-pulse as a function of the C-T interval was estimated using the scaling techniques developed by Yeomans (1975). The effectiveness curve has been interpreted to reflect the excitability cycle of the directly stimulated reward relevant neurons (Yeomans, 1975; 1979; Bielajew et al., 1981; Bielajew et al., 1982; Bielajew & Shizgal, 1982).

Using the same subjects and stimulation fields, electrophysiological recordings of single-unit action potentials were then made at sites anterior to the stimulation electrodes. Thus, the same cells ought to have been stimulated in the behavioural and recording phases of the experiment.

The recording sites were chosen in part on the results of (a) existing psychophysical data, (b) anatomical data including those collected using the 2DG technique, and (c) previous electrophysiological recording studies. The refractory period estimates derived in the behavioural phase were used to select candidate reward fibres. That is, electrophysiologically recorded, single-units that were directly activated at intensities used in the behavioural phase and that exhibited refractory period estimates within the behavioural range were considered as candidate reward neurons.

In previous research, the behavioural experiments and electrophysiological recordings were often performed in different subjects. Consequently different stimulation fields were used. The use of the same subjects in both phases of the current experiment was expected, in at least two ways, to tighten the constraints on the choice of candidate neurons. First, the effect of variability due to differences between stimulation sites was reduced. Second, it may be assumed that a directly driven cell monitored in the recording study was directly driven by rewarding stimulation since the stimulation fields in both cases were the same.

Method

Behavioural experiments

Subjects

The subjects were nine male, hooded rats (Royal Victoria strain) obtained from the Canadian Breeding Farms, Ltd. They were individually housed in Plexiglas cages with food (Purina lab chow) and water available ad lib. A reverse cycle 12-hour night/12-hour day was maintained in the animal colony where the subjects were housed.

Apparatus

Electrodes. Three different types of electrodes were used: (a) fixed stimulating electrodes, (b) moveable stimulating electrodes, and (c) current return electrodes.

Fixed stimulating electrodes were made by crimping a male Amphenol pin to one end of a stainless steel wire, 12 mm in length and 250 µm in diameter. The wire was insulated up to the Amphenol pin with Formvar. The tip was bared of insulation in the process of honing it to a hemispherical shape.

Moveable stimulating electrodes and their corresponding leads were purchased from Kinetrods Inc. (Ottawa, Ontario). The design of the electrode permitted it to be moved vertically without twisting by attaching a calibrated driver. A quarter turn of the driver resulted in a movement of approximately 80 µm of the electrode.

Current return electrodes were made from stainless steel wire, 250 µm in diameter, and flexible, insulated wire. The insulation at the tips of the flexible wire was chemically removed with Strip-X (GC Electronics). One end of the flexible wire was soldered to the middle of a 20 mm length of stainless steel wire. Male Amphenol pins were crimped to the end of the flexible wire and to one end of the stainless steel wire. The stainless steel wire was then insulated with Formvar from the tip to the soldered joint. (The rationale for this flexible extension was that during surgery the current return electrode could be lowered into the skull and the stainless steel wire cut just above the soldered joint. The flexible extension then enabled the Amphenol pin to be placed where it would not interfere with the subsequent surgery for the recording session.) The stainless steel wire was shortened so that the distance from the solder joint to the tip was approximately 7 mm in length; the tip was honed flat, and 2-3 mm of insulation was scraped from the tip.

Equipment for the behavioural experiments. The equipment for the behavioural experiments was of two types: (a) hand-operated equipment and (b) computer-operated equipment. The hand-operated equipment was employed to determine whether the stimulation was rewarding. The computer-operated equipment was used to stabilize the subject's performance and to gather the behavioural data used to estimate recovery

from refractoriness in the neurons responsible for the rewarding effect.

The test chambers controlled by the hand-operated equipment were wooden boxes measuring approximately 28 cm X 27 cm X 69 cm high with a Plexiglas front. Two rodent levers (Lehigh Valley Electronics, 121-05) were mounted 4.5 cm above a wire mesh floor on opposite sides of the chamber. To allow the animal to circle in the test chamber without twisting its lead, the connection between the electrode and the stimulator was routed through a seven-channel slip ring commutator (Airflyte Electronics Co., CAY-652). Digital pulse generators (Mundl, Note 1) controlled all temporal parameters of the stimulation whereas constant current amplifiers (Mundl, 1980) controlled the current intensity. When no stimulation pulse was present, the outputs of the stimulators were shorted through a one kiloohm resistor to prevent an accumulation of charge at the brain-electrode interface. An oscilloscope (D61a Telequipment) was used to monitor the stimulation current by reading the voltage drop across a one percent precision, one kiloohm resistor in series with the electrode.

The test chambers controlled by the computer-operated equipment were entirely constructed from Plexiglas and measured approximately 25 cm X 25 cm X 70 cm high. Two rodent levers (Lehigh Valley Electronics, 121-05) were mounted 6 centimeters above the Plexiglas floor on opposite sides of

the chamber. A 1.5 cm "jewel" light was located 8.5 cm above each lever. Above the rear lever the lens was yellow whereas above the front lever the lens was red. In this experiment, only the rear lever was used. The light over the lever was turned on whenever stimulation was available. As in the hand-operated equipment, the connection between the electrode and the stimulator was routed through a seven-channel slip ring commutator (Airflyte Electronics Co., CAY-652). Wooden boxes (50 cm X 50 cm X 90 cm high) insulated with 2.5 cm thick Styrofoam encased each of the test chambers. The front panel of each wooden box was removable and had a double, clear Plexiglas window so that the activity of the rats could be monitored by video from an adjacent room. In the center of the ceiling of each wooden box was mounted a 40-Watt light bulb that was turned on when each trial began and turned off when each trial ended. Each wooden box was equipped with a 11 cm fan that assured a constant flow of air and provided masking noise.

A dedicated microprocessor equipped with a custom-built interface set the experimental parameters for each rat. The parallel port of the dedicated microprocessor controlled the relays that determined which of the electrodes would be used for stimulation. A digital to analog converter supplied the voltage necessary to control the amplitude of the pulses produced by the constant current amplifiers (Mundl, 1980). When no stimulation pulse was present the outputs of the

stimulators were shorted by means of transistor switches through a one kiloohm resistor to minimize accumulation of charge at the brain-electrode interface. The current intensity was monitored by reading the voltage drop across a one percent precision, one kiloohm resistor in series with the rat.

Procedure

Surgery. Subjects weighed between 300-450 grams at the time of surgery. Each subject was anesthetized with sodium pentobarbital (Somnotol, 65 mg/kg injected intraperitoneally) and placed in the stereotaxic device so that lambda and bregma were in the same horizontal plane. Five subjects were implanted with fixed stimulating electrodes aimed at the lateral hypothalamus (LH) and the ventral tegmental area (VTA). The LH coordinates used were 2.8 mm behind bregma, 1.7 mm lateral to the midline, and 9.0 mm below the surface of the skull. The VTA coordinates used were 5.8 mm behind bregma, 0.6 mm lateral to the midline, and 8.7 mm below the surface of the skull. Four subjects were implanted with a fixed stimulating electrodes aimed at the LH and a moveable stimulating electrode aimed at the VTA. The LH coordinates used were 2.8 mm behind bregma, 1.7 mm lateral to the midline, and 9.0 mm below the surface of the skull. The VTA coordinates used were 5.8 mm behind bregma, 0.6 mm lateral to the midline, and 7.8 mm below the surface of the skull. All coordinates were obtained from the stereotaxic atlas of

Paxinos and Watson (1986). The stimulating circuit was completed via a current return electrode aimed 3.5 mm anterior to bregma, 4.0 mm lateral to the midline, and 5.5 mm below the surface of the skull. For recording, a ground reference was provided by an electrode identical to that for the current return, aimed 2.0 mm anterior to bregma, 3.5 mm lateral to the midline, and 7.5 mm below the surface of the skull. The location of the ground reference was selected so that the recording sites were as close as possible to the predicted position of zero potential surfaces between a stimulation electrode and the current return. In principle, this should minimize the stimulation artifact registered by the recording electrode (Ranck, 1981).

Four stainless steel jeweler's screws (skull screws) were fixed in the skull, two near the AP level of the current return and the other two lateral to the stimulating electrodes. A current return for the marking lesions used to locate the stimulation sites and the penetrations made by the recording electrodes was made by wrapping a flexible, stainless steel wire around the anterior skull screws. Except for an area approximately 5 mm long and 10 mm wide between the current return and the LH electrode, the exposed surface of the skull was covered with dental acrylic to cement the stimulating electrodes and the current return electrodes to the skull and skull screws. The area free of dental acrylic was later used to gain access to the record-

ing sites. Both stimulating electrodes and the current return were located in the left hemisphere whereas the ground reference was located in the right hemisphere.

For the initial subjects, the cranial bone of the area free of dental acrylic was removed, covered with mineral oil, and then covered with Gelfoam (Upjohn Company). In addition, a nut soldered to a 22-gauge wire was placed in this 'well' which was then filled with sticky wax. However, when this wax 'cork' was subsequently removed during the surgery prior to the recording session, it was noted that a white, translucent mucous developed that obscured inspection of the superficial vasculature. To overcome this problem in subsequent subjects, the cranial bone was not removed during the initial surgery. The nut and wire were placed on the bone and then the sticky wax was poured into the well formed by the dental acrylic. When the wax cork and cranial bone were removed using this revised technique, the superficial vasculature was easily discerned.

The subjects were allowed a one-week period prior to initial training and selection to recover from surgery.

Initial training and selection. In the first training phase, subjects were shaped to bar press for stimulation in the hand-operated equipment. Trains of pulses were delivered upon successively closer approximations to a bar press until the desired behaviour was manifested. Each stimulation train lasted for 0.500 sec. and consisted of 50 cathodal pulses

(100 Hertz), 0.100 msec. in duration, and 400 µA in amplitude. If subjects exhibited disruptive motor responses or aversive reactions, they were eliminated from the experiment. If the remaining subjects showed little tendency to approach the lever, then the current was incremented in 100 uA steps until either the desired behaviour was manifested or else a maximum current of 1800 µA was reached. If the subject showed little tendency to approach the lever for stimulation of a moveable electrode, the electrode was lowered approximately 320 µm and the subject was retested 24 hours later. Subjects that could not be shaped on either electrode were eliminated from the experiment.

Subjects were next taught to initiate bar pressing after being primed by five non-contingent trains of stimulation. Each bar press during a 30 sec. period following priming resulted in the delivery of a stimulation train. Subjects had to maintain a rate of at least 50 bar presses per minute to pass into the stabilization phase of training.

Stabilization. Before data were gathered, the ability of each subject to respond consistently for stimulation of fixed parameters was ensured. This was done by using a computer operated stabilization procedure that repeatedly determined the number of pulses required to produce a half-maximal response rate of lever pressing (hereafter referred to as the required number). Determination of the required number of single pulses involved first plotting the number

of responses (bar presses) within the 30 sec. trial against the logarithm of the number of pulses to yield a rate-number curve. The required number of single pulses for the current employed was the antilogarithm of the x-coordinate on the rate-number curve corresponding to a y-coordinate of half the maximum response rate. A subject's performance was considered stable when the range of required number values varied by no more than 0.1 $\log_{10}$ units for two consecutive stabilization sessions. Each session consisted of approximately 15 determinations of the required number.

Initial starting values for the number of pulses, current, and maximum and minimum number of pulses that had resulted in vigorous bar pressing during the initial screening served as seed values for the computer operated stabilization procedure. The algorithm described below was used by the computer to determine the rate-number curve.

First, the computer determined an estimate of the upper limit of the rate-number curve. Using the manually derived parameters, a 30 sec. trial was run. If the subject self-stimulated, then the number of pulses per train was increased by 0.1 $\log_{10}$ units and a new trial was run. This procedure yielded an estimate of the asymptotic performance by continuing until the number of bar presses on the last trial did not exceed the number of bar presses on the previous trial by more than 10 percent. If, however, the subject did not respond on the initial trial, then the number of pulses

was increased by 0.3 $\log_{10}$ units and a new trial was run. This procedure was continued until eith : (a) the rat responded, at which time 0.1 $\log_{10}$ increments in the number of pulses were employed to define the upper asymptote as outlined above or (b) the maximum number of pulses defined by the experimenter was attained, at which time a new determination would begin using the manually derived parameters. The maximum number of pulses per train was limited to ensure that the subject would not receive an aversive level of stimulation.

Having determined the upper asymptote, the computer next determined an estimate of the lower limit of the rate-number curve. To accomplish this, the computer set the number of pulses to a value 0.1 $\log_{10}$ units below the first point at which the subject had responded and a new trial was run. If the subject had not responded at all, the computer then set the number of pulses to the manually derived value. This procedure yielded a lower asymptote by continuing to decrease the number of pulses in 0.1 $\log_{10}$ unit steps until two consecutive trials yielded response rates less than 10 percent of the maximal response rate.

<u>Refractory period test</u>. In a manner similar to the stabilization procedure, a series of determinations of the required number of pulses was carried out with the current, pulse duration, and train duration held constant. On some determinations, trains of pulse pairs were delivered rather

than trains of single pulses. The first pulse (C-pulse) conditioned the neurons within the field of excitation by firing them thus assuring that they all were in the same state; the second pulse (T-pulse) tested the state of excitability of the neurons at a given interval following the delivery of the C-pulse. The length of time between the C- and T-pulse is designated as the C-T interval. Test trials during the refractory period experiment involved determining the number of pulse pairs required to produce a half-maximal rate of lever pressing (hereafter referred to as the required number of pulse pairs) while the C-T interval was systematically varied.

Subjects with two electrodes supporting self-stimulation were tested at 15 different C-T values ranging from 0.15 msec. to 10.0 msec. (short schedule) while those subjects with only one electrode that supported self-stimulation were tested at 26 different C-T values ranging from 0.15 msec. to 10.0 msec. (long schedule). Given the amount of time that would have been required to test all of the C-T values in the long schedule, two sessions of 13 different C-T values arranged in a pseudo-random order were required to yield one full refractory period curve. Two pseudo-randomly ordered versions of the short schedule were used.

Each session for both the long and short schedules, comprised approximately five determinations of the required number of single pulses and a series of determinations of

the required number of pulse pairs, each carried out at a different C-T interval.

The required number of single pulses was determined using the procedure outlined above. These were then averaged to yield a single estimate of the required number of single pulses for the session. The required number of pulse pairs for each C-T interval was also determined using the procedure outlined above except that the number of responses (bar presses) within the 30 sec. trial was plotted against the logarithm of the number of pulse-pairs instead of against the logarithm of the number of pulses.

The following formula developed by Yeomans (1975) was used to calculate the effectiveness (E) of the T-pulse as a function of the C-T interval:

$$E = (RN_{SP} - RN_{C-T}) / RN_{C-T}$$

where E = effectiveness of the T pulse

RN_{SP} = required number of single pulses

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

The formula relates the required number of pulse pairs to the required number of single pulses. If the T-pulse in each pair was not effective in exciting the neurons responsible for the rewarding effect, the calculated E value would be equal to zero. If, on the other hand, each T-pulse was as

effective as each C-pulse, then half as many pulse pairs would be required as single pulses. In that case, the calculated E value would be equal to one. The curve that results from plotting the E values against the C-T interval is believed to reflect a weighted average of the excitability cycles of the directly stimulated neurons responsible for the rewarding effect (Yeomans, 1975).

Subjects were run approximately six to ten times on each of their respective C-T schedules. At the end of the experiment, the data for all the sessions for each rat were grouped and the maximum and minimum E values at each C-T interval were eliminated. The remaining E values at each C-T interval were averaged and a final curve relating the mean E value to the corresponding C-T interval was plotted. The curve relating mean E value to C-T interval will be hereafter referred to as a recovery curve.

Data analysis

Using the Biomedical Computer Programs (BMDP), a data analysis procedure was developed to estimate the C-T intervals at which the recovery curve attained its minimum and maximum asymptotic values. The procedure used the iterative, derivative-free nonlinear regression program (AR program) to estimate a least squares three-segment recovery curve. The first segment, an exponential function, was used to describe the decay of the LPS. The second segment, a linear function, was used to describe the recovery from refractoriness. The

third segment, a constant, was used to describe the maximum asymptotic value of the curve. Seed values for the analysis were derived from visual inspection of the recovery curve.

Recording sessions

Subjects

The nine subjects used in this experiment were the same as those run in the behavioural experiments.

Apparatus

Electrodes. The recording assembly consisted of three lacquer insulated tungsten microelectrodes (Frederick Haer and Co.), two made of tungsten and one of stainless steel. The tungsten microelectrodes were fine-profile electrodes (that is, 50 µm from the tip, the shank diameter was 10 µm) with tip impedances of 9-12 megohms. The stainless steel microelectrode was a coarse-profile electrode (that is, 50 µm from the tip, the shank diameter was 20 µm) with tip impedances of 2-5 megohms. The tungsten electrodes were glued such that the tip of one was approximately 500 um above the tip of the other. Rompre and Shizgal (1986) determined that this was an effective configuration for obtaining differential cancellation of the stimulation artifact without cancellation of unit responses. The stainless steel microelectrode was glued approximately 500 µm above the shorter tungsten electrode and was used to lesion brain tissue so as to mark the position of the electrode track via deposition of iron ions. This electrode will

be hereafter referred to as the lesioning electrode. The relative positions of the tips of the electrodes were measured prior to the recording session. Thus the approximate location of a recorded cell could be estimated given the histologically determined location of a lesion stain. About 17 mm above the tip of the longer tungsten microelectrodes, the shanks of the three microelectrodes were glued to a no. 3 stainless steel insect pin so that the assembly could be rigidly mounted on a stereotaxic electrode carrier.

Recording equipment. Integrated circuit pulse generators connected to constant-current amplifiers (Grass CCU1) via radio frequency isolators and a custom-built transistor interface produced cathodal rectangular pulses, 0.1 msec. in duration. Both a Fluke 8060A True RMS multimeter and a Tektronix Type 502A dual-beam oscilloscope were used to monitor the stimulation currents by reading differentially the voltage drop across a one percent precision, one kiloohm resistor in series with each electrode. The oscilloscope was used in adjusting the stimulation current in those situations when an approximate value could be used. When a more precise estimate was desired, the stimulation electrodes were switched out of the circuit and replaced by resistors; a direct current was passed and the multimeter was used as an ammeter. To minimize the stimulation artifact, Wagner grounds were connected via transistor gates across the outputs of the constant-current amplifiers (Ranck, 1981).

This configuration made it possible to use a separate Wagner ground with each of the two stimulation electrodes. A slope/height window discriminator (Frederick Haer and Co.) was used to trigger the stimulation pulse when collision tests were carried out.

Headstage amplifiers were made from precision FET-input operational amplifiers (AD515J or AD545J), configured as unity-gain voltage followers. The headstage amplifiers were mounted approximately 10 cm above the recording electrode. After passing through separate headstage amplifiers and separate variable gain amplifiers and filters, the signals from the two tungsten microelectrodes were combined by a differential amplifier (Tektronix 3A9) so that the stimulation artifact could be cancelled. Using this arrangement, it was possible to compensate for small differences in the amplitude and shape of the stimulation artifacts recorded by the two electrodes. A digital storage oscilloscope (Gould OS4020) displayed the output of the differential amplifier. The output was stored on FM tape whenever any cells were being monitored.

