Chapter 5

Experiment I: Intensity and shuttling behaviour

Several studies have shown that both ON and OFF time may be considered decreasing functions of current intensity (Section 2.2). This finding represents one of the three features of shuttling behaviour that any model must explain. If OFF time, a time during which no stimulation is received, can be considered a decreasing function of the intensity of stimulation, then two possible sources of dependence may be identified. The decrease is either due directly to the intensity increase or is due to the decreased duration that also occurs.

5.1 Experiment Ia: Mapping of response measures

5.1.1 Introduction

The present experiment was designed to examine in detail the relationship between the intensity of stimulation and shuttling behaviour. The investigation of this relationship was approached from three directions. Firstly, the experiment was designed to verify existing data concerning the functional dependence of ON time, OFF time and crossing rate, on the intensity of stimulation. Secondly, the relationship between ON and OFF time was to be examined in terms of the statistical correlation that may be computed between ON and OFF time: and, thirdly, the relationship was to be examined in terms of changes that might occur in terms of derived variables such as charge, proportion, or total time.

Correlations

Conflicting evidence exists regarding the extent of the correlation between ON and OFF time (Section 2.2.1). This conflict may have come from three sources. First, a correlation based on mean ON and OFF time (taken over subjects or trials), may not be the same as a correlation based on within-trial ON and OFF times. Second, because both ON and OFF time decrease as intensity is increased, the correlation between them might also depend on the stimulus intensity. No studies have investigated this possibility.

Third, different methods of calculating the correlations in the above studies may have contributed to the different results. Atrens and Becker (1975) used a rank order correlation. Atrens (1970) used a Pearson product-moment correlation, while Atrens et al. (1983) and Schmitt et al. (1981) transformed ON and OFF times logarithmically before calculating Pearson product-moment correlations. The use of the same method at each intensity level should clarify the degree of correlation that exists between these two variables and how the correlation may, or may not, change with intensity.

If a correlation between within-trial ON and OFF time existed, the correlation might be between each ON time and the succeeding OFF time. That is, OFF time may be viewed as a consequence of the stimulation (perhaps due to 'forcement' or 'priming' factors). Alternatively, a significant correlation might exist between each ON time and the preceding OFF time (i.e., due to 'anticipation' or 'expectation' of the stimulation, or as implied in the term 'latency to initiate'). Both of these possibilites will be examined.

Derived variables

The relationship between the intensity of stimulation and ON and OFF time might be better expressed in the form of a combined variable such as a ratio (Liebman, 1983) or total time (Valenstein & Myers, 1964). In this regard, a number of variables may be derived to describe shuttling behaviour that have not been considered in detail previously, or the results of which have been disputed. For instance, Valenstein and Myers (1964) found that the total time spent ON was considerably altered by intensity whereas Atrens (1970) found that this variable was not altered by intensity.

Other possible measures include: proportion of time spent ON, mean charge accepted per cross, total charge accepted per trial, and various correlations. These

measures are not all independent; however, a more detailed description of the response of these measures and a clearer understanding of their inter-relation (see Cane, 1961; Ludlow, 1976) might make more apparent what aspects of shuttling behaviour (under continuous reinforcement) are most important and which are most closely related to the independent variable of intensity. The results and discussion sections to follow distinguish between the dependent measures in terms of 'primitive' (i.e., number of crosses, ON time, OFF time), 'derived' (i.e., proportion, total time, charge), and 'correlation' variables (i.e., within-trial correlation between ON and OFF time, regression line slope).

Limitations of existing data

Previous studies have examined the relationship between intensity and ON and OFF time (Section 2.2), but have at least three limitations when applied to the present research. These limitations are: the number of intensity increments used, the number of ON/OFF periods used, and the site of stimulation.

Most previous studies have used a small number of intensity increments. Atrens (1970), Atrens and Von Vietinghoff-Riesch (1972), Valenstein and Valenstein (1964) and Montgomery et al. (1981) used three levels — either low, medium and high (Atrens, 1970; Atrens & Von Vietinghoff-Reisch, 1972, Valenstein & Valenstein, 1964) or 1, 2.5 or 4 times a base level of intensity (Montgomery et al., 1981). A greater sampling of values might provide a clearer image of the underlying functional relationship between intensity and ON and OFF time. Schmitt et al. (1981) used from four to six levels for each of the parameters: intensity, pulse duration and IPI. These authors, however, used trade-off techniques to assess the relative contribution of each parameter in producing a constant level of behavioural output. The present concern is with how shuttling behaviour changes as a function of intensity, hence, input-output functions are more applicable. Only Montgomery et al. (1981) have combined data into group curves.

In some of the above studies, only a small number of crosses were allowed during an observation period. For instance, Montgomery et al. (1981) used five crosses as their basic measurement unit; Schmitt et al. (1981) used ten. Earlier studies (e.g., Atrens, 1970; Valenstein & Valenstein, 1964) used 15-minute trials, which, considering that animals may shuttle 80 times or more during a 10-minute period (Mendelson, 1969), provides a better basis for computing summary statistics.

Another limitation with existing data is that the VTM has not been considered in the studies cited. Valenstein and Valenstein (1964) used hypothalamic and septal placements; Atrens (1970, 1973), Schmitt et al. (1981) and Montgomery et al. (1981) all used hypothalamic placements. Olds et al. (1960) have shown that RIFs for the lever press response vary considerably with electrode placement. Consequently, RIFs need to be established for animals with electrodes located in the VTM region. In more general terms, RIFs should be produced in reasonable detail for the particular region being studied, the particular stimulus parameters being employed, and the particular testing apparatus being used. This will establish bounds within which the independent and dependent variables can be expected to vary.

Frequency and sequence effects

Previous studies have also varied widely with regard to the stimulus waveform that has been used. For example, Atrens (1973) used 50 Hz sine wave, whereas Valenstein and Valenstein (1964) used 100 Hz biphasic, 0.2 msec rectangular pulses. Because the stimulus wave form has been found to have significant effects on ICSS behaviour (e.g., Gengerelli, Priddy, & Averill, 1963; Kling, Brownlow, Menich, & Velozo, 1979), any results that may be found in the present experiment may be peculiar to the particular choice of stimulus parameters. The extent to which the results might be generalizable would be improved by showing that they do not depend on a particular set of parameters. Therefore rats were randomly assigned to either a 100 Hz or a 200 Hz group; the stimulus conditions prevailed throughout all screening, training, and testing procedures.

The determination of RIFs may be significantly altered by contrast effects when different intensities follow each other closely (e.g., Atrens et al., 1973; Koob, 1977). For example, Atrens et al. (1973) found that when test sessions were spaced at one hour intervals both ON and OFF time showed positive contrast effects. The procedure adopted here was to test a rat one to three times daily with at least two hours between any two test sessions (Section 4.4.3). This procedure was adopted in order to reduce the likelihood of 'carry-over' effects from one session to another. However, as a check on the possibility of order effects in ICSS test sessions as used here, a sequence condition was included in the experimental design.

5.1.2 Subjects and apparatus

A total of 28 male, albino rats were implanted with stainless steel, bipolar electrodes. The animals and experiments concerned with this series of operations have been referred to as the M-Series. The apparatus was the standard shuttlebox. See the General Method for all details concerning care and surgery and the apparatus.

