Chapter 1

Background, definitions and the VTM

1.1 Introduction

The question of why an animal behaves as it does is intimately linked to the concept of reinforcement. For example, the apparent purpose and direction, or 'sustained, functional cohesiveness' of behaviour (Gallistel, 1980, p. 406) may be explained by the identification of reinforcers which have strengthened behaviour appropriate for a particular environment or appropriate for a particular state of the animal (Baer, 1982; Davis, 1980; Staddon, 1980; Valenstein, 1966). As another example, motivated behaviour has been described as having the property that it leads to reinforcement (Stellar & Stellar, 1985).

The concept of reinforcement, as usually expressed, makes no commitment to the nature of underlying process(es) by which the reinforcer might influence behaviour. Yet a reinforcer must act on the nervous system and behaviour must result from the activity of that nervous system. This indicates neurobiological involvement in the linkage between reinforcement and the direction, intensity and coherence of behaviour. Because direct electrical stimulation of the central nervous system can have powerful reinforcing effects, the opportunity exists for an explanation of reinforcement and behaviour in terms of a mediating neurobiological process. Processes that underlie electrically induced behaviour might also provide the basis for an account of the organisation and control of natural behaviour.

There are several approaches to the study of electrically reinforced behaviour:

each has advantages and disadvantages (Liebman, 1983). Behavioural measures available from a consideration of the self-regulation of the duration of stimulation have been argued to represent valid measures of the reinforcing effect of electrical brain stimulation (e.g., Liebman, 1983; Popov, Parsons, & Levitt, 1983), but some assumptions underlying the use of these measures have not been adequately examined. One aim of the present research program has been to examine the assumption that the self-selected duration of intracranial self-stimulation (ICSS) and the self-selected duration of no stimulation are independent. Differing explanations for ICSS 'shuttling' behaviour have been proposed (Atrens, Sinden, & Hunt, 1983; Deutsch & Hawkins, 1972; Liebman, 1983; Mendelson, 1969; Stein, 1962) and a further aim of the present program is to assess their relative potential to form the basis for a model of that behaviour. The development of an analytical and theoretical model able to explain the basic properties of the behaviour produced by ICSS could provide a foundation for a more general understanding of neurobiological bases for reinforcement and behaviour (see, for example, Ludlow, 1976; Sibly, 1980).

1.2 Background and definitions

Olds and Milner (1954) made the initial discovery that rats with chronically implanted electrodes would work to obtain electrical stimulation of their own brains. Subsequent research has shown that the phenomenon remains the same despite variation in the species of animal, electrode material, site of stimulation, wave shape of the stimulus, or the type of work required. Given the opportunity to apply an electrical stimulus to their own brains, many animals will perform almost any response within their behavioural repertoire in order to obtain that stimulation. The animal works to obtain a stimulus from its environment which has the characteristics of being precisely defined and of being delivered directly into the central nervous system (CNS).

The phenomenon has been termed 'electrical stimulation of the brain', 'brain-stimulation reward', 'self-stimulation', and 'intracranial self-stimulation'. The latter term has gained greatest acceptance and will be used here. 'Electrical stimulation of the brain' does not describe the particular reinforcing property of this type of stimulation, 'brain-stimulation reward' does not capture its self-administering property, and 'self-stimulation' alone does not emphasise the CNS. An animal that responds to ICSS stimulation is referred to as a 'self-stimulator' (SS).

The term ICSS does not distinguish between electrical self-stimulation of the

brain and various chemical stimulants which may also be self-administered directly into the brain (e.g., Olds & Williams, 1980: Wise, 1978). However, convention has been to use ICSS to imply electrical ICSS and to place the onus on those researchers using chemical ICSS to make the distinction. The term ICSS may also be used in a narrower sense to refer to the stimulation itself. That is, the electrical stimulation that produces the behaviour described above as characteristic of ICSS, may also be referred to as ICSS.

Following the approach of Liebman (1985) and Stellar and Stellar (1985), the terms 'reward' and 'reinforcement' are used interchangeably throughout this thesis, although preference is for 'reward' (Wise, 1985). 'Reward' may be interpreted as referring to an hedonic-affective quality of the stimulation (Atrens, 1984; De Witte, 1982), however, this is not intended here. Both terms (and 'reward value' and 'reward effect') are used to imply that there is some characteristic of this type of stimulation for which animals will work.

1.2.1 Response measures

Brief contingent ICSS stimulation can be used to reinforce almost any response within an animal's behavioural repertoire. For example, responses such as lever pressing (Olds & Milner, 1954), runway activity (Gallistel, 1969), shuttling (Atrens, 1970), immobility (Paxinos & Bindra, 1970; Routtenberg, 1976), handedness (Hernandez-Mesa & Bureš, 1985), and heart rate (Miller, 1969) have been conditioned by rewarding brain stimulation.

The use of any particular response measure is likely to have advantages and disadvantages (see Liebman, 1983, for review), however, as almost all of the literature reviewed here refers to either the lever press response or the shuttle response, each of these will be defined and discussed briefly.

The lever press response

The lever press response is a manipulative response that usually allows the animal control over the initiation of stimulation only. That is, contingent on each depression of the response lever, a burst of stimulation of arbitrary length (but usually one-half second), is applied to the brain through the implanted electrode. The measure of ICSS behaviour is usually the number of responses per unit time (e.g., 'lever presses/minute'), although the distribution of response periods and inter-response

intervals has occasionally been investigated (e.g., Katz & Wagner, 1984; Terman, Terman, & Kling, 1970).

The lever press measure has the advantage of being convenient and economical to use, and "contains the logical appeal that the length of time permitted to elapse between responses reflects the intensity of the desire for the reward" (Valenstein, 1964, p. 416). However, difficulties with interpreting a change in response rate as indicating a change in reward value have led to an increased use of alternative response measures (Liebman, 1983).

The shuttle response

Many animals that respond for ICSS stimulation will also learn a response to terminate that same stimulation. Usually, once the animal has terminated the stimulation it will also reinitiate it. When an animal that responds for ICSS stimulation is placed in a situation where it has control over both the initiation and termination of stimulation an oscillating sequence of on/off responses often occurs which has been termed 'shuttling' (Atrens, 1970, 1973; Mendelson, 1969).

The shuttle response is essentially a locomotor response which requires a simpler level of sensorimotor integration than that required for the lever press response (Atrens & Becker, 1975; Schiff, Rusak, & Block, 1971; Valenstein & Meyers, 1964). It has been found that animals learn the shuttle response more readily than the lever press response (Atrens, 1970; Schiff, 1976), although not all animals that shuttle for ICSS will also lever press for it (Atrens & Becker, 1975; Margules, 1966). Despite this, the shuttle response has been found to occur at many diverse locations in the reward system (Atrens, 1970, 1973; Atrens, Von Vietinghoff-Reisch, & Der-Karabetian, 1973; Steiner, Bodnar, Ackermann, & Ellman, 1973; Valenstein & Valenstein, 1964), that, in general, correspond to the distribution of ICSS sites as determined by the lever press response.

The shuttle response allows measurement of the amount of time the stimulation is left on ('latency to terminate' or ON time) and the amount of time the simulation is left off ('latency to initiate' or 'OFF time'). Shuttling behaviour may be readily described across the entire period of observation by allocating for each 0.1 sec (say) whether the behaviour is ON or OFF. 'Free-shuttling' is used to describe shuttling behaviour under continuous reinforcement (CRF) when the rat has control over both the initiation and termination responses. The term 'preferred duration' refers to the

average ON time selected by an SS during a free-shuttling period.

Measures similar to the lever press rate measure are also possible (e.g., rate of initiation, rate of termination). However, a rate measure determined over some period of observation (e.g., 20 responses/minute) does not use the information contained in the relationship between the response/no response periods. In effect, each response is treated as a point in time having no duration (Baum & Rachlin, 1969). A particular rate may be arrived at by any number of response/no response periods, and does not predict the onset or offset of the response/no response periods (Collier, 1982).

The use of ON and OFF times as measures of the effect of an experimental manipulation might therefore confer an advantage over the use of a rate measure alone if it could be shown that the times were independent. If they were independent then each measure could contribute towards the assessment of the effect of an experimental manipulation. On the other hand, if the two times were stongly related there would be no advantage in using both measures since the knowledge of one would effectively predict the other. A rate measure might be sufficient.

Liebman (1983) outlined several other advantages associated with the shuttle response, including the ease of acquisition, and precision in response measures. Some disadvantages include a more evident increase in baseline thresholds over months of testing, and, in general, a lower experimental 'capacity'. This term refers to the economy of data gathering. With the shuttle response a greater period of observation is required to obtain a reasonable number of data points for statistical analysis. Liebman also suggested that a greater number of implanted animals may be rejected with this method if comparable baselines are required for ON and OFF times. Problems associated with interpretation of shuttling behaviour are considered in a later section (Section 2.3.1).

For ease of later reference three related procedures are briefly defined here. A 'single lever' procedure allows an animal to initiate stimulation by depressing a lever and to terminate stimulation by releasing the lever. In a 'single lever escape' procedure, a rat can only terminate stimulation. A 'two lever' procedure allows the animal to press and release one lever in order to initiate stimulation and then to traverse a short distance to press and release a second lever to terminate that stimulation. The 'single lever' procedure usually allows a measure of ON time only and the 'two lever' procedure usually allows a measure of both ON and OFF times.

The relationship among the measures obtained from these procedures (i.e., lever press, single lever, two lever, and shuttling) is not clearcut. Not all rats that shuttle

will also lever press (Atrens, 1970; Margules, 1966). The extra ON time recorded in a two lever situation compared to a single lever situation is not explainable in terms of the extra distance required to be traversed (Valenstein, 1964). Lever press response rate is correlated most with OFF time in the shuttling procedure (Atrens, 1970; Schmitt & Karli, 1984). ON time determined from the two lever procedure correlates with ON time determined from shuttling (Atrens & Becker, 1975), but the respective OFF times do not.

The principal empirical question to be examined in the present research program is the extent to which ON and OFF time in shuttling behaviour (and under continuous reinforcement) are independent. The assessment of the effect of an experimental manipulation that measures these times might then rest on firmer ground.

1.2.2 Modelling of behaviour

A model is a representation of how something (i.e., a system) is supposed to work. It is a set of assumptions about the relations among the various components of the system that allows it to operate (Bartholomew, 1982; Sibly, 1980).

Models have been proposed for ICSS behaviour when that behaviour has been represented by the lever press response (e.g., Gallistel, Shizgal, & Yeomans, 1981; Mogenson, 1982; Swanson & Mogenson, 1981; Wise & Bozarth, 1984). However, insufficient attention has been given to the development of a model capable of explaining the basic properties of shuttling behaviour. The problems posed by the initiation and termination properties, and the termination property in particular, for the development of a satisfactory model, are worthy of more consideration. The termination response and subsequent reinitiation response must reflect the activity of the neural substrate being stimulated, and an understanding of the factors which contribute to the timing of these responses might assist in understanding more fully how the neural substrate is organised.

The technique of modelling behaviour has become well developed in several research fields (e.g., Bartholomew, 1982; Sibly, 1980) and to a certain extent a set of equations could probably be developed that would describe shuttling behaviour to within some acceptable margin of error simply by following a relatively standard set of procedures. This approach risks becoming a sophisticated description with only an appearance of explanation. As expressed by O'Neil (1987),

> Good descriptions, especially when put in precise, replicable quantitative terms, are of basic value but because they enable the inference of the facts they summarise they should not be confused with explanations (p. 131).

The approach adopted here is to examine the theoretical and experimental evidence in order to select an explanatory concept as a basis for a model of ICSS behaviour that goes further than just sophisticated and detailed description.

1.3 Anatomy and the ventral tegmental area

Not all areas of the brain will support the phenomenon of ICSS. In fact, one of the more crucial variables in the study of ICSS involves the anatomical localisation of the electrode tip. Several comprehensive reviews of the relevant anatomical and neurochemical data have been made elsewhere (e.g., Clavier & Routtenberg, 1974; Fibiger, 1978; Gallistel, 1973; German & Bowden, 1974; Olds, 1976; Olds & Fobes, 1981; Rolls, 1974; Stellar & Stellar, 1985; Wise, 1978). The experiments reported here do not require a detailed neuroanatomical or neurochemical treatment for their interpretation, however, an explanatory model of shuttling behaviour has to be consistent with what is known in these areas. Since the ventral tegmental area (VTM) was the site for the present series of experiments this area will be considered in some detail. The present discussion serves as a general framework, emphasising the complexity of the neural structure underlying ICSS and the consequent need for theoretical interpretations to take this into account.

The VTM was chosen as the stimulation site because it is closely associated with several other ICSS regions of the brain, because it is a site from which vigorous self-stimulation behaviour can be obtained, and because it has been well studied experimentally. The VTM is also a site from which feedback circuitry to the nucleus accumbens has been proposed and the VTM has already been given a prominent role in some existing ICSS models.

1.3.1 The ventral tegmental area

The ventral tegmental area and its associated dopaminergic systems have been the subject of considerable neurophysiological and neurochemical research (e.g., German, Dalsass, & Kiser, 1980; Nakamura & Nakamura, 1976; Phillips & Fibiger, 1976;

Phillips & LePiane, 1986; Shizgal, Bielajew, Corbett, & Skelton, 1980; Wang, 1981; Yim & Mogenson, 1980). The VTM has also figured as a central component in theoretical formulations concerning ICSS circuitry (Crow, 1971, 1972; Mogenson, 1982; Routtenberg, 1968; Wise & Bozarth, 1984). The importance of the VTM in ICSS research has come about because of the region's anatomical connections, neurochemical composition, and because of the vigour of the self-stimulation behaviour (both electrical and chemical) that is obtainable from this region. Stimulation of the VTM can produce ICSS behaviour at least as vigorous as that produced by stimulation of the lateral hypothalamus (Corbett & Wise, 1980; Crow, 1972; Gratton & Wise, 1983; Miliaressis & Cardo, 1973). The VTM and its projections have been implicated in several other functions, including the initiation of locomotion (Crow, 1976; Mogenson, 1982; Swanson & Mogenson, 1981), fear or flight reactions (Deutsch & Howarth, 1963; Gallistel, 1973), the integration and organisation of complex behaviour, the pathogenesis of schizophrenia, and in the therapeutic action of anti-psychotic drugs (Nakamura & Nakamura, 1976; Wang, 1981; Yim & Mogenson, 1980). Activation of the dopaminergic cells of the VTM has been argued to be important for the rewarding effects obtained from ICSS, from the self-administration of opiates, and from natural rewards (Wise, 1982a, 1982b, 1985; Wise & Bozarth, 1984).

The VTM (or group A10 - Dahlström & Fuxe, 1964) lies close to the floor of the rat midbrain, lateral and somewhat dorsal to the interpeduncular nucleus (IP), about 2–3 mm caudal to the lateral hypothalamus (LH), and from 0.5–2.0 mm lateral to the midline (Corbett & Wise, 1980; Pellegrino & Cushman, 1967; Shizgal et al., 1980; Yim & Mogenson, 1980). The region is particularly noted for its high concentration of dopamine (DA)-containing cell bodies (Corbett & Wise, 1980; German, 1982; Mogenson, 1982; Stellar & Stellar, 1985; Wise, 1982b), which appears concentrated in the dendrites of these cells (German et al., 1980). The axons extend rostrally into the medial forebrain bundle (MFB) to terminate primarily in either the limbic system (the mesolimbic dopaminergic system), or in the cortex (the mesocortical dopaminergic system) (German et al., 1980; Nieuwenhuys, Geeraedts, & Veening, 1982; Veening, Swanson, Cowan, Nieuwenhuys, & Geeraedts, 1982; Wang, 1981; Yim & Mogenson, 1980).

The ascending DA fibres of the mesolimbic system have been shown to terminate "massively" in the nucleus accumbens (NA), the olfactory tubercle, and the bed nucleus of the stria terminalis (Nieuwenhuys et al., 1982, p. 59-60). Other projections terminate in the hypothalamus, amygdala, hippocampus, thalamus, septum,

olfactory bulb, and nucleus of the diagonal band of Broca (Nieuwenhuys et al., 1982).

The anatomical connections of the VTM are also characterised by reciprocal, descending, innervation from many of these same regions. The VTM receives efferent fibres from the medial septal nucleus, the diagonal band of Broca, the interstitial beds of the anterior commissure and stria terminalis, nucleus accumbens and substantia innominata (see Nieuwenhuys et al., 1982; Stellar & Stellar, 1985, for detailed descriptions). The descending fibres enter the MFB at various levels and then pass caudally through the LH before terminating in the VTM. Other brain stem nuclei implicated in ICSS (e.g., dorsal and medial raphe nuclei, locus coeruleus) also receive descending input from many of the same anterior structures (Nieuwenhuys et al., 1982). The reciprocal interconnection between the VTM and the nucleus accumbens, along with evidence from single cell recording studies, have suggested the existence of feedback regulatory mechanisms between these two structures (Bunney, 1983; Mogenson, 1982; Stellar & Stellar, 1985).

The VTM has been argued to be directly linked to the LH (e.g., German & Holloway, 1973; Hand & Franklin, 1983; Phillips, 1984; Shizgal et al., 1980; Szabo & Milner, 1973; Wise & Bozarth, 1984). For example, Shizgal et al. (1980) found that antidromic-orthodromic collision effects could be obtained between ICSS sites in the LH and the VTM; which (with other evidence) suggested to the authors that ICSS sites in the two regions were most likely connected by direct, descending, long-axon linkages. The fibres of passage directly excited by the stimulating electrode and identified by the Shizgal et al. collision technique have become known as the 'first stage' neurons of ICSS (Gallistel et al., 1981; Wise & Bozarth, 1984). It has been hypothesised that the first stage neurons synapse with a second stage set of neurons which may be catecholaminergic (Gallistel et al., 1981) and may, at least in part, be located in the VTM (Gratton & Wise, 1983; Wise & Bozarth, 1984).