Procedure

Surgery. A half hour prior to the first injection of urethane, each subject received an injection of atropine sulphate (0.6 mg/kg) to reduce bronchial secretions. Each subject was anesthetized with urethane (ethyl carbamate - 1.2 g/kg injected intraperitoneally). Due to the volume of

anesthesia injected, the dose was divided into three equal parts and injected at half hour intervals. Throughout the recording session, each subject was tested periodically to assess the level of anesthesia. If the subject reacted to a pinch of the tail, a supplemental injection of urethane was administered. The subjects were mounted in a stereotaxic frame (Kopf 1700) so that a position as similar as possible to the position during the initial surgery was obtained. The wax plug was then removed. As noted previously, some subjects had had a rectangular portion of the frontal and parietal bones removed during the initial surgery. For those subjects with the cranial bones still intact, a rectangular portion as large as possible given the constraints imposed by the surrounding dental acrylic was removed. For all subjects, the dura mater was removed and the pial surface was covered with Ringer's solution. Throughout the recording session, the subjects' body temperature was maintained at 37°C through the use of a heating pad connected via a negative feedback loop to an anal thermometer.

Electrophysiological recording. At the beginning of each penetration, the recording electrodes were advanced to the starting dorsal-ventral coordinates using the stereotaxic manipulator. To allow for dissipation of any compression of neural tissue at this time, the electrode was left at the initial coordinates for approximately 30 minutes. A hydraulic microdrive (Narishige MO-8) was then used to slowly lower

the electrode. When it was calculated that the electrode tip was near the base of the brain, the penetration was ended. If the activity of some cells had been recorded, then a lesion was made at the bottom of the electrode track by passing a 100 µA direct current for 15 sec. with the lesioning electrode serving as the anode and the skull screws serving as the cathode. Occasionally, when the electrode assembly was being removed from the brain, a further lesion was made near the starting point so that any shrinkage of the brain tissue after the perfusion was performed could be measured. Upon terminating the session, the stimulation sites were lesioned in a similar manner.

During the initial recording sessions, only neurons driven by the current used during behavioural testing were noted. In subsequent sessions, all unit responses with signal-to-noise ratios of at least 2:1 were monitored.

The preliminary test applied to a monitored response involved determining whether the activity of the cell was affected by the stimulation. If it was affected, then one of two procedures was employed. The first procedure was used when the cell was spontaneously active at a rate greater than 0.2 Hertz. In that case, the procedure involved triggering the stimulator shortly after the spontaneous, orthodromic action potentials. If the stimulation-induced action potentials were eliminated but restored by increasing the S-C interval, then it was assumed that they were antidromic.

The second procedure was used when the cell was either not spontaneously active or spontaneously active but not at a sufficiently high rate. In that case, the cell was stimulated at a moderate to high rate (20 - 200 Hertz). If the response latency was constant or nearly so, then it was assumed that antidromic action potentials were being recorded.

Next, the current was varied to determine whether the signals being monitored were single-unit or compound action potentials. If the amplitude of the response varied with the current rather than showing the characteristic all-or-none response typical of a single cell, the response was assumed to be a compound action potential.

Finally, the gain of the recording circuit was reduced and recordings of activity registered by the short and long electrodes alone were obtained (single-ended recordings) to determine which electrode was recording the activity of the cell. Although the stimulation artifact was large, it was possible to distinguish, in the majority of cases, which electrode was recording the unit response.

Since recording sessions tended to run 20-24 hours, a team approach was used. Typically, one member of the team arrived early in the morning, performed the surgery, and started the first penetration. Another member arrived later in the day to continue the recording session. Finally, a third member arrived in the evening, took over the recording, and subsequently perfused the subject.

Refractory period experiment. One of two procedures was used for estimating the refractory period of antidromically driven single-units. The first or standard procedure involved delivering pulse pairs at a frequency of one Hertz and varying the C-T interval. The refractory period was defined as the range of C-T values bounded at the lower extreme by the longest C-T interval that consistently failed to produce a response to the T-pulse in at least nine out of ten trials and bounded at the upper extreme by the shortest C-T interval that consistently produced a response to the T-pulse on nine out of ten trials. The second or Swadlow procedure (Swadlow, 1982) was used when the cell was spontaneously active. This procedure also involved delivering pulse pairs and varying the C-T interval. In this case, however, the C-pulse was delivered after a spontaneous response occurred. In this manner, the antidromic action potential induced by the C-pulse never reached the recording site because of collision with the spontaneously induced orthodromic action potential. Rather than delivering the C-pulse immediately following detection of the spontaneous response, the antidromic latency was determined. The C-pulse was set to follow the spontaneous response at an interval equal to one half the antidromic latency.

The same criteria used in the standard procedure were applied in determining the range of the refractory period using the Swadlow procedure. The currents and pulse dura-

tions used for both procedures were the same as those that had been used in the behavioural experiments.

Histology

At the end of the recording session, the deeply anesthetized subjects were killed by exsanguination. After perfusion with 0.9% saline solution followed by a Prussian Blue solution (3% potassium ferricyanide, 3% potassium ferrocyanide, and 0.5% trichloroacetic acid dissolved in 10% formalin), the brains were removed and then soaked in the Prussian Blue solution for approximately 12 hours. The brains were then stored in 10% formalin for several days prior to sectioning. The brains were then blocked in the plane of electrode insertion and sliced in sections 40 µm thick on a cryostat (at −18°C). Brain slices were mounted on gelatine coated glass slides and stained with thionin. The stereotaxic atlas of Paxinos and Watson (1986) was used to localize the electrode tips.

To estimate the location of the cells recorded, the blue spot resulting from the reaction of the Prussian Blue solution with the iron ions deposited during the lesion was noted. The difference between the estimated termination site and the estimated recording site according to the micrometer readings was calculated. This difference and the spatial relationship between the tip of the lesioning electrode and the electrode used to record the cell could be used to yield an estimate of the location of the recording site.

Results and Discussion

The results are presented in three sections. The data obtained from the behavioural and recording phases are examined separately and then combined to select candidate reward neurons.

Behavioural phase

The behavioural phase was designed to provide characteristics of the reward-relevant fibres that neurons in the recording phase would have to match to be considered candidate reward neurons. This section first presents the refractory period data and then the location of the stimulation electrodes.

Refractory period data

Figures 2 - 12 show the plots of the mean E values versus the C-T intervals for the 11 behavioural curves that were determined. A logarithmic scale is used along the abscissa so that the changes in the mean E values at short C-T intervals will be more apparent. The error bars about each mean E value denote a confidence interval of plus or minus one standard error.

The initial decline of the curve at short C-T intervals has been interpreted as being due to local potential summation (Yeomans, 1975). One might expect that there would be a C-T interval at which the T-pulses fail to fire any reward-relevant neurons. However, at MFB placements, a zero T-pulse effectiveness is seldom found, probably because recovery

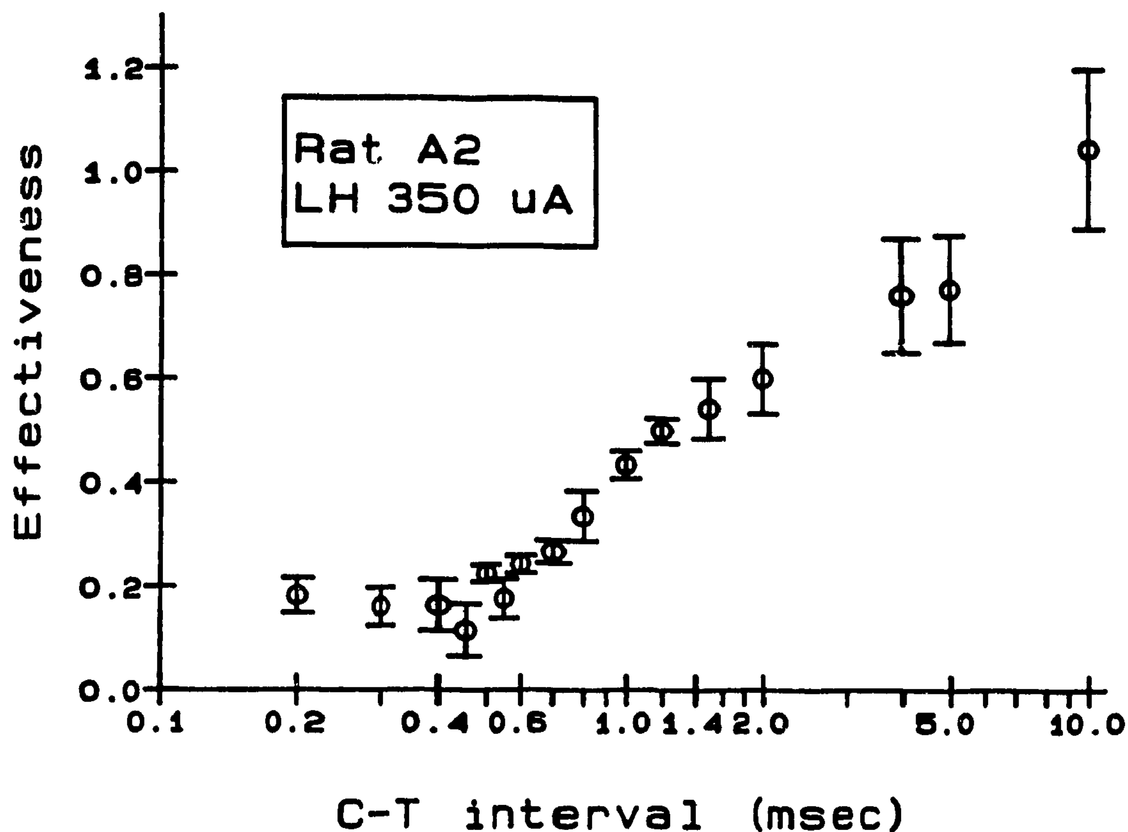


Figure 2. Behaviourally derived refractory period curve plotted with a logarithmic abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error.

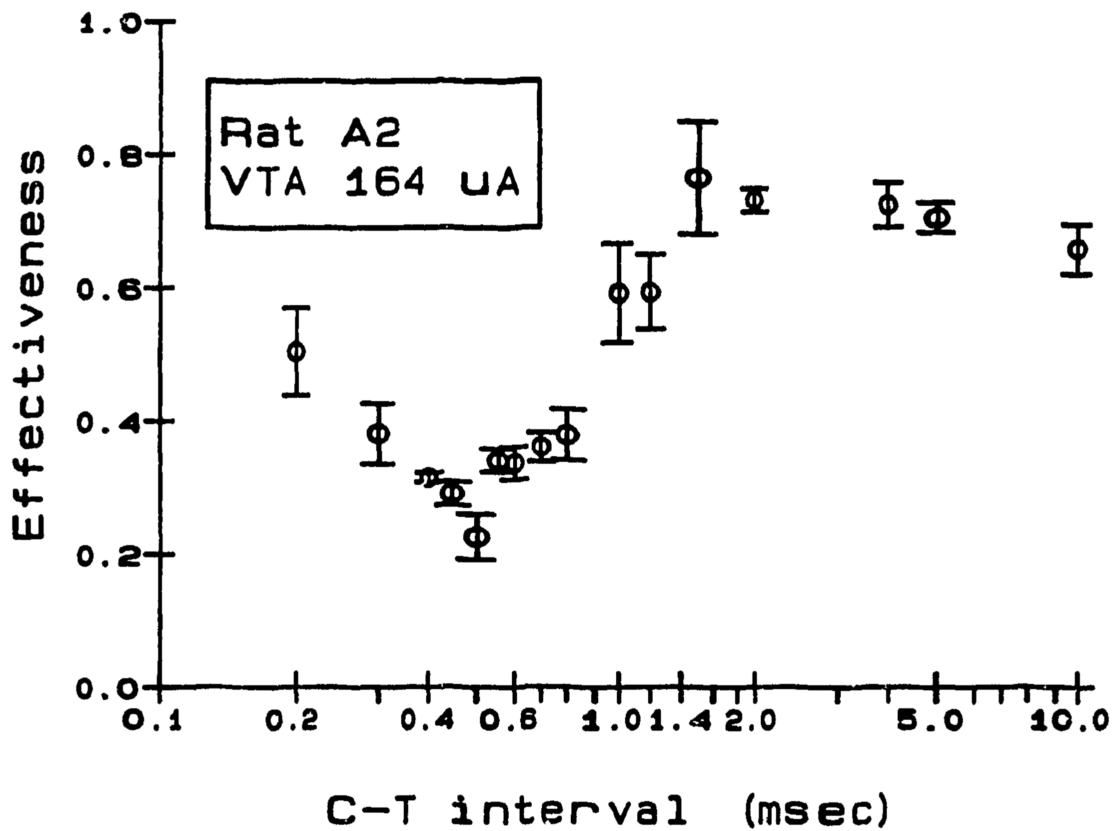


Figure 3. Behaviourally derived refractory period curve plotted with a logarithmic abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error.

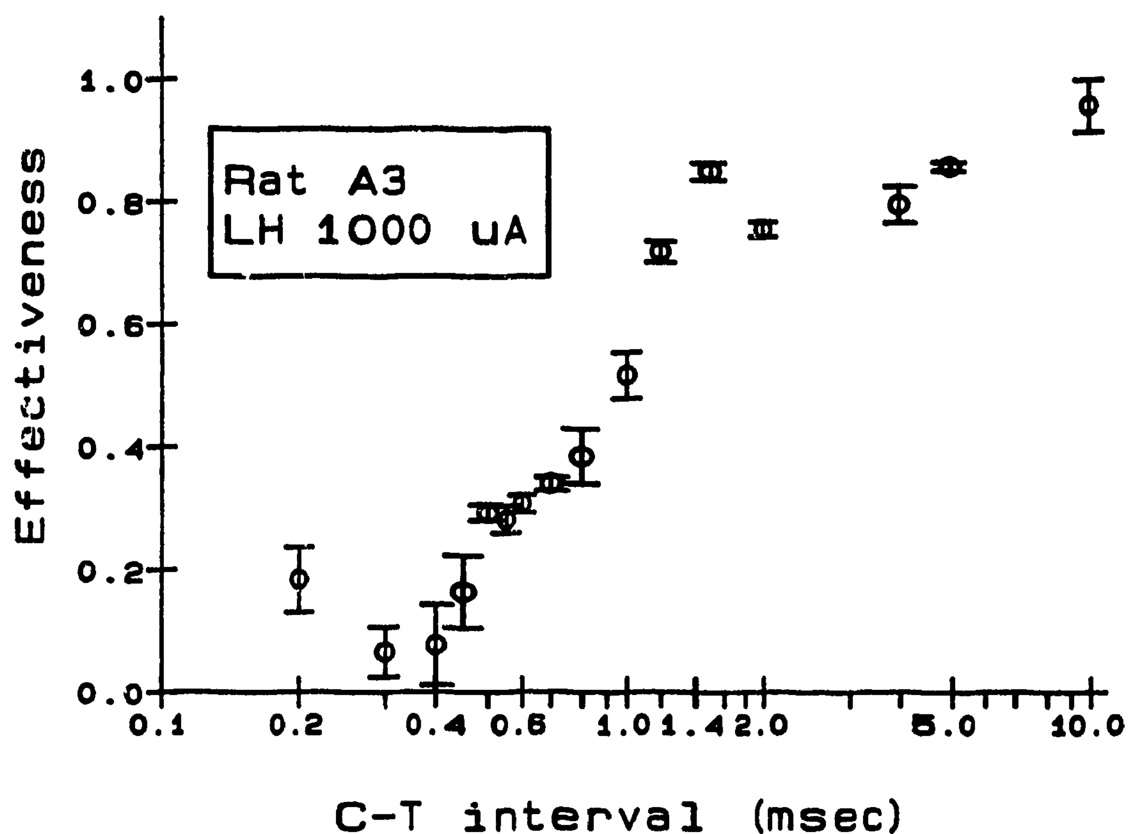


Figure 4. Behaviourally derived refractory period curve plotted with a logarithmic abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error.

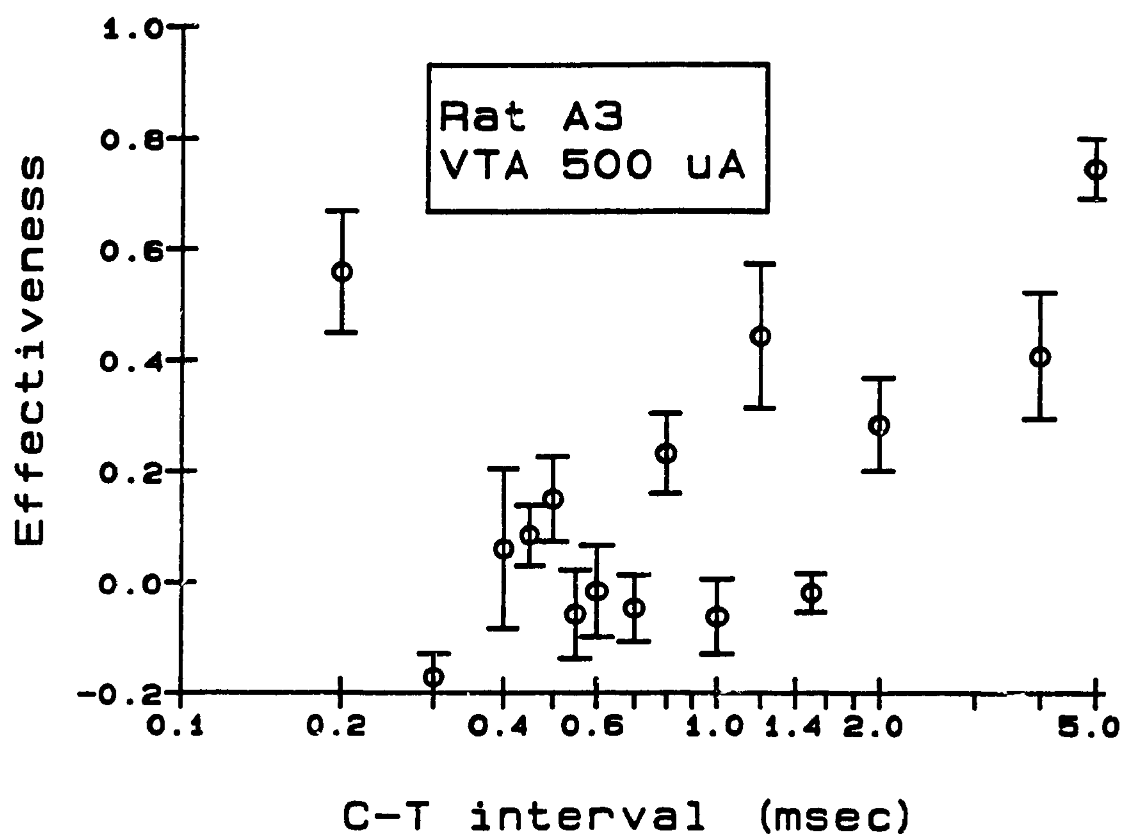


Figure 5. Behaviourally derived refractory period curve plotted with a logarithmic abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error.