5.1.3 Method

The 28 rats were randomly assigned to one of two groups before implantation of electrodes. These two groups were a 100 Hz group and a 200 Hz group. All subjects in the 100 Hz group received 100 Hz, biphasic, rectangular pulses (zero IPI) throughout training, screening and testing. The 200 Hz group received 200 Hz biphasic, rectangular pulses (zero IPI) at all stages of training, screening, and testing.

After general training and pre-screening as described in the General Method, RIFs were determined for all possible SSs. Intensities were usually presented in ascending order, with each intensity presented for one 10-minute trial. Intensities used included zero intensity (two trials), 5 μa (one trial), 10 μa (one trial), and then from 4–6 higher intensities (one trial each). The last 4–6 intensities varied for each animal and were determined from the pre-screening procedure to be at approximately equal intervals. Animals were defined as either self-stimulators or non-self-stimulators on the basis of the derived RIF. An SS was defined as a rat that made 40 or more crosses in 10 minutes at two consecutive intensity levels without obvious interference from motor artifact. See General Method for more details.

From each RIF, a steep rise in crossing rate between two consecutive intensities was considered an indication that the animal was most sensitive and responsive to that particular range of intensity. The midpoint of this range then estimated the intensity that produced the highest rate of change in crossing rate (referred to as I_c). If the I_c value did not correspond to a rate of crossing greater than, or equal to, 40 crosses/10-minutes, the animal was not continued into the experiment.

Alternatively, a maximum rate could have been defined and intensities chosen as a percentage of that maximum (e.g., as used by Koob, 1977). However, the locus of rise of the RIFs was found to be more reliable and more easily definable (also see Edmonds & Gallistel, 1974; Stellar & Stellar, 1985). A curve fitting method in which the inflection point could be defined as common to all animals (e.g., as proposed by Coulombe & Miliaressis, 1987) was considered. This method presupposes the

form of the RIF. At present not enough evidence is available to determine this form satisfactorily, particularly with regard to crossing rate rather than lever press rate. The use of 'many' line segments, as described here, estimates rate of change without the necessity for assuming the mathematical form of the curve.

Seven current intensities were calculated for each rat based on the intensity identified as I_c. The seven intensities ranged from -15% to +15% of the I_c value. Each rat completed one 10-minute trial at each of the seven intensities in either an ascending, descending, or randomised sequence of presentation. See Table 1 for a summary of the experimental design.

5.1.4 Definitions of variables and statistics

The variables analysed were: number of crosses (N_c), mean ON time ($\overline{X}$), mean OFF time ($\overline{Y}$), proportion of cross time spent ON ($\overline{P}$), total time spent ON (TT), mean charge accepted per cross ($\overline{Q}$), total charge accepted per trial (TQ), correlation between ONs and succeeding OFFs (Corr (X, Y)), correlation between ONs and preceding OFFs (Corr (X, Y_{-1})), correlation between ONs and succeeding OFFs for the (first-order) differenced series (Corr $D(X, Y)$) and the correlation between ONs and preceding OFFs for the first-order differenced series (Corr $D(X, Y_{-1})$).

Some of these variables were mean values (i.e., $\overline{X}$, $\overline{Y}$, $\overline{P}$, $\overline{Q}$) based on the number of completed crosses for each trial (N_c). Number of crosses may also be considered a rate variable (i.e., per 10-minute trial) and more strictly speaking was the number of initiations per trial (see Method, Section 4.2.2). Because some variables normally require large numbers of observations (say, > 25) for valid results, within-trial correlation must be viewed with some caution when based on only a few crosses. However, in most cases, these values were still retained because they provided the best estimate available for a particular animal in a particular condition. Finally, some variables ($\overline{X}$, $\overline{Y}$, $\overline{Q}$, and TT) were transformed before analysis by the Log10 transformation to stabilise variance and to normalise the frequency distribution (Atrens et al., 1983; Cryer, 1986; Mueller, 1949; Winer, 1962). Number of crosses, total charge, and correlations were not transformed because they did not show the degree of skewness evident with the preceding variables (correlations were not constrained by being near $+1$ or -1). Proportions were transformed by the arcsine transformation (Winer, 1962).

Subscript i is used to indicate that values for that variable were calculated for each cross in the trial and then averaged over $N_c - 1$ crosses. Subscript j indicates

Table 1: Experimental design I

Seven current intensities were calculated for each animal. The seven intensities were calculated as a percentage of I_c, where I_c estimated the point on the rate-intensity function showing the greatest rate of change.

Current Intensity — as percentage of I_c						
1	2	3	4	5	6	7
85%	90%	95%	100%	105%	110%	115%

For example, 95% represents 95% of the estimated critical current intensity I_c.

Using the code that:
A = ascending sequence, D = descending sequence, R = randomised sequence, one-third of the animals from each of the 100 Hz and 200 Hz groups were assigned to the sequences i)—iii):

i)	A	R	D
ii)	D	A	R
iii)	R	D	A

As an example for i), two (randomly assigned) animals from the 100 Hz group were presented with the seven intensity levels in ascending order, (individually) randomised order, and descending order. Another two animals from the 200 Hz group followed the same pattern. (One animal in the 100 Hz group repeated one of these patterns).

that only one value for that variable was obtained for that trial.

Analysis of Variance (ANOVA) with repeated measures on two dimensions (Intensity (7) and Sequence (3)), and two independent groups on a third dimension (Frequency) was performed using the BMDP program BMDP2V.

5.1.5 Results

The procedures used to define a self-stimulator initially defined 15 SSs. The added requirement for this experiment that the calculated value for I_c be equal to, or greater than, 40 crosses/10-minutes, eliminated two more animals. Of the remaining 13 animals, 7 were from the 100 Hz group and 6 were from the 200 Hz group. Figure 3 gives the individual rate curves for the 13 animals defined as SSs. The figure indicates the estimate of the critical intensity used in the main experiment (also Table 2). Responses to the two zero-intensity trials have been averaged.

Because the stimulator could only be adjusted in 1 μa steps, the intensity calculated from Figure 3 could only be approximated to the nearest whole unit. Similarly, in calculating increments or decrements the nearest integer value had to be used. (Increments or decrements were calculated on the original theoretical value obtained from Figure 3 — not the nearest integer approximation). This meant that for some animals not all the seven intensity increments were distinct, and for the group results not all the increments were equally stepped. This did not substantially detract from the main group results. Finally, no significant differences existed between the two independent groups as regards the identified critical intensity ($t = 0.695, df = 11, p > 0.05$). The intensities calculated were similar for all animals except rat M11. The intensities used for this rat were considerably higher than those used for the remaining twelve, however, its behaviour was not noticeably different to the others and was therefore still included.