The relationship between the DA cells of the VTM and the reward effect of ICSS stimulation is still not fully resolved. For example, Corbett and Wise (1980) used a moveable electrode technique to determine the relationship between ICSS sites and ascending dopaminergic neurons in the ventral tegmental area. With this technique, electrode placements could be systematically incremented in the vertical plane by 0.25 mm over a 2.0 mm range. At each placement rats were tested for ICSS. Corbett and Wise found that the lowest self-stimulation thresholds and the highest response rates were elicited from those areas traversed by dopaminergic fibre bundles, and that current thresholds and response rates were proportional to the density of

dopaminergic elements surrounding the electrode tip.

However, since it has been shown that the directly stimulated substrate is most likely non-catecholaminergic (Gallistel et al., 1981), Wise and Bozarth (1984) proposed that first stage neurons, directly stimulated by LH or VTM ICSS, synapsed with the DA-containing (and opioid-containing) neurons in the ventral tegmentum (and substantia nigra zona compacta). The DA-containing neurons, in turn, communicated with the nucleus accumbens. Wise and Bozarth suggested that activation of the fibres of the DA cells of the VTM was essential for the rewarding effect of ICSS stimulation.

As it stands, the model of brain reward circuitry proposed by Wise and Bozarth (1984) cannot account for ICSS shuttling behaviour since it does not explain the termination and subsequent reinitiation of the stimulation. Some form of feedback circuitry between the neurons of the NA and the VTM (e.g., as proposed by Bunney, 1983; Mogenson, 1982) might provide the modulation necessary to explain the more dynamic features of shuttling behaviour.

Other evidence suggests caution is needed about accepting the proposal that activation of DA-containing cells is necessary for the direct reward effect of ICSS, despite the fact that dopamine is strongly implicated. Studies of single cell recordings have shown that iontophoretic application or systemic injection of dopamine releasers or agonists (e.g., amphetamine, apomorphine) cause the DA cells of the VTM to decrease their firing. Conversely, application of DA receptor blockers (e.g., haloperidol, chlorpromazine) to DA cells of the VTM cause the same cells to increase their firing rates (Cools & Van Rossum, 1976; German et al., 1980; Stellar & Stellar, 1985; Yim & Mogenson, 1980). Since dopamine releasers increase ICSS response rates while dopamine blockers decrease ICSS rates (e.g., Nazzaro & Gardner, 1980; Zarevics & Setler, 1979), the action of DA-containing cells must be predominantly inhibitory on some other group of neurons more directly associated with the reward effect. German et al. (1980) have suggested that DA neurons might be collaterally inhibited by nearby DA neurons, or might even exhibit self-inhibition.

Another possibility is that there are two types of DA neurons — one excitatory and one inhibitory. Cools and Van Rossum (1976) suggested that DA-loaded structures in the mammalian brain, such as the caudate nucleus, putamen, and nucleus accumbens, contained at least two distinct types of DA-receptors: a pre-synaptic excitatory receptor, and a post-synaptic inhibitory receptor. It might be that the reward effect is produced by stimulation of the excitatory receptor. Although other

reports have supported the existence of different types of DA receptors (Creese, Sibley, Hamblin, & Leff, 1983; Joyce, 1983; Stellar & Stellar, 1985) this possibility as it applies to ICSS, has not been experimentally supported.

Finally, a further detail is that non-dopaminergic neurons and pathways have been found in the VTM and shown to project to the nucleus accumbens (Wang, 1981) and from the NA to the VTM (Nazzaro & Gardner, 1980). Evidence also indicates that gamma aminobutyric acid pathways from the NA might exert an inhibitory modulation effect on VTM dopaminergic cells (Kelley, Stinus, & Iverson, 1980; Nazzaro & Gardner, 1980; Stellar & Stellar, 1985; Swanson & Mogenson, 1981).

1.4 Organisation of chapters

The above review has highlighted some of the factors involved in defining and measuring ICSS shuttling behaviour and also examined the neural substrate of the VTM. The next two chapters are concerned with issues of interpretation. In general the approach to be adopted is to consider ICSS shuttling behaviour as the output from a system that is responding to a limited, known set of input (i.e., stimulus) variables. An examination of what is known about the input variables and what is known about their distribution and inter-relationships should establish the conditions necessary for developing a satisfactory model of ICSS shuttling behaviour.

Three areas need to be approached. First, the relative roles of the main input variables need to be evaluated. In particular, an assessment of the relative contributions of intensity and duration in the determination of the timing of the shuttle response would allow the model to be based on the most influential variable. Secondly, a fundamental explanatory concept is required to form the basis for the model. Here a reward/aversion model and a reciprocal inhibition model are given consideration. Thirdly, the mathematical relationships among the various dependent variables need to be clearly understood. Of prime importance here is the correlation between ON time and OFF time and under what conditions this correlation may be influenced. Of secondary importance is the relationship among a series of ON times and a series of OFF times (i.e., autocorrelation). Once these relationships have been clearly determined the selection of suitable system components can be limited to those which reproduce the same properties.

The chapters of this dissertation are organised as follows: Chapter 2 is a review of the relative importance of the main stimulus variables affecting ICSS shuttling and of

existing knowledge about the relationship between intensity and ON and OFF times in shuttling behaviour. Some of the problems associated with the interpretation of shuttling behaviour are also considered. In Chapter 3 theoretical approaches which have been advanced to explain shuttling behaviour are examined. In Chapter 4 details are provided concerning the animals, methodology and apparatus used in the experiments.

Chapters 5, 6, and 7 are devoted to the three series of experiments forming the empirical research program. Chapter 8 gives the results of the histological analysis. In Chapter 9 some examples of shuttling data from a 'black box' modelling approach are briefly examined and discussed. Chapter 10 is a summary, discussion and conclusion.

Chapter 2

Input and output factors

To develop a satisfactory model of shuttling behaviour an examination is required of the main input variables to the system. What is known about these variables can provide clues about how to structure the model or at least about the characteristic response of the system and its various physiological limits. A closer examination of the output variable (i.e., shuttling behaviour) can also help clarify the requirements for an appropriate model.

2.1 Stimulus factors

Numerous input variables can influence ICSS response vigour. Of interest here are the factors to do with electrical stimulation. All other state and environmental variables are assumed constant (or subject only to random fluctuation). The various stimulus factors include: electrode size, material and configuration; stimulus source (e.g., constant voltage or constant current); wave shape (e.g., sine wave or 'square' wave); and, for any particular wave shape, the parameters: frequency, amplitude, and duration. For the experiments to be reported here, the type, size and configuration of electrodes, the stimulus source, and wave shape have been kept constant. In shuttling behaviour the train durations (ON time) and the time between them (OFF time) are the dependent variables. Therefore, the independent stimulus variables relevant to the present study are pulse amplitude, pulse duration and pulse frequency.

To a large extent pulse amplitude, duration and frequency can be considered interchangeable (Gallistel, 1976, 1978; Keesey, 1962; Ward, 1959; Wetzel, 1971). A decrease in response vigour produced by a decrease in one of these parameters may be compensated for by an increase in either or both of the others. Although this

may be due in part to a lack of sensitivity in the response measurement, it may also reflect the spatial and temporal integrating characteristics of the underlying neural substrate. In particular, the almost linear relationship between current intensity and the reciprocal of frequency in maintaining a constant behavioural output might indicate that the second-stage neural network is indifferent to the spatiotemporal distribution of axon potentials generated in the first-stage, directly stimulated axons, whenever a constant strength stimulus is used (Edmonds, Stellar, & Gallistel, 1974; Gallistel, 1974, 1976, 1978; Gallistel et al., 1981).

When ICSS response rates are considered as a function of the time over which a given stimulation is applied (pulse duration, train duration) rather than as a function of amplitude or frequency, response rates show relatively limited changes. Therefore, an increase in the time over which stimulation applies is a less efficient means of delivering a certain quantity of charge than is an increase in either intensity or frequency (Barry, Walter, & Gallistel, 1974; Gallistel et al., 1981; Goldstein & Keesey, 1969; Huston, Mills, & Huston, 1973; Keesey, 1962; Wetzel, 1971). Short pulse durations (e.g., 0.1 msec) require the least charge transfer to maintain a constant running speed (Barry et al., 1974).

Physiological limits exist for frequency and pulse duration parameters in that high frequency may lead to electrothermal damage to CNS tissue and long pulse durations may lead to electrolytic damage (Lilly, 1961; Lilly Hughes, Alvord, & Galkin, 1955; Miller, Jensen, & Myers, 1961; Olds, Travis & Schwing, 1960). But given the acute nature of ICSS work, pulse widths of less than about 10 msec, and of low intensity, and frequencies of 200 Hz or less should not produce tissue damage if the stimulus is presented in biphasic form.

In summary, intensity and frequency are the more efficient input variables. Because intensity has generalisation properties similar to external stimuli (Coleman & Berger, 1978; De Witte, 1982b; Stutz, Hastings, Rossi, & Maroli, 1978) and appears to be the more dominant perceptual dimension (Colpaert, Maroli, & Meert, 1982), the present series of experiments has concentrated on the manipulation of intensity, with frequency being kept constant at 100 Hz.

2.1.1 Intensity

An increase in the intensity or amplitude of the stimulating pulse implies an increase in the suprathreshold region around the electrode tip (e.g., Frank, Preshaw, & Stutz,

1982; Gallistel, 1973; Gallistel et al., 1981; Olds, 1958b; Olds et al., 1960; Stein 1962; Stellar & Stellar, 1985; Valenstein 1966; Yeomans, 1975). An increase in intensity can therefore excite a greater number of neurons to threshold, can stimulate a higher proportion of high threshold neurons (Ranck, 1975; Yeomans, Mercouris, & Ellard, 1985), or cause some neurons to fire more than once (Aidley, 1978; Stein & Ray, 1959).

The spread of current

Some theoretical interpretations of the relationship between intensity and ICSS behaviour argue that current spread from the electrode tip activates neuronal elements that differ in terms of their relevance for ICSS. For example, the termination response has been explained as current spread to eventually activate aversive systems located nearby (e.g., Atrens, 1970; Stein, 1962).

However, the question of how far the current provided by a 'typical' ICSS electrode spreads through the neural and non-neural tissue surrounding the electrode tip has not been fully resolved (Fouriezos & Wise, 1984; Olds et al., 1960; Ranck, 1975; Stark, Faxio, & Boyd, 1962; Szabó, 1973; Valenstein & Beer, 1961; Valenstein, 1966; Wetzel, 1970; Wise, 1972; Yeomans et al., 1985). Fouriezos and Wise (1984) have stated, nonetheless, that there is consensus on the claim that the current required to fire a neuron increases as the square of the distance between the electrode and the neuron increases.

Early reports argued for an effective region of stimulation of about 1 mm (Olds et al., 1960; Valenstein, 1966; Valenstein & Beer, 1961; Wetzel, 1970). Ranck (1975) has extensively reviewed evidence suggesting that currents around 100 μa (pulse width= 0.2 msec, monopolar, cathodal pulse) may excite tissue up to 1.2 mm from the electrode tip. However, ICSS studies that use 0.2 msec pulses often report intensities in the range 400–1500 μa (e.g., Schenk, Prince, & Shizgal, 1985; Schmitt, Sandner, & Karli, 1981). On the basis of Ranck's data, a 1000 μa pulse would excite cells up to about 2 mm from the electrode tip. Wise (1972) has reported evidence indicating that current spread appears to be confined to around 0.125 mm for intensities in the order of 25 μa (60Hz sine wave). Ranck (1975) stated that this was consistent with his data if small fibres were being stimulated. More recently, Fouriezos and Wise (1984) suggested that "for currents that are routinely used in ICSS studies" (p. 88) effective stimulation may only spread to tissue in the "radii of a few tenths of 1 mm"

(p. 88).

In summary, the effective spread of current should be about 0.1–0.3 mm for wave forms similar to 60 Hz sine wave and at intensities near 25 μa. Because many studies use much shorter pulse durations and consequently higher intensities, the estimate of 0.1–0.3 mm would be a minimum distance in ICSS studies.

2.1.2 Intensity and response rate

Early experiments established that there was an important relationship between stimulus intensity and response rate (Ellman, Ackermann, Bodnar, Jackler, & Steiner, 1975; Keesey, 1962; McIntire & Wright, 1965; Olds & Milner, 1954; Olds et al., 1960; Reynolds, 1958; Sidman, Brady, Conrad, & Schulman, 1955; Wauquier, Niemegeers, & Geivers, 1972). Reynolds (1958) showed that lever press response rate was a non-monotonic function of increasing stimulus voltage. At first rates increased with increasing voltage, and thereafter declined. The change in rate could not be attributed to "a temporal cumulative effect" (p. 196) since the same inverted U-shaped function was reproduced when voltages were presented in descending order.

Rate-intensity functions

Rate-intensity functions (RIFs) describe the relationship between response rate and the independent variable of intensity. It has been argued that RIFs provide valuable information regarding the identification of "the sensitive range in which behaviour is able to change in response to manipulations that alter stimulation effectiveness" (Stellar & Stellar, 1985, p. 92). Caution is required, however, in interpreting an increase in response rate as an increase in reward value (De Witte, 1982; Liebman, 1983; Stellar & Stellar, 1985; Valenstein, 1964). Liebman (1983) suggested that a change in response rate reflected a change in the reward value of stimulation only "within a range where rate is proportional to intensity" (p. 46). That is, if some portion of the RIF is approximately linear then over that range a change in response rate may be a reasonable reflection of a change in reward value.

Rate-intensity functions are obtainable from all ICSS brain regions (e.g., Ellman et al., 1975; Olds et al., 1960; Schenk et al., 1985) and it has been argued that different ICSS substrates generate differently shaped RIFs (e.g., septal versus MFB RIFs — Bradley, 1974; Hodos & Valenstein, 1962; Olds et al., 1960; Stutz et al., 1978). Olds et al. (1960) reported results of an extensive mapping study of ICSS

sites in the hypothalamus and adjacent regions and categorised RIFs into either "steep", "square", or "undulating" functions on the basis of whether response rates continued to rise with each increment in intensity, rose to a maximum and then plateaued, or rose to a maximum and then fell. Keesey (1962) also determined RIFs from stimulation of the hypothalamic region and concluded that the "undulating" form of the RIF was the most common.

The prefrontal cortex (PFC) has been reported as being particularly unresponsive to intensity change (Hand & Franklin, 1983: Robertson, Laferrière, & Franklin, 1981). However, Schenk et al. (1985) showed that although intensities needed to be quite high compared to MFB ICSS, and that response rates were low, the rewarding effect of PFC stimulation continued to increase as intensity was increased (as measured by the trade-off technique). Conflict with the previous reports may have been due to response 'ceiling' effects or to the fact that PFC rate-intensity functions tend to be shallower than those derived from MFB sites and hence require larger increments in intensity for comparable effects with MFB ICSS.

In general, increases in stimulus intensity produce increases in response rate up to a certain maximum level, beyond which rates may remain steady, fall again, or show a pattern of increases and decreases. It appears more common that rates increase to some maximum and then decline.

Response rate changes at high intensity

The reason why response rates tend to decline at high intensities might provide some indication of the mechanism by which intensity exerts its effects. For example, Reynolds (1958) and Stein and Ray (1959) argued that high intensities of stimulation summate to bring an aversive system to threshold. Olds et al. (1960) also argued that high intensities increased the size of the suprathreshold electrical field and were therefore more likely to excite an aversive system. "Steep" and "square" RIFs were explained as the result of spread of current to extra volumes of tissue that were either positive or neutral with regard to the reward value of the stimulus. The "undulating" (or, inverted U-shape) RIF was considered a result of a spread of current to tissue which, when excited, contributed aversive effects to the rewarding effects produced by neurons closer to the electrode tip. The total reward value of the stimulation was therefore less and rates declined.

Detailed mapping studies with moveable electrodes (Corbett & Wise, 1979, 1980:

Gratton & Wise, 1983; Wise, 1981) suggest that although many RIFs tend to decline at higher intensities, there is no tendency for positive ICSS sites to be surrounded by aversive sites. Gratton and Wise (1983) did find that high intensity stimulation of the medial and lateral boundaries of the MFB was associated with increased aversive and motor effects respectively, but the response rate decline was attributed to lowered response ceilings rather than actual aversiveness. Other authors have also suggested that the most likely explanation of the decrement in response rate at high intensities is due to interference from competing behaviours, particularly motor artifact (Atrens, 1970; Hodos & Valenstein, 1962; Terman et al., 1970).

If motor artifact is controlled, some evidence indicates that intensity of stimulation itself does not become aversive. For example, Hawkins and Pliskoff (1964) allowed stimulation to occur only during the second member of a two-member behavioural chain. The first member of the chain was a VI 30 schedule which did not produce stimulation. Its completion resulted in the presentation of a second lever which did allow self-stimulation. The rat worked to gain access to the second lever which then allowed five presses at the selected intensity. In this way interference from motor artifact or seizures could not hinder response rate on the first member of the chain at any intensity. The results indicated that although response rates for CRF stimulation tended to decrease at higher intensities, response rate on the first member of the chain continued to increase. Hawkins and Pliskoff argued that the rewarding effect of ICSS continued to increase in strength over the range of intensities which produced lower response rates.