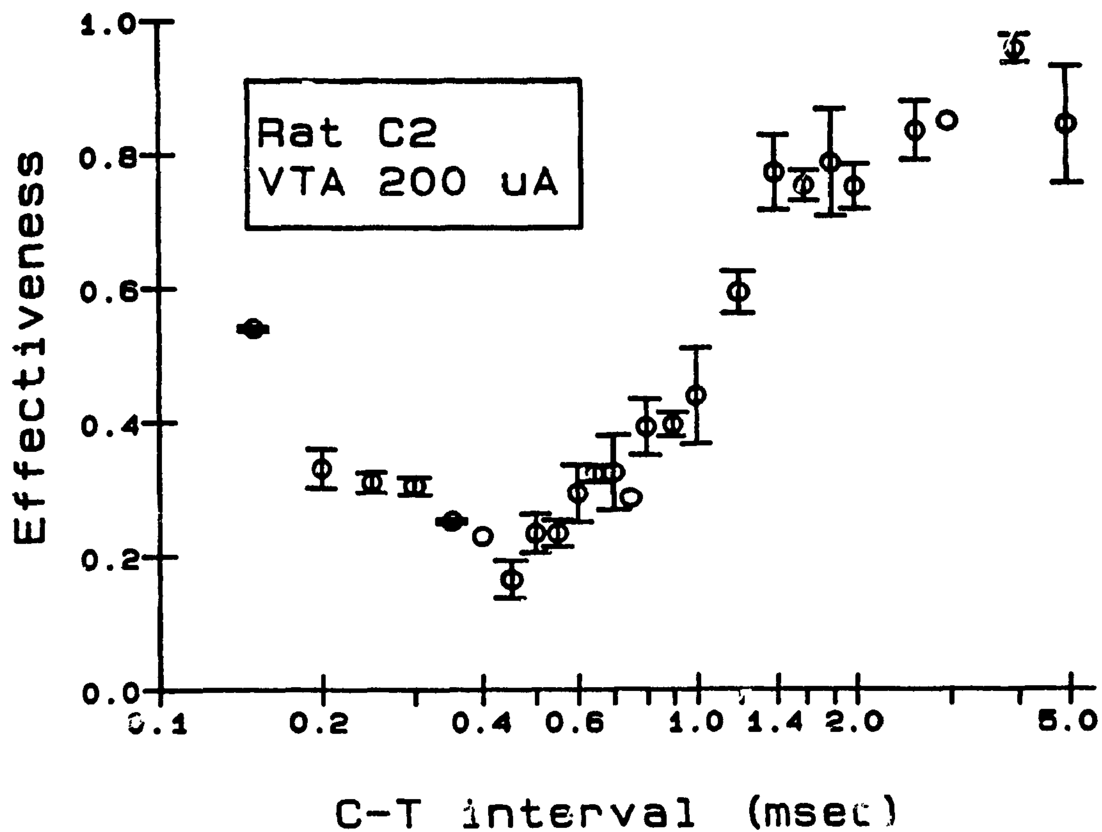


Figure 6. Behaviourally derived refractory period curve plotted with a logarithmic abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error.

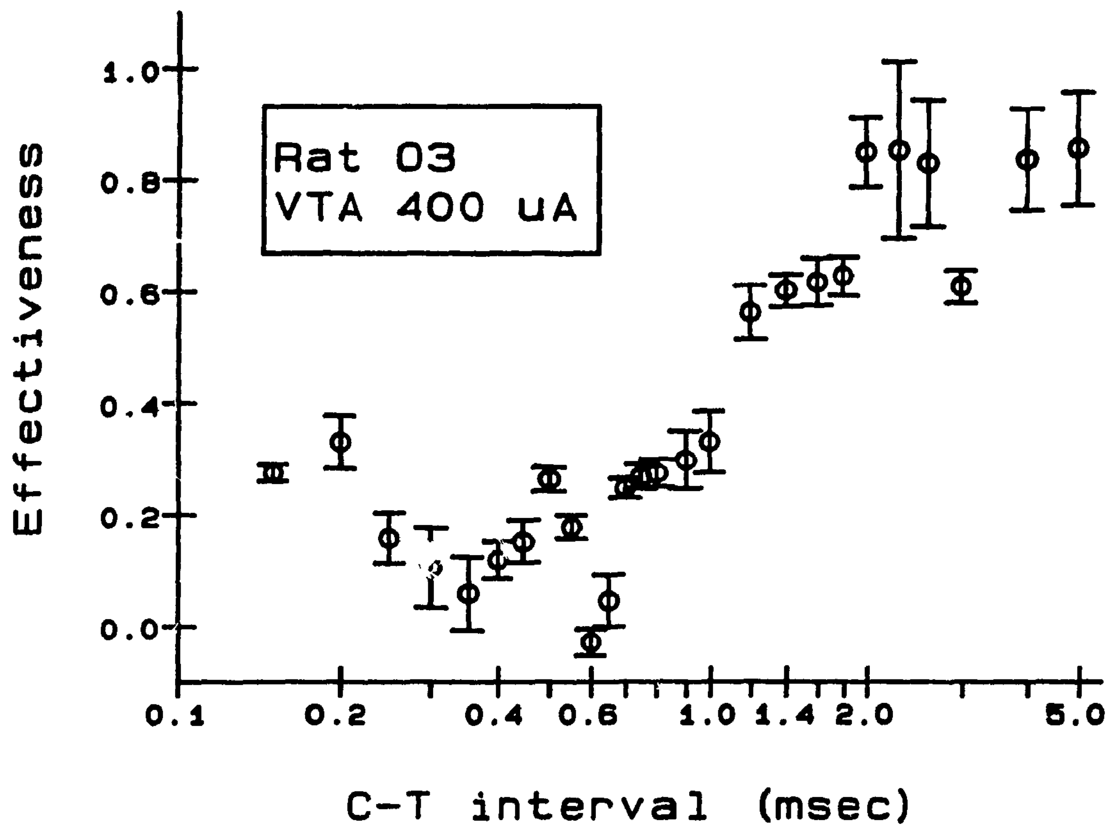


Figure 7. Behaviourally derived refractory period curve plotted with a logarithmic abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error.

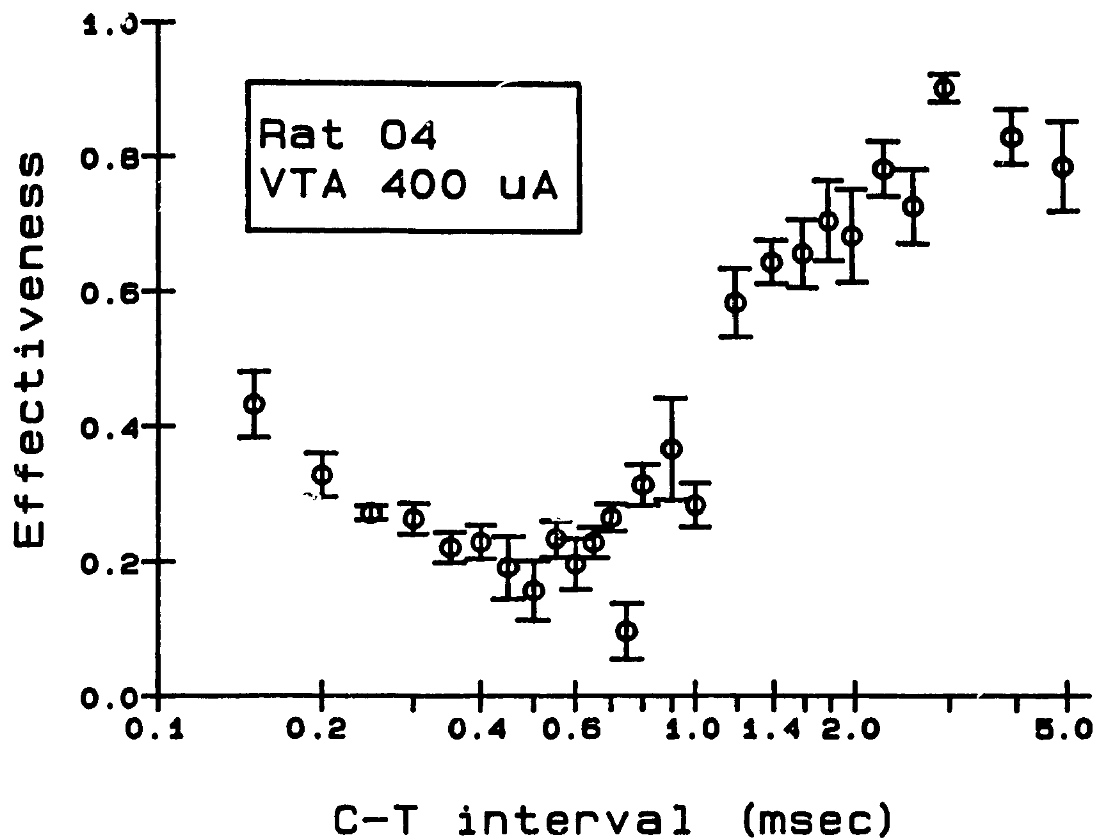


Figure 8. Behaviourally derived refractory period curve plotted with a logarithmic abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error.

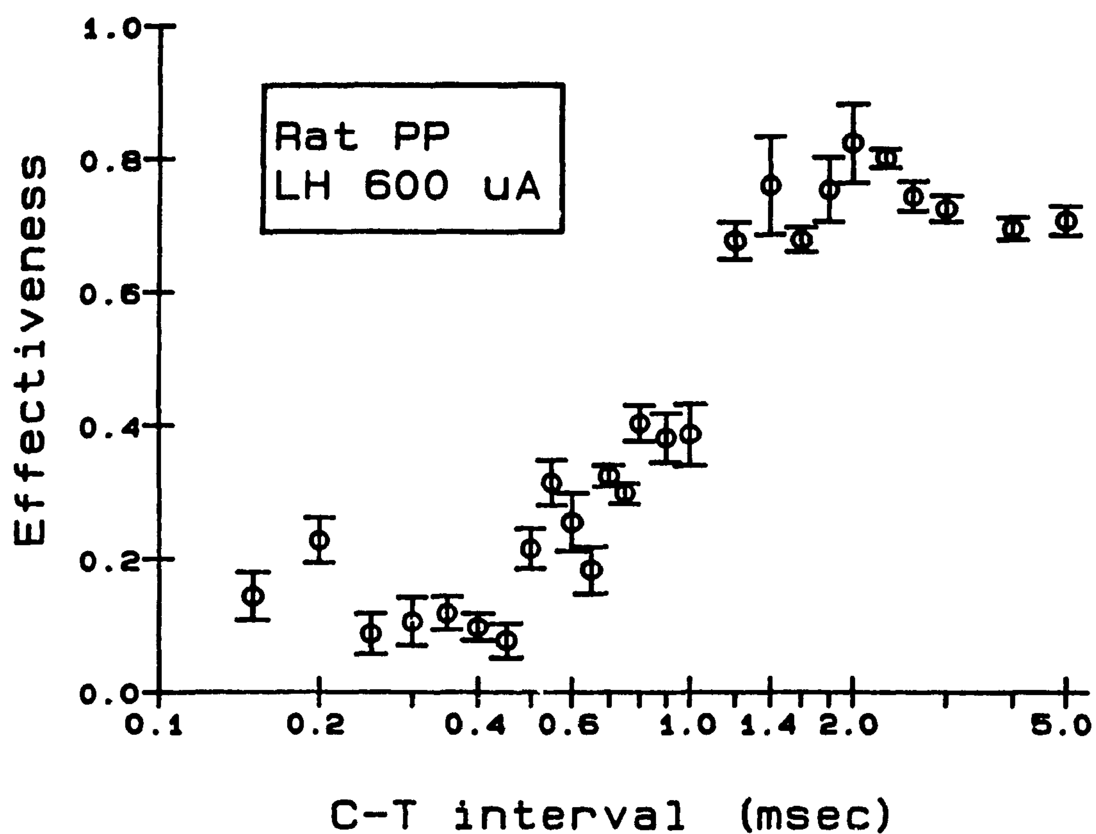


Figure 9. Behaviourally derived refractory period curve plotted with a logarithmic abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error.

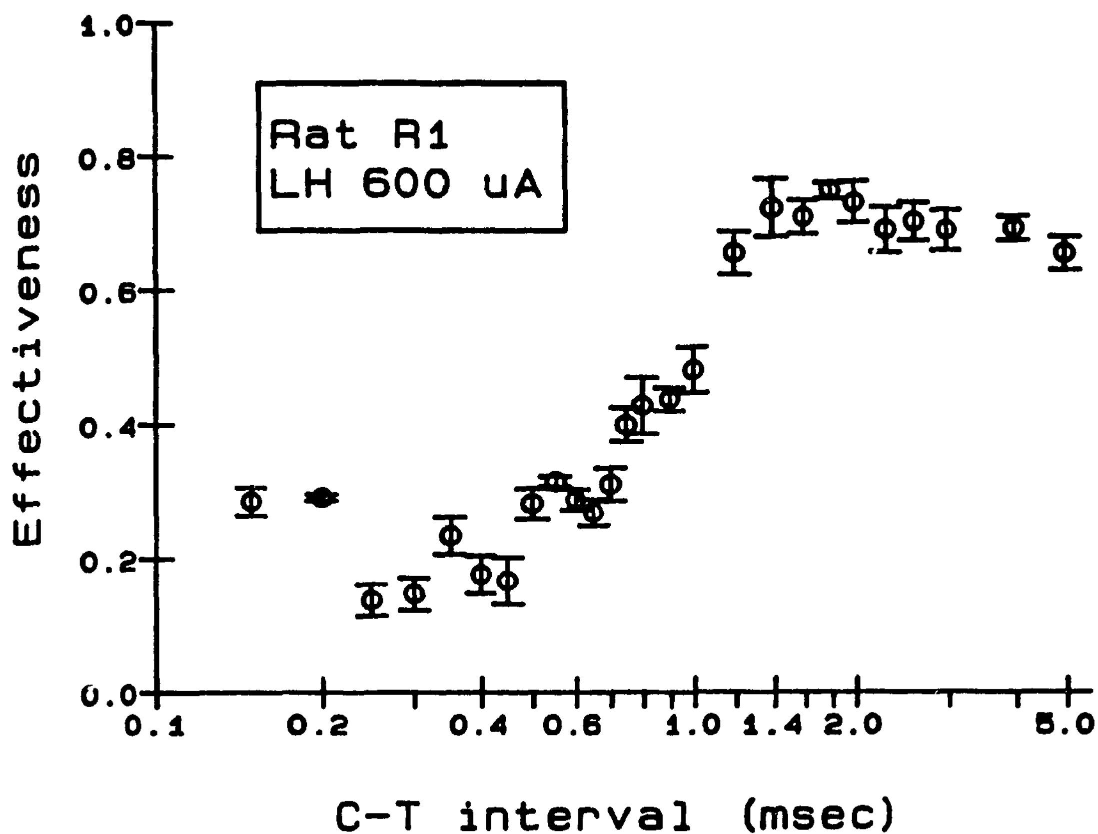


Figure 10. Behaviourally derived refractory period curve plotted with a logarithmic abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error.

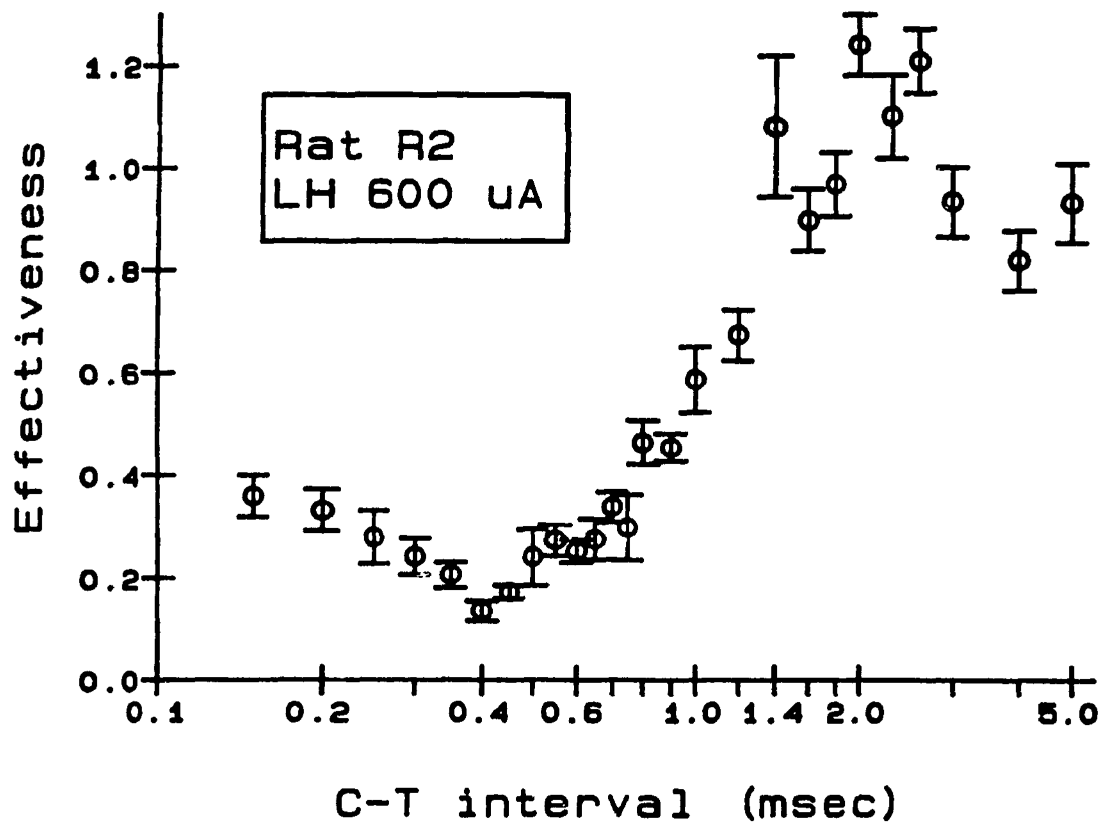


Figure 11. Behaviourally derived refractory period curve plotted with a logarithmic abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error.

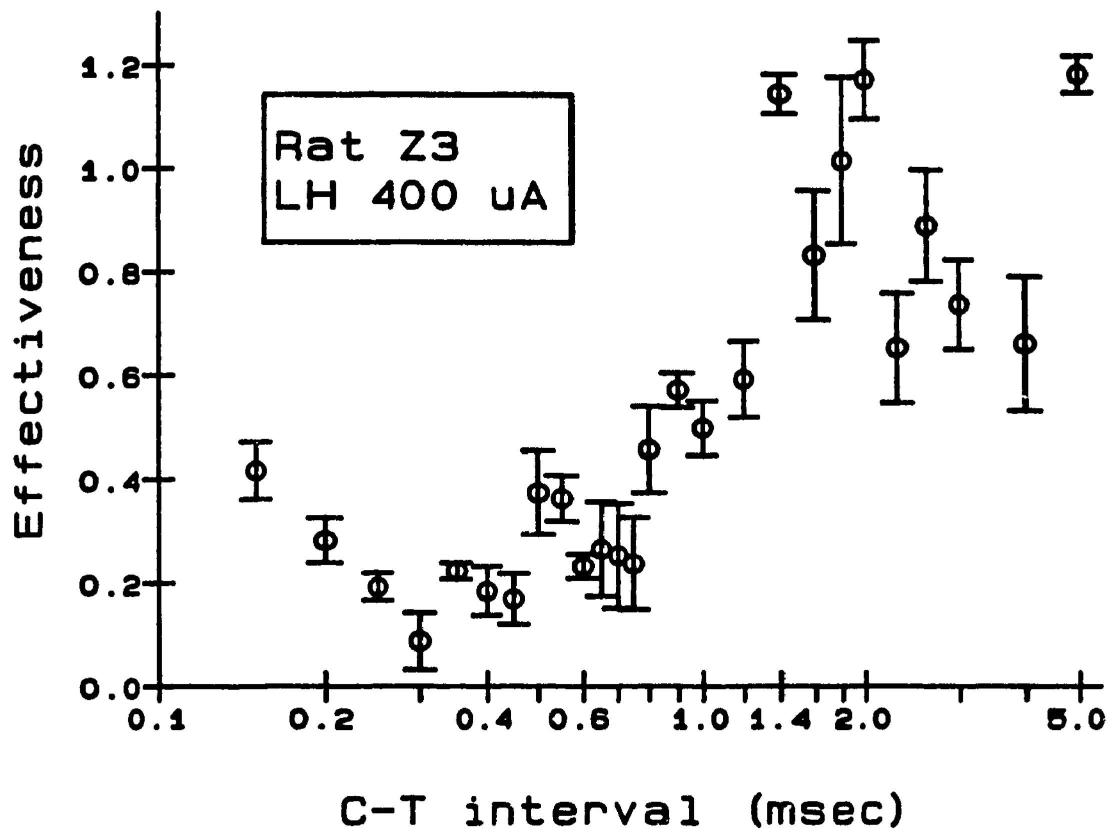


Figure 12. Behaviourally derived refractory period curve plotted with a logarithmic abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error.

from refractoriness in the most excitable elements begins before local potential summation ends.