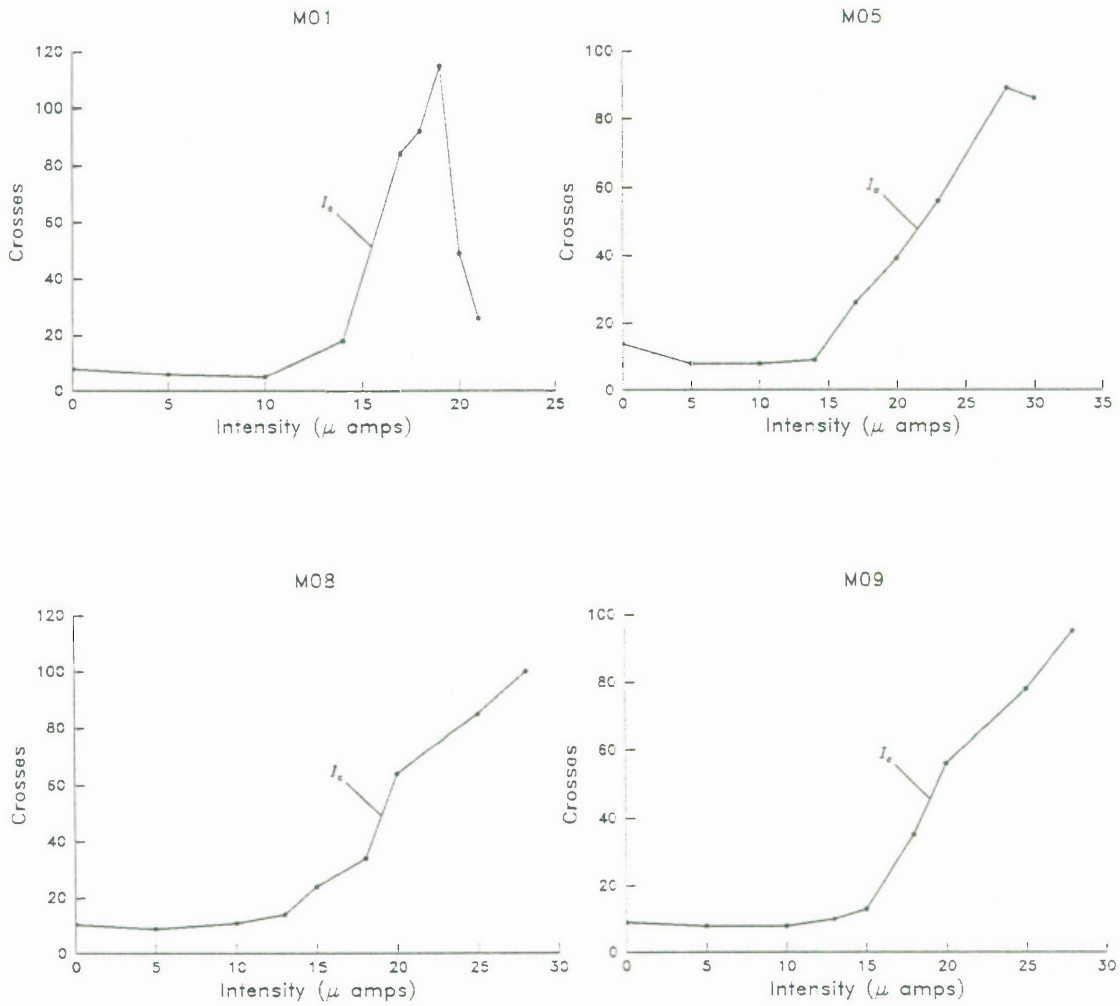

Figure 3: Rate-intensity functions for M-Series animals. I_c indicates point at centre of steepest-rising line segment.

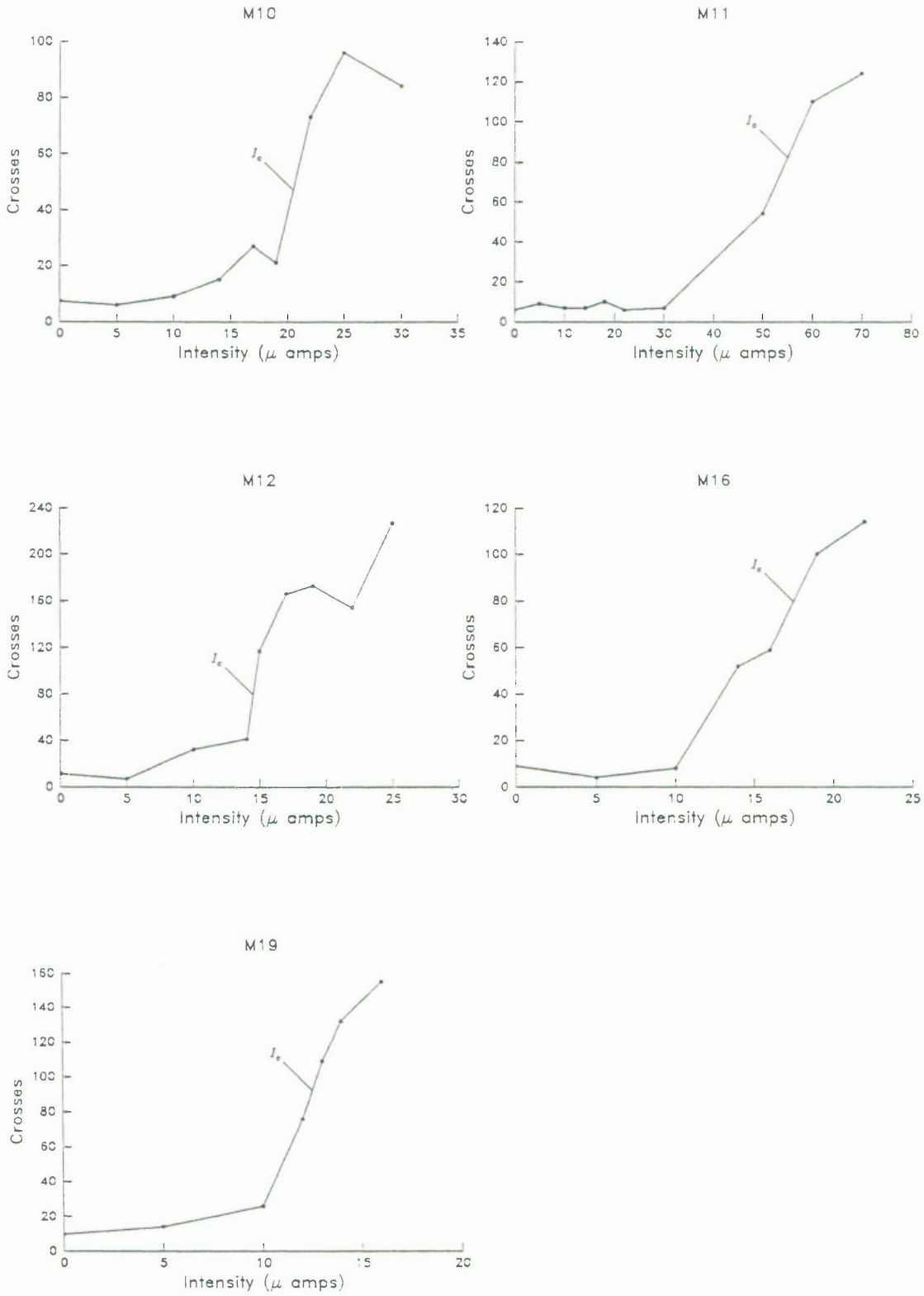

Figure 3 [cont'd]: Rate-intensity functions for M-Series animals.