If high intensity ICSS stimulation is aversive, rats given the opportunity to regulate the intensity of stimulation, should maintain it below convulsion-producing levels. Stein and Ray (1959) using a two lever increment-stepping procedure showed that rats could learn to control the intensity of the stimulation, whereas Bradley (1974), using the same procedure, concluded they could not. However, even Stein and Ray observed that the self-selected intensity levels were "usually higher than most experimenters would care to assign under the conventional fixed-intensity procedure" (p. 571). Intensities of 13 to 20 milliamps were cited (0.25 sec train, 50 Hz of Lilly-type pulse pairs, pulse width 50 μsecs separated by 200 μsec interval). Bradley (1974) found that rats would continue to increase the intensity to convulsion-producing levels using the two lever procedure, but with a two platform shuttling procedure, self-regulation occurred without any special training and at intensities well below convulsion-producing levels. Stein and Ray also found that the ability

to control the intensity of stimulation may be site-dependent. One animal with an anterior electrode just rostral to the septum and a posterior electrode in the midbrain tegmentum increased intensity on the anterior electrode to convulsion-producing levels (40–50 ma), but self-regulated "expertly" at the tegmental site.

More recently, Zarevics and Setler (1979) used a modified version of Stein and Ray's procedure (referred to as 'auto-titration'), in which after five responses on the first of two levers, the intensity of stimulation automatically decremented (rather than incremented, as in the Stein and Ray (1959) procedure) by some specified amount (e.g., 20 μa). The animal could reset the stimulation to its initial value at any time by a single press on the second lever. The second lever itself, however, did not produce stimulation. Therefore, a measure of reward threshold could be obtained at the point where reset responses occurred. Although positive and negative contrast effects might be present with this procedure (Liebman, 1983), the autotitration technique provides a sensitive rate-independent measure of the relative reward value provided by different stimulus combinations.

Zarevics and Setler (1979) confirmed that response rates increased to a maximum and then declined at higher intensities. However, an analysis in terms of the charge consumed indicated that beyond the point where response rates had started to decline a constant rate of charge consumption was being maintained. Zarevics and Setler suggested that at higher intensities the decrease in response rate occurs because maximal stimulation of the reward system can be achieved with less behavioural output. However, below a critical level an increase in behavioural output cannot achieve this maximal activation.

In summary, the argument that response rate declines at higher intensities because of current spreading so as to eventually activate neighbouring aversive systems has not been strongly supported. Stronger evidence exists to suggest that the decline occurs because of interference from competing behaviours, or because, beyond a critical level at which the reward system can be maximally activated, a constant rate of charge input is being maintained. It cannot be discounted that some aversive effects occur due to excessive autonomic arousal (e.g., Ángyán, 1976; Sadowski, 1976), or due to convulsions, but high intensity itself (within the RIF range) may not be aversive.

2.2 Intensity and shuttling behaviour

Much of the early evidence on the relationship between intensity and ICSS behaviour rested on the relationship between intensity and lever press response rate. More recently the shuttle response and ON and OFF time measures have found increasing use in the ICSS literature (see Liebman, 1983, for review) and in the evaluation of drug effects in particular. Until now, certain basic assumptions underlying the use of the shuttle response under CRF, such as the independence of the ON and OFF times, and the relationship between these times and the parameters of stimulation have not been fully analysed.

Intensity and ON and OFF times

Keesey (1964) allowed rats to regulate the duration of stimulation by both the use of a single lever, and later a two lever procedure. It was found that as intensity or frequency was increased, the selected ON duration decreased to some minimum value (approximately 0.5–0.6 seconds on the single lever). The shape of the obtained curves differed in that the frequency/duration curve showed a smooth decelerating decline from 1.4 secs to 0.6 secs, whereas the intensity/duration function showed a gradual decline initially followed by a more rapid decline to asymptote. Work and Elder (1964) also showed that an increase in stimulation frequency would produce a decrease in self-selected ON time (also single lever). Valenstein and Valenstein (1964) used a two lever design and Hodos (1965) used a two compartment design to produce essentially similar results.

Valenstein and Valenstein (1964) found that although stimulation was terminated more quickly at high intensities, it was also reinitiated more quickly. Both the time the stimulation remained ON and the time the stimulation remained OFF were decreasing functions of current intensity. Valenstein and Valenstein found that at intermediate intensities, mean ON time was 4.3 secs and the mean OFF time was 3.2 secs (averaged over all sites and over 15 min periods). At high intensities, mean ON time was reduced to 2.8 secs and mean OFF to 2.6 secs. These authors also reported that the above relationships held for 22 diverse ICSS-supporting structures, including sites located in the hypothalamus, septum, amygdala, and hippocampus.

Margules (1966) found some ICSS sites near the base of the hypothalamus for which an increase in the intensity of stimulation did not produce a decrease in ON time ('pure positive' sites). Stein (1962) found two forebrain sites that evoked longer

ON times as intensity was increased. So it is possible that not all ICSS sites follow the pattern of decreased duration as intensity is increased. Because Margules (1966) used a two lever procedure and Stein (1962) a single lever procedure, the finding of longer or unchanged ON times at higher intensities may also be due to the use of manipulative responses (Atrens & Becker, 1975; Bradley, 1974; Valenstein, 1964; Valenstein & Valenstein, 1964; Section 1.2.1). Atrens (1973), using a shuttle response, found no pure positives from stimulation of the same brain regions as Margules (1966).

Atrens (1970) also examined the relationship between ON and OFF time as a function of increased intensity. It was found that ON time was a linear decreasing function of intensity, yet the total amount of time an animal left the stimulation ON over the test period was unrelated to intensity. Valenstein and Myers (1964), using a form of interrupted stmulation, had earlier reported that total ON time was markedly altered by changes in stimulus intensity and proposed total time as an index of reinforcement strength. Poschel (1966) also found that total time spent on a positive platform increased with intensity. More recently, Atrens et al. (1983) have argued that "The total time that the stimulation is left on in the shuttlebox .... is unlikely to be a reliable measure of anything" (p. 792). Although the use or non-use of interrupted stimulation may be an important factor here (Liebman, 1983), the relationship between total time and intensity remains unresolved.

Montgomery, Apicella, Inzerillo, and Sinnamon (1981) studied ON and OFF time measures elicited from ICSS sites from the ventral midbrain to the rostral diencephalon. In general, their results showed that ON and OFF times decrease with increasing current intensity. Moreover, Montgomery et al. found that OFF times were less sensitive to manipulations of intensity than were ON times. However, Montgomery et al. employed a lever press response for initiation and a locomotor response for termination, and so the times may not be directly comparable. Schmitt et al. (1981) have also confirmed that both ON and OFF times decrease as a function of increasing intensity.

To a large extent, intensity may be substituted by pulse duration or pulse frequency in a manner similar to that described previously for the lever press response. In particular, Schmitt et al. (1981) have shown that ON and OFF times can be differentially manipulated by varying intensity and interpulse interval (I/IPI) combinations. They noted that to obtain ON time equal to OFF time (and both times equal to 6.0 secs), the most common I/IPI combinations were a rather high stimulation intensity with a rather long IPI. When IPI was increased both ON time and OFF

time increased, although OFF time more so than ON time. When IPI was decreased both ON time and OFF time decreased, with OFF time again decreasing more by comparison. In contrast to the results of Montgomery et al. (1981), OFF time was found to be the more sensitive to stimulus changes.

Schmitt et al. (1981) also found that for a constant ON time produced by either high intensity plus low frequency (and therefore many neurons) or by low intensity plus high frequency (and therefore few neurons), differential effects could be established with OFF time. The direction of change in OFF time appeared dependent upon the site of stimulation with a shift from stimulation of many neurons to stimulation of few neurons (while maintaining a constant ON time) decreasing OFF time.

In conclusion, the stimulus dimension of intensity may be used to increase or decrease the vigour of ICSS behaviour observed in the shuttlebox. In general, an intensity increase causes an increase in the rate at which rats will initiate stimulation but also increases the rate at which the stimulation is terminated. These findings are relevant to the selection of a model of shuttling behaviour (Chapter 3).

2.2.1 The independence of ON/OFF times

In addition to the evidence above that ON and OFF times are separately manipulable, it has been found that the two measures respond differently to a reduction in stimulus intensity, with only OFF times showing a negative contrast effect (Atrens et al., 1973). OFF times at certain hypothalamic sites are more sensitive to food deprivation (Atrens & Sinden, 1975) and to the effects of catecholaminergic blockers (Hunt, Atrens, Chesher, & Becker, 1976). ON times have been shown to be more sensitive to the effects of narcotic agonists (Baltzer, Levitt, & Furby, 1977; Gerhardt, Prowse, & Liebman, 1982; Levitt, Stilwell, & Evers, 1978; Liebman, 1985). Shizgal and Matthews (1977) have shown that the two measures respond differently when the stimulation train consists of controlled bursts and inter-burst intervals.

These results suggest that the two measures comprising shuttling behaviour are relatively independent in terms of their response to experimental manipulation. However, a more conclusive approach to determining the independence or otherwise of ON and OFF times would be to examine their correlation.

The correlation between ON and OFF times

The statistical relationship between ON and OFF times may be considered in terms of mean ON and OFF times taken over a number of subjects, or over a number of trials. Alternatively, the relationship between individual ON and OFF times within a particular trial (termed 'within-trial' ON and OFF times) may be considered. This second possibility has received little attention.

Existing evidence on the statistical relationship between ON and OFF time suggests that there is no significant association between these two variables (Atrens, 1970, 1973; Atrens & Becker, 1975; Atrens et al., 1983; Atrens & Von Vietinghoff-Reisch, 1972). For instance, Atrens (1973) reported a non-significant ($r = -0.26$) Pearson product-moment correlation coefficient between ON and OFF times in 36 animals with hypothalamic electrodes. Atrens and Becker (1975) reported a non-significant Spearman rank-order correlation of $r = -0.47$. In both these studies the correlation coefficient was obtained from a correlation between mean ON time and mean OFF time, and not from within-trial ON and OFF times. Atrens (1970) reported a non-significant correlation between 'mean minimum latency to terminate' and 'mean minimum latency to initiate'. The correlation of $r = 0.70$ (N= 9) was not significant at the 0.01 level (it is significant at the 0.05 level, however). In any case, the relationship between 'mean minimum' ON and OFF times may not be the same as that between within-trial ON and OFF times.

In contrast to these results, Schmitt et al. (1981) reported that for most stimulation sites a positive correlation existed between ON and OFF times. Average correlations ranged from 0.44 to 0.79 for the logarithmically transformed ON and OFF times. The data description indicates that mean ON and OFF times were again being used in the analysis.

The differences between the correlations reported by Atrens (1973) or Atrens and Becker (1975), and those reported by Schmitt et al. (1981) are quite substantial. The reported correlation between ON and OFF times in the shuttlebox situation has ranged from -0.47 to $+0.79$. Whether these differences reflect the different mathematical transformations used, the different testing procedures used, the different stimulation sites, or whether the times were recorded at different intensities of stimulation, is not clear.

Atrens et al. (1983) have described correlations between specific ON times and the preceding, and succeeding, OFF times as well as correlations between successive

ON times and between successive OFF times (with all times transformed by Log10 before analysis). The analysis showed that orderly correlation patterns were obtained when three initiations and terminations were allowed per block of trials. ON times became progressively less correlated with the preceding OFF time over the three trials within each block (0.354, 0.266, and 0.099, respectively). Even the last correlation was significant (because based on larger numbers than the correlations of -0.47 and 0.70 described earlier). Two examples of the correlation between each ON time and the immediately succeeding OFF time are also obtainable from these data. The second OFF time with the first ON time yields $r = 0.422$ and the third OFF time with the second ON time yields $r = 0.169$. Therefore, these data also describe a positive, but declining correlation. It cannot be determined from these data whether the observed decline in correlations over the first three ON and OFF times would continue to decline to zero or beyond, or level out at some positive value. Moreover, the correlations were calculated from blocks of three trials repeated many times and not from a long series of within-trial ON and OFF times.

The evidence concerning the degree of correlation between ON and OFF time therefore remains inconclusive. No study has systematically examined the within-trial correlation between ON and OFF time as a function of intensity or of stimulus duration. The existence or not of a significant relationship between ON and OFF time would indicate the most appropriate form for a model of shuttling behaviour, and would also establish the utility or otherwise of using ON time and OFF time independently as measures of the effect of some experimental manipulation.

2.3 Output factors

This section briefly examines some of the problems associated with interpreting shuttling behaviour in terms of intervening variables. In particular, the extent to which a change in behaviour can be interpreted as being due to a change in reward or a change in performance is examined.

2.3.1 The interpretation of shuttling behaviour

Changes in the qualitative or quantitative characteristics of a reward stimulus may only be inferred from observed alterations in an animal's performance on some arbitrarily chosen response. However, a change in performance does not necessarily imply

a change in reward value; hence a basic difficulty with ICSS research has been the problem of distinguishing a change in performance due to a change in reward value from a change due to other factors. For example, the degree of manipulative skill, the amount of effort or learning required in the task, or the animal's general health and level of arousal might significantly influence the observed performance and subsequently be interpreted as a change in reward value. Liebman (1983) has suggested that the inference of a change in reward value from the observation of a change in performance has usually been arrived at by a process of elimination. If performance changes while all other factors have been held constant, the change must have been due to a change in reward value.

Which factors should be kept constant is not always so obvious. Seemingly trivial aspects of a test situation might alter performance on the chosen response measure and the alteration wrongly attributed to a change in reward value. Schiff (1976) reported that bilateral caudate lesions abolished a lever press response for LH ICSS. This could be interpreted to suggest that this region of the brain was essential for the reward effect of LH stimulation. However, later work with another group of animals, also with bilateral caudate lesions, showed that 45% of the lesioned group would learn the lever press response if the lever was relocated at 64 mm above the floor rather than the 10 mm in the original test. The height of the bar in the Skinner-box had been a significant factor in assessing the reward value of stimulation.

In a self-regulatory situation, a change from a single lever procedure to a two lever procedure resulted in an increase in ON time of several orders of magnitude (Valenstein, 1964). The differences were not explicable in terms of the time taken to traverse the distance between the two levers. Apparently the rats had difficulty inhibiting the motor reactions which forced them off the single lever (also see Gallistel, 1973; Poschel, 1966; Valenstein & Valenstein, 1966). Therefore, if the duration of self-regulation is taken as the measure of reward value, the type of response required to initiate and terminate becomes an important factor.

More generally, different response requirements may not reflect the same reinforcement property. Beyra (1976) and De Witte and Bruyer (1980) have shown that the reinforcing effect of ICSS is composed of a complex hierachy of factors. De Witte and Bruyer (1980) employed 38 different parameter combinations in 28 different experimental situations with 3 'motivational procedures' (feeding, sexual, and aggressive behaviour). Included were: choice and cost methods, consummatory

methods (i.e., 'amount' of reward in terms of charge and time), extinction, and escape procedures. Statistical methods were then used to partial out the contribution of various factors to ICSS reward.

The analysis identified five main factors which could be further clustered into three second-order factors. One of these three factors included a cluster of consummatory behaviours including the amount of charge and time spent allocated to the stimulation, frequency of feeding, and frequency of initiation in a self-regulatory procedure. Another factor included methods wherein the animal was required to discriminate between brain stimuli on the basis of the quantity or quality of reward, such as percent choice on a two lever procedure, number of lever presses on continuous or fixed ratio schedules, and extinction. Finally, a third factor included the solitary measure of 'aggressive dominance behaviour'. In particular, therefore, choice measures of ICSS may not measure the same reinforcing property as do consummatory measures.

Part of the difference between using alley choice measures or ON time consummatory measures may lie in the fact that most choice procedures use the first ON time only (and the OFF time that precedes that ON time). Later ON and OFF times in a long period of interaction, as is more typically used in CRF shuttling procedures, may not reflect the same underlying properties as the very first OFF time and the very first ON time (Atrens et al., 1983).

Pure positive reinforcement and performance

Another important aspect in the measurement of ICSS reward has been the distinction between an arousal, incentive, or forcement component, and a 'pure' positive reinforcement component (Atrens, 1984; Atrens et al., 1983; Bindra, 1968; De Witte, 1982; Gallistel, 1969, 1973; Katz & Wagner, 1984; Liebman, 1983; Trowill, 1976; Trowill, Panskepp, & Gandelman, 1969). Liebman (1983) describes incentive as a "nonspecific energizing effect of brain stimulation" (p. 46). Atrens (1984) describes forcement as including the skeletal, autonomic and endocrine responses directly elicited by the stimulation.

Gallistel (1973) found that incentive had a potentiating effect on the immediately following response. The performance of a well-trained rat responding for ICSS could be enhanced by non-contingent pretrial stimulation (or 'priming'). Priming was cumulative because running speed was greater after six priming trains than after three. A single 0.5 second priming train of stimulaton could provide a relatively large

incentive. The after-effect of the single train dissipated within 30 seconds to a few minutes, while the after-effect of 10 to 20 such trains lasted up to 15 minutes.

Atrens et al. (1983), using shuttling behaviour, have also demonstrated the strong influence of previous responses over succeeding ones. A strong facilitation of responding occurs during the early part of a block of trials, (especially using CRF), because the stimulation train not only reinforces the previous response but also elicits or forces the next one. Atrens et al. have suggested that for the shuttle response "hypothalamic stimulation produces forcement processes which become the principle determinant of stimulation initiation under conditions of continuous reinforcement" (p. 790). The build-up of forcement that occurs during CRF schedules may be alleviated by the spacing of discrete trials or by providing only intermittent reinforcement.