The subsequent rise of the curve at longer C-T intervals has been interpreted to reflect the recovery from refractoriness in the directly stimulated, reward-relevant fibres (Yeomans, 1975). In principle, there ought to exist a C-T interval at which the T-pulse would fire every reward-relevant fibre that had been fired by the C-pulse. In fact, a T-pulse effectiveness value of 1.0 is seldom observed, and no a˙ ˙uate explanation has been found for this phenomenon.

The data from Figures 2 - 12 are replotted in Figures 13 - 22 along with the fitted three-segment functions. A linear scale has been used along the abscissa to facilitate visual comparison of the fitted functions to the behavioural data. The error bars about each mean E value denote a confidence interval of plus or minus one standard error. It was not possible to derive a three-segment function for the data obtained from the VTA placement of subject A3. As may be seen from Figure 5, the curve relating T-pulse effectiveness to C-T interval does not show the same trend as the curves for the other subjects. It is possible that the electrode assembly was not cemented properly. If so, movements of the tip might account for the large variance. ce the data from the VTA placement of subject A3 could not be fit adequately by a three-segment line, these data will not be considered in any of the subsequent presentations.

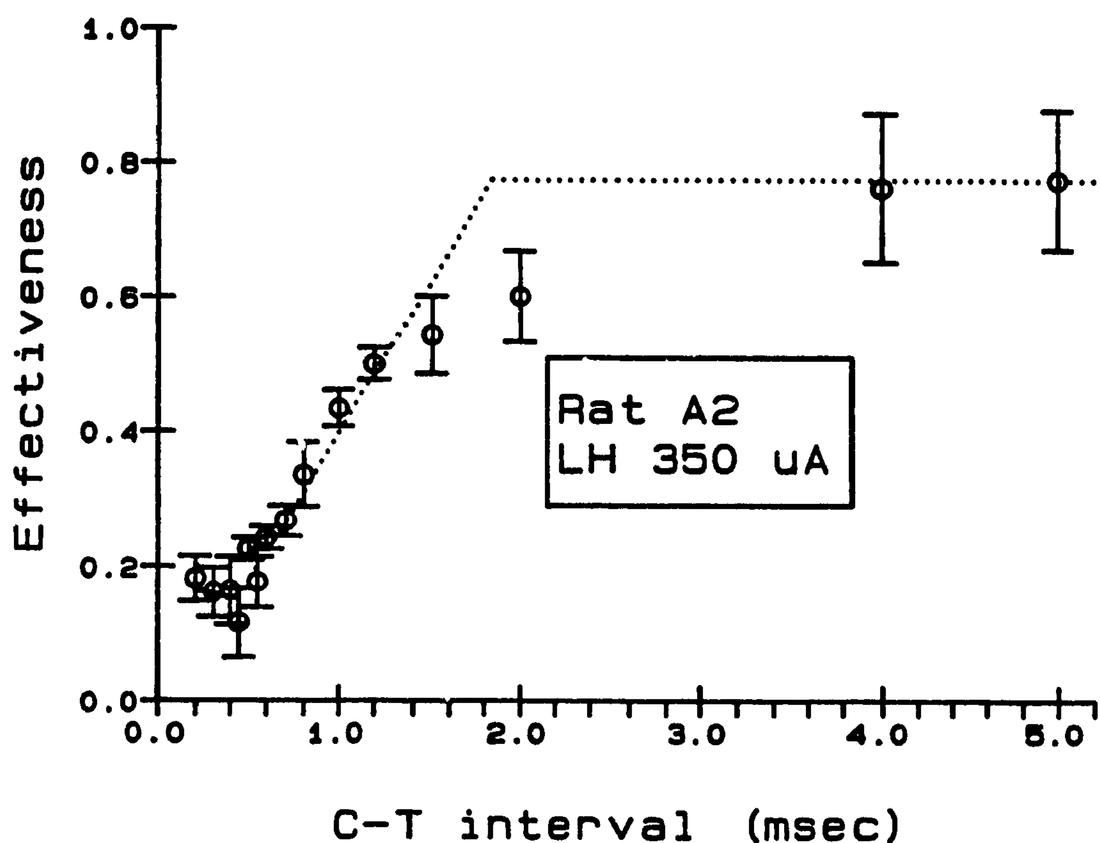


Figure 13. Behaviourally derived refractory period curve plotted with a linear abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error. In addition, the fitted, three segment line used to estimate the C-T intervals at which the recovery curve attained its minimum and maximum asymptotic values has been superimposed on the behavioural data.

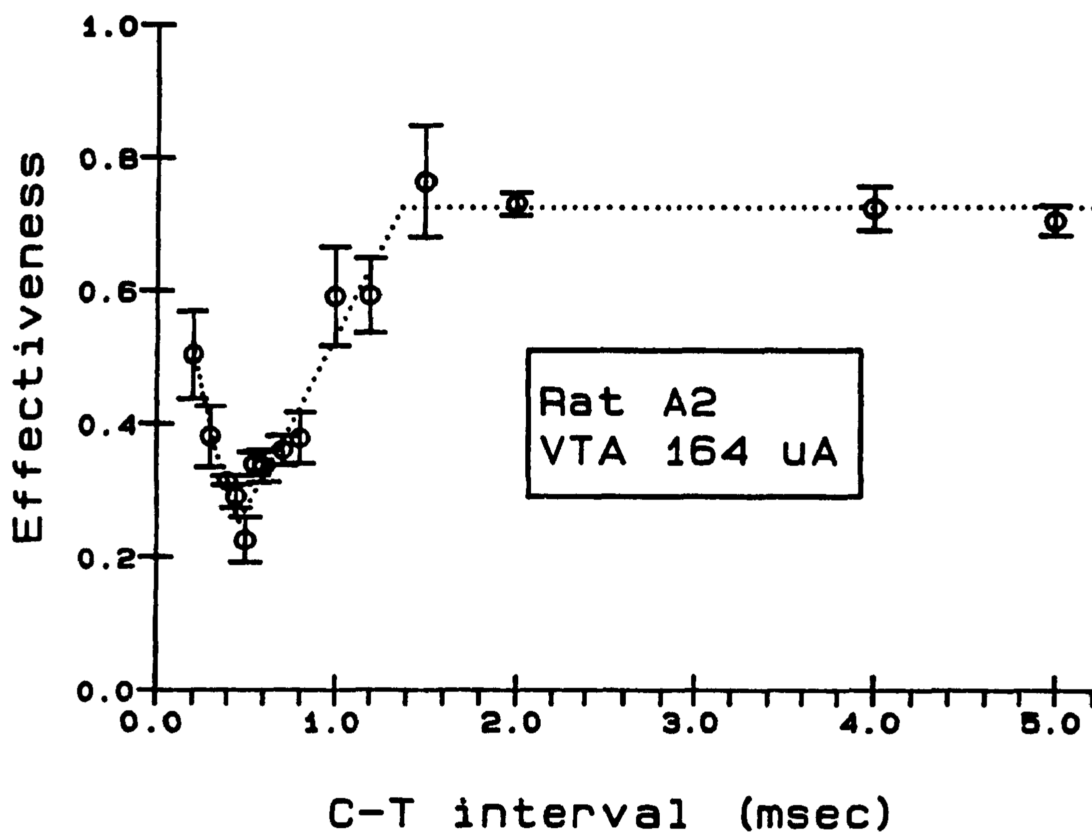


Figure 14. Behaviourally derived refractory period curve plotted with a linear abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error. In addition, the fitted, three segment line used to estimate the C-T intervals at which the recovery curve attained its minimum and maximum asymptotic values has been superimposed on the behavioural data.

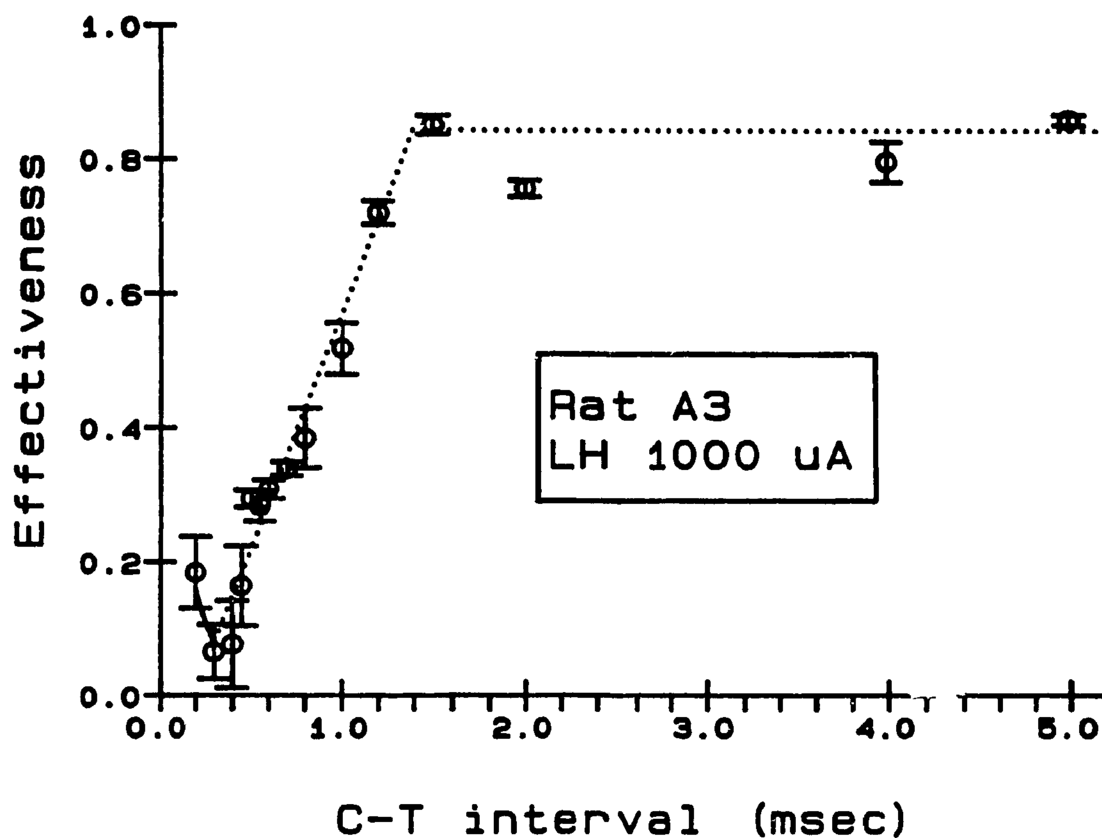


Figure 15. Behaviourally derived refractory period curve plotted with a linear abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error. In addition, the fitted, three segment line used to estimate the C-T intervals at which the recovery curve attained its minimum and maximum asymptotic values has been superimposed on the behavioural data.

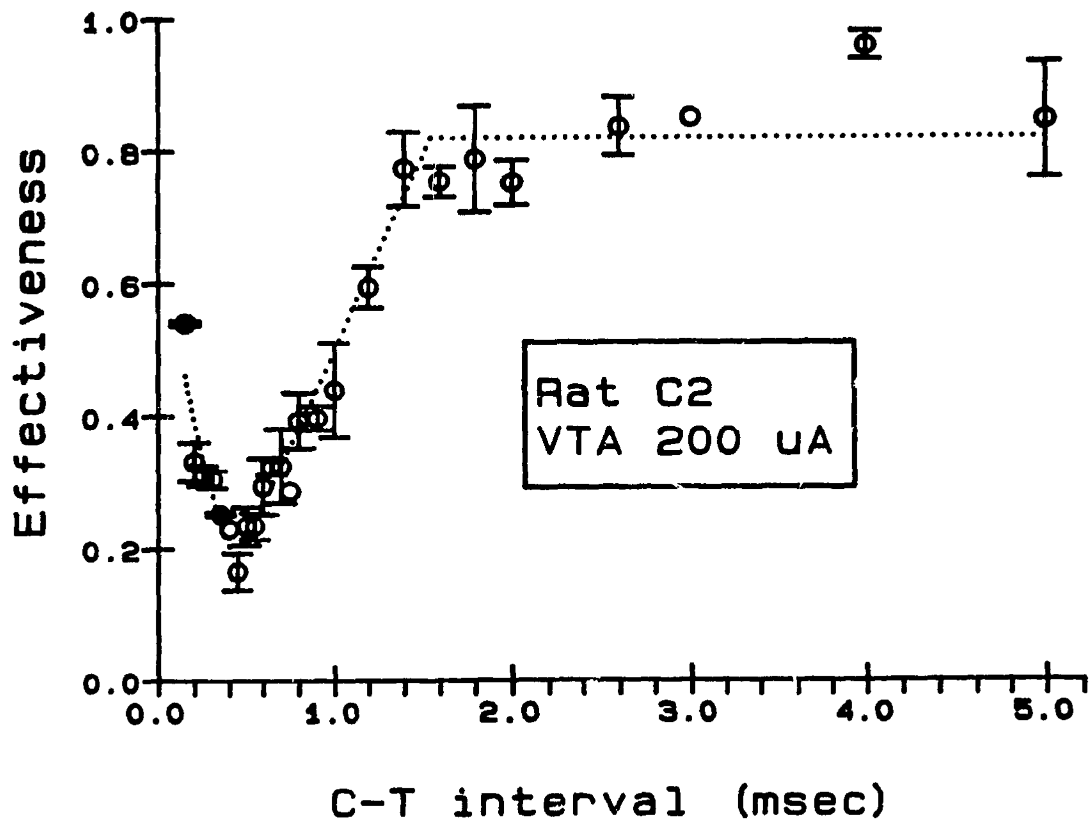


Figure 16. Behaviourally derived refractory period curve plotted with a linear abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error. In addition, the fitted, three segment line used to estimate the C-T intervals at which the recovery curve attained its minimum and maximum asymptotic values has been superimposed on the behavioural data.

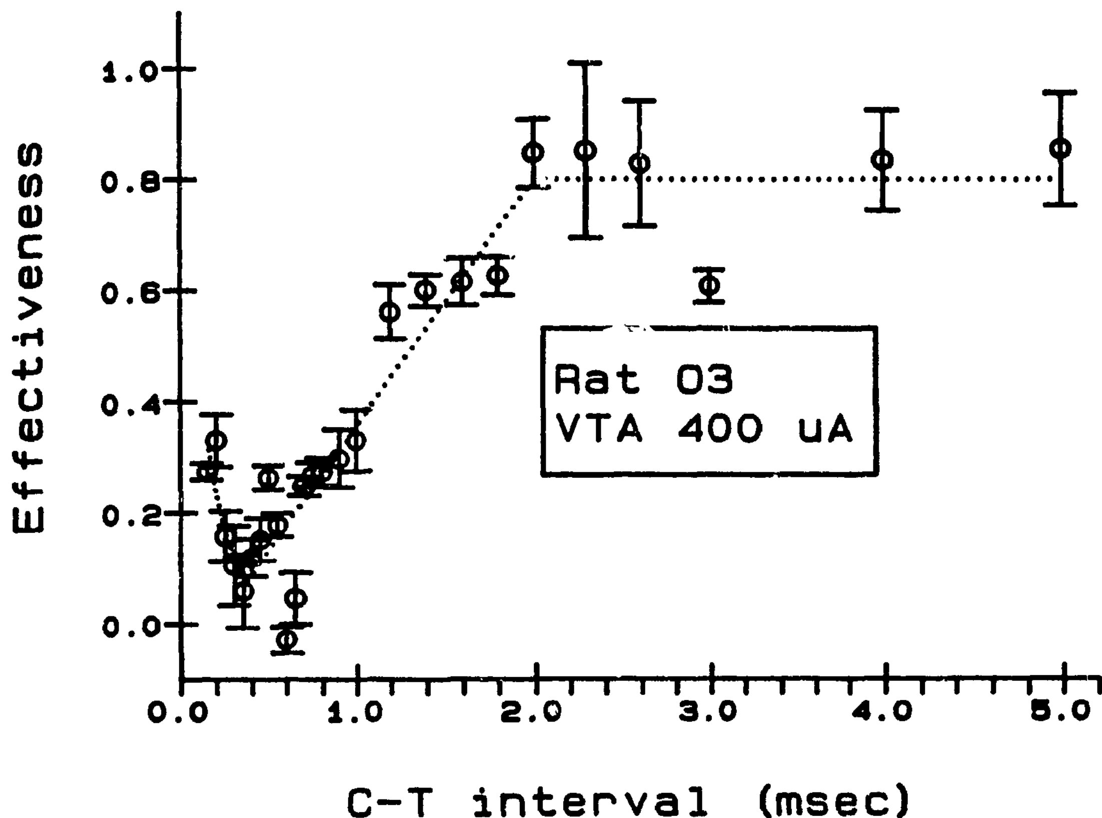


Figure 17. Behaviourally derived refractory period curve plotted with a linear abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error. In addition, the fitted, three segment line used to estimate the C-T intervals at which the recovery curve attained its minimum and maximum asymptotic values has been superimposed on the behavioural data.