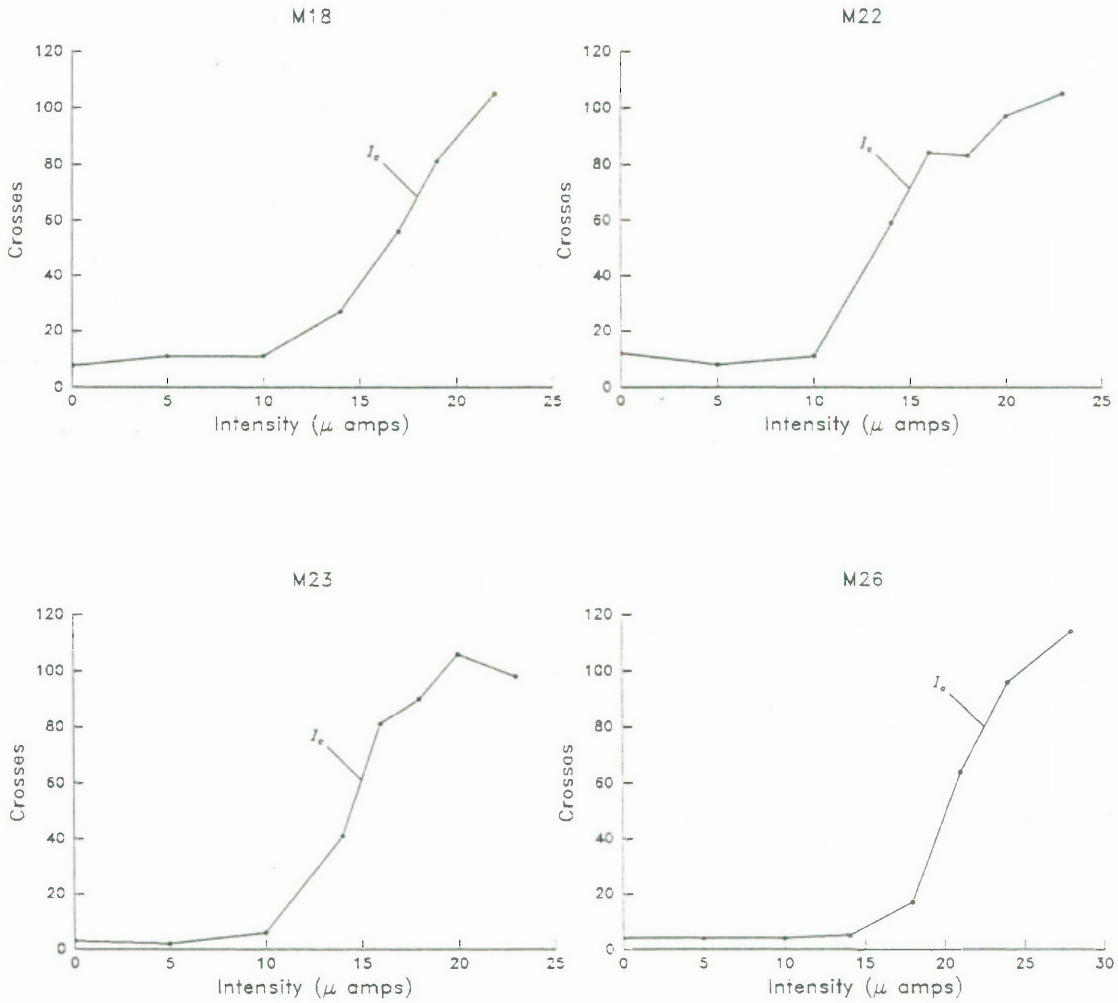

Figure 3 [cont'd]:Rate-intensity functions for M-Series animals.

Table 2: Current intensities used for each subject (μa).

Group	Subject	Intensity level						
		-15%	-10%	-5%	I_c	+5%	+10%	+15%
100 Hz	M01	13	14	15	16	16	17†	18
	M05	18	19	20	21†	22	23	24
	M10	17	18	19	21	22	23†	24
	M11	47	50	52	55	58†	61	63
	M12	12	13	14†	15	15	16	17
	M22	13	14	14	15	16	17†	17
	M23	13	14	14	15†	16	17	17
	Means	19.0	20.3	21.1	22.6	23.6	24.9	25.7
200 Hz	M08	16	17	18	19	20†	21	22
	M09	16	17	18	19	20†	21	22
	M16	15	16	17†	18	18	19	20
	M18	15	16	17	18	18	19†	20
	M19	11	12	12	13†	14	14	15
	M26	19	20	21†	23	24	25	26
	Means	15.3	16.3	17.2	18.3	19.0	19.8	20.8
Overall Means		*17.3*	*18.5*	*19.3*	*20.6*	*21.5*	*22.5*	*23.5*

† indicates intensity used in Experiment Ib.

Sequence and frequency effects

No significant effect was found for sequence or frequency on any variable. For this reason, all data was collapsed across these two dimensions to produce a matrix of 39 observations at seven intensity levels. The seven intensity levels will be referred to as Level 1 (i.e., lowest intensity), through Level 4 (i.e., central level, or I_c) to Level 7 (i.e., highest level).

The results for the combined data are depicted graphically in Figures 4– 12. The raw data may be found in Tables A.1–A.12 in Appendix A. Table 3 summarises the main findings.

Table 3: Summary data for dependent variables at successive intensity levels

Variable	Intensity Level						
	-15%	-10%	-5%	I_c	+5%	+10%	+15%
Intensity (μa)	17.3	18.5	19.3	20.6	21.5	22.5	23.5
Crosses/10 minutes	22.9	32.2	39.8	58.2	67.5	82.9	88.4
Mean ON time (secs)	16.96	16.26	14.22	9.41	7.47	6.00	5.84
Mean OFF time (secs)	30.02	16.81	13.67	4.73	3.58	2.20	2.03
Paired t-value	2.789*	0.193	-0.234	-4.357*	-4.786*	-6.613*	-7.048*
Correlation	0.452*	0.602*	0.609*	0.455*	0.224	0.050	0.022
Proportion of time ON/Cross	0.544	0.612	0.626	0.696	0.715	0.730	0.744
Total time ON (secs)	280.6	344.9	350.7	412.9	425.8	439.9	448.1
Mean charge/cross (μC)	300.7	303.7	264.6	196.6	154.8	134.3	139.9
Total charge (mC)	5.10	6.38	6.74	8.47	9.02	9.88	10.46
Regression line slope							
— ON time	0.881	0.698	0.425	0.290	0.097	0.034	0.029
— OFF time	2.986	1.797	1.842	0.219	0.165	0.034	0.035
Within-trial correlations							
Correlation (X,Y)	0.203	0.147	0.092	0.126	0.028	0.041	0.081
Correlation (X,Y_{-1})	0.045	0.145	0.050	0.086	0.029	0.027	0.065
Correlation D(X,Y)	0.084	-0.010	-0.019	-0.036	-0.110	-0.088	-0.077
Correlation D(X,Y_{-1})	-0.139	0.043	-0.010	-0.054	0.007	-0.034	-0.005

Data represent means for 13 subjects each with 3 10-minute trials (i.e., 39 observations at each intensity level). * indicates $p < 0.05$.