Atrens (1984) has argued that forcement processes invalidate virtually all CRF measures of positive reinforcement. The present research specifically investigates CRF procedures, and, because of this, an interpretation of shuttling behaviour in terms of positive reinforcement may not be warranted. It is assumed that a mechanistic approach to modelling ICSS behaviour under CRF can provide a basic organising principle for understanding the behaviour. For example, a reciprocal inhibition model (Section 3.2.1) or a positive feedback model (Houston & Sumida, 1985) are established models that could form the basis for such a mechanism. A distinction between positive reinforcement and forcement has not been attempted.

Liebman (1983) has also questioned the necessity for discriminating between 'pure' reinforcement and performance factors in understanding ICSS behaviour and has pointed out that useful results have been obtained by research that has not attempted to dissociate the two. Liebman emphasised that "The unique value of ICSS lies in its potential insight into brain reward mechanisms" (p. 46). If the "nonspecific energizing effects of brain stimulation" or 'forcement' may be considered part of the phenomenon of ICSS then there may be no need to dissociate these effects from 'pure' positive reinforcement effects in all situations.

In summary, different behavioural measures may be confounded to different degrees by relatively specific factors such as motoric side effects or the ability to perform the required response, or by relatively non-specific factors such as incentive or forcement. Moreover, consummatory measures may not measure the same reinforcing property as do choice measures. The present series of experiments has used CRF stimulation throughout and therefore some caution is warranted in interpreting a

change in a behavioural measure in terms of a change in a pure positive reinforcement factor or a change in some performance factor. The term 'reward value' is therefore used as a generic term to encompass both factors, and is defined empirically (over the linear range of the RIF) as the rate of shuttling. A higher rate of shuttling indicates a higher work rate and therefore a higher 'reward value'.

Chapter 3

Theoretical perspectives

The fact that many animals will terminate ICSS stimulation has been known since Bower and Miller (1958) and Roberts (1958a,b) first demonstrated the phenomenon. From their observations, these authors proposed that ICSS was initially rewarding but that prolonged stimulation became aversive. Animals therefore terminated the stimulation in order to escape from an excessive accumulation of aversion. As an alternative, Stein (1962) suggested that the rewarding effect might rapidly adapt to a low level rather than actually becoming aversive. Therefore, an animal's termination of stimulation allows the reward system time to recover and the full rewarding effect to be regained on the next initiation.

Several other proposals have been offered to explain why an animal should terminate ICSS stimulation, including the proposal that both responses are positively reinforcing (Hodos, 1965), or that termination is respondent (i.e., involuntary — Fibiger, 1978). No proposal has received unequivocal support (Liebman, 1983).

Any explanation as to why animals terminate ICSS stimulation must also account for two other features of shuttling behaviour. First, despite the fact that the stimulation may be terminated, the same stimulation is also readily reinitiated. Second, any explanation must also account for the finding that both ON and OFF time decrease as the intensity of stimulation is increased.

The present chapter examines the hypotheses concerning the termination response in terms of how well they also account for reinitiation and the changes with intensity. The term 'hypothesis' is used to refer to an explanation of the termination response. However, the requirement that any explanation of shuttling behaviour must also explain reinitiation and the changes with intensity has been emphasised by reference to a 'model' of shuttling behaviour. The present review deliberately excludes a large

volume of literature on neurochemical aspects of shuttling behaviour since the series of experiments reported here did not investigate this aspect of the reward system. Only where neurochemical findings are particularly relevant to a theoretical point will these be brought into the discussion.

3.1 The reward/aversion model

Some differences may be found in the literature concerning how ON and OFF times should be interpreted in terms of reward and aversion. Gallistel (1973) proposed that "the selection of a preferred train duration reflects primarily the reinforcing effect of [ICSS]" (p. 202). That is, ON time was interpreted as a measure of ICSS reward. De Witte and Bruyer (1980) used a single lever escape procedure as a measure of ICSS behaviour and assumed that: "the less frequently a brain stimulus was switched off, the more rewarding it was" (p. 387). That is, longer ON times indicated greater reward.

More typically, ON time has been interpreted as a measure of aversion. Atrens and Sinden (1975) described the ON time measure as "a straightforward index of aversion" (p. 226). Others have also referred to ON time as an index of aversion or as a 'latency to escape', or an 'escape' measure — terms implying stimulus aversion (e.g., Frutiger, 1986; Schmitt & Karli, 1984; Schmitt et al., 1981; Shizgal & Matthews, 1977; Sinden & Atrens, 1983). The latter interpretation of ON time has gained greater acceptance and will be followed here.

With this interpretation of ON time, OFF time, or the interval between stimulations, has usually been regarded as the index of reward (e.g., Atrens, 1970; Atrens & Becker, 1975; Atrens, Von Vietinghoff-Reisch, Der-Karabetian, & Masliyah, 1974; Liebman, 1983; Mendelson, 1969; Schmitt et al., 1981; Valenstein, 1964). For example, Liebman (1983) has concluded: " [These findings] further support the position that the initiation latency is inversely related to reward value of stimulation" (p. 61). The evidence for these proposals and their implications will be examined under the three features that are required to be explained.

The termination response

Initial explanations for the termination response in terms of aversion implied that an animal terminated stimulation once that had actually become aversive. In other

words, termination represented an escape response. More recently, however, Liebman (1983) has suggested that experienced self-stimulators learn to terminate the stimulation because of impending aversiveness. That is, they have some forewarning of the increasing aversiveness and effectively avoid punishing levels of aversion. Termination by well trained animals is, therefore, essentially an avoidance response (although some minimal amount of aversion may be experienced).

Liebman (1983) has argued that evidence from several sources favours an aversion hypothesis including: overt signs of 'flight-like escape' behaviour, differential effects of drugs and parametric manipulation on ON and OFF times, precision in the regulation of duration, and strain differences.

Not all of these lines of evidence will be re-reviewed here. However, the demonstration by Gerhardt et al. (1982) that various anxiolytic drugs such as pentobarbitol, chlordiazepoxide, and diazepam preferentially increased ON time while leaving OFF time virtually unchanged is significant (also, Stein, 1962). Since this class of drugs is known to relieve anxiety, the increase in ON time could be accounted for by a decrease, or delayed onset, in the aversive component of the stimulation. It is also possible that some anxiety is induced by the stimulation or by the impending aversiveness and this is relieved by these drugs. The observation that 'flight-like escape' behaviour often occurs early in training but then quite rapidly disappears with further training, could also indicate that animals learn to avoid punishing levels of stimulation.

Further support for the view that well-trained self-stimulating animals normally terminate before aversiveness reaches punishing levels may be found in Atrens et al. (1983). On partial reinforcement schedules, initiation rates increased as train durations were increased from 1 second to about 5 to 10 seconds but thereafter declined for durations up to 40 seconds. Thus, durations greater than about 10 seconds could become increasingly aversive. For many of the same animals, an average preferred duration of about 4.6 seconds may be calculated (Atrens et al., 1983, p. 794). Therefore, in the CRF shuttling situation, termination is likely to occur before aversiveness has developed to any significant extent.

Several early reports show that durations in excess of the durations at which rats themselves terminated the stimulation did not produce lower response rates (Hodos, 1965; Keesey, 1964; Keesey & Lindsley, 1962; Valenstein & Valenstein, 1963). For example, Hodos (1965) used a progressive ratio schedule in which each rat was required to make a progressively increasing number of lever presses for successive reinforcements. The breakpoint at which the animal would no longer work to obtain reinforcement was

then taken as a measure of reinforcement strength. Under these conditions, durations of stimulation from 0.15 to 10 seconds were presented as reinforcement. It was found that all rats continued to make an increasing number of responses as the duration was increased. Long durations of ICSS did not appear to become aversive, but instead increased in total reward value. For one rat Hodos presented a stimulus of 2 minutes duration and found that a substantial rate of responding was still maintained at this duration. Keesey (1964) found similar results on a variable interval schedule for durations up to twice that previously self-selected.

Taken together the above results might indicate that initiation rates continue to increase for stimulus durations up to about the preferred duration, or to about twice the preferred duration (or about 10 secs) but thereafter decline. This would suggest that the duration of stimulation provides an increment in reward value over and above that provided by intensity. OFF times should decrease as duration of stimulation is increased from some low value to about the preferred duration. However, these findings may only apply to partial reinforcement schedules, the importance of duration in CRF shuttling behaviour is unclear.

The reinitiation response

Superficially, at least, it would appear unlikely that an electrical stimulus that had most recently been aversive would be reinstated within such a short period of time. Bower and Miller (1958) could not train rats to avoid the place where they had been stimulated, yet it is more usual for animals to avoid places where punishment has been received (Deutsch, 1973; Roberts, 1958a; Stein, 1964). At least two possibilities can be proposed: the aversion may be experienced and hence motivate an escape response but then has no effect on reinitiation behaviour (Deutsch, Roll, & Wetter, 1976; Roberts, 1958a), or an animal terminates before aversion has accumulated to punishing levels (Liebman, 1983).

Whether or not an aversive event will have an effect on subsequent operant responding depends on several factors (Church, 1963; Hanson & Stone, 1964). For example, Bower and Miller (1958) followed 1 sec of ICSS with footshock from which the rat could escape by running to a safe compartment. When the footshock was gradually increased from a low voltage (e.g., 25v – 200v) over 3 sec, rats learnt to escape, but still not avoid, the place where ICSS was obtained. However, if the

same voltage was increased rapidly (e.g., over 1 sec) or began at the high voltage, the rats would learn to avoid the place where they had been stimulated. Valenstein (1965) showed that if rewarding LH stimulation was immediately followed by aversive tegmental stimulation rats would continue to initiate.

Roberts (1958a) suggested that evidence that animals could not learn to avoid the place where they had been stimulated, when that stimulation was presumed to have become aversive, could indicate an amnesic effect similar to that observed following electroconvulsive shocks. Any amnesic effect would be expected to interfere with the elimination of errors during the initial learning of the escape response, but Roberts found there was "excellent" performance of the escape response. Deutsch et al. (1976) found, in a choice situation, that stimulus durations above one to two seconds were equally rewarding. They argued that longer durations should have been preferred if the aversiveness was not remembered since the total reward value of the longer durations was greater.

Some evidence does indicate a disruptive learning effect from ICSS stimulation. Colpaert et al. (1982) found that when ICSS was used as the conditioned stimulus for footshock in a two-way avoidance task, an increase in the train duration from 0.1–1.9 secs significantly increased the number of avoidance errors. This might indicate a disruption of the memory for the avoidance task. Stein and Hearst (1958) reported that discrimination learning was severely retarded if ICSS was paired with performance of the required response. Bull (1968) could not train rats to learn a T-maze if only correct responses were accompanied by LH stimulation, and Steiner et al. (1973) found that there was little transfer of learning from an escape response produced by rewarding dorsal brainstem stimulation to an escape response produced by rewarding LH stimulation.

In summary, the proposal that prolonged ICSS stimulation might have some disruptive learning effect cannot be discounted. But even if further research verified the existence of such an effect, it is unlikely to be strong enough to account for the continued reinitiation that is characteristic of shuttling behaviour. However, as a consequence of the hypothesis that aversion is experienced but has no effect on reinitiation behaviour, the period of time required for reinitiation reflects only the reward value provided by the stimulation. The shorter the reinitiation latency the greater the reward value.

The hypothesis that experienced self-stimulators terminate before aversion actually reaches punishing levels requires no additional assumptions in order to explain

reinitiation. Since an SS does not experience the aversiveness, or only a minimal amount, the period of time required for reinitiation again reflects (inversely) the reward value of the stimulation.

Changes with intensity

A reward/aversion model may explain the decrease in both ON and OFF time if it is further assumed that a rat terminates brain stimulation whenever a certain, fixed level of aversiveness has been reached. Therefore, as intensity is increased, the decrease in ON time occurs because an increased rate of accumulation of aversion reaches the fixed level more rapidly. The decrease in OFF time reflects only the increased reward value provided by the increase in the intensity of stimulation.

The difference between the hypothesis that an SS terminates because the stimulation has already become aversive and the hypothesis that an SS terminates because of impending aversiveness may be interpreted as lying in the point at which the fixed level of aversiveness is assigned; after punishing levels have been reached or at some earlier stage when only some just acceptable level has been reached. The point in time at which the aversion reaches the fixed level is presumed to occur before the preferred duration (to allow some time for the actual termination response to occur). In well trained rats, and under CRF, the preferred duration should be quite close to this point in time.

To choose between the two variants of the reward/aversion model it is helpful to consider the plausibility of the assumptions of each. In particular, if animals are placed in a situation in which they have control over an impending aversive event it would seem unlikely that after some experience they would allow that aversive event to occur. An SS would be more likely to learn to avoid the aversion. The assumption that animals cannot remember the aversion that occurred some few seconds previously, but can remember the reward value which occurred prior to the aversion also seems unlikely. Finally, although some evidence may be found to indicate a disruptive learning effect from prolonged periods of ICSS stimulation the effect is unlikely to be strong enough to account for reinitiation. This cumulative evidence indicates that the model variant that shuttling animals escape aversiveness but that the aversiveness does not influence the reinitiation response, need not be considered further.

3.1.1 Source of the aversion

An important requirement for any model of shuttling behaviour is its compatability with anatomical information. In the case of the 'reward/aversion' model, an important requirement should be the identification of the source of the hypothesised aversion. Again, several possibilities have been proposed.

Current spread

Current spread from the stimulating electrode may progressively activate neurons located near the electrode (Atrens, 1970: Gerhardt et al., 1982; Olds, 1958, 1960; Olds et al., 1960; Schmitt & Karli, 1984). The physical spread of current is relatively fixed since this is determined by the physical characteristics of the electrodes, brain tissue and by the parameters of stimulation (Stein, 1962). The radius of the effective current field is of the order of 0.1–0.3 mm for 60 Hz sine wave (see Section 2.1.1). Two possibilities as to how a different set of neurons may be activated are encompassed in the terms 'latent temporal addition' and 'temporal summation' (Gallistel, 1973).

Latent temporal addition refers to the property that a neuron may be activated by subthreshold stimulation if there is sufficient residual depolaristion remaining from previous subthreshold stimulations for the additive effect to be greater than threshold. Latent temporal addition only occurs in directly stimulated neurons (Gallistel, 1973). Therefore, directly stimulated but high threshold neurons, or neurons on the fringe of the effective current field (Ranck, 1975) may be activated by long durations of stimulation.

Alternatively, post-synaptic neurons may be activated by temporal summation. Work by Smith and Coons (1970) and Ungerleider and Coons (1970) showed that synaptic events in the reward system decayed very gradually (a time constant of decay at least 1.2 secs — Gallistel, 1973). Thus, it might be possible for postsynaptic neural networks to summate incoming impulses over a long period, and hence, to an aversive level. Gallistel (1973) suggested that the long decay time (relative to the stimulus duration) might be due to either (or both) a relatively slow deactivation of neurotransmitter on the post-synaptic membrane or to a relatively slow mobilisation of transmitter substance in the presynaptic terminals.

Perhaps because of different usage of the term (Gallistel, 1973), evidence about the temporal summation properties of the CNS remains unclear. For example, Valenstein (1964) argued that the proposal that stimulation spreads to an aversive system "might

be appropriate to durations in the order of 1 sec but would require a considerable extension of neurophysiological data to be applied to durations above 10 seconds." (p. 422). Gallistel et al. (1981) suggested that temporal summation occurred in the reward system up to 2 secs. Shizgal and Matthews (1977) found that the neural substrate responsible for OFF responding (i.e., ON time) integrated activity over at least several seconds. Atrens et al. (1983) suggested that temporal summation occurred up to 10 secs.

Under continuous reinforcement an animal terminates before the stimulation actually becomes aversive, therefore temporal summation is only required to occur over some duration less than about 10 seconds. Since Atrens et al. used shuttling behaviour, and since this estimate does not conflict with Valenstein (1964) above, temporal summation may be supposed to occur up to about 10 secs. Therefore, either postsynaptic, or directly stimulated, neurons may constitute the source of aversion.

Shizgal and Matthews (1977) found that when burst-width and inter-burst interval were manipulated over the stimulus duration, the neural substrate that determined ON time integrated activity over at least several seconds. However, the neural substrate that determined OFF time reached its maximal effect within 1 sec of stimulus initiation (at the particular frequency and pulse durations used). These results are consistent with the interpretation that the initiation of stimulation rapidly activates the reward system but that neighbouring aversive system(s) are more slowly recruited, perhaps due to greater distances from the electrode, because of higher thresholds (Ranck, 1975), because of different spatial distributions (Shizgal & Matthews, 1977; Stein, 1962), or because of slowly decaying synaptic processes (Gallistel, 1973).

The idea that neural elements in the medial hypothalamus might be involved in producing the aversive effect has received some attention since it is from this region that the lateral hypothalamus and the ventromedial hypothalamus have been conceived to be "functioning reciprocally or in balanced opposition" (Atrens & Von Vietinghoff-Riesch, 1972, p. 229; also Hoebel, 1984; Olds & Olds, 1962; Rolls & Rolls, 1982; Schmitt & Karli, 1984; Valenstein & Valenstein, 1964). Schmitt and Karli (1984) found interactive effects of lateral and medial hypothalamic stimulation which they suggested were best accounted for by being related to the affective (i.e., rewarding and aversive) properties of the stimulation. Atrens (1973), however, found that strong reward effects were found near the midline in the paraventricular region of the hypothalamus, although this region traditionally has been associated

with aversion. Gratton and Wise (1983) found that stimulation of more medial sites in the LH could evoke behaviour that was indicative of aversiveness and this was most obvious at high intensities.