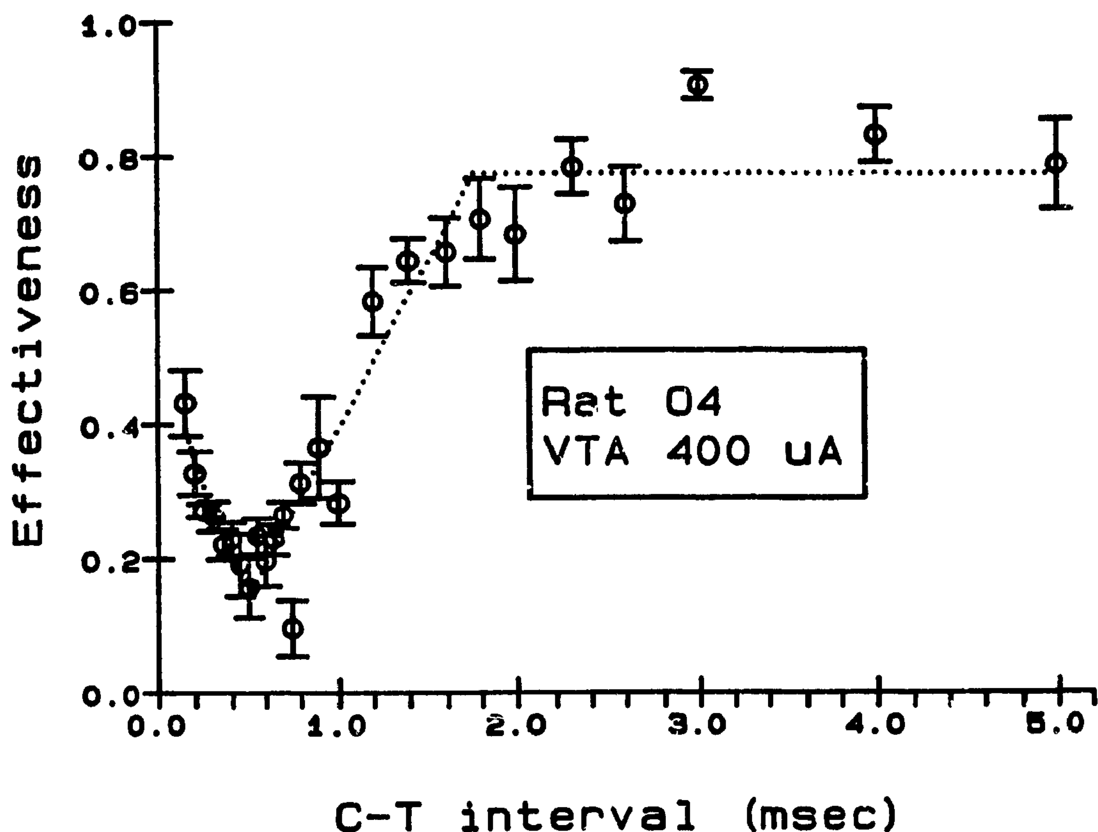


Figure 18. Behaviourally derived refractory period curve plotted with a linear abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error. In addition, the fitted, three segment line used to estimate the C-T intervals at which the recovery curve attained its minimum and maximum asymptotic values has been superimposed on the behavioural data.

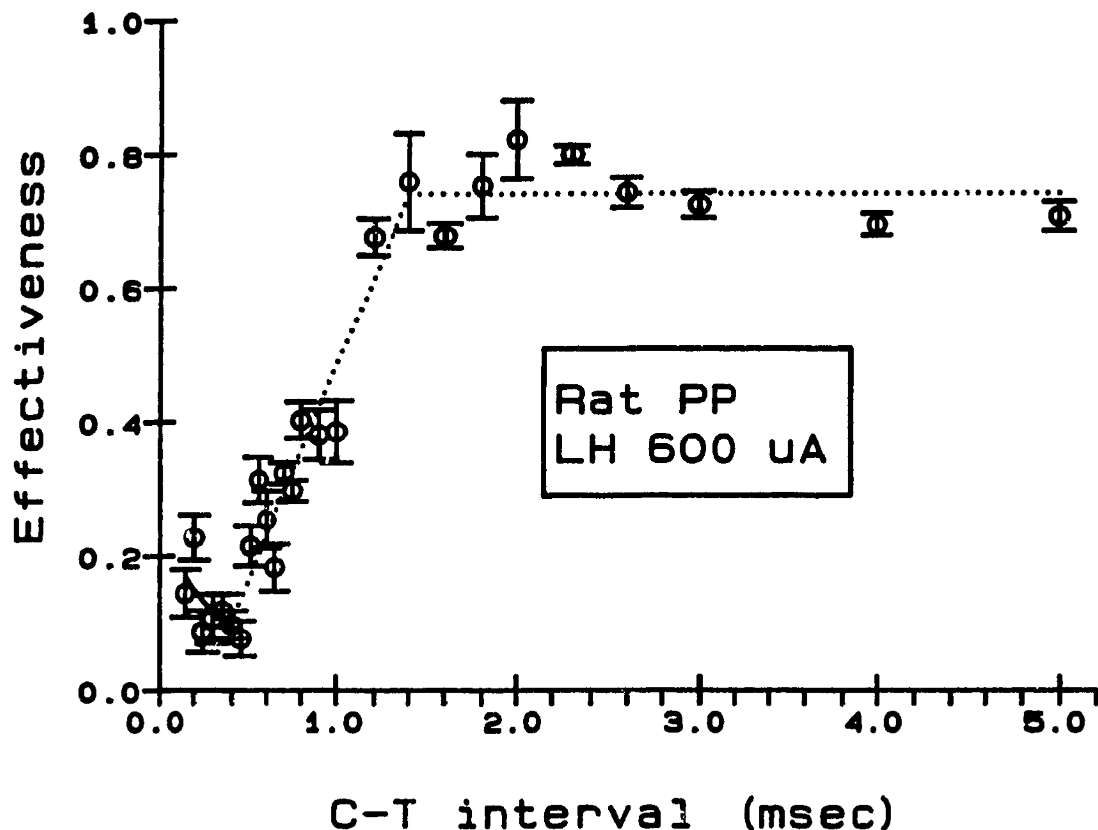


Figure 19. Behaviourally derived refractory period curve plotted with a linear abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error. In addition, the fitted, three segment line used to estimate the C-T intervals at which the recovery curve attained its minimum and maximum asymptotic values has been superimposed on the behavioural data.

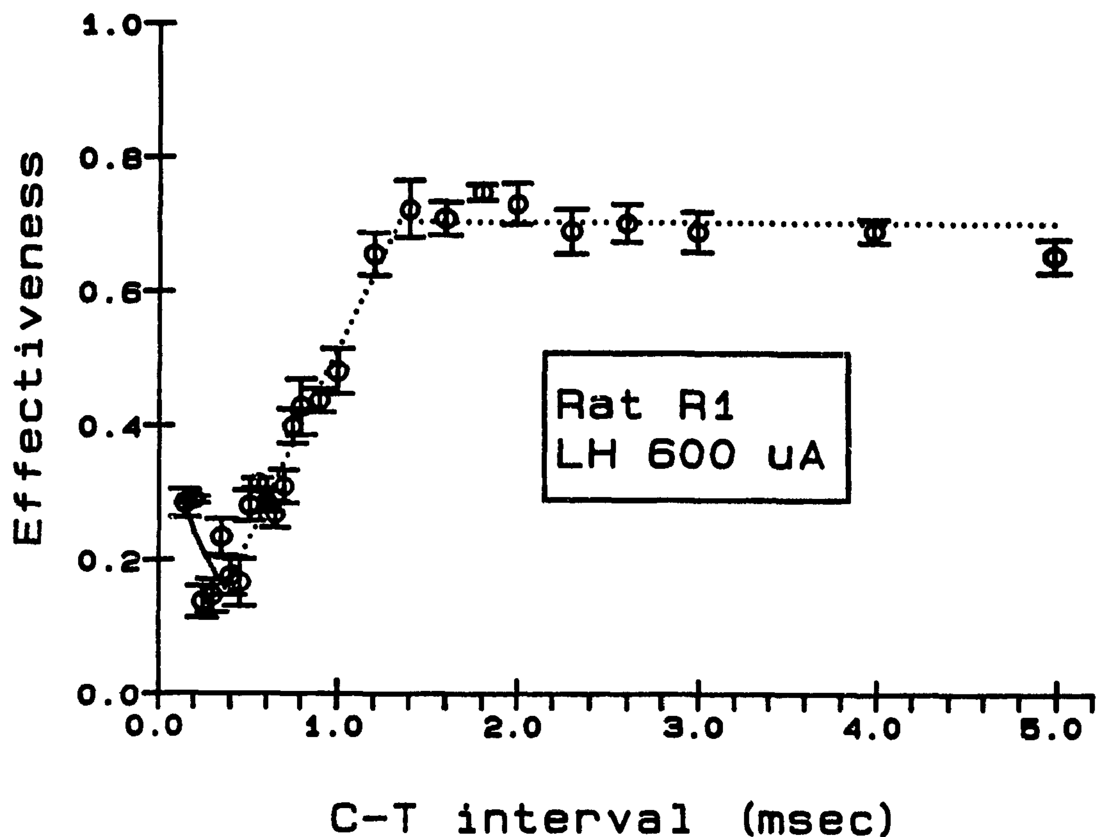


Figure 20. Behaviourally derived refractory period curve plotted with a linear abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error. In addition, the fitted, three segment line used to estimate the C-T intervals at which the recovery curve attained its minimum and maximum asymptotic values has been superimposed on the behavioural data.

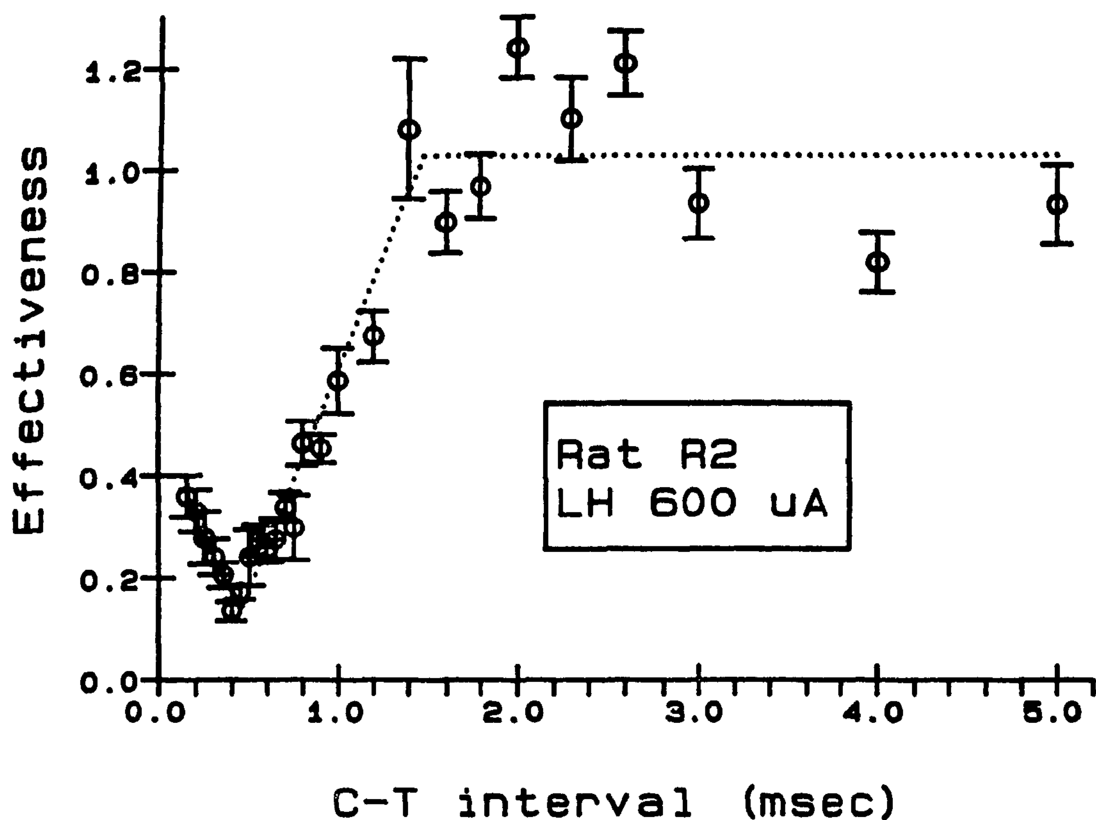


Figure 21. Behaviourally derived refractory period curve plotted with a linear abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error. In addition, the fitted, three segment line used to estimate the C-T intervals at which the recovery curve attained its minimum and maximum asymptotic values has been superimposed on the behavioural data.

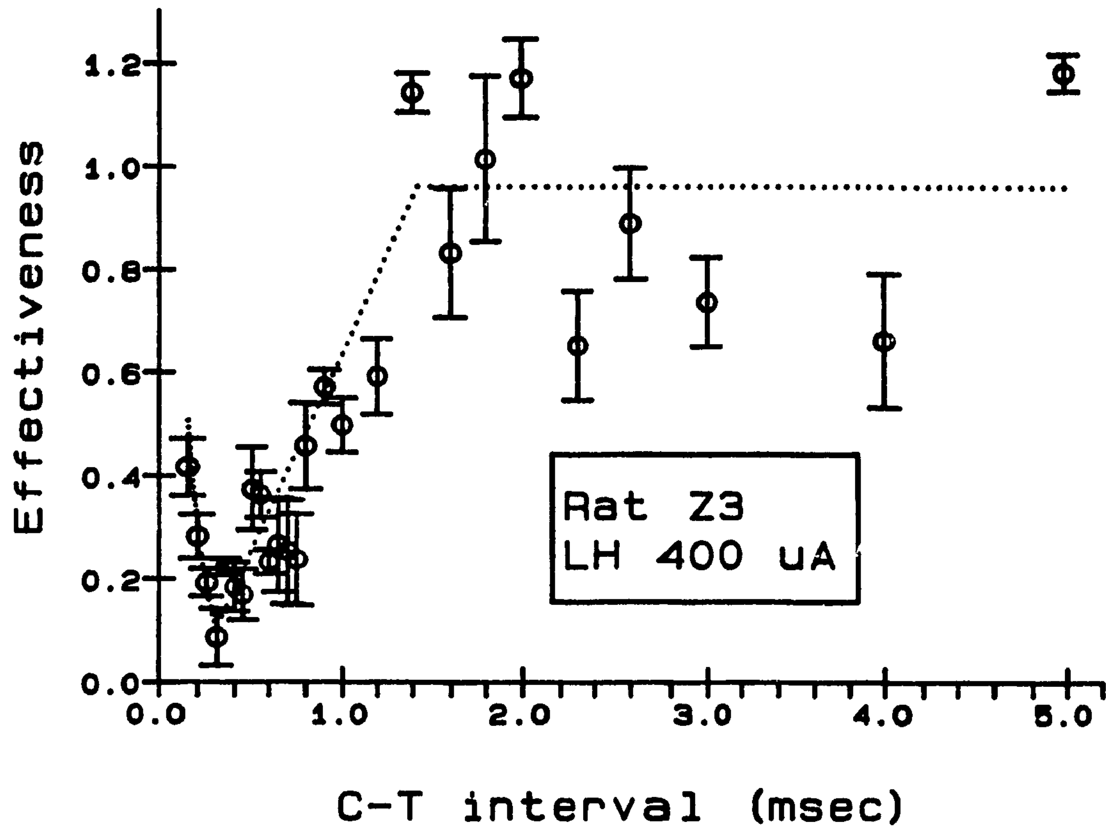


Figure 22. Behaviourally derived refractory period curve plotted with a linear abscissa. The mean effectiveness (E) value at each C-T interval tested is indicated by the open circles. The error bars about each mean E value denote a confidence interval of plus or minus one standard error. In addition, the fitted, three segment line used to estimate the C-T intervals at which the recovery curve attained its minimum and maximum asymptotic values has been superimposed on the behavioural data.

Table 1 shows the estimates of the first point of recovery, the C-T interval corresponding to the minimum E value, and the last point of recovery, the C-T interval corresponding to the asymptotic E value, derived by the curve-fitting procedure. The median value for the first point of recovery is 0.35 msec. (range 0.25 - 0.45 msec) while the median last point of recovery is 1.4 msec. (range 1.0 - 2.0 msec).

In the majority of cases, the first point of recovery from refractoriness appears to accurately reflect the data. In some cases, there are small differences between what would be visually perceived as the C-T interval corresponding to the minimum E value versus the calculated value. These differences are probably due to the error associated with the mean E values, and/or to systematic deviations of the population means from the fitted three-segment function.

With respect to the last point of recovery from refractoriness, the calculated value appears to accurately reflect the data in the majority of cases. The one exception is subject A2 for which a value of 1.85 msec. was obtained even though the curve continues to rise up to a C-T interval of 10 msec. Given the relatively large variability at long C-T intervals for this subject and the constraints imposed by the curve fitting procedure, it is not surprising that the mean E values for the five longest C-T intervals were treated as indiscriminable.

Table 1

The first point and the last point of recovery. Estimated C-T intervals corresponding to the first point and the last point of recovery for the refractory period curves obtained from electrodes aimed at the LH and the VTA. All values are in milliseconds.

Lateral hypothalamus

Rat no.	First point of recovery	Last point of recovery
A2	0.45	1.85
A3	0.31	1.39
PP	0.39	1.40
R1	0.37	1.34
R2	0.46	1.48
Z3	0.30	1.43

Ventral tegmental area

Rat no.	First point of recovery	Last point of recovery
A2	0.47	1.38
C2	0.44	1.55
O3	0.37	2.00
O4	0.52	1.76

Histology

Figure 23 shows the histologically determined placements of electrode tips aimed at the LH and VTA that supported self-stimulation. Five electrodes aimed at the LH yielded useful behavioural data. All of these electrode tips were located in the LH except in the case of subject R2 whose electrode tip was located on the border between the lateral hypothalamus and the tuber cinereum. The five electrodes aimed at the LH were located between 2.30 to 2.80 mm caudal to bregma (Paxinos & Watson, 1986). Four electrodes aimed at the VTA yielded useful behavioural data. The electrode tip for subject A2 was located on the ventral portion of the medial lemniscus. The electrode tip for subject C2 was located in the VTA while the electrode tip for subject O4 was located on the upper border of the VTA below the rostral interstitial nucleus of the medial longitudinal fasciculus. All of these electrode tips were located between 4.80 to 5.80 mm caudal to bregma (Paxinos & Watson, 1986). No histological result is available for the electrode aimed at the VTA for subject O3.