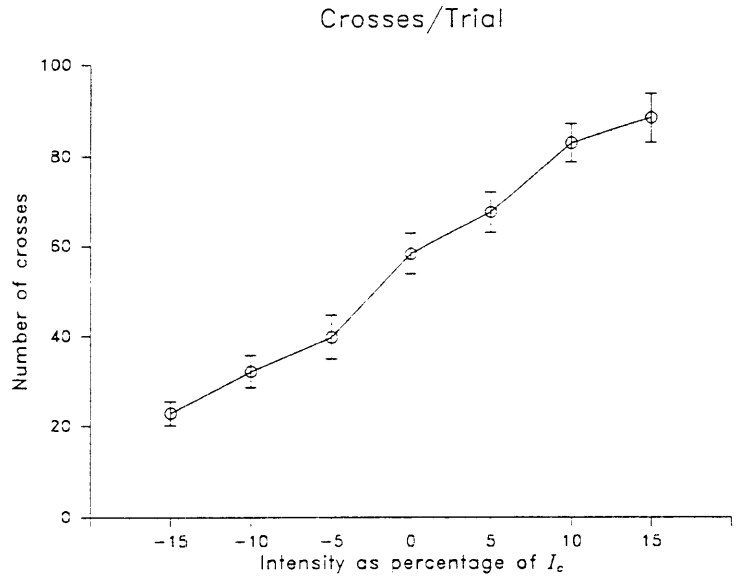

Figure 4: Mean number of crosses per 10-minute trial at successive intensity levels. Error bars indicate ± Standard Error of the Mean (S.E.M.).

Primitive variables

Number of crosses

An increase in intensity resulted in a significant increase in crossing rate ($F = 40.16, df = 6, 66, p < 0.0001$). No other independent variable or interaction neared significance.

A test for trend using orthogonal components (Winer, 1962) indicated a linear component only in the crossing rate data (Table 4). Visual inspection of Figure 4 also indicates that the procedures followed effectively captured the linear region of interest in the RIFs.

Mean ON and OFF time

An increase in intensity resulted in significant decreases in both mean ON time and mean OFF time. ANOVA results for the transformed data revealed a significant effect for intensity only ($\text{Log}(\overline{X})$: $F = 35.08, df = 6, 66, p < 0.0001$;$\text{Log}(\overline{Y})$: $F = 39.01, df = 6, 66, p < 0.0001$). A marginally significant Intensity × Sequence × Frequency interaction for OFF time was also found ($F = 2.08, df = 12, 132, p < 0.05$). Without the Log transformation the corresponding F-values for ON and OFF time were 8.90 and 10.74. Although the standard deviations for ON and OFF time were not statistically analysed, they showed similar changes to the mean ON and OFF time data (see Tables A.3 and A.5, Appendix A). Standard deviations for ON and

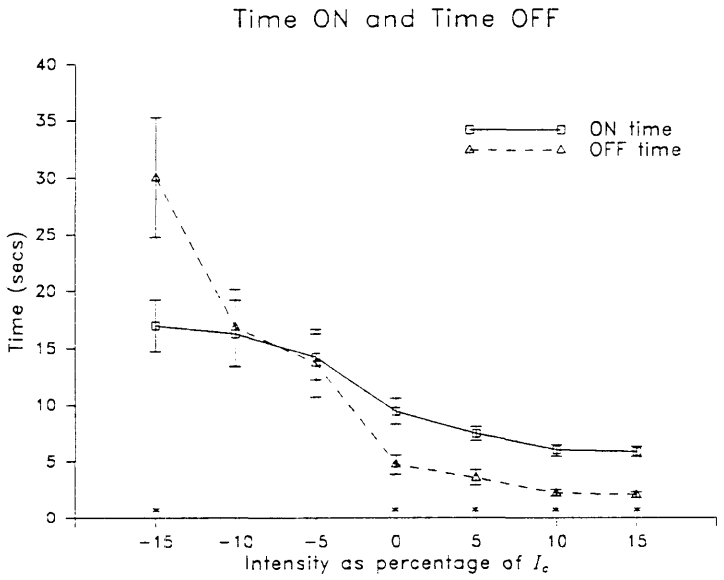

Figure 5: Mean ON and OFF time per 10-minute trial at successive intensity levels. Error bars (and on following graphs) indicate $\pm$ S.E.M. * indicates a significant difference between mean ON time and mean OFF time at that intensity level ($p < 0.05$, matched t-tests).

OFF time therefore show a strong proportional dependence on the mean (Mueller, 1949).

Log ($\overline{X}$) showed a significant linear and cubic trend, while Log ($\overline{Y}$) showed a significant linear and quadratic trend (Table 4). Note that for the sake of interpretability, the raw data is graphed in Figure 5 and in all subsequent figures — not the transformed data. (This procedure was adopted by Atrens et al., 1983).

OFF times were significantly higher than ON times at the lowest intensity level, were effectively equal at the second and third levels and were significantly lower than ON time at the four highest levels (Figure 5 and Table 3). Pearson product-moment correlations between the mean ON and mean OFF times for the 39 observations showed a significant positive correlation for the four lowest intensity levels which declined to near zero at the two highest levels (Table 3). Paired t-tests and correlations were carried out on the raw (i.e., untransformed) scores.

Proportion

ON and OFF times may also be expressed as proportions (i.e., $P_i = X_i/(X_i + Y_i)$, where Y_i is the OFF time immediately following each ON_i). The results show (Figure 6) that as intensity was increased the proportion of cross time spent with the stimulation ON also increased ($F = 22.71, df = 6, 66, p < 0.0001$).

Table 4: Results of trend analysis

Table entries are F-values calculated by the method of orthogonal components (Winer, 1962).

Trend	Variable		
	N_c	Log($\overline{X}$)	Log($\overline{Y}$)
Linear	236.56*	209.76*	225.75*
Quad	0.602	0.475	5.869*
Cubic	1.707	4.901*	1.047

* indicates $p < 0.05$.

The difference between the two lines in Figure 6, is due to the fact that a proportion may also be calculated from mean ON and mean OFF times (giving a proportion of trial time spent ON — the dotted line in Figure 6). For this calculation, the last cross was not used (see Method, Section 4.3.1). Over a large number of crosses the two proportions should be approximately equal (as in the three highest levels), however, at lower intensity levels, the last cross makes an appreciable difference. When the last cross is used (i.e., mean ON and OFF time calculation), total OFF time must increase for the same number of initiations, thereby decreasing the calculated proportion.

Alternatively, the ratio of say OFF time to ON time might have been considered (Liebman, 1983). This descriptor tended to show large variation within a trial with outliers having an overwhelming effect on a majority of 'well-behaved' values. Although mean values often show a consistent ratio, within-trial ratios can show considerable variation. For this reason, ratios have not been considered further.

Total time

As intensity was increased, total time spent with the stimulation ON increased significantly ($TT_j = \overline{X}_j \times N_{cj}$, Figure 7). ANOVA results for the Log transformed data indicated a significant effect for intensity only ($F = 14.75, df = 6, 66, p < 0.001$). Because total time ON may be expressed as a proportion of the total time available in a trial (i.e., 600 secs), total time and proportion of trial time spent ON (i.e., dotted line in Figure 6) are essentially equivalent.