In general, evidence from detailed mapping studies with moveable electrodes (e.g., Corbett & Wise, 1979, 1980; Gratton & Wise, 1983; Wise, 1981) does not indicate that positive ICSS sites are surrounded by negative sites even when the depth of the stimulating electrode was moved 0.125 to .250 mm at a time (i.e., within the effective current field). Instead response rates and thresholds change gradually as the electrode is lowered. A difficulty with these mapping studies is that only lever press responding was tested. The results of a mapping study that tested for shuttling behaviour would be unlikely to give exactly the same distributions (Margules, 1966).

Margules (1966) suggested that the progressive activation of aversive systems was particularly likely in areas of the diencephalon that border on the thalamic-hypothalamic-central grey areas. However, Atrens (1973) did not support this. Stein (1962) suggested that evidence that negative cell groups were located posteriorly in the rat brain (Olds, 1958, 1960) favoured an aversion hypothesis in regions such as the hypothalamus and midbrain. However, Atrens et al. (1983) found that rats with more anterior LH electrodes terminated readily in a choice situation. This was interpreted as indicating that aversion was more likely to occur at the more anterior hypothalamic sites.

The 'pain' system of the CG region has also been implicated as a possible source of aversion (e.g., Olds & Olds, 1962; Schmitt et al., 1981; Stein, 1964; Valenstein, 1965). Schmitt et al. (1981) found that the mechanisms implicated in the performance of an escape response elicited from the 'pain' systems of the CG had similar excitability properties to those mechanisms responsible for the termination response elicited from the medial or lateral hypothalamus. However, similar excitability properties may only indicate that similar neural mechanisms are involved whenever a rat interrupts an applied stimulation, they do not specifically support the interpretation that the interruption occurs because of aversion.

Conclusive evidence to support the idea that the termination response occurs because of progressive activation of nearby aversive systems is lacking. A lesion placed between an ICSS site and the presumed aversive system should predominantly eliminate the termination response. However, there is almost no experimental work concerning the effects of lesions on shuttling behaviour.

Autonomic aversion

Still another explanation of the termination response is possible in terms of punishment concepts. Prolonged periods of stimulation might induce a discomforting buildup of autonomic activity, so that the stimulation is terminated in order to gain respite from excessive shifts in heart rate, body temperature, respiratory rhythm and numerous other physiological states known to be produced by ICSS (Ángyán, 1976; Atrens, 1970, 1973; Halperin & Pfaff, 1982; Ikegami & Kawamura, 1981; Mendelson, 1969; Sadowski, 1976; Valenstein & Valenstein, 1964). Ángyán (1976), for example, recorded blood pressure and respiration rate from cats responding for ICSS in either a lever press or single lever procedure. The results showed that the cats stopped responding when autonomic responses increased to a certain level and started to respond when they decreased below a certain level. Moreover, an initial warm-up period in responding correlated with the time lags inherent in peripheral autonomic responses. After the initial warm-up period, the cats would maintain a fairly constant level of autonomic activity.

Sadowski (1976) suggested that ICSS changed the set point of basic regulatory mechanisms and, in particular, the set point of energy homeostasis (i.e., temperature set point). The activities of both the sympathetic and parasympathetic divisions of the autonomic system were suggested as balancing at a higher level.

Although evidence exists to support the idea that there is a relationship between peripheral and central mechanisms during ICSS, a causal role has not been identified. Ward and Hester (1969) found that ICSS in cats was unaffected by sectioning of autonomic outflow. Ball (1974) showed that subdiaphragmatic vagotomy did not abolish ICSS, although it did significantly raise threshold. This evidence suggests that ICSS is essentially due to central processes, perhaps modulated by peripheral feedback.

3.1.2 Conclusions

Several lines of evidence indicate that aversiveness is involved with the termination response at many brain sites. Moreover, the hypothesis that motivation for termination derives from aversiveness, either impending or actually experienced, can be developed to explain the three major features of shuttling behaviour. The most parsimonious basis for a reward/aversion model includes the hypothesis that experienced rats normally terminate before aversiveness has accumulated to punishing levels.

Several aspects of the reward/aversion model are still not clear from the above review. In particular, the fate of the initial reward effect has not been specifically considered. The reward effect gained on initiation may remain at its initial value (Deutsch et al., 1976; Shizgal & Matthews, 1977) and eventually be outweighed by the increasing aversiveness; or the initial reward effect may decline in some way (adapt) as the aversiveness is increasing (Atrens et al., 1983). The latter possibility would require less aversion to accumulate than the former. Also, the basis for what leads an animal to terminate needs to be considered. Increasing aversiveness alone could be compared to some fixed criterion of acceptable aversion, or a comparison made between the residual reward effect, the increasing aversiveness, and the fixed criterion. The latter possibility suggests a more dynamic process in which a rat shuttles in order to titrate the reward effect against the aversive effect and hence manage a conflict situation (Liebman, 1983, 1985).

The present research is aimed at finding a means of describing the basic features of shuttling behaviour in a way that is amenable to modelling and simulation. To this end the above interpretation of the reward/aversion model satisfactorily accommodates the three features described previously. But more information is required in order to define the reward/aversion model more clearly, particularly with regard to the relationship between ON and OFF time and the relationship between these times and intensity.

3.2 Adaptation

Roberts (1958b) and Stein (1962) suggested that ICSS stimulation might be terminated because the initial rewarding effect had adapted to some near zero level. Animals therefore reinitiate in order to restore the full reward effect on the next initiation. Adaptation is a property of nerve fibre that has been well studied in sensory neural systems and isolated nerve preparations which describes the finding that frequency of action potentials in the stimulated nerve tends to decline while ever a constant rate and intensity of stimulation is applied. Some time after the stimulation has been removed, the responsiveness of the nerve fibre returns to normal resting levels (Gray, 1959; Katz, 1966; Kuffler & Nicholls, 1976; Ochs, 1965). Adaptation may be a property of all excitable cells (Katz, 1966).

In sensory systems, the rate or degree of adaptation varies considerably depending on which system is being stimulated. Touch receptors adapt readily; muscle spindle

receptors tend to adapt quite slowly (Ochs, 1965). In nerve-muscle preparations, the level to which frequency of firing adapts is related to the intensity of stimulation, such that greater weights applied to the muscle result in a higher adapted level of frequency (Gray, 1959). Ochs (1965) has also stated that the rate of impulses excited in the sensory fibres is related to the intensity of the applied stimulus in a regular and consistent way, which is approximated by the logarithm of the applied stimulus (which in turn may be interpreted to imply the maintenance of a constant signal-to-noise ratio — Rushton, 1961).

The termination response

Adaptation in the neural system that subserves ICSS might be demonstrated by techniques that have been used to test adaptation in sensory or peripheral systems. If the neural substrate responsible for the reward effect adapts to a steady level of stimulation, then a rapid increase in the intensity or frequency of stimulation (without withdrawing the stimulation) should reinstate the reward effect. Because an increase in intensity would activate new, unadapted neurons in CNS tissue, experiments that have tested for adaptation in the CNS use increased frequency rather than increased intensity.

Deutsch and Hawkins (1972) found that after rats were given one second of stimulation at 100 Hz in the startbox of a Y-maze, they would usually prefer a further one second of 200 Hz stimulation rather than terminate the stimulation or receive a further one second of the same stimulation. However, if the same rats were given a choice between a further one second of 100 Hz stimulation and termination, they tended to prefer the termination. This suggested that the increase in stimulation to 200 Hz induced a significant increment in reward value over that provided by the continuation of 100 Hz or over that provided by immediate termination. The authors interpreted these results as being more consistent with the hypothesis that positive rewarding stimulation adapts. The fact that animals significantly preferred termination of stimulation to continuation at the same frequency was explained as poorer learning since the next reward stimulation could not occur until the start of the next trial.

Atrens et al. (1983) criticised the use of a period of only one second of forced stimulation and one second of increased frequency in the Deutsch and Hawkins (1972) experiment. They argued that if aversive effects did develop from the stimulation,

they may not become apparent until after one second. Atrens et al. (1983) repeated the experiment with 5 secs of forced 100 Hz stimulation and 5 secs of 200 Hz stimulation. Results showed that animals with more anterior hypothalamic electrodes chose termination while animals with more posterior electrodes chose increased stimulation. The direction of choice was not correlated with each animal's preferred duration. For example, of two rats which had previously selected a 3 sec ON time, one animal chose a further 5 sec of 200 Hz stimulation on 90% of occasions, while the other chose termination on 90% of occasions. Adaptation might explain the termination response but only in those animals stimulated in more posterior locations of the hypothalamus.

Finally, Deutsch et al. (1976) have pointed out that even if the reward effect does adapt, all rats may not necessarily terminate the stimulation at the same level of adaptation. Some rats may maintain the low adapted level significantly longer than others. This suggests that ON time under an adaptation hypothesis would be far more variable than under the assumption than ON time represents the control of an aversive system.

The reinitiation response

If a rat terminates in order to reinstate the full reward effect contingent on the next initiation, the period of time with the stimulation OFF largely reflects the time for the underlying neural substrate to recover from adaptation. OFF time must also reflect to some extent the reward value of stimulation. For example, at higher intensities, and under CRF, the reward effect obtained on initiation may be sufficient to outweigh the necessity for full recovery from adaptation (Atrens, 1984).

The implication that the termination response is reinforced or maintained by the reward obtained on the subsequent reinitiation would suggest that if the opportunity for reinitiation was not provided the rat would learn to maintain the low adapted level of reward. Mendelson and Freed (1973) allowed rats only one initiation per test session, yet despite the fact that the next opportunity for stimulation was at least two hours away, the rats showed no signs of extending the single duration of stimulation.

Changes with intensity

A significant argument against the hypothesis of adaptation is provided by the finding that both ON and OFF times tend to decrease as the intensity of stimulation is increased. At higher intensities the stimulation would be expected to take longer to

adapt or adapt to a higher level (Gray, 1959). In either case, if animals terminate the stimulation because the rewarding effect has adapted, at higher intensities the stimulation should remain on for longer periods (Deutsch, Chisholm, & Mason, 1980; Stein, 1962; Valenstein & Valenstein, 1964). However, this has usually not been the case (Section 2.2).

Deutsch et al. (1980) have argued that adaptation does take a longer time to occur if intensity or frequency of stimulation is increased. At low reward values (i.e., low intensity or low frequency) rats showed no preference for 1 sec stimulation over 0.5 sec stimulation in a choice procedure. However, at high reward values (i.e., high intensity or high frequency) rats significantly (with two animals) preferred the 1 sec reward. This was interpreted as indicating that at higher intensities the reward effect takes longer to adapt since only then does the reward last sufficiently to provide discrimination. This still does not explain why rats select shorter ON times at higher intensities in free-shuttling situations. Because rate of adaptation was independent of whether high reward was produced by high intensity or high frequency, Deutsch et al. (1980) also argued that rate of adaptation is due to properties of the neural substrate after the stimulation has been translated into reward. This might imply that adaptation occurs in post-synaptic networks. However, if adaptation does occur in CNS, it must at least occur in the directly stimulated substrate.

Atrens (1984) has argued that "the magnitude of positive reinforcement, and the rate at which positive reinforcement adapts, are independent" (p. 241). Although inferences about 'positive reinforcement' may not be sound when CRF procedures are used (Section 2.3.1), the extent to which an increase in intensity provides an increase in the magnitude of positive reinforcement indicates that rate of adaptation and reinforcement must be related. If ON time under CRF may be accounted for by adaptation, the decrease in ON time as intensity is increased indicates faster adaptation at higher intensities.

Several reports indicate that the reward effect of stimulation reaches a maximum after about one to two seconds (Deutsch et al., 1976, 1980; Gallistel, 1974, 1978; Shizgal & Matthews, 1977). If this reward effect then adapts, a gradual shortening of ON times should occur with training to a level commensurate with the rat's ability to discriminate the stimulus durations. If the animal terminates ICSS in order to reinstate the full positive stimulation, and at the same time could not discriminate durations greater than say, two seconds, then the chance occurrence of shorter durations would be differentially reinforced because the reward state would be reinstated

sooner than would otherwise occur (Anger, 1956). ON times should approach an approximate value of 2 seconds. Others have reported that ON time stabilises for each rat after a number of sessions, but usually at values greater than two seconds (Atrens, 1970, 1973; Atrens et al., 1983; Mendelson, 1969). A problem here is that choice measures used to estimate the duration of the reward effect do not correlate well with consummatory measures (Atrens et al., 1983; De Witte & Bruyer, 1980; Deutsch et al., 1976; Section 2.3.1). Atrens et al. (1983) have shown that the very first ON time is the shortest so the estimate of one to two seconds might only apply to the first ON time.

A greater difficulty with accepting an absolute time for the duration of the reward effect is that both ON and OFF times decrease as intensity is increased. A reference to duration of ON or OFF time also requires reference to where on the intensity-duration function the measure was taken. Animals also vary quite markedly in the relative allocation to ON or OFF time depending on electrode location and stimulus parameters (e.g., Atrens, 1970, 1973; Schmitt et al., 1981). More useful comparisons among animals might be obtained by some measure other than simply ON time or OFF time, such as the charge accepted, or the ratio of ON time to OFF time (Liebman, 1983). Part of the present research is concerned with determining the most appropriate measures of shuttling behaviour under CRF.

3.2.1 The reciprocal inhibition model

The difficulty encountered by the adaptation hypothesis in explaining the decrease in ON time as intensity is increased, may be overcome by postulating the arousal of an inhibitory system which actively contributes to the declining reward value. That is, rather than firing rate of stimulated neurons passively declining, increased activation of an inhibitory system might actively contribute to the decline. An increase in intensity could activate the inhibitory system more quickly and hence the greater inhibition suppresses the reward value more quickly and ON time decreases.

A possible model of neuronal interaction that might satisfy these conditions is referred to here as a 'reciprocal inhibition' model (Furman, 1965; Gregson, 1983; Harmon, 1964; Ludlow, 1976, 1980; McDougall, 1903; Reiss, 1962; Selverston, Miller, & Wadepuhl, 1983) and has been described, in a general way, with reference to ICSS behaviour by Stein (1964) and Routtenberg (1968).

The reciprocal inhibition (RI) model[1] has been used to describe a number of brain-behaviour relationships, including the simulation of muscle contractions (Reiss, 1962), retinal image-enhancement (Furman, 1965), rhythmic gastric contractions (Selverston et al., 1983), and more recently as a decision maker in the allocation of feeding, drinking, singing and preening bouts (Ludlow, 1976, 1980). Stein (1964) used the RI model, in a general way, as an explanation for the interaction between pain and reward systems.

The RI model (see Figure 1) has usually been described in terms of two interacting neurons (e.g., Reiss, 1962). However, this is not a necessary requirement; Ludlow (1976, 1980) used the RI model while assuming the two components represented interacting systems or subsystems of neurons. For the present discussion the operation of the RI model will be interpreted in terms of two neurons.

The essential feature of the model is the ability for switching behaviour to develop, so that for a suitable choice of inhibitory parameters G(a) and G(b), and a constant input frequency, Neuron A may be caused to fire for a period of time, while Neuron B remains suppressed. After a period of repeated firing by Neuron A, fatigue (Reiss, 1962) or adaptation (Ludlow, 1980) develops, so that its ability to keep Neuron B suppressed also gradually weakens. Eventually, Neuron B reaches a critical level of excitability and begins to fire. Because of the reciprocal inhibition, Neuron A will then be suppressed until fatigue (or adaptation) begins to reduce the firing capacity of Neuron B. Cyclic periods of dominance of one neuron over the other develop which continue indefinitely while ever a constant rate of input stimulation is reaching both neurons (Ludlow, 1980; Reiss, 1962).

The parameters G(a) and G(b) refer to the inhibitory connections between the two neurons (i.e., the connections with filled circle endings in Figure 1). Both of these parameters need to be greater than one for switching behaviour to develop. Also, in the model as described by Reiss (1962), other properties of neurons were modelled; including the responsivity of each neuron to the incoming excitatory stimuli, the rate of decay of the 'membrane' after each 'firing', and time delays for transmission time between neurons. Each of these properties requires at least one other parameter to be specified. For the present discussion all of these parameters are assumed to remain constant.

Further developments of the basic RI unit described in Figure 1 have been made by

[1]The author is indebted to Professor R.A.M. Gregson for the initial suggestion of a reciprocal inhibition model.