Relation to earlier studies

Refractory period estimates for reward-related neurons projecting through the LH and the VTA have been obtained in numerous studies (for example, Bielajew et al., 1982; Bielajew & Shizgal, 1982; Macmillan, Simantirakis, & Shizgal, 1985; Yeomans, 1975; 1979). In general, the first point of

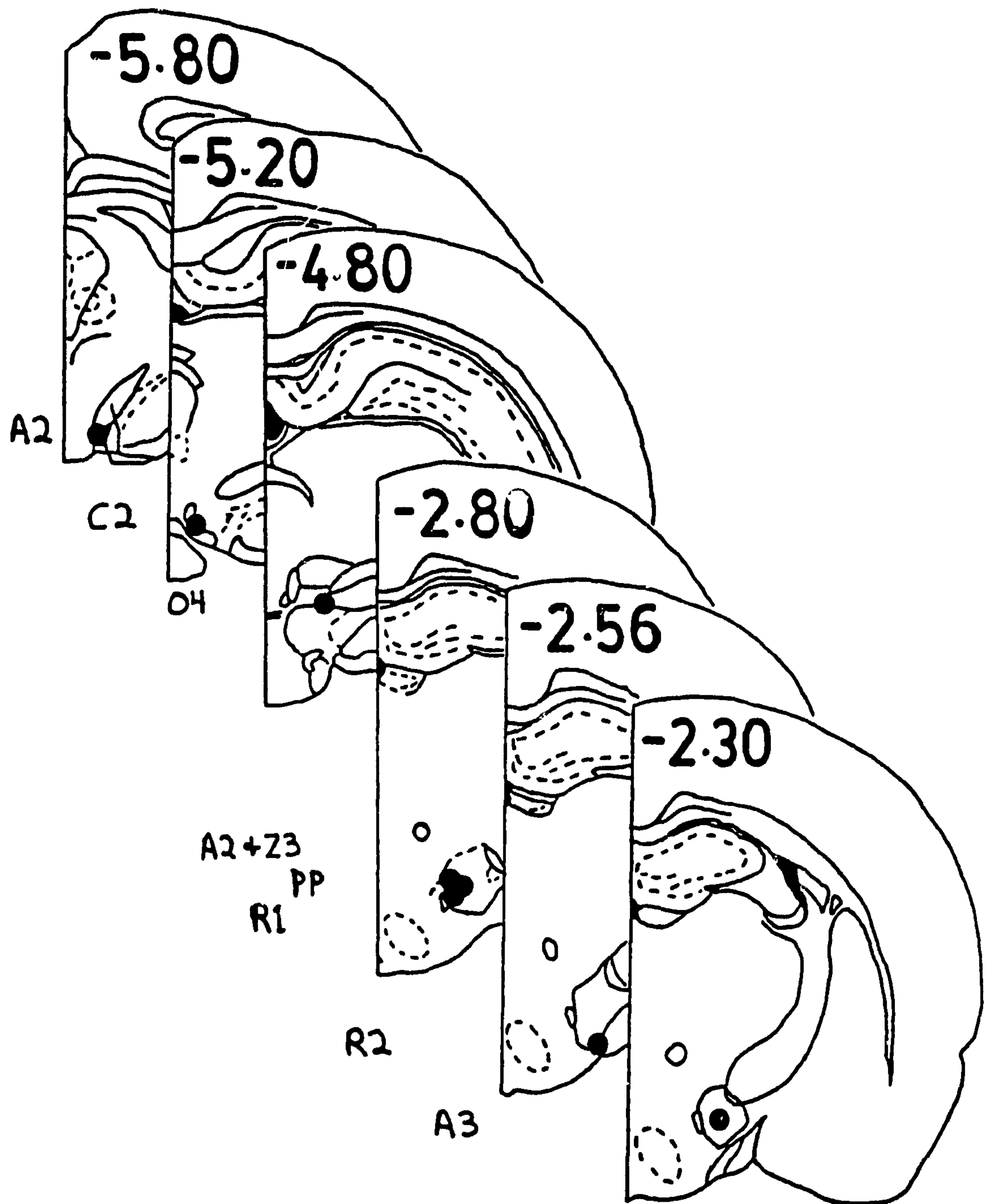


Figure 23. Location of the tips of the stimulation electrodes aimed at the lateral hypothalamus and the ventral tegmental area. Placements for all subjects are shown on tracings from the Paxinos and Watson (1986) stereotaxic atlas. The electrode tip for subject no. 03 could not be located.

recovery tends to occur between 0.4 - 0.6 msec. while the last point of recovery tends to occur between 1.0 - 2.0 msec. However, there are some cases where the curves have continued to rise up to 4.0 msec. and even 10.0 msec. The present data are consistent with these results. Thus, it would appear that the subjects used and the data obtained are typical of behavioural refractory period studies carried out at LH and VTA self-stimulation sites.

Recording phase

In this phase, the activity of single-units was recorded from those subjects tested in the behavioural phase. For each subject, the current, pulse duration, and stimulation electrode employed for the refractory period tests was the same as in the behavioural phase. Refractory period tests were performed when the recorded responses met the following set of criteria: (a) the response was consistent with somatic as opposed to axonal activity, (b) the response was all-or-none, and (c) the response was antidromically-driven rather than synaptically-driven. Each of these criteria will be treated separately below. Subsequently the results of the refractory period tests performed on directly driven, single-units will be presented.

Somatic or axonal action potentials

The relative sizes of the electrical field generated by the somatodendritic region and the axon favours recording of somatic activity. Nonetheless, it is possible to record

axonal action potentials via extracellular electrodes. Thus, some way must be established to determine whether an action potential is of axonal or somatic origin. Two criteria have been used in previous work. First, the action potential duration provides a means to distinguish between somatic and axonal responses (Humphrey, 1979; Lemon, 1984). Action potential durations in the present study were greater than 0.7 msec: such values are more characteristic of cell body responses than of axon responses. Second, the prominence of an initial segment/somatodendritic (ISSD) break has been considered evidence that the recorded activity originates near the soma of the neuron (Nelson & Frank, 1964; Terzuolo & Araki, 1961). This feature is illustrated in Figure 24, in which frequency-dependent changes affect the form of the response for cell 104 of subject R1.

Each trace represents the first few responses to a one-second train of stimulation pulses delivered at the frequency indicated to its left. Note that on all electrophysiological traces the stimulation artifacts have been truncated and designated by filled circles. The first and second traces show, respectively, the responses following 50 Hertz and 100 Hertz stimulation. The third trace shows the first four responses following a 200 Hertz burst of stimulation. Compared with the first two responses of this trace, the third response shows a decrease in amplitude and an increase in duration. This change in form has been attributed to

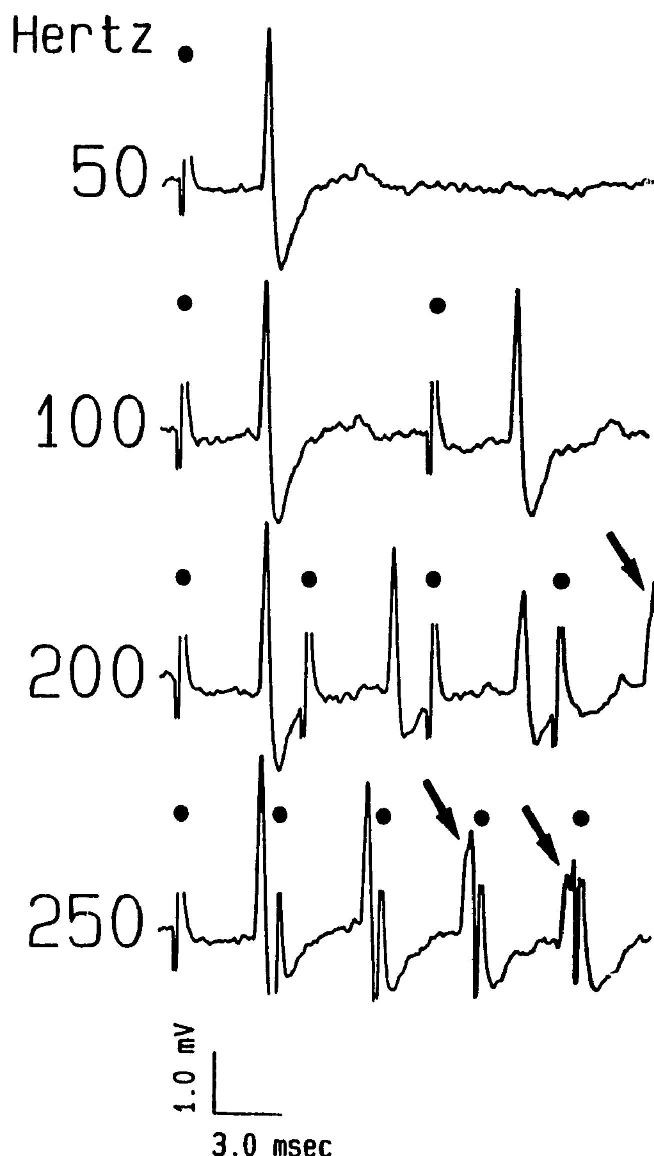


Figure 24. Frequency following. Each of the tracings represent the responses of cell 104 of subject R1 recorded during the first 22 msec. of a one-second train of stimulation. The stimulation artifacts have been truncated and designated by filled circles. As the frequency increases from 50 to 250 Hertz, the ability of the somatodendritic component to follow the stimulation is attenuated, delayed, and broadened. The notch, indicated by the arrow, is believed to result from the delay between the initial segment (IS) and the somatodendritic (SD) components of the response. This notch, the ISSD break, is a characteristic of somatic responses.

differences in the excitability cycles of the initial segment and the somatodendritic portions of the cell. At low frequencies, both portions of the response follow the stimulation well. However as the frequency increases, the somatodendritic component cannot follow faithfully; resulting in its attenuation, delay, and broadening. Thus in the fourth response, a notch, indicated by the arrow, appears on the rising portion of the response. This notch is believed to result from the delay between the initial segment and the somatodendritic components of the response. This differentiation becomes even more apparent in the fourth response to a 250 Hertz burst of stimulation in the fourth trace.

A similar change in form can be seen in Figure 25. In this case, a refractory period estimate was obtained using the standard procedure for cell 104 of subject R1. In the first trace, the T-pulse was set to follow the C-pulse by 2.00 msec. Note the decrease in amplitude, increase in duration, and the notch on the rising portion of the response to the T-pulse. As the C-T interval was reduced from 1.2 msec. to 1.1 msec., the response to the T-pulse became probabilistic. That is at this C-T interval, the T-pulse elicits an action potential on an occasional rather than a continual basis. The probabilistic nature of the response is shown by superimposing two traces, one at which the T-pulse elicited a response and another, obtained at the

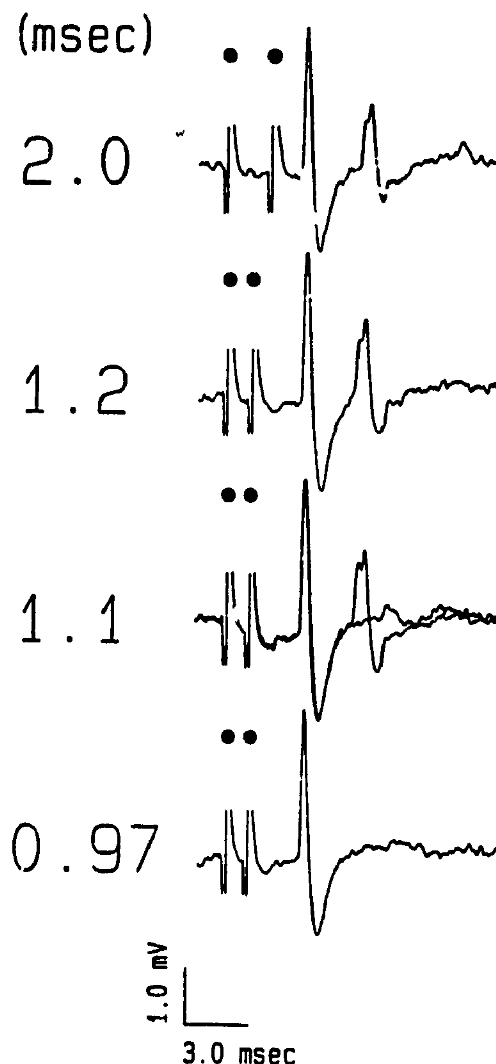


Figure 25. Responses recorded during a standard refractory period test showing ISSD break at short C-T intervals for cell 104 of subject R1. The stimulation artifacts have been truncated and designated by filled circles. The C-T interval in milliseconds is indicated to the left of each tracing. By reducing the C-T interval from 1.2 msec. to 1.1 msec., the response becomes probabilistic. The probabilistic nature is shown by superimposing two tracings, one at which the T-pulse elicited a response and one at which the T-pulse failed to elicit a response. By further reducing the C-T interval from 1.1 msec. to 0.97 msec., the response to the T-pulse was abolished. The response to the T-pulse illustrates the initial segment/somatodendritic response typical of somatic responses.

same C-T interval, at which the T-pulse failed to elicit a response. Finally at intervals less than or equal to 0.97 msec., the T-pulse consistently failed to elicit an action potential.

Usually, the ISSD break could be clearly seen in large responses (of at least 1 millivolt) at either high frequencies or at short C-T intervals. In contrast, the signal-to-noise ratios of many smaller responses was too low to discern an ISSD break. However, given the relatively long duration of the smaller responses, it was likely that they too were of somatic origin. Nonetheless, the possibility cannot be ruled out that some of the smaller responses were axonal action potentials of unusually long duration.

Single-unit versus compound action potentials

The criterion employed to distinguish single-unit activity from compound action potentials was invariance in the amplitude of the response in the face of suprathreshold increases in the stimulation current. Electrophysiological data were obtained only from responses that fulfilled this criterion.

Directly driven or synaptic action potentials

At least one of three criteria was employed to distinguish directly driven from synaptically evoked action potentials (Humphrey, 1979; Lemon, 1984). They were (1) near-constant latency at threshold intensity, (2) the ability to follow high stimulation frequencies, and (3) collision be-

tween spontaneous and evoked action potentials. The collision test is generally considered the most crucial test to determine whether a cell is directly driven. However, the cell must be spontaneously active for the collision test to be employed. When a cell was spontaneously active a collision test was run.

The majority of responses did not occur spontaneously. Thus, the criteria of near-constant latency at threshold intensity and the ability to follow high stimulation frequencies were employed to determine whether the cell was directly driven. Figure 24 shows these two criteria applied to cell 104 of subject R1. While this cell was chosen for illustrative purposes because of the good signal-to-noise ratio, this pattern of results is typical of all directly driven cells. This cell shows very little variation in response latency even over a range from 50 - 250 Hertz and shows no difficulty in following stimulation frequencies up to 250 Hertz (see Figure 24). While the majority of cells recorded were able to follow 100 Hertz, other cells were only able to follow 40 Hertz. If a cell was unable to follow at least a 20 Hertz burst of stimulation, it was considered to be synaptically driven.

Figure 26 shows a collision test performed on cell 106 of subject R2. In the first trace, the stimulation pulse (C-pulse) was set to follow the spontaneously occurring action potential (S) by 2.30 msec. This S-C interval appears

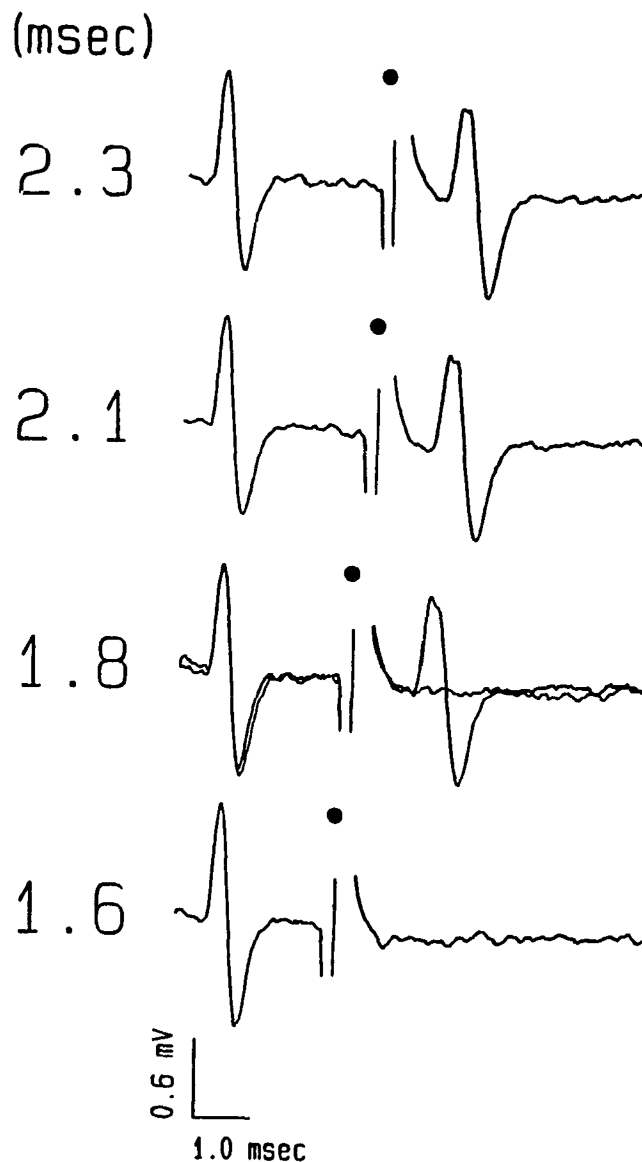


Figure 26. Collision test for cell 106 of subject R2. The stimulation artifacts have been truncated and designated by filled circles. The S-C interval in milliseconds is indicated to the left of each tracing. By reducing the S-C interval from 2.1 msec. to 1.8 msec., the response becomes probabilistic. The probabilistic nature is shown by superimposing two tracings, one at which the C-pulse elicited a response and one at which the C-pulse failed to elicit a response. By further reducing the S-C interval from 1.8 msec. to 1.6 msec., the response to the C-pulse was abolished.

to have allowed sufficient time for the spontaneously occurring action potential to propagate past the stimulation site and for the neuron to recover from refractoriness prior to the application of the stimulation pulse since an antidromic action potential was registered by the recording electrode. As the S-C interval was reduced from 2.10 msec. to 1.80 msec. the response to the stimulation pulse became probabilistic. That is, at this S-C interval, the C-pulse elicits an action potential on an occasional rather than a continual basis. The probabilistic nature of the response is shown by superimposing two traces, one at which the C-pulse elicited a response and another, obtained at the same S-C interval, at which the C-pulse failed to elicit a response. Finally at intervals less than or equal to 1.60 msec., the stimulation-induced action potential consistently failed to reach the recording site presumably due to collision with the spontaneously occurring action potentials.

A successful collision test tends to support the hypothesis that the recorded response was directly driven by the stimulation. However, Barillot et al. (1980) have pointed out that a monosynaptic input can display collision-like effects when the somatic refractory period is greater than the latency. Of 12 cells for which collision tests were performed, eight had somatic refractory periods less than the response latencies. Thus by using the collision test as a criterion, one can be reasonably confident that the recorded

response was directly driven by the stimulation only for eight of the 12 spontaneously active cells.

When the collision test could not be applied, the ability of the cell to follow high stimulation frequencies was employed as a criterion to determine whether the cell was directly activated. However given that the frequency following ability of a cell is an even less reliable indicator than the collision test, it is possible that some responses were incorrectly classified.

Single-unit responses

The activity of 180 single-units was recorded. Of these, 83 single-units showed spontaneous but not stimulation-dependent activity while another 16 single-units were synaptically driven. Thus, 81 responses met the criteria for antidromic, single-unit action potentials. Sixty-eight of these units were driven by electrodes aimed at the LH while nine units were driven by electrodes aimed at the VTA. Four units were driven by both the LH and the VTA electrodes. These results are consistent with the work by Nieuwenhuys et al. (1982) indicating that many MFB fibres arise in forebrain structures. These results also agree with those of Rompré and Shizgal (1986).

Refractory period data

One of two procedures was employed to determine the refractory period of a directly driven response. If the cell was not spontaneously active, only the standard procedure

was run. If the cell was spontaneously active, then both the standard and the Swadlow procedure (Swadlow, 1982) were run. The purpose of running both the standard and Swadlow procedures whenever possible was to investigate the differences these procedures yield when estimating the refractory period. Swadlow (1982) and Rompré and Shizgal (1986) found that the standard procedure tended to over-estimate the refractory period when the site of stimulation is axonal. The results of the refractory period tests performed during the recording phase will focus initially on the comparison of the standard and Swadlow procedure and then on a summary of the refractory period data.

Given the probabilistic nature of the response at some C-T intervals, it was not possible to assign a single C-T value to the refractory period. Instead, it was decided to use a pair of values: (a) an upper estimate indicating the shortest interval at which a response to the T-pulse was elicited on at least nine of ten trials and (b) a lower estimate indicating the longest interval at which a response to the T-pulse was elicited on no more than one of ten trials.