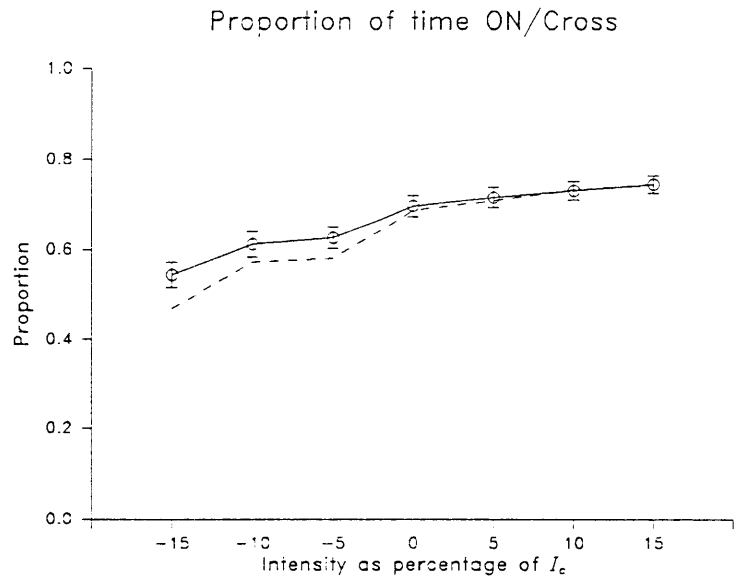

Figure 6: Mean proportion of each cross time spent with stimulation ON. Dotted line indicates proportion of trial time spent ON (for explanation, see text).

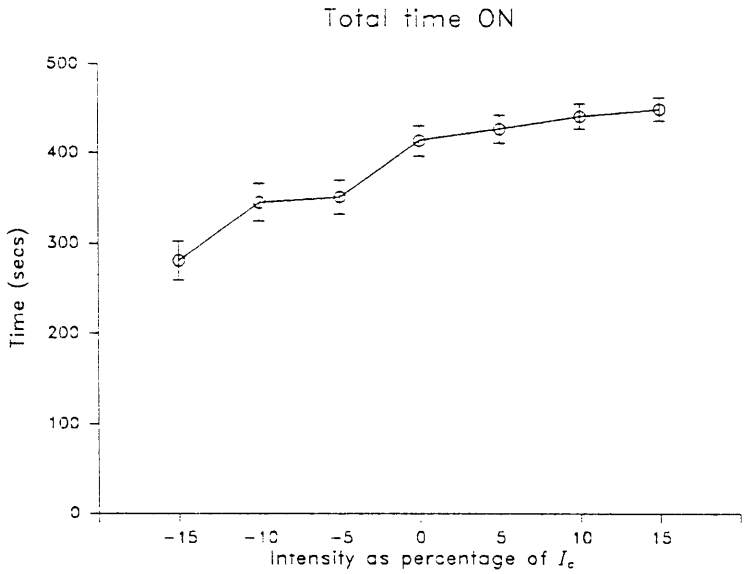

Figure 7: Total time spent with stimulation ON. Note congruence with dotted line in Figure 6. ($TT_j = \overline{X}_j \times N_{cj}$).

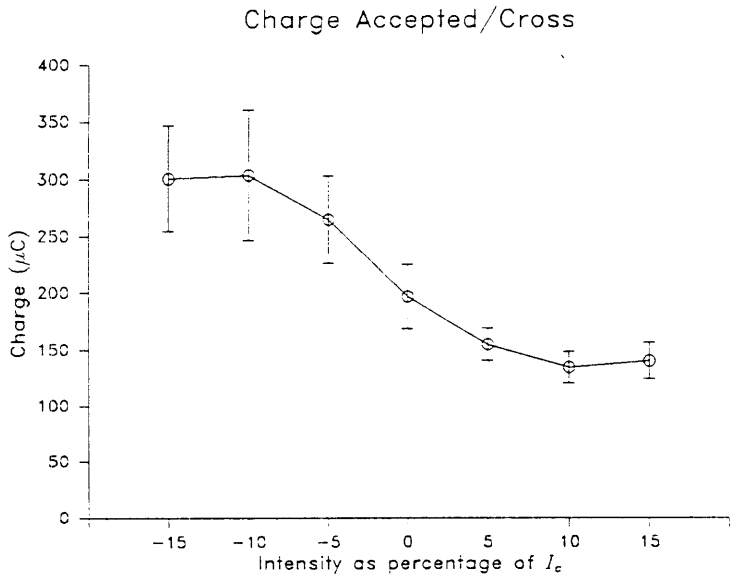

Figure 8: Mean charge accepted per cross at successive intensity levels ($\overline{Q}_i = X_i \times I_i$).

Charge

Charge accepted per cross ($\overline{Q}_i = X_i \times I_i$) decreased significantly as intensity was increased ($F = 19.51, df = 6, 66, p < 0.001$: Figure 8). No other effects, nor interactions, were significant. Both positive and negative phases of the stimulating pulses were considered equally in the determination of charge intake.

Total charge accepted per trial (i.e., $TQ_j = \overline{X}_j \times I_j \times N_{cj}$) was not transformed before the analysis of variance was computed (because the standard deviation and mean data did not exhibit the proportionality typical of previous variables — Mueller, 1949). Again, a significant effect for intensity was found ($F = 41.67, df = 6, 66, p < 0.0001$). No other variable, nor interaction, was significant (Figure 9).

Correlation variables

Regression line slope

The slope of the regression line calculated between ON time and cross number and between OFF time and cross number was used as an estimate of the rate of change of ON and OFF time across the observation period. Preliminary work had indicated a tendency for both ON and OFF time to increase over a 10-minute period. A gradual rise in mean level of a series of observations is described as a source of nonstationarity and can be removed by first-order differencing (Box & Jenkins, 1976: Chapter 9).

Although a regression line may be calculated between as few as two points, in

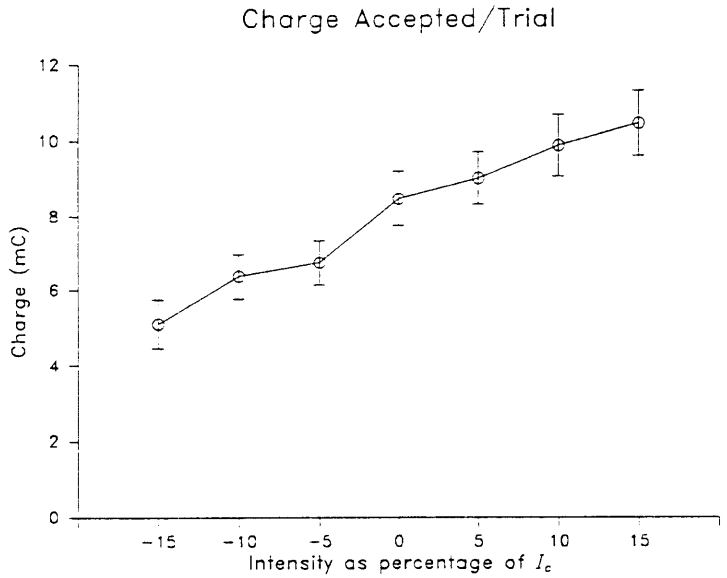

Figure 9: Total charge accepted per trial at successive intensity levels (i.e., $TQ_j = \overline{X}_j \times N_{cj} \times I_j$).

order to lessen the effect on the mean of a few extreme values in low cross trials, the minimum number of data points required was arbitrarily set at five (i.e., $N_c = 6$). In those trials in which five or fewer crosses occurred, the slope of the regression line was estimated from the data of the remaining animals in that group and in that sequence. Despite this, some extreme values remained.