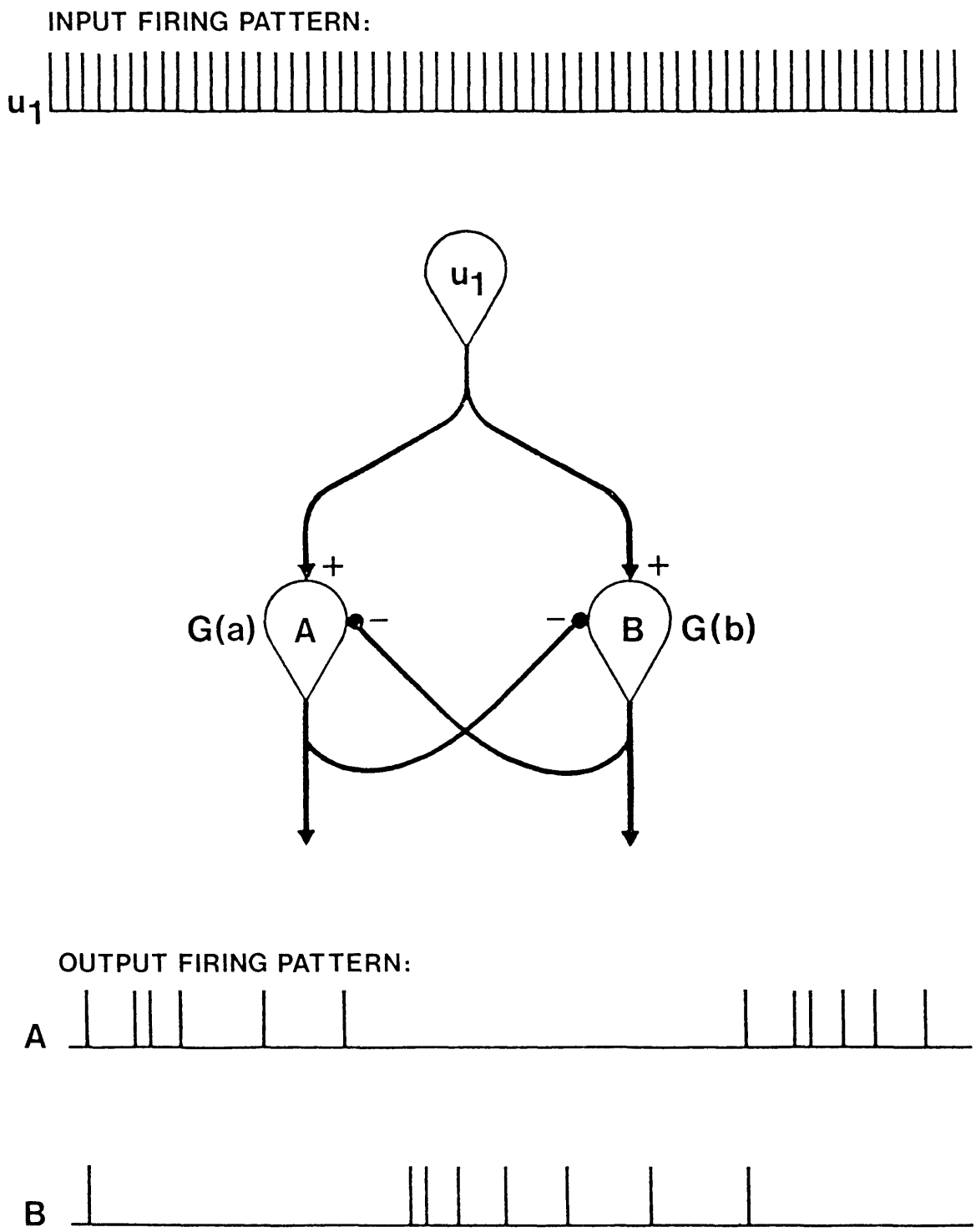

Figure 1: The reciprocal inhibition model.

Furman (1965), Selverston et al., (1983), Harmon (1964) and Ludlow (1980). Furman, for instance, investigated the effect of combining 100 of these units under four different coupling arrangements. A shunting condition was developed to incorporate properties of current flow in neurons when both excitatory and inhibitory endings terminated on a particular neuron.

Ludlow (1976, 1980) used the RI model as a decision making unit in a larger model which incorporated the concept of interacting systems or subsystems. Ludlow (1980) was interested in determining when particular behaviours (e.g., eating, drinking, singing, preening) would dominate, and the magnitude and length of the periods of dominance. Ludlow showed that a simple neuronal (or system) concept could be used to approximate quite complex behaviour patterns.

The termination and reinitiation responses

The simplest form of the reciprocal inhibition model, and as described here in relation to shuttling behaviour, assumes that the timing of both the initiation and termination response is involuntary. Although this is speculative, the RI model can explain the basic features of shuttling behaviour examined previously for the reward/aversion model.

Fibiger (1978) has argued that the termination response is a respondent or 'involuntary' response which is controlled by a neural system separate from that which controls the operant or 'voluntary' initiation response. Atrens et al. (1983) have shown that there is a large reduction in termination responding if ON time is fixed at 10 sec. Therefore, the actual termination response is unlikely to be completely respondent. Because rats will also lever press, enter another arm of a maze, or withdraw a nosepoke, in order to terminate rewarding brain stimulation (e.g., Atrens & Becker, 1975; Roberts, 1958b; Schallert, 1985), the complex skeletal muscle responses necessary to do these things are unlikely to be involuntarily elicited by the stimulation.

But under CRF schedules, the timing of the termination response, or the timing of the signal to terminate, may be involuntaily elicited rather than the response itself. A rat may have little control over the timing of the termination response once that response has been well learnt. Atrens (1984) has argued that the termination response includes a significant element of elicitation.

If the timing of the termination response can be described as involuntary, then

it may be possible to describe the timing of the initiation response as involuntary. With CRF schedules, Atrens et al. (1983) have claimed that: "each stimulation train not only reinforces the previous response, it also elicits or forces the next one" (p. 790), and "hypothalamic stimulation produces forcement processes which become the principle (sic) determinant of initiation behaviour" (p. 790). Gallistel (1969) has also argued that each stimulation has a significant effect on subsequent response performance. To the extent that the timing of the termination and reinitiation responses under CRF may be considered involuntary, the RI model may provide a reasonable approximation.

Changes with intensity

Reiss (1962) showed that the periods of dominance of one neuron over the other were dependent on the frequency of the input stimulation. Ludlow (1980) showed that the magnitude of the stimulation could also be used to modulate the periods of dominance. For example, (from Reiss, 1962), for a particular set of parameters, an input frequency of 350 pulses/sec results in Neuron A being dominant for some 85 msec and Neuron B for 110 msec. At 450 pulses/sec, the dominant periods were reduced to 40 msec and 55 msec respectively.

For cyclic periods of dominance and for changes with intensity both to occur, the RI model requires a continuous input of stimulation to both neurons. In shuttling behaviour, the animals alternate between a period of time in direct contact with the stimulation and a period with no contact. If the RI model were to apply to shuttling behaviour, an hypothesis proposing continuous input to the RI unit would be needed. This may not be so difficult; a number of theories have proposed neural feedback or reverberatory mechanisms that could accomplish this (Bunney, 1983; German et al., 1980; Grastyan, 1968; Routtenberg, 1968; Szabó, 1973).

3.2.2 ICSS and inhibition

There is no direct evidence for the control of behaviour, and in particular, ICSS behaviour, in terms of an RI model. However, the evidence for inhibitory CNS mechanisms has received considerable support (e.g., see McGeer, Eccles, & McGeer, 1978). For example, dopamine has been argued to be an important neurotransmitter in ICSS behaviour and the release of dopamine has a predominantly inhibitory effect on the firing rate of DA neurons. German et al. (1980) have suggested that DA

neurons in the VTM can be self-inhibited or collaterally inhibited. The concept of inhibition has also figured prominently in some theoretical explanations for ICSS (e.g., Endröczi & Lissák, 1966; Grastyan, 1968; Routtenberg, 1968). Routtenberg (1968) specifically proposed a reciprocal inhibition model for ICSS to describe the interaction of two arousal systems (generally, the reticular activating system and the 'limbic' reward system).

More specific evidence for the involvement of inhibitory mechanisms in ICSS behaviour has been described by Hamburg (1971), Ito (1972, 1976), Ito and Olds (1971), Olds (1973), and Olds and Fobes (1981). Olds (1973) reported that many neurons of the reward system located near an ICSS electrode were inhibited by the stimulation, while neurons farther removed were excited. Catecholaminergic drugs that counteracted the rewarding effects of ICSS were effective against the local inhibition, but had little effect on distant excitation. Also, neurons switched off by ICSS stimulation were also switched off during normal feeding behaviour. Olds (1973) therefore claimed that the data suggested: "that local inhibitions might be more importantly related to brain reward than were the more distant excitatory actions" (p. 38).

Although conclusions drawn from unit recordings must be considered tentative (Olds & Fobes, 1981), involvement of inhibitory mechanisms in ICSS is indicated.

3.2.3 Conclusions

The RI model is proposed as a mechanism, and conceptual basis, for explaining the reinitiation of stimulation and the decrease in both ON and OFF times with increasing intensity, characteristic of shuttling behaviour. The model incorporates the neural property of adaptation (Deutsch, 1973; Katz, 1966), combined with an active, mutually inhibiting mechanism which can explain oscillatory behaviour. The adaptation hypothesis of Deutsch (1973) accounts for ON time by a process of passive decline in responsivity. Reciprocal inhibition, on the other hand, proposes that active inhibition also contributes to the determination of ON time. An increase in the intensity or frequency of input serves to increase the activity of both components, with each component becoming alternately dominant and suppressed at a faster rate. The point at which the switch-over occurs could provide a signal to the animal to initiate or terminate. Under CRF procedures, the timing of the switch-over might be approximated by the timing of the two responses.

The RI model, like the reward/aversion model, has difficulty accommodating

the results of the detailed mapping studies of Wise and his colleagues because the moveable electrodes would be expected to pass through regions responsible for the inhibitory effect and hence be unresponsive to ICSS stimulation.

3.3 Other hypotheses

Instead of behaviour being reinforced by the withdrawal or avoidance of an aversive stimulus, the termination response may be accounted for by positive reinforcement (Roberts, 1958b; Keesey, 1964; Hodos, 1965; Atrens et al., 1983). There might be something positively reinforcing about the offset of stimulation, as well as its onset; and both types might be quite different. Roberts (1958b) suggested that both the onset and offset of stimulation were rewarding, but that the onset reward effect had a lower threshold than the offset reward effect.

Perhaps the period of no stimulation, or the expectation of a period of no stimulation acts as positive reinforcement. Stein (1965) and Margules and Stein (1968) provided data consistent with the view that the adaptive operant component of avoidance behaviour depends on positive incentive for its maintenance. By a rebound effect, the positive reinforcement system goes through a brief period of increased activity upon removal of the aversive stimulus. Moreover, "the positive reinforcement system serves as a common mechanism for the facilitation of all operant behaviour, whether controlled by positive or negative reinforcement" (Margules & Stein, 1968, p. 182).

If a common mechanism of reinforcement exists in the CNS, it may function in a manner similar to that described for the opponent-process (Solomon, 1980; Stellar & Stellar, 1985). The opponent-process has been advocated as a basic property of the nervous system (e.g., Hurvich & Jameson, 1974) and describes the induction of powerful motivational states by the repeated arousal of affect of opposite sign. Thus, aversive consequences might be integrally contingent on the activation of reward (Montgomery et al., 1981).

Finally, there may be an explanation for ICSS behaviour in terms of the finding that animals will alternately switch on and off any stimulus placed under their control (e.g., Davis, 1958; Kavanau, 1964; Roberts, Marx, & Collier, 1958). For example, Davis (1958) showed that rats will lever press for weak light reinforcement and response rates increase with food deprivation.

The predominant explanations for why an animal terminates apparently rewarding brain stimulation have been the aversion and adaptation hypotheses (Liebman, 1983). Two positive processes, opponent processes, or still other processes, may explain the three features of shuttling behaviour but they are not considered further here.

3.4 Single or dual system substrates

The issue of whether a single neural substrate is sufficient to explain ICSS behaviour or whether two or more simultaneously activated systems are required, has not been resolved. Phillips (1984) has suggested that anatomical and pharmacological evidence implicates multiple independent substrates.

A single system may be invoked under an aversion hypothesis if it is assumed that brief stimulation produces one affective state while prolonged stimulation of the same system produced an opposite state (e.g., as an opponent process). However, proposals for an aversive hypothesis have usually supposed that current eventually spreads to activate aversive fibre systems located at some distance from the electrode, and therefore at least two systems are involved. The RI model would also require the identification of two systems.

By contrast, Deutsch and Albertson (1974) and Skelton and Shizgal (1980) have argued that the adaptation hypothesis relies on one affect only and may therefore be accommodated by the existence of a single fibre system. Evidence for a single or dual system substrate has come from at least three sources. Each will be discussed briefly.

Anatomy

Anatomical evidence shows no differentiation of the neural substrate into two distinct components. For example, Szabó, Lénárd and Kosaras (1974) found no difference in the diameter spectra of myelinated axons, regardless of the distance of the analysed sample from the electrode tip. They also found no evidence to indicate two different sets of fibres in the VTM compared to the LH. Gratton and Wise (1983) accurately mapped hypothalamic ICSS sites and found that: "the present data suggest a single substrate of MFB ICSS" (p. 28).

Excitability

Refractory period estimates and chronaxie/rheobase measurements have also not satisfactorily resolved the question of whether one or two neural systems determine ON and OFF responding. For example, Deutsch and Albertson (1974) measured refractory periods of neurons involved in initiation and termination responses and reported no difference. This suggested that one set of fibres supported both types of responses and, more likely, an adaptation hypothesis. However, Schmitt, Sandner, and Karli (1976; cited in Skelton & Shizgal, 1980) reported similar but not identical refractory periods for the directly stimulated fibres, and consequently proposed that two different systems supported shuttling behaviour.

Skelton and Shizgal (1980) used frequency threshold-scaling methods (Yeomans, 1975) and concluded that the refractory periods for both the initiation and termination responses were the same, supporting a one-system view. However, differences in the magnitude of local potential summation (LPS) effects for the ON and OFF responses indicated different spatial densities of the neurons involved. Skelton and Shizgal (1980) argued that this was sufficient evidence to support a two-system substrate. The significant LPS effects were in opposite directions suggesting that more research is required into this finding.

In any case, a lack of differentiation on anatomical grounds, or on the grounds of different excitability characteristics, does not imply a functional differentiation. Fibres with the same excitability characteristics or the same dimensions may still be associated with different behavioural functions.

Collision effects

Bielajew and Shizgal (1980) used the two electrode collision technique described earlier (Section 1.3.1; Shizgal et al., 1980), to argue that different populations of directly stimulated fibres were responsible for the ON and OFF responses. Four rats, each with two electrodes (in the LH and the VTM) and of which three had previously shown collision effects for lever press ICSS, were tested for escape on a single lever. Three of the four rats failed to show collision effects. Since the same animals had shown these effects for lever press ICSS (at the same parameters), a different population of neurons was held responsible for the escape response.

These results must be viewed with some caution. Single lever escape measures do not always correlate well with shuttling measures (Atrens & Becker, 1975; Keesey,

1962; Valenstein, 1964), and the automatic presentation of stimulation may not necessarily be equated with automatic termination (e.g., Faircloth, 1974; Steiner, Beer, & Shaffer, 1969). Evidence for this may in fact be shown by Bielajew and Shizgal's own report, in which "seizures began to develop during the stimulation-escape tests and eventually forced their termination" (p. 710).

Other evidence

Several lines of evidence (Section 2.2.1) suggest that ON and OFF time are separately manipulable. Such evidence would most readily be interpretable within a two system view — both systems activated simultaneously but with different response characteristics. The two systems may or may not be completely independent.

Shizgal and Mathews (1977) manipulated the temporal properties of the stimulus delivered during ICSS so that the duration of application was broken into bursts of pulses separated by intervals of no stimulation. In a two lever procedure, trade-off functions between burst width and intensity, and between interburst interval and intensity were derived for both ON and OFF time measures. Shizgal and Matthews hypothesised that if there were two systems underlying the ON and OFF responses, the two may be differentially sensitive to such manipulations. Rate of buildup of activity in the system with slow response characteristics should be most affected by a shortening of burst width, since this system might not be able to respond quickly enough to accumulate activity to a level sufficient to affect behaviour. The converse should apply to a system that responded vigorously with each burst of stimulation. Shizgal and Matthews found that the intensity increase required to maintain ON responding was consistently smaller than that needed to maintain OFF responding. Therefore, the 'reward' system (ON responding) was identified as a rapid accumulator of excitation, and the 'aversive' system (OFF responding) as a slow accumulator of excitation. The authors claimed that at very short burst widths, OFF responding ceased entirely while ON responding continued (this should only be interpreted to mean that ON time exceeded 20 seconds, since at that point stimulation was automatically terminated and another cycle begun).

In summary, evidence for a single system substrate or a two system substrate remains equivocal. The evidence is probably best interpreted to suggest a two system arrangement, but with the same excitability characteristics. Skelton and Shizgal

(1980) suggested that ON and OFF responding were mediated by different populations of directly stimulated neurons with similar distributions of excitability characteristics but different spatial distributions.

3.5 Conclusions and current research

For shuttling behaviour to be modelled, three features require explanation: the termination response, the reinitiation response, and the finding that both ON and OFF times decrease as intensity of stimulation is increased. On this basis, two alternative models may be developed which satisfactorily accommodate these features. These are referred to here as the reward/aversion model and the reciprocal inhibition model. Neither of these models can unequivocally satisfy all the available evidence but they may, at least, provide a starting point for further investigation.

The present research recognises the approach of Routtenberg (1968), Swanson and Mogenson (1981) and Wise and Bozarth (1984) by attempting to integrate evidence from several directions which might eventually be incorporated into an overall model. Routtenberg (1968) suggested that "An important direction for research would consist in attempting to model the type of 'theoretical explanation' and determine whether there is any relation between the model and the biological system" (p. 72). Such an approach, he argued, would permit quantification of the degree of influence of the respective components on the system as a whole. Routtenberg (1968) also stressed the importance of a consideration of the time course of events in the behaviour of the system under study. With particular reference to his own two-arousal theory, Routtenberg proposed that "the organism regulates its behaviour such that there exists a dynamic balance of activity between these two systems" (p. 63). The two systems were conceived to be "in constant activity, and that reciprocal suppression allows for the two systems to be in a dynamic equilibrium; first one is active then the other" (p. 63).

Because of considerable gaps in the literature concerning the time course of ICSS behaviour and the relationship between ON and OFF time measures, the present research program merely attempts to define more clearly what data any model, reciprocal inhibition or otherwise, must explain. The actual process of matching theoretical models to experimental data and formalising into mathematical relationships has not been considered here. Most likely the development of any model would over-simplify the actual mechanisms involved. Ludlow (1980) has warned against the dangers of

"over-simple reductionism" (p. 276) when attempting to apply simple models to the complex processes of behaviour.