Figures 27 - 28 show a comparison of the two procedures using cell 106 from subject R2. Figure 27 presents a refractory period estimate obtained using the standard procedure. At 1.80 and 2.00 msec., both the C- and T-pulses elicit a response. At 1.60 msec., the response to the T-pulse becomes

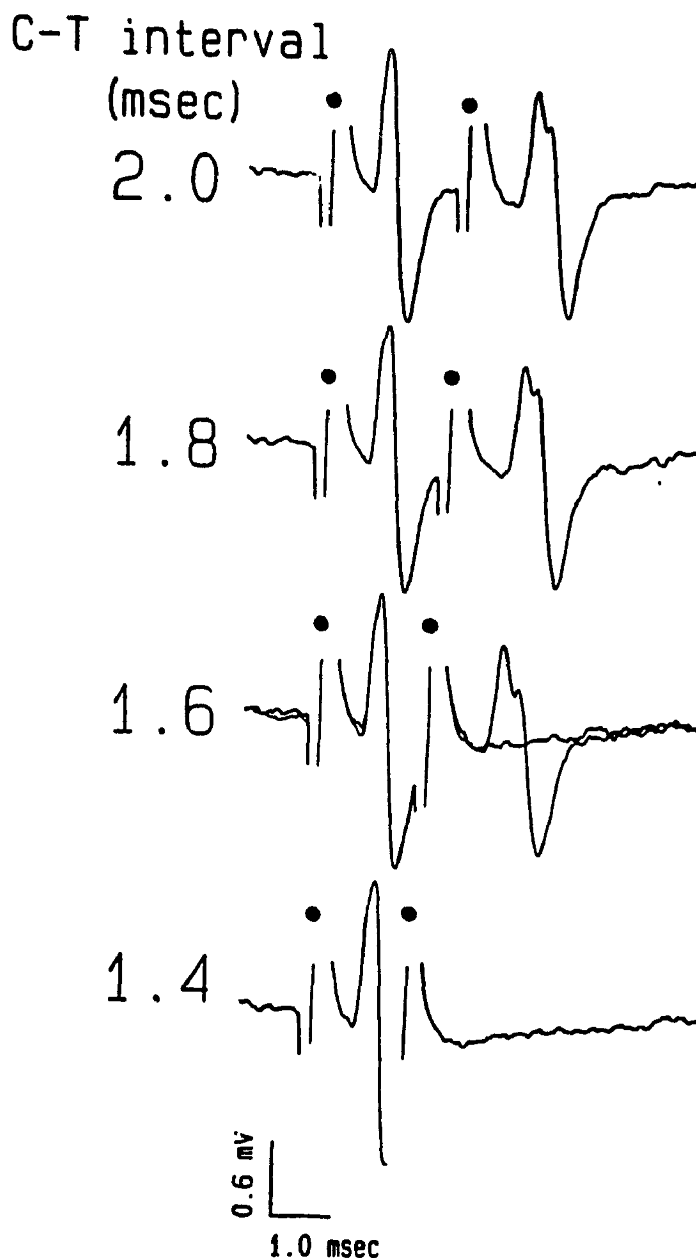


Figure 27. Results of standard refractory period test for cell 106 of subject R2. The stimulation artifacts have been truncated and designated by filled circles. The C-T interval in milliseconds is indicated to the left of each tracing. By reducing the C-T interval from 1.8 msec. to 1.6 msec., the response becomes probabilistic. The probabilistic nature is shown by superimposing two tracings, one at which the T-pulse elicited a response and one at which the T-pulse failed to elicit a response. By further reducing the C-T interval from 1.6 msec. to 1.4 msec., the response to the T-pulse was abolished.

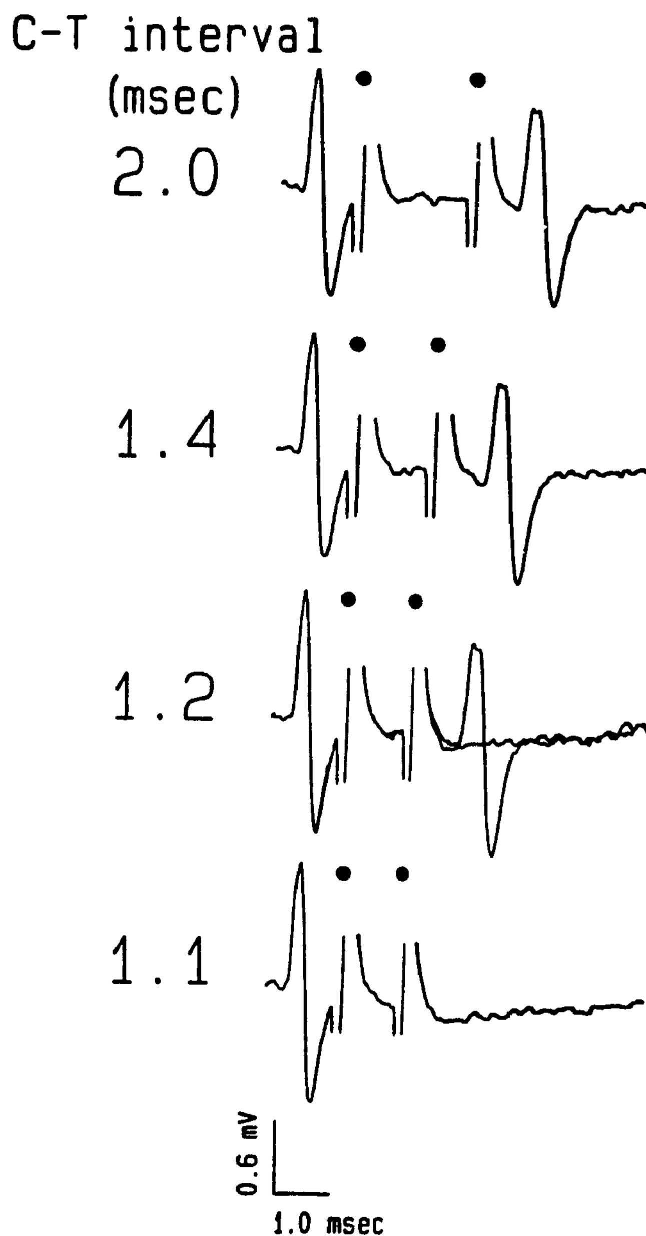
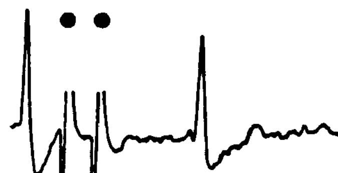
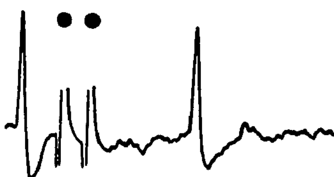


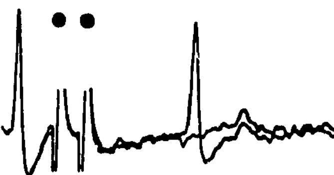
Figure 28. Swadlow test for cell 106 of subject R2. Note that the C-pulse was delivered shortly following a spontaneous spike; the action potential triggered by the C-pulse is cancelled by collision. The stimulation artifacts have been truncated and designated by filled circles. The C-T interval in milliseconds is indicated to the left of each tracing. By reducing the C-T interval from 1.4 msec. to 1.2 msec., the response becomes probabilistic. The probabilistic nature is shown by superimposing two tracings, one at which the T-pulse elicited a response and one at which the T-pulse failed to elicit a response. By further reducing the C-T interval from 1.2 msec. to 1.1 msec., the response to the T-pulse was abolished.

probabilistic. Finally, at a C-T interval of 1.40 msec., the T-pulse is unable to elicit a response. In this case, the upper estimate is 1.80 msec. while the lower estimate is 1.40 msec. Figure 28 shows a refractory period estimate obtained using the Swadlow procedure. In this case, the apparatus is configured so that a spontaneous action potential triggers a C-pulse. Note that there is never any response to the C-pulse. This is due to the collision of the spontaneous orthodromic action potential with the stimulation-induced antidromic action potential. Using this procedure, the T-pulse reliably elicits a response at 1.40 msec. At 1.20 msec. the response becomes probabilistic while at 1.10 msec. there is no response to the T-pulse. In this case, the longest C-T interval at which a response is reliably recorded using the Swadlow procedure is less than or equal to the shortest interval at which no response is reliably recorded using the standard procedure. Thus, there is no overlap between the two estimated refractory period intervals.

Figures 25 and 29 show refractory period estimates obtained using cell 104 from subject R1. The results in Figure 25 were obtained using the standard procedure while those in Figure 29 were obtained with the Swadlow procedure. In this case, the standard procedure yields a refractory period estimate ranging from 0.97 - 1.20 msec. while the Swadlow procedure yields an estimate ranging from 0.98 - 1.02 msec. Thus, these two refractory period estimates overlap.







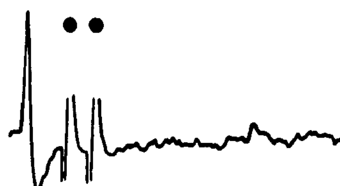


Figure 29. Swadlow test for cell 104 of subject R1. Note that the C-pulse was delivered shortly following a spontaneous spike; the action potential triggered by the C-pulse is cancelled by collision. The stimulation artifacts have been truncated and designated by filled circles. The C-T interval in milliseconds is indicated to the left of each tracing. By reducing the C-T interval from 1.02 msec. to 1.0 msec., the response becomes probabilistic. The probabilistic nature is shown by superimposing two tracings, one at which the T-pulse elicited a response and one at which the T-pulse failed to elicit a response. By further reducing the C-T interval from 1.0 msec. to 0.98 msec., the response to the T-pulse was abolished.

In the present study, ·fractory period estimates were derived using both procedures for 13 cells. These estimates are shown in Table 2. The values in the column labelled 'Difference' indicate the difference between the upper estimates for both procedure. These range from 0.00 to 1.30 msec. with a median of 0.19 msec., values compatible with those obtained by Swadlow (1982) for corticotectal axons of the rabbit and by Rompré and Shizgal (1986) for forebrain nuclei. In principle, refractory period estimates obtained using the Swadlow procedure reflect the excitability characteristics of the axon, the site of stimulation in the behavioural experiments, whereas estimates obtained using the standard procedure may preferentially reflect the excitability characteristics of the soma. To render the standard procedure estimates more comparable to the Swadlow procedure estimates, the lower and upper estimates of the refractory periods based solely on the standard procedure were reduced by 0.20 msec. when comparisons with the behavioural data were carried out (Rompré and Shizgal, 1986).

Of the 81 directly driven single-units, it was not possible to obtain refractory period estimates for 15 single-units because either the single-units ceased to respond to the stimulation during the tests or else the response size diminished to the point where it became impossible to discern the response from the background noise. Thirty refractory period estimates were obtained from 28 cells since es-

Table 2

Comparison of refractory period estimates. Comparison of refractory period estimates using the standard and the Swadlow procedures. For each procedure, the C-T range in milliseconds over which the cell was refractory is indicated. The values in the column marked 'Difference' indicate the differences between the upper limits of each procedure. That is, the C-T interval at which the stimulation elicited a response nine out of ten times. The standard procedure yields a median upper estimate of 0.19 milliseconds greater than the Swadlow procedure.

Subject no.	Cell no.	Stimulation electrode	Estimate-standard procedure	Estimate-Swadlow procedure	Difference
A2	102	LH	3.20 - 6.00	3.00 - 6.00	0.00
	401	LH	1.20 - 1.50	1.00 - 1.30	0.20
A3	212	LH	0.85 - 1.00	0.85 - 0.91	0.09
	404	LH	0.66 - 2.00	0.59 - 0.70	1.30
C2	401	LH	7.00 -10.00	5.00 - 7.00	3.00
O3	102	LH	2.20 - 2.80	2.00 - 2.60	0.20
	111	LH	1.40 - 1.80	1.40 - 1.80	0.00
O4	302	LH	4.30 - 6.00	3.40 - 6.00	0.00
	405	VTA	1.40 - 2.00	0.67 - 0.75	1.25
R1	104	LH	0.97 - 1.20	0.98 - 1.02	0.18
	108	LH	2.00 - 2.10	2.00 - 2.10	0.00
R2	106	LH	1.40 - 1.80	1.10 - 1.40	0.40

timates were obtained at both the LH and the VTA stimulation sites for two cells. The reason for working with only 28 cells rather than the 66 directly driven, single-units will be presented in the section below.

Selecting candidate reward neurons

At this point, the behavioural data from the present experiment and previous experiments (Shizgal et al., 1980; Bielajew & Shizgal, 1986) regarding the anatomical and electrophysiological characteristics may be used to filter the results from the recording phase of the current experiment. Three sets of criteria or filters are applied to determine candidate reward neurons. The first criterion, that the responses were directly driven, single-units has already been discussed. The two additional filters are presented below

The second criterion applied concerns the stimulation site and intensity. It was stated earlier that responses from 180 single-units were recorded. Sixty-six of these fulfilled the criteria for directly driven, single-unit responses and showed an adequate signal-to-noise ratio for a long enough time to allow collection of refractory period estimates. However, since the subjects had previously participated in the behavioural phase of the experiment, data existed concerning the site of stimulation (that is, which of the two implanted electrodes supported self-stimulation) and the stimulation intensity employed. Using this informa-

tion as additional criteria, it was possible to eliminate one group of 14 cells because their thresholds were greater than the current used in the behavioural phase. That is, since the threshold current required to activate these cells was greater than the behavioural current, these cells would not have contributed to the rewarding effect of the stimulation. A second group of 24 cells was eliminated because they were activated by an electrode that did not support self-stimulation. Thirty refractory period estimates were obtained from the remaining 28 cells since estimates were obtained at both the LH and the VTA stimulation sites for two cells.

The third criterion applied concerns the behaviourally derived, refractory period estimates for each subject. Single-units with a refractory period overlapping with the behavioural values are considered candidate reward neurons. For instance, Figure 30 shows the refractory period curve for subject R1 reproduced without the error bars. In addition, a vertical line cuts the curve at the C-T interval at which the asymptote of the recovery curve was approached (approximately 1.20 msec). To be considered as a candidate, the lower estimate of the refractory period obtained during the recording session for a given cell had to be lower than this C-T interval. That is, such cells might have contributed to the increase of the T-pulse effectiveness in the corresponding behaviourally derived curves. When the lower estimates were greater than this C-T interval, then the

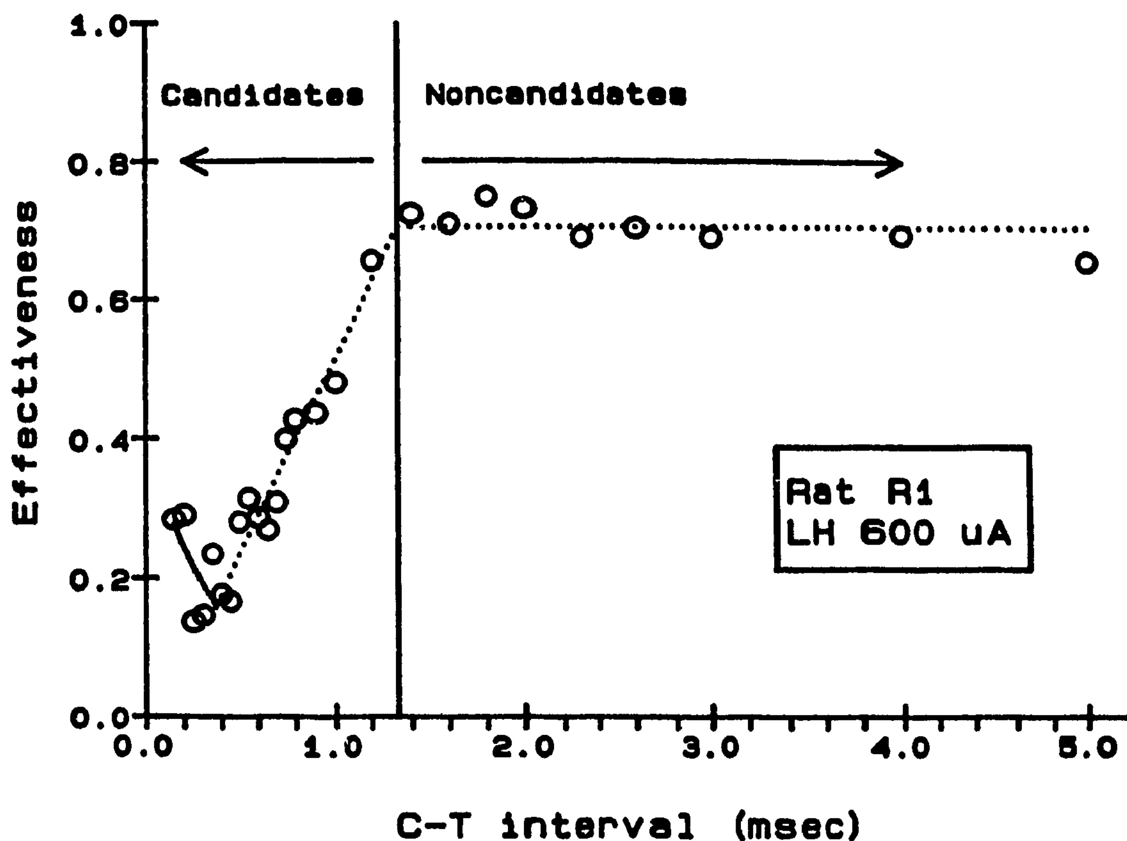
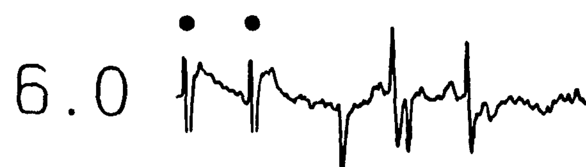
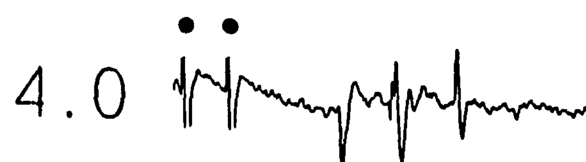


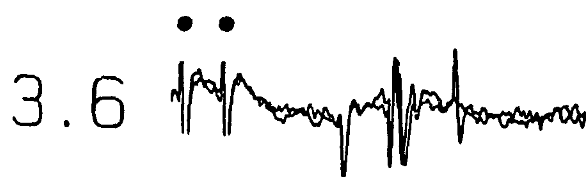
Figure 30. Schematic representation of behavioural data of subject R1. The fitted three-segment line used to estimate the C-T intervals at which the recovery curve attained its minimum and maximum asymptotic values has been superimposed on the behaviourally derived data. A vertical line cuts the recovery curve at the maximum asymptotic value. To be considered a candidate, the lower estimate of the refractory period of a single-unit must be lower than this C-T interval.

cells were considered to be unlikely candidates. Figure 31 shows the refractory period estimate for cell 103 for this subject. At both 4.00 and 6.00 msec., a response is obtained for each T-pulse. At 3.60 msec., the response to the T-pulse has become probabilistic while at 3.20 msec. the T-pulse fails to elicit a response. Given the difference between the minimum C-T interval at which a response may be obtained during the recording session and the C-T interval at which the behavioural curve asymptotes, it is unlikely that this neuron contributed to the rewarding effect of the stimulation. Figure 29 shows the recorded response for another cell from the same subject (cell 104), obtained using the Swadlow procedure. In this case, there is no response to the T-pulse at a C-T interval of 0.97 msec. but there is always a response at 1.02 msec. This range is well within the range over which the behaviourally derived curve rises. As such, this cell is classified as a candidate reward neuron.