The slope of the regression line gives an indication of the rate of change of successive time periods across the observation period. For example, a slope of 0.01 for ON time indicates that ON times were tending to increase by 1/100th of a second each time the stimulation was initiated. If one hundred crosses were made, then the average ON time towards the end of the trial was about one second higher than the average ON time near the beginning of the trial. In general terms, the animal was slowing down. A negative slope indicates a trend toward shorter times as the trial proceeded.

Figure 10 indicates that ON and OFF time tend to increase during a trial. At lower intensity levels, OFF times tend to increase more during a trial than the corresponding ON times. However, the apparent differences between the slopes at the lower intensity levels were not statistically significant. Wilcoxon matched pairs tests, with normal approximation (Welkowitz, Ewen, & Cohen, 1976), produced $z = 1.84, 1.81$, and 1.60, for the first three levels respectively. (Estimated values were not included in the Wilcoxon test).

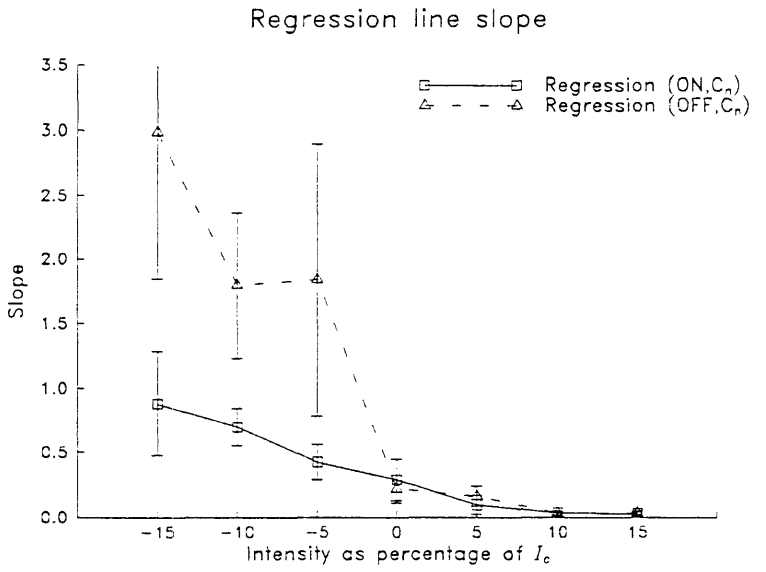

Figure 10: Slope of regression line calculated against cross number per 10-minute trial for both ON time and OFF time. C_n indicates cross number.

Correlations

All within-trial correlations were calculated from the raw (i.e., untransformed), within-trial ON and OFF times. Assumptions of bivariate normality should not be critical for N_c greater than about 25–30 (Welkowitz et al., 1976). Therefore, in order to keep the analysis on the same level as the data, the Log10 transformations was not used. Also, no transformations were carried out before the analysis of variance was computed. The raw data may be found in Tables A.9–A.12, Appendix A. Figures 11– 12 give a graphical presentation of the main results.

As intensity was increased a significant decline in the correlation between each ON time and the succeeding OFF time was found (i.e., for Corr (X, Y): $F = 3.17, df = 6.66, p < 0.01$). Also, the overall mean for Corr (X, Y), was significantly different from zero (mean $= 0.103, F = 10.86. df = 1, 11, p < 0.01$). The overall mean for the Corr (X, Y_{-1}) data was also significantly different from zero (mean $= 0.064, F = 9.66. df = 1, 11, p < 0.05$) but intensity had no significant effect. The only other significant result for the correlation data was a Sequence $\times$ Intensity interaction for the Corr $D(X, Y_{-1})$ data ($F = 2.04, df = 12, 132, p < 0.05$).

There was no significant difference between the overall mean for Corr (X, Y) and the overall mean for Corr (X, Y_{-1}) ($t = 1.578, df = 272, p < 0.05$, matched t-test). There were also no significant differences between Corr (X, Y) and Corr (X, Y_{-1}) at any intensity level (matched t-tests).

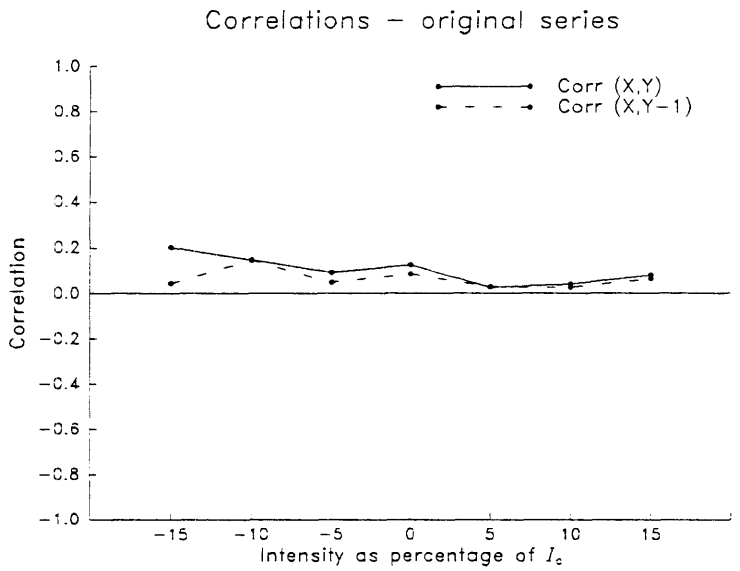

Figure 11: Mean correlation between ON time and succeeding OFF time (i.e., Corr (X, Y)) and ON time and preceding OFF time (i.e., Corr (X, Y_{-1})). The original data series was used.

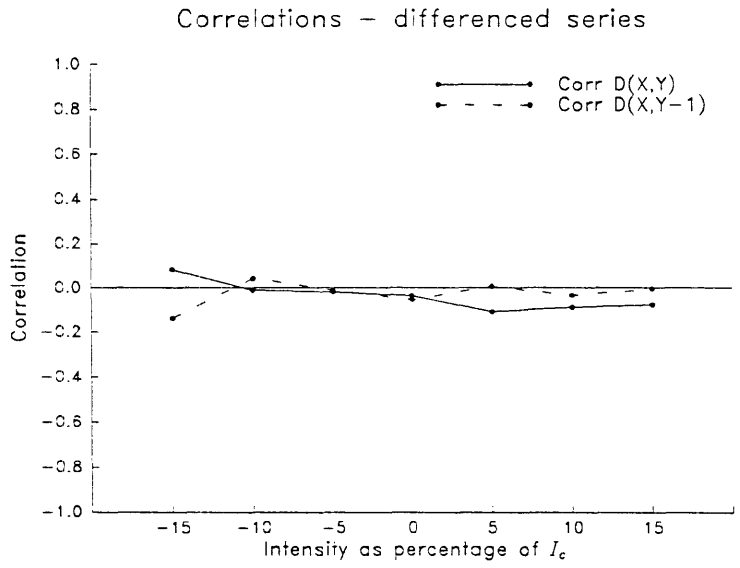

Figure 12: As for Figure 11 but with the original data series differenced once to remove trend.