Current research

In order to define more clearly the data to be explained, the feature that both ON and OFF time decrease as intensity is increased needs to be examined more closely. Both of the models considered here assume an increase in intensity produces decreased ON times. However, the decreased OFF time may be due to either the increase in intensity or the decreased ON time that also occurs. OFF time might decrease directly because of the intensity increase or indirectly because of the prior decrease in duration of stimulation. A large part of the current research is directed towards differentiating the effects of intensity and duration on OFF time (under CRF).

If OFF time were determined principally by the duration of stimulation, a significant correlation should be demonstrable between the two times. A significant correlation would support a single system view of the neural substrate which, in turn, would not support either of the two models being considered here. OFF time might then be due to a rebound effect of the stimulated substrate. Evidence reviewed above suggests this is not the case, however, the evidence is inconclusive and not encompassing enough for the possibility to be dismissed.

Alternatively, OFF time might be directly determined by intensity. This alternative would be supported by default, since if the period of time over which the stimulation is applied has no effect on OFF time, and if all other variables are held constant, OFF time must be primarily determined by intensity *per se*. In this case, a two system view of the neural substrate of ICSS would receive stronger support.

It is also of theoretical interest, in either of the above cases, to try to determine which OFF time, in a series of ON/OFF times, is most related to intensity. For example, it may be that the immediately following OFF occurs as a consequence of the previous stimulation (because of priming or forcement effects). It may also be the preceding OFF time which is most related to the stimulation, that is, an 'expectancy' or 'anticipatory' effect occurs (Stein, 1964), as implied in the term "latency to initiate". Also, within a particular period of ICSS interaction, the relationship between successive ON times, or successive OFF times, might reveal the extent of the contribution of previous ON and OFF times on the current ON or OFF time (i.e., autocorrelation). It should be possible to differentiate the effects of past responses from the effect of the

immediate stimulus (by the use of Box-Jenkins time series methods).

Experiment I (Chapter 5) examines in detail the relationship between the three variables: ON time, OFF time and intensity. Experiment II (Chapter 6) examines the changes in OFF time that occur as the duration of stimulation is increased at a fixed intensity. As a control, and with another group of animals, OFF time was increased over a similar range while ON time was free to vary. Finally, Experiment III (Chapter 7) examines the relationship between ON and OFF time as a function of within-trial changes in stimulus parameters. Within-trial changes in stimulus parameters were used in order to assess which OFF time was most strongly associated with a particular set of stimulus parameters.

On the basis of the behavioural changes that occur as a result of experimental manipulations, two models will be examined and discussed in order to assess their potential for explaining the observed changes. If a simple, plausible model could be found for shuttling behaviour then the model might suggest alternative approaches and concepts in the further study of ICSS.

Chapter 4

General method

A total of 98 animals were involved in the two series of experiments to be reported. Procedures adopted for the selection of subjects, housing and care of subjects, surgery, early training and screening, and several other factors were constant for the two series. These procedures and also details concerning the apparatus used are described here. Specific details for each experiment are described in the Method section for each experiment.

4.1 Subjects

4.1.1 Care and housing

Subjects for each of the two series of experiments were male, albino rats (Wistar strain), weighing from 280 grams to 520 grams at the time of operation. The rats were obtained from the University animal house, where they had been in colony cages, and transferred to individual cages. They were maintained under conditions of 12 hours light/dark cycle and constant temperature (22° C ±2°). Food (Fielders Stock Food) and tap water were available *ad libitum*. The diet was supplemented with bread, fruit and vegetables.

In general, the animals were in good condition for the duration of the experiments (up to seven months with electrode implants) but there were some problems with mites, a skin condition, and a respiratory complication (pneumonitis).

The mites, which burrowed into the skin to cause scratching, bleeding and loss of fur, could be eradicated with several bathings in Mala-Vet (a flea and tick insecticide). This was carried out whenever such symptoms were found. The skin disorder

appeared to be a vitamin deficiency, since the yellowing skin and slight loss of fur, could be prevented by supplementing the diet with bread, fruit and vegetables (this was done regularly). The respiratory disorder (breathing congestion, running eyes) could not be relieved, hence animals suffering from this condition in anything more than a very mild way were not included in the testing program and were instead destroyed. The condition was brought from the breeding colonies and veterinary advice indicated little could be done to cure the problem.

The condition of an animal did not appear to produce changes in performance unless the animal was in quite poor condition, in which case the data were discarded and testing was not continued again until the condition was relieved.

4.1.2 Surgery

Surgery did not commence until a few days to a week after the animals had been individually housed under the conditions described above. This allowed some time for the animals to adjust to the new conditions.

All operations were conducted under Sodium Pentobarbitol anaesthesia (60 mg/kg — TM Abbott laboratories, Sydney) administered by intraperitoneal injection. Once the anaesthetic had taken effect (as evidenced by a lack of response to tail pinch), the scalp was shaved and the anaesthetised animal placed in the stereotaxic frame (Model 400 Student Rat Stereotaxic Instrument, David Kopf Instruments, California). An incision made down the midline approximately 10–13 mm long, with the aid of curved haemostats, exposed the skull. Coordinates for the electrode site were determined from the bregma landmark and the electrode carrier adjusted and positioned at the site for implantation. The locus was lightly marked with a soft grade pencil.

The electrode carrier was then temporarily removed while four holes were drilled in the skull, one for the electrode and three for stainless steel jeweller screws. After the screws were fixed, the electrode carrier was pivotted back into position and lowered to the correct depth.

Dental cement, placed around the electrode and jeweller screws, secured the electrode into position. Finally, an antibiotic spray (Neotracin, Ethnor, Sydney) was sprayed around the wound area to reduce the risk of infection. The animals were then returned to their individual cages. All surgical instruments, electrodes and jeweller screws were sterilised with 10% Zephiran solution before use.

Two series of operations were performed. These included 28 animals in the first

series and 70 in the second series. The two series of operations were known as the M-Series and R-Series respectively.

4.1.3 Electrodes

All electrodes were twisted, bipolar stainless steel (Plastic Products, Roanoke, USA), 250 μm in diameter, and insulated at all points except the cross-sectional area of the tips. The separation of electrode tips was approximately 0.5 mm.

The orientation of the tip of the electrode for the M-series was random, that is, no attention was paid to this aspect of implantation. In the R-series, approximately one half of the electrodes were aligned transversely to the midline suture, and one half were aligned longitudinally. This was to test the claim that bipolar electrodes in the VTM and LH stimulated the reward system more effectively (with 60 Hz sine wave), when oriented in the medio-lateral direction than in the anterior-posterior direction (Szabo & Milner, 1973).

4.1.4 Coordinates

Electrodes were aimed at a block of tissue 4.0–4.3 mm posterior to bregma (AP), 0.4–0.7 lateral to the midline (L), and 8.5–9.5 below the skull surface (V). The incisor bar was set at -3 mm above the intra-aural line. This region of the brain corresponds to the area known as the ventral tegmentum (VTM). The coordinates used were obtained from Miliaressis and Cardo (1973) and Miliaressis, Thoa, Tizabi, and Jacobowitz (1975).

4.2 Apparatus

Two different types of apparatus were used in the experiments to be reported. The 'standard shuttlebox' was used with the M-Series animals, and the 'T-maze/shuttlebox' with the R-Series animals. The T-maze/shuttlebox could be converted into a configuration similar to that described for the standard shuttlebox and it was this form that was used in all experiments reported. The following sections describe both types of apparatus.

4.2.1 Standard shuttlebox

What will be hereafter referred to as the 'standard shuttlebox' consisted of an aluminium box 60 cm long, 25 cm wide, and 40 cm deep with a clear perspex front. The floor comprised of a central grid section with a flat aluminium section at either end of 14 cm in length.

Two photobeams situated 14 cm from each end (coinciding with the end of the aluminium sections) crossed the width of the box. This meant that the two beams were 32 cm apart. Interruption of the photobeam on one side of the box initiated a continuous train of electric current. The subsequent interruption of the photobeam on the other side of the box terminated the stimulation. The breaking of a photobeam triggered the computer recording of elapsed time.

The constant current stimulator was an instrument constructed by the department's technical section and delivered biphasic, rectangular pulses with zero interpulse intervals. In other words the stimulus waveform consisted of a continuous sequence of biphasic rectangular pulses. In effect, this meant that it was the pulse width that was being altered since the stimulator did not allow for interpulse interval to be anything other than zero. The stimulator was constructed to allow current intensity to be selected in 1 microampere (μa) steps from zero to 200 μa. Frequency could be altered from 40 Hertz to 200 Hertz (Hz). The waveform was continuously monitored on an oscilloscope (Serviscope, Telequipment, London). All changes in current intensity and frequency were effected by manual adjustment of dials on the stimulator itself.

The current passed through two long, light, flexible leads attached to the ceiling (1.35 m above the floor of the shuttlebox) and then to the electrode assembly on the rat's head. These leads could take a considerable amount of twisting without restricting the performance of the shuttle response in any way.

4.2.2 Definition of dependent variables

Three measures of shuttling behaviour were available using the apparatus described above; these were an ON time measure, an OFF time measure and the number of crosses completed in a fixed time period.

ON time was defined as the time from initiation of stimulation (by crossing the ON photobeam) to termination of stimulation (by crossing the OFF photobeam).

OFF time was defined as the time from termination of stimulation to the next

initiation of stimulation.

CROSS time was defined as an ON time plus the immediately following OFF time. A 'cross' or 'shuttle' constituted the response of crossing to the ON side of the shuttlebox and back to the OFF side of the shuttlebox. A completed cross included an ON time and the immediately following OFF time.

Number of crosses per trial was defined as the number of initiations that occurred during that trial (the number of times the rat crossed to the ON side of the shuttlebox). During Time trials, the number of terminations could be one less than the number of initiations (see under 'Time trials', Section 4.3.1, for more details). With reference to ON time, OFF time and number of crosses as mathematical variables, the symbols X, Y, and N_c were used respectively.

Recording of data

A FORTRAN-language program recorded the ON and OFF time for each cross and immediately displayed that value on the screen of a computer terminal. Each value was also recorded on disk under the particular identification code for that animal. The time and date of the trial were also recorded in the data file.

The program allowed the experimenter to specify the length of the trial (1 minute or greater). At the completion of the specified time the trial would terminate regardless of the animal's behaviour. Elapsed time was always determined from the time of the first initiation. The rat was always placed in the OFF side of the box at the start of a trial. At the completion of a trial the program displayed mean ON and OFF times. The number of crosses could be counted from the screen. Manual records were kept of these three measures.

4.2.3 T-maze/shuttlebox

What will be hereafter referred to as the 'T-maze/shuttlebox' (Figure 2), included a startbox (30 cm wide, 30 cm long, and 15 cm high) and an alley (17 cm wide, 104 cm long, and 15 cm high) which ended in a T-junction. Each arm at the end of the T-junction (each arm was 19 cm wide, 12 cm long, and included a rear wall 35 cm high and a diagonally-cut front wall from 15 cm to 35 cm) entered a shuttlebox (25 cm wide, 78 cm long, and 35 cm high). The walls were made of 3 mm white plastic sheeting while the floor was 1 cm^2 wire mesh.

Six infra-red light cells were placed along the walls of the alley and shuttleboxes

and could be programmed to trigger relays or to record times when the light beam was broken. One light was placed near the entrance to the alley from the startbox, another was placed near the end of the alley and two were placed in each shuttlebox to mark the ON and OFF sides as described previously under the standard shuttlebox. All light cells were centered 3.8 cm above the mesh floor.

In each shuttlebox, the OFF light cell was placed 24 cm from the rear wall (the wall closest the T-maze entry) and the ON light cell was placed 14 cm from the front wall. This meant that the two light cells were 40 cm apart. The front wall of each shuttlebox had the top half made in clear perspex so that the animal could be observed from a distance.

The ON and OFF time measures were defined in the same way as for the standard shuttlebox (see Section 4.2.1). ON time constituted the time from when the ON light cell was triggered to when the next OFF light cell was triggered (a minimum distance of 40 cm). OFF time constituted the time from when an OFF light cell was triggered to when the next ON light cell was crossed (also a minimum distance of 40 cm). Buffers in the switching circuits ensured that two or more consecutive ON lights or OFF lights could not be triggered in less than 0.5 secs. This was to eliminate the occasional errors that occurred in the standard shuttlebox when a rat carried its tail high enough to interrupt a light beam with its tail after the body had interrupted the beam at the other end. The increased inter-light cell distance (i.e., 40 cm compared to 32 cm), also helped eliminate this source of error.

The arms of the T-maze were incorporated to allow for experiments on an animal's choice of stimulus conditions. Different sets of parameters (intensity, pulse interpulse interval and the order of each of these) could be set independently for each of the two shuttleboxes. However, for the experiments reported here, only the left side shuttlebox was used.

The design of the left side shuttlebox was basically the same as that described for the standard shuttlebox, and as that described by Atrens (1970) and Mendelson (1969), but there were some differences. The apparatus was designed to enable the animal to enter the OFF side of the shuttlebox from the left arm of the T-maze. As a consequence, the area of the OFF side was larger than the area of the ON side. Specifically, the area of the OFF side (defined as the area from the rear wall to the OFF-side light cell) was 645 cm^2 whereas the area of the ON side (defined as the area from the front wall to the ON light cell) was 350 cm^2. This compares to the standard shuttlebox area of 350 cm^2 for both ON and OFF side.

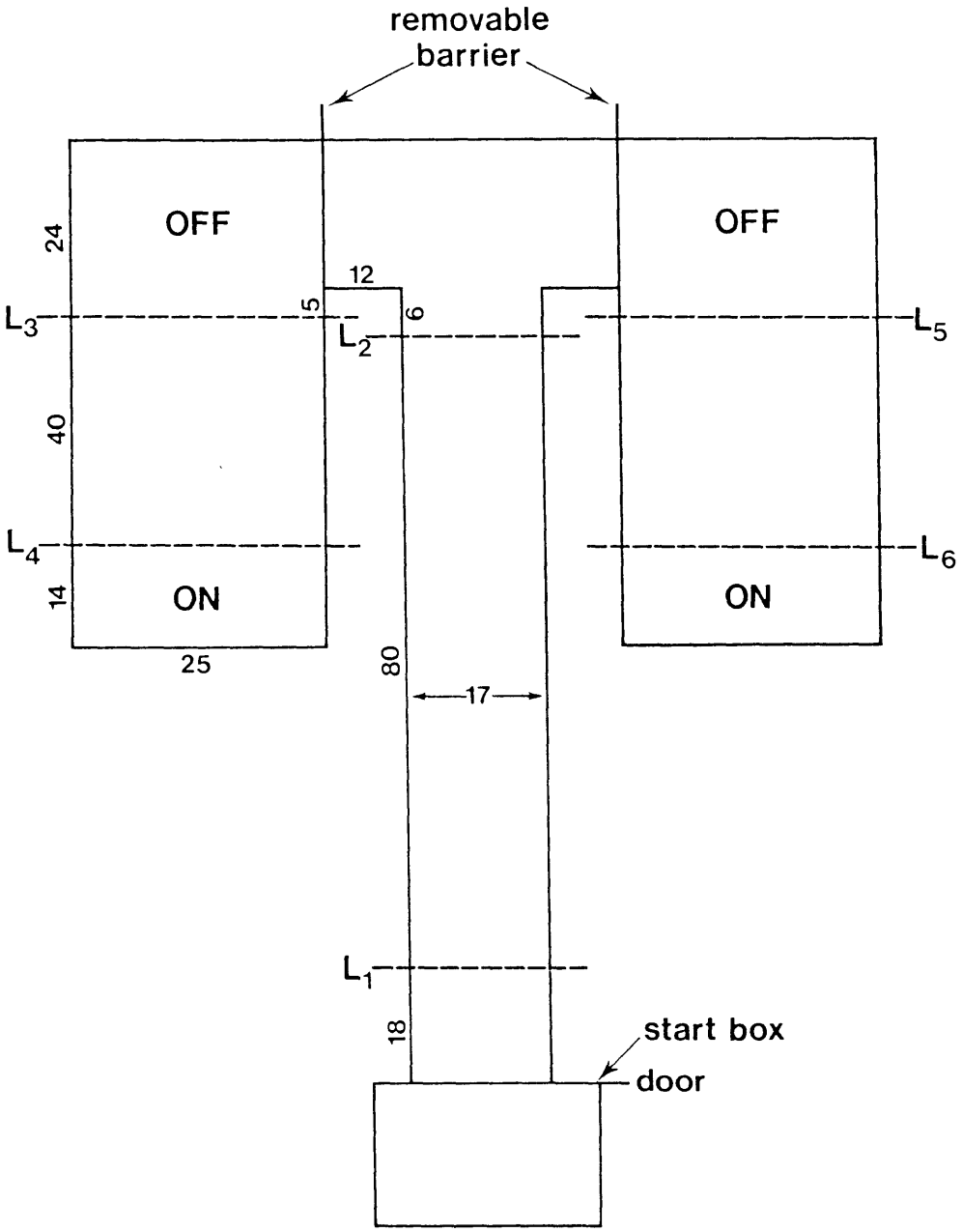

Figure 2: T-maxe-shuttlebox

Another difference was that with the standard shuttlebox photo-cells, an obvious light signalled to the animal where the ON and OFF triggers were. (To a lesser extent, the floor of the shuttlebox signalled these divisions as well.) During early training in the standard shuttlebox animals appeared to use the light signals as cues to determine how far to travel before the stimulation would turn ON or OFF. However, in the T-maze/shuttlebox the infra-red light cells no longer produced visible light to clearly mark where the simulation would turn ON or OFF. Casual observation suggested that rats initially had difficulty associating a particular response with the termination and initiation of stimulation. In order to improve the cues that might be available for animals to distinguish parts of the box, green electrical tape was placed around the holes for the infra-red beam. The OFF-side beam was indicated by horizontal strips of tape and the ON-side beam was indicated by vertical strips. This did appear to improve the association between the stimulation and the required response although it never seemed as strong an association as when the light beams were being used. In any case, once an animal became reasonably experienced at controlling the initiation and termination of stimulation it did not seem to have difficulty, and presumably was using cues of some sort to differentiate sections of the shuttlebox.