In principle, a C-T interval might exist at which the T-pulse effectiveness is zero. In such a case, the upper estimate of the refractory period obtained during the recording session for a given cell ought to be greater than this C-T interval for the cell to be considered a candidate. However since most behavioural curves displayed increases in T-pulse effectiveness at very short C-T intervals, it was not possible to accurately estimate a lower limit for the recovery curve (see Figures 13 - 22). Thus, for the purposes







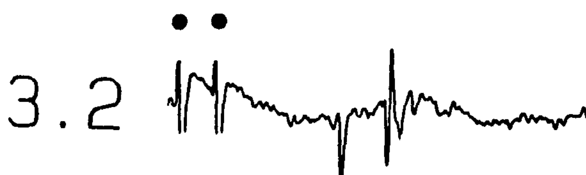


Figure 31. Results of the standard refractory period for cell 103 of subject R1. The stimulation artifacts have been truncated and designated by filled circles. The C-T interval in milliseconds is indicated to the left of each tracing. By reducing the C-T interval from 4.0 msec. to 3.6 msec., the response becomes probabilistic. The probabilistic nature is shown by superimposing two tracings, one at which the T-pulse elicited a response and one at which the T-pulse failed to elicit a response. By further reducing the C-T interval from 3.6 msec. to 3.2 msec., the response to the T-pulse was abolished.

of this study only the upper limit of the recovery curve was used as a criterion.

By using the behavioural data as a filter to discriminate between those cells that are more likely to have played a role in behaviour versus those that probably have not, the number of candidate reward neurons can be reduced from 28 to 12.

Histology

Figure 32 shows the location of the recording sites of all cells for which refractory period data were obtained. The filled circles and filled barbells represent the locations of single-units that are considered candidate neurons. That is, these cells were directly driven, single-units activated by an electrode that supported self-stimulation, had a threshold current less than or equal to the behavioural current, and had a refractory period that overlapped with the behavioural range. The open circles and open barbells represent the noncandidate neurons. That is, these cells were directly driven, single-units activated by an electrode that supported self-stimulation, had a threshold current less than the behavioural current, and had a refractory period that was outside the behavioural range. In some cases, it could not be determined whether the shorter or longer microelectrode recorded the response. Thus, the locations of both recording electrodes are indicated by the barbell symbols. All sites were estimated to be in a region

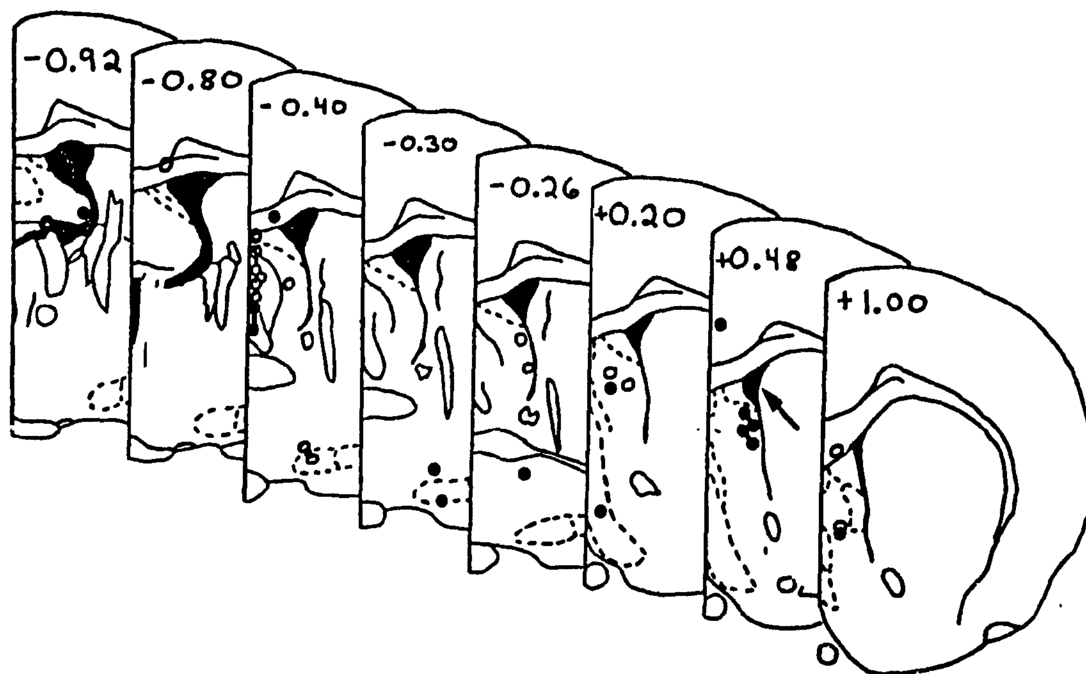


Figure 32. Estimated location of recording sites. Placements are shown on tracings from the Paxinos and Watson (1986) stereotaxic atlas. The filled circles and filled barbells represent the estimated locations of single-units that are considered candidate reward neurons. The unfilled circles and unfilled barbells represent the estimated locations of the noncandidate reward neurons. In some cases, it was impossible to determine whether the recorded response was being monitored by the shorter or longer microelectrode. The barbell symbols indicate the locations of both electrodes. The arrow indicates the estimated location of two noncandidate single-units.

between +1.00 mm rostral and -0.92 mm caudal to bregma (Paxinos & Watson, 1986). The majority of the single-units (20-21 out of 28), candidate and noncandidate reward neurons, were in the region classified by Swanson and Cowan (1979) as the septal complex (lateral and medial septal nuclei, diagonal band complex, and the bed nucleus of the stria terminalis). 84% of the noncandidate neurons were found in this region, while 58% of the candidate neurons were localized to this region.

When interpreting the results in Figure 32, the error prone nature of the procedure for localizing the cells should be kept in mind. To estimate the location of a cell, the blue spot indicating the location of the tip of the lesioning electrode had to be determined. If a blue spot for a given penetration could not be located, the calculations were based on the position of the blue spot from either a previous or a subsequent penetration. Since the configuration of the tips of the recording electrodes with respect to the lesioning electrode was determined prior to the recording session, any change in this configuration from one penetration to another would increase the error associated with determining cell location on some subsequent penetrations.

Overview

These results agree with the summary of the neuranatomical research summarized by Nieuwenhuys et al. (1982) which noted different groups of cell bodies in the forebrain that

send projections through the LH to the VTA. These results also overlap with the findings of Rompré and Shizgal (1986) who recorded the activity of candidate reward neurons in the SE, DBB, APL, ACB, and the insula Calleja magna complex. However contrary to the results of Rompré and Shizgal (1986), a greater percentage of the noncandidate neurons were found in the septal complex in the present study.

General discussion

Developed from the four-stage paradigm proposed by Gallistel et al. (1981), the present experiment sought to locate the somata of neurons likely to subserve the rewarding effects of MFB stimulation. To recapitulate, the following are the stages of the approach: (1) trade-off functions obtained by manipulating various stimulation parameters are derived to place quantitative constraints on the choice of a candidate substrate; (2) the trade-off functions are interpreted in physiological and anatomical terms; (3) electrophysiological recordings are performed of cells activated by the same stimulation electrodes and parameters employed in stage one; (4) the behavioural results serve as criteria for deciding which of the neurons studied in stage three are to be considered candidate reward neurons. Although previous studies have employed one or more of these stages, the present experiment is the first to embody the complete paradigm in which behavioural and recording data are acquired from each subject. The partial implementation of this approach has meant that the behavioural filter used in step four has not been as selective as it might have been. A weak behavioural filter tends to increase (a) the number of likely candidate reward neurons that are overlooked and (b) the number of unlikely candidate reward neurons that are chosen. For the most part, the weakness of the behavioural filter

results from the fact that the data from the behavioural experiments and the recording sessions were derived using two separate groups of subjects. As illustrated below, this may lead to problems of interpretation when attempting to classify candidate reward neurons.

First, consider the situation where it has not been determined, prior to the recording session, whether the stimulation sites support self-stimulation. It is true that the stimulation sites may be localized through histological analysis and, on the basis of previous findings, this knowledge can be used to estimate the likelihood that self-stimulation would have been supported. However, histological localization of electrode tips is error prone and does not indicate the exact path of current flow. Thus, it is possible that some of the placements might not have supported self-stimulation. If the stimulation site had not been behaviourally screened, then a cell might be considered a likely candidate if it exhibited anatomical and electrophysiological characteristics similar to those derived from the results of previous behavioural experiments. However, if the same stimulation site had been screened behaviourally and was found not to support self-stimulation, then the neuron in question would not be regarded as a candidate. While the absence of self-stimulation cannot be interpreted to indicate an absence of reward-relevant fibres, it could be argued that the

likelihood of finding reward-relevant fibres in such a case would be decreased.

A second point concerns the variance in the behaviourally derived refractory period curves that arises due to differences in electrode placement, stimulation parameters, and subjects. At least two situations could arise that would increase the probability of incorrectly labelling a neuron as either a likely or an unlikely candidate. First, consider the case in the present experiment, where the VTA refractory period curves asymptote at different C-T intervals for subjects A2 and O3 (Figures 14 and 17). For subject A2, any cell for which the lower estimate of the refractory period was 1.39 msec or more would be considered as an unlikely candidate since the behavioural curve has reached a maximum at that C-T interval. The same could not be said for subject O3 since the behavioural curve continues to rise even up to a C-T interval of 2.00 msec. Therefore, if behavioural data such as those obtained for subject A2 were applied in analyzing the electrophysiological results obtained from subject O3, some neurons would be incorrectly rejected as unlikely candidates. Second, consider the case in the experiment of Rompré and Shizgal (1986), where a C-T interval of 1.20 msec was used as a criterion to differentiate likely and unlikely candidates. Here again, some neurons for subject O3 would be rejected as unlikely candidates even though the behavioural curve indicates that some reward neurons are still recover-

ing from refractoriness between 1.20 and 2.00 msec and, hence, contributing to the effectiveness of the T-pulse. Thus, it is only when the behavioural and recording data are obtained from the same subject that the power of the behavioural filter is maximized.

By applying the four-stage paradigm to each subject, the present study sought to overcome some of the difficulties noted above and thereby increase the 'resolution' of the behavioural filter. To accomplish this, single-unit refractory period estimates were compared to the behaviourally derived refractory period curves for the same subjects. In addition, the same stimulation electrodes and parameters were used in both phases. By using the stimulation fields in both phases, one ensures that the same neurons are activated.

A third point concerns the comparability of the data derived from the behavioural experiments and from the recording sessions. As noted above, it follows from Swadlow's work that electrophysiological estimates derived using these two procedures may differ. For instance, consider a hypothetical VTA-activated cell monitored during the recording session for subject A3 for which the lower estimate of the refractory period was 1.40 msec. At 1.39 msec, the recovery of the behavioural curve is complete (Figure 15). If the refractory period for the cell had been obtained using the Swadlow procedure, this cell would be considered as an un-

likely candidate. However, if the refractory period had been determined using the standard procedure, the same would not be true since estimates derived in this manner tend to overestimate the true refractory period. Therefore, if comparisons are made without considering the conditions under which the estimates were derived, then some cells will be incorrectly rejected as unlikely candidates.

Whenever possible in the recording sessions, refractory period estimates were derived using the procedure described by Swadlow (1982) so that they would be as comparable as possible to estimates derived during the behavioural phase (Rompré & Shizgal, 1986). However, this procedure could be carried out in only 12 of 28 cases. In future experiments, the use of a multi-barreled glass micropipette might compensate for the lack of spontaneous activity. In such a situation, one of the barrels would contain an excitatory transmitter substance such as glutamate that could activate the cell. By activating the cell with the excitatory transmitter, a collision test could be performed to determine whether the cell was directly driven and the refractory period could be determined using the Swadlow procedure.

Although a majority of the directly driven responses were recorded in the septal complex, a response was almost as likely to be classified as a noncandidate as it was to be classified a candidate. One might ask what is the significance of the failure to find a particular forebrain struc-

ture that exhibits concentrations of likely candidate reward relevant neurons. Certainly, the results of the current experiment are frustrating for the researcher who was hoping that the reward system(s) would be clean and neat. However, there is no a priori reason to believe that a single forebrain nucleus would be involved. On the contrary, there are survival advantages for an organism possessing some redundancy in a system subserving appetitively motivated behaviours. Rather, the present results can be considered encouraging in many respects. Rompré and Shizgal (1986) found 76 responses that met the criteria of antidromic, single-units. By using only the refractory period as a behavioural constraint, 34% of these responses were considered candidate reward neurons. In the present experiment, the behavioural filter was improved by increasing the constraints imposed on the candidate fibres. By increasing the constraints, only 18% of the directly driven, single-units could be considered as candidate reward neurons.

The ability to reduce the number of candidate reward neurons can be attributed to the increase in the number of criteria imposed. In the present experiment, stimulation sites were behaviourally screened and the same current employed in the behavioural and recording phases. Thus, the proportion of neurons satisfying the constraints can be reduced by increasing the number of criteria. More stringent criteria might further restrict the number of candidate

nuclei. The possibility of increasing the number of criteria has been discussed by Rompré and Shizgal (1986). They suggest that collision data impose additional constraints on candidate neurons over and above those already imposed when the same subjects are used within both the behavioural and recording phases and when an electrophysiological characteristics such as the refractory period data is used to filter candidate reward neurons. If the number of candidate forebrain nuclei were reduced, then these nuclei could be mapped more thoroughly.

The four-stage paradigm proposed by Gallistel et al. (1981) constrains the choice of candidate reward-relevant neurons from among the neurons activated by the stimulation electrodes but it cannot prove that a given neuron was definitely involved in the rewarding effect. Given the structural and functional diversity of neurons within the MFB, it is possible that there is a subset of neurons that share similar anatomical and electrophysiological properties and yet subserve different functions. Thus, it may eventually be shown that some of the neurons that are currently being labelled as candidate reward neurons actually subserve totally unrelated functions.

Once candidate nuclei have been located, these nuclei could then be lesioned in subsequent behavioural experiments to determine their role in reward. In addition, candidate neurons could be stained intracellularly, during the record-

ing experiments, with the goal of tracing their projections. When the synapses of the reward neurons have been located pharmacological interventions aimed at determining the neurotransmitter(s) involved could be performed. If the results of these experiments converge on a subset of MFB fibres, then it is likely that these fibres play a role in the rewarding effect of the stimulation. When the trajectories and destinations of these fibres have been fully mapped, the first step in establishing a circuit diagram of the reward system(s) will have been completed.

Update

Since the experimental work for the thesis was completed, a number of studies have investigated the role of forebrain neurons in MFB reward Shizgal, Schindler, and Rompré (in press) have increased the number of subjects tested using the combined behavioural and recording paradigm from nine to 23. Given the larger sample, a clearer picture of the distribution of candidate and noncandidate forebrain reward neurons has emerged. We found that 82-87% of noncandidate cells were recorded in the septal complex while only 51% of candidate cells were found in this region. These findings are comparable to the results obtained in the present study. However, it was found that 23% of the candidate cells were lateral, ventral or caudal to the septal complex while only 0-3% of noncandidate cells were localized to this

region. These findings are compatible with the notion that some forebrain nuclei give rise to descending fibres having electrophysiological characteristics similar to MFB reward neurons and that there is some anatomical segregation of candidate and noncandidate neurons.

As noted above, lesioning of candidate nuclei can help evaluate their role in reward. Several lesion studies have in fact been performed using different techniques such as neurotoxin, knife cuts, and electrolytic lesions. Ibotenic and kainic acid, neurotoxins, are hypothesized to selectively destroy cell bodies within a region but to spare fibres of passage. Some researchers (e.g., Lestang, Cardo, Roy, & Velley, 1985; Nassif, Cardo, Libersat, & Velley, 1985; Velley, Chaminade, Roy, Kempf, & Cardo, 1983; Sprick, Munoz, & Huston, 1985) have employed these neurotoxins to investigate the role of neurons intrinsic to the MFB in self-stimulation at LH sites. Although some of the ibotenic acid studies have been interpreted to support the hypothesis that neurons intrinsic to the MFB are sufficient to elicit self-stimulation, these results need to be viewed with caution. First, the studies use rate measures to determine the effects of the lesions. However, a rate measure cannot discriminate between changes in the subject's ability to perform the behaviour and changes in the rewarding effect of the stimulation. Second, Waraczynski and Stellar (1987) found that ibotenic acid not only destroys cell bodies but

also produces demyelination. Further, Stellar, Waraczynski, and Hall (1988) have shown the ability to detect changes in performance depends on whether the demyelination zone reaches the electrode tip.

Janas and Stellar (1984) found that knife cuts anterior to the MFB from the anterior commissure to the base of the brain had little effect on reward while knife cuts through the lateral preoptic area (LPO) had some effect. Waraczynski (1988) tested the effects of basal forebrain knife cuts on MFB reward. She found that knife cuts of the outputs of the septal complex had little effect on MFB stimulation. Knife cuts in the LPO decreased the rewarding effects of stimulation if there was considerable rostrocaudal tissue damage. Waraczynski (1988) noted that LPO knife cuts by Janas and Stellar (1984) that attenuated the rewarding effects of MFB stimulation were also associated with rostrocaudal damage. Munoz, Keller, and Huston (1985) lesioned the LPO and found that LH self-stimulation behaviour was attenuated but recovered to prelesion levels in 1 to 2 weeks. Murray and Shizgal (1988) looked at the effects of anterior lesions on LH and VTA collision. Ineffective lesions damaged fibers in compartment 'c' of the anterior MFB as defined by Nieuwenhuys et al., (1982). Lesions that affected collision tended to destroy fibers in the more lateral compartments 'a', 'd', and 'e'. These findings suggest that some forebrain fibers can play a role in MFB reward. Additional sup-

port for a role for LPO fibres has come from the work of Bielajew, Thrasher, and Fouriezos (1987). These authors have obtained collision between the LPO and LH. The range over which recovery from refractoriness occurred for fibers in the two regions overlapped.

Of particular interest for mapping MFB reward fibres is the work by Glimcher and co-workers. Gallistel, Glimcher, and Miselis (1989) used a retrogradely transported tracer to ascertain which regions would be labelled by both the medial LH and the dorsal anterior VTA. Among the structures labelled by injection in both sites was the dorsomedial hypothalamic area (DMHA). They found that lesions of the DMHA led to permanent attenuations of VTA stimulation. Subsequently, Miselis and Glimcher (1989) have identified the afferents and efferents of the DMHA by using horseradish peroxidase. Afferents arise in the medial prefrontal cortex, lateral septum, bed nucleus of stria terminalis, medial preoptic area, hypothalamic paraventricular nucleus, amygdala, subiculum, parabrachial nuclei, central gray, and periventricular thalamus.

Taken together these findings seem to suggest that even though the septal complex gives rise to descending fibres having electrophysiological characteristics similar to MFB reward neurons, these fibres do not appear to contribute to the rewarding effects of MFB stimulation. However, both the preoptic area and the DMHA deserve further investigation. At

this point, one might inquire as to the next step in the mapping of MFB self-stimulation. I would suggest using the behavioural and recording paradigm to identify candidate reward neurons in both the preoptic and the dorsomedial hypothalamic areas. Candidate neurons could then be injected with dye to more readily trace candidate pathways. This could then be followed by more lesion studies and pharmacological interventions aimed at determining the neurotransmitter(s) involved. Thus, to some extent the cycle would start over but with more "attractive" candidates.

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