Stimulation was conveyed to the animal by means of two long, light leads from the stimulator to the electrode assembly on the rat's head. The leads passed through a metal hoop (3 cm in diameter) which was approximately 1.35 metres above the floor of the shuttlebox. The metal hoop could slide freely along a glass rod (1.2 metres in length) centred above the alley in the T-maze. This arrangement allowed considerable freedom of movement for the animal travelling along the alley and into either shuttlebox. Also, in each shuttlebox, no hindrance to an animal's movements occurred except in quite long trials or when the particular animal exhibited quite vigorous circling behaviour (for instance this might happen at high intensities). In these cases the leads could become twisted and eventually shorten to such an extent that the animal's behaviour was restricted. If this did happen, the trial was discarded and the trial re-run (this sometimes meant that the leads had to be twisted in the opposite direction before they were connected to the electrode assembly). It should be stressed that these problems were rare and in the overwhelming number of trials the arrangement described above proved adequate and reliable.

Modifications to the stimulator described under the standard shuttlebox allowed the stimulator to be controlled by computer commands. Also, interpulse interval

could now be selected although over a restricted range (see under Frequency, Section 4.3.2, for more details). The stimulus waveform was continuously monitored on an oscilloscope (Type 585, Tectronix Inc., Portland, U.S.A.).

Recording of data

All data were recorded automatically while the experiment was in progress. Whenever a cross had been completed the relevant values for that cross (current intensity, pulse width, interpulse interval, ON time, OFF time, box number) were immediately sent to disk and recorded under the particular identification code for that animal. Subsequent analysis of the data required the writing of FORTRAN programs to open and read these data files and perform the necessary calculations.

4.3 Computer control of stimulus parameters

All operations of the T-maze/shuttlebox were controlled from a Video Display Unit. A FORTRAN-language program allowed the experimenter to select stimulus conditions from the keyboard or from prepared data files. It was possible to control (albeit, not all at the one time) current intensity, pulse width, interpulse interval, the sequence of each of these, whether the trial was a time trial or an event trial, and whether initiation or termination were at the control of the experimenter or the animal (stimulus contingency). Intensity and frequency could also be controlled manually from the hardware, however, for all experiments with the T-maze/shuttlebox the conditions of stimulation were determined from the keyboard. Details and limitations of each stimulus condition are outlined separately.

4.3.1 Modes of operation

The length of an experimental trial could be determined by either an elapsed time criterion or by an elapsed number-of-crosses criterion. This allowed two alternative modes of operation for the T-maze/shuttlebox each of which had advantages and disadvantages.

Time trials

When an animal was placed in the shuttlebox (always placed in the OFF side), timing for the trial did not begin until the animal had crossed to the ON side for

the first time. However, the trial immediately terminated once the prescribed time had elapsed. In other words, the start of any trial was contingent upon the animal crossing to the ON side but the termination of the trial was program-dependent. This was the same situation as that described under the standard shuttlebox. Time trials have been used by Atrens (1970, 1973), Mendelson (1969), and Valenstein and Valenstein (1964). The time interval could be selected in integer values of 1 minute or greater.

Because a time trial would terminate regardless of what part of the box the animal was in, the last cross in a time trial was always incomplete. Although this was only a minor factor in the majority of circumstances, it did have more of an effect at low rates of crossing. Hence, the following procedure was adopted: the last (incomplete) cross was in fact counted as a cross for the purposes of calculating the rate measure for that time period, no matter how incomplete the cross. (The 'number of crosses' measure was the number of times the animal crossed to the ON side of the shuttlebox, or the number of initiations). Mean ON and OFF times were both calculated by dividing the total amount of time spent with the stimulation ON or OFF by the number of crosses (as determined above). This meant some bias existed in mean times at low rates of crossing, particularly with mean OFF time, however, it was one way to give meaning to the occasional cases where an animal crossed to the ON side and stayed there for the full time period. In these cases, the number of crosses was one (hence, the minimum number of crosses was always one), mean ON time was 600.0 secs (for a 10-minute trial), and mean OFF time was 0.0 secs. However, the last cross was not considered in any other statistic; only the previous $N_c - 1$ crosses were used. Where only one cross occurred, number of crosses, mean ON time, and mean OFF time were the only measures that could be calculated.

The above procedure ensured that mean ON time ($\overline{X}$) plus mean OFF time ($\overline{Y}$) multiplied by the number of crosses (N_c) was equal to the total time allowed for the trial. For a 10-minute trial:

$$(\overline{X} + \overline{Y}) \times N_c = 600.0$$

Event trials

One way of circumventing the problems described above with the incomplete last cross was to terminate the trial when the last cross had been completed. In this

case the operating program allowed the experimenter to specify how many complete crosses were to be made before the trial would terminate. Every ON time was therefore followed by a complete OFF time, and every recorded value could be used for statistical purposes. Another advantage was that all animals completed the same number of crosses regardless of how quickly they responded. This was useful for statistical purposes because the data for each animal was based on the same sample size. Also, each animal covered approximately the same distance and therefore expended a similar amount of energy. Such trials were termed 'event' trials. It was possible to specify an integer value of one or greater. Event trials have been used by Montgomery et al. (1981), Schmitt et al. (1981), and Schmitt and Karli (1984).

The main disadvantage was that the time it took for an animal to complete, say, 60 crosses was indeterminate and in some cases a considerable amount of time could elapse waiting for an animal to complete a trial. Because only one animal could be tested at a time, this could become a severe restriction. For practical reasons, time trials were used whenever the rate of crossing could become awkwardly slow, including training, screening and determination of rate-intensity functions. Event trials were used whenever an animal was well trained and was responding to a current intensity that should have produced a reasonable rate of crossing.

4.3.2 Stimulus waveform

The wave form used for stimulation was generated by the PDP 11/23 computer via a separate callable subroutine. The subroutine could be used to build wave shapes of almost any description. Sine wave, triangular- or crescent-shaped waves, monophasic or biphasic or some combination of any or all of these could be constructed with this subroutine. For the experiments reported here biphasic, rectangular pulses were used.

The use of the PDP 11/23 to produce the waveform allowed considerable versatility in selecting the parameters of stimulation. However, because the 11/23's clock was used for both producing the waveform and recording data it was only possible (at this stage of program development, anyway) to produce a rectangular pulse of integer millisecond (msec) dimensions. Pulse width and interpulse interval could only be selected in integer msec increments.

Current intensity

Current intensity could be selected in the range from zero to 199 μa in 0.1 μa steps. Because the stimulator was only accurate to within $\pm 0.3\mu$a, intensities less than an accuracy of 0.5 μa were not specified. Periodic testing of current intensities was carried out with the use of a multimeter.

Frequency

Frequency (F) of pulse presentation, pulse width (PW) and interpulse interval (IPI), were related by:

$$F = 1000 \div (2 \times PW - IPI)$$

Pulse width could only be selected in integer millisecond increments of 1 msec or greater. Interpulse interval could be selected in integer msec increments of zero or greater. This meant that the most rapid presentation of pulses available was 500 Hertz. The notation PW/IPI= 3/4 is used to indicate that pulse width was 3 msec (for both positive and negative phases) and interpulse interval was 4 msec.

The restriction on PW and IPI dimensions has been explained earlier (see Waveform, Section 4.3.2) as being due to the use of the 11/23's clock to produce both the waveform and to record data. The waveform used here was unusual (i.e., PW/IPI = 5/0, or 3/4), when compared to that reported in the literature. However, previous work in this laboratory has shown that PW/IPI = 5/0 (i.e., 100 Hz), biphasic square wave, is comparable to 100 Hz sine wave which has been commonly used in the ICSS literature. Evidence from a number of sources (e.g., Lilly, 1961; Lilly et al., 1955; Miller et al., 1961; Olds et al., 1960; Section 2.1) suggests that even 60 Hz sine wave (i.e., 8.3 msec hyperpolarising and depolarising phases) is not injurious when used in acute ICSS experiments. The 3/4 setting was chosen after an experiment comparing stability of crossing rate under three conditions (i.e., PW/IPI = 1/8, 3/4, 5/0) found no differences among the three conditions. Consequently, a central value was chosen. (This experiment is not reported in greater detail).

4.3.3 Contingency of stimulation

Intensity and frequency could be selected in both operating modes (i.e., time trials or event trials). However, the following options could only be selected in the event trial mode.

The more usual conditions under which an animal shuttles in response to electrical stimulation are those in which the initiation and termination of stimulation are contingent upon the animal performing a specific response such as crossing to one side of a box or pressing a lever. The operating program used here allowed the contingency of stimulation to be altered in a number of ways. These were: the withdrawal of stimulus termination contingency, withdrawal of stimulus initiation contingency, and withdrawal of both contingenices.

Control of termination response

The program allowed the experimenter to select an option which removed the control of the OFF response from the animal to the program. In effect this meant the experimenter specified the amount of time the stimulation was to be left ON. The animal had to cross the ON beam to initiate the stimulation. ON times could be selected in 0.1 second units from zero.

Control of initiation response

Similarly, the program allowed the experimenter to select an option which removed the control of the ON response from the animal to the program. The experimenter determined how long the stimulation would stay OFF but the animal had to cross the OFF beam to terminate the stimulation. OFF times could be selected in 0.1 second units from zero or greater.

An important point here was that the very first initiation was under the control of the animal since this first cross determined the start of recording for that trial. Hence, the animal did determine when the stimulation first came ON, although all subsequent 'initiations' were determined by the prepared data file.

Control of both initiation and termination responses

The program also allowed the selection of an option which removed the control of both the ON response and the OFF response from the animal to the program. This meant the experimenter could select both how long the stimulation was ON and how long it was OFF. Regardless of the behaviour of the animal the stimulation would come ON for a specified time and then go OFF for a specified time until the prescribed number of times. This option allowed the experimenter to play back the times previously

recorded by the animal under normal 'free-shuttling' conditions. The ON and OFF times could be selected in 0.1 second units from zero or greater.

Again, the animal had control over the very first initiation response as this started the recording of a trial. After that, the animal's behaviour was irrelevant to the onset and offset of stimulation.

4.3.4 Stimulus control files

Any one of the parameters: current intensity, pulse width, interpulse interval, time ON, time OFF, and both time ON and OFF could be controlled internally from a data file when in the event-trial mode. All remaining parameters were fixed for the trial's duration. Data files were constructed separately and called from within the program. The time ON and time OFF aspects of the stimulus could only be controlled by the use of data files; they could not be entered directly from the keyboard.

Stimulus control files could be used to specify the order of presentation of any one of the above parameters. This feature was employed in experiments investigating the effects of within-trial variation in the electrical stimulus (Chapter 7).

4.4 Behavioural testing

4.4.1 Training and pre-screening

Animals were allowed at least 2 weeks to recover from surgery before testing began. An animal chosen for testing was connected to the lead and placed in the OFF side of the shuttlebox and allowed to start the trial by crossing to the ON side of the box for the first time.

The first two or three sessions were of variable length (but of at least 10 minutes) in which very gradual increments in current intensity (e.g., 2–5 μa) were made every three or four minutes. These early stages of training were used to ensure that animals became accustomed to the handling procedure and testing apparatus. Eventually intensities were increased to a level which the animal just appeared to notice.

Subsequent training and screening sessions were of 10 minutes duration unless otherwise specified. Only one animal could be tested at any one time and each animal was tested from one to three times daily while participating in an experiment or training procedure.

During the next three to four sessions intensity was increased to a level which had some effect on the behaviour of the rat. Criteria for deciding that intensity was having an effect included a consistent change in behaviour whenever the current was turned ON (either by force or voluntarily). The behavioural change may have included a change in alertness, the presence of sniffing or exploratory behaviour, the just discernible ocurrence of motor effects such as circling, rising on back legs, head dipping, closing eyes, or jumping. Once the animal returned to the OFF side of the box the above behaviours would disappear.

As intensity was increased still further (in 2–5 μa steps), any behaviours induced by the stimulation were made more definite and pronounced, including obvious and quick termination of stimulation or escape behaviour (in the sense of trying to escape from the box).

At this point any animals actively terminating the stimulation (when forced ON) were discarded and any animals showing at least some resemblance to shuttling behaviour were continued into the rate-intensity function determination. Criteria for discarding animals included an obvious effect of the stimulation (e.g., quite pronounced motor artifact) and/or the animal would stay in the OFF side, or, would immediately return to the OFF side when forced to the ON side. Often animals in this group were retested a number of times under different intensities, pulse widths or frequencies before they were completely discarded. Nonetheless, these continued attempts were never successful in finding a parameter combination that would sustain self-stimulation.

4.4.2 Definition of self-stimulation

Following completion of the training and pre-screening procedures all possible SSs were systematically presented with increasing intensity over a series of test sessions. These test sessions were to determine the approximate shape of the rate-intensity function for each animal. For the M-Series experiments, the RIF sessions were seven to nine 10-minute intervals, incrementing from zero to a point which was determined by the appearance of vigorous motor artifact or obvious escape behaviour (in the sense of trying to escape from the box). For any 10-minute interval only one intensity prevailed. The intensities were generally presented in ascending order.

The number of crosses in each trial were recorded and formed the basis for the final screening decision. This was that the animal had to obtain 40 crosses or more

in 10 minutes at two consecutive intensity levels without motor artifact problems.

RIFs were not determined in the same detail for the R-Series experiments. Instead, four to five 20-cross trials were used in which intensities were chosen to represent the central portion of the RIF, as described in the results of Experiment Ia (approximately, Levels 4–6). If an animal completed two 20-cross trials in less than five minutes at two consecutive intensity levels it was defined as a self-stimulator.

Occasionally animals could be encouraged to cross more than 40 times at high intensities but if motor interference (such as circling) interfered with the termination response these animals were also discarded. In general terms, a self-stimulator was required to shuttle fairly rapidly and freely over a 10-minute interval.

The reason for choosing a rate measure as the criterion for self-stimulation rather than a particular ON or OFF time (e.g., as used by Atrens, 1970) was that it was the relationship between the ON and OFF times which was the subject of the current research. To arbitrarily put a limit on one of these two components would unnecessarily restrict one of the variables of interest. It was also important for statistical purposes to have a reasonable number of crosses in each trial (alternatively, to complete a certain number of crosses in a reasonable time). It therefore seemed logical for the purposes of the present study to use a criterion of rate and to then look at the two components of that rate, namely the ON time and the OFF time, and determine the factors that caused the animal to apportion its time in that particular way.

4.4.3 Testing procedure

The testing procedure varied depending on the aims of the experiment, however, some procedural aspects were the same for all experiments.

The animal was always placed in the OFF side of the shuttlebox and allowed to start the recording for the trial by crossing to the ON side of the shuttlebox for the first time. Test sessions for any animal were never closer together then about two to three hours and never more than two days apart. The usual testing procedure was one to three test sessions per day every day until that program had been completed. The order of animals tested on any day was continually varied to balance out possible diurnal factors in the behaviour of the animals. Also, a one to two week rest period was provided for all animals between experiments. Each new experimental program, after the rest period, began with the use of 'warm-up' trials.

Staggered groups

The testing for all experiments reported here was staggered over time. This meant that where, for example, twenty animals in two groups of ten were to be tested, ten animals completed the test program before the second ten were started on the program. The first ten animals and the second ten animals included 5 animals from each of the two groups. In other words, approximately one half the subjects (with this half being made up of half the subjects in each subgroup) were put through the experimental procedure first and after that had been completed, the other half were put through exactly the same procedure.

Staggering of the testing program meant that experimental groups were controlled for health or hormonal variations over the testing period. Also, time since implantation and the degree of training and experience could be balanced across groups and subgroups.

4.5 Histology

At the completion of all testing, animals were sacrificed by overdose with Sodium Pentobarbitol. The brains were removed and stored in normalised (i.e., 0.9% saline) formalin for several days, after which the formalin was replaced with 70% alcohol and again stored until sectioning. The brains from the animals were eventually embedded in wax, sectioned on a microtome (Model "820" Spencer, American Optical Corporation) at 7–8 microns, stained with cresyl violet and mounted on slides. The slides were then examined through a miscroscope under low power.

Other methods of preparing brain sections for histological examination were tried, including alternative stains such as thionin or haemotoxylin, or potassium ferrocyanide. The alternative stains were not as successful as cresyl violet in producing clear and contrasting sections.

Another method used photographs of fresh sections to obtain a quick, yet permanent, record of electrode location. Brains were blocked around the electrode tract and sectioned on a freeze microtome with the aid of pressurised carbon dioxide. The most representative of the (unstained and unmounted) sections were then immediately photographed two to three times under a low-power microscope with black and white film. The tip of the electrode could then be determined from the photographs. However, practical difficulties of setting up equipment and the inability to re-examine

the original section meant that this method was not very successful.

Whichever method was used for determining the stimulation site, the results were collated onto diagrams schematically redrawn from Pellegrino and Cushman (1967).