Table 5: Results of ANOVA for intensity main effects — correlation data

a) – All animals –

Variable	F-value	*df*	Tail probability
Corr (X, Y)	3.17	6,66	0.0085*
Corr (X, Y_{-1})	1.19	6,66	0.3236
Corr $D(X, Y)$	1.99	6,66	0.0793
Corr $D(X, Y_{-1})$	2.22	6,66	0.0515

b) – With M10 omitted –

Corr (X, Y)	3.37	6,60	0.0063*
Corr (X, Y_{-1})	1.39	6,60	0.2322
Corr $D(X, Y)$	2.25	6,60	0.0500
Corr $D(X, Y_{-1})$	1.92	6,60	0.0919

* indicates $p < 0.01$.

Because the data for rat M10 contained up to four estimated values (out of a possible 21 values) and because this animal was a rather ambivalent self-stimulator, the above analyses were repeated with the data for M10 omitted. This procedure did not alter the major results (i.e., intensity was significant for Corr (X, Y) only, $F = 3.37, df = 6, 60, p < 0.01$; the overall mean for Corr (X, Y) was significant, mean $= 0.108, F = 10.58, df = 1, 10, p < 0.01$; the overall mean for Corr (X, Y_{-1}) was also significant, mean $= 0.067, F = 8.99, df = 1, 10, p < 0.05$). For Corr $D(X, Y)$, intensity main effects produced a tail probability equal to 0.05 ($F = 2.25, df = 6, 60$), which, although technically still not a significant result, was very close to being considered significant. Also, a significant Intensity × Frequency interaction appeared for the Corr $D(X, Y)$ data ($F = 2.64, df = 6, 60, p < 0.05$), while the Sequence × Intensity interaction found when M10 was included, disappeared with this analysis. See Table 5 for a summary of these analyses.

5.1.6 Discussion

The finding that order of presentation of intensities does not significantly alter any variable indicates that the procedures adopted (i.e., counterbalanced groups, 10-minute test sessions, one to three test sessions per day) eliminates contrast effects (cf., Atrens et al., 1973; Koob, 1977).

The finding that the frequency of stimulation (i.e., 100 Hz and 200 Hz, zero IPI) did not significantly alter any variable indicates that the rate of alternation between positive and negative phases of a 'continuous' wave form does not alter the behavioural measures considered here. The results therefore have some generality in terms of the stimulus used.

Discussion of the relationship between intensity of stimulation and ON and OFF time is approached by a discussion of the results for each particular behavioural measure.

Primitive variables

Number of crosses

The results for the N_c variable indicate that the methods followed effectively captured the region of interest from the rate-intensity functions. That is, the central, steeply rising, approximately linear, portion of the curve. This portion of the curve is unlikely to be significantly constrained by 'floor' or 'ceiling' effects. Moreover, because the only significant component in the data was linear, crossing rate over this parameter region should provide a reasonable reflection of reward magnitude or reward value (Liebman, 1983: Section 2.3.1).

A significant linear component in the composite curve does not necessarily imply that individual curves were also linear. All the individual curves could in fact have been sigmoid functions for instance, and still have produced a linear shape when averaged over a large number. Visual inspection of the individual data (Figure 3) in fact suggests that a sigmoid shape probably best represents the individual graphs (Coulombe & Miliaressis, 1987) but that in the averaging process this has been lost. The obtained RIFs show a strong resemblance to RIFs obtained when the number of lever presses per unit time have been measured (e.g., Olds et al., 1960). Some RIFs show "undulating" or "steep" forms, but no "square" RIFs, in which response rate fails to increase beyond a certain level, were found.

Individual RIFs (Figure 3) show little evidence for a true threshold in rate of responding (Stellar & Stellar, 1985; Valenstein, 1964; Wetzel, 1971), where a true threshold may be defined as some point of discontinuity in the RIF below which virtually no increases occurred and beyond which very rapid increases occurred. Instead the locus of rise of the RIFs was approached with a gradually increasing gradient.

Mean ON and OFF time

The present results confirmed that both mean ON time and mean OFF time are decreasing functions of current intensity. Both the amount of time animals spend with the stimulation ON and the amount of time they spend with the stimulation OFF, significantly decrease as the stimulus intensity is increased.

The shape of the ON and OFF curves indicates that ON and OFF time differ in their responsivity to intensity increase. OFF times were generally greater than ON times at low intensities, but decreased rapidly to eventually be less than ON times at higher intensities. These results are similar to those reported by Schmitt et al. (1981) for hypothalamic placements. There the authors showed that, for individual animals, a cross-over in mean ON and OFF time occurred as a function of interpulse interval.

The steeper decline seen with OFF time indicates a general feature of OFF time. This variable was generally more unstable, or more weakly controlled, than the ON time variable under conditions which produce low rates of crossing (e.g., low intensity, or low charge density — also see Schmitt et al. 1981). The unstable nature of OFF time data was evident within trials at low intensities, and by the appearance of some step-like functions for mean OFF time (e.g., M01 (A), M22 (A), M19 (A,D,R), M23 (A.D.R) — Table A.4, Appendix A). The evidence for step-like functions in the OFF time data gives some support for the notion of a threshold for OFF time. An increase in intensity rapidly induces a high degree of control over OFF time. Schmitt et al. (1981) were led to similar conclusions: "the approach response increased more rapidly than did the escape response from a low or even zero performance to a maximum level of performance" (p., 76).

Atrens (1970) reported no significant quadratic terms in his analysis of ON and OFF time data for nine animals with a variety of sub-cortical, limbic electrode placements. The present results found a significant quadratic term in (Log) OFF time and a significant cubic term in (Log) ON time as well as significant linear components in both (Table 4). At least three reasons may be provided for this discrepancy. Firstly, the electrode sites used by Atrens (1970) sampled a number of telencephalic and diencephalic structures which did not include the VTM. Secondly, Atrens (1970) did not transform the ON and OFF time data by Log10 before analysis to stabilise variance and reduce positive skewness. This transformation markedly increased the calculated F-values in the present study. Only then may the (marginally) significant

trends in the data be identified. Lastly, no mention was made in the Atrens (1970) study of tests for cubic trend.

Derived variables

Examination of the data for the derived variables (i.e., $\overline{P}$, TT, $\overline{Q}$, TQ), revealed that all were significantly modified by intensity. The proportion variable showed that SSs spend a significantly greater proportion of the time for each cross with the stimulation ON. This was due to OFF time decreasing more rapidly than ON time as intensity was increased.

The significance of the total time variable lies in the availability of a comparison with other reported data. Atrens (1970) suggested that the positive results found by Valenstein and Myers (1964) with this variable may have been due to the latter authors' use of interrupted trains of stimulation (i.e., 0.5 sec trains separated by 0.5 secs for LH stimulation and separated by 1.5 secs for septal stimulation). Atrens (1970) had used a continuous, uninterrupted, sine wave stimulus. Interrupted trains of stimulation might delay the onset of the aversive component responsible for motivating the termination response. The present experiment used an uninterrupted square wave stimulus and found that total time with the stimulation ON was significantly greater at higher intensities.

Several reasons may be offered to account for the discrepancies between the present results, those of Valenstein and Myers (1964) and those of Atrens (1970). Firstly, each of the three studies employed considerably different stimulus parameters. Valenstein and Myers used 100 Hz biphasic square pulses of 0.2 msec duration, with a 0.2 msec delay between positive and negative pulses; Atrens (1970) used 60 Hz sine wave, and the present study used both 100 Hz and 200 Hz biphasic square wave with zero interpulse interval. Although the present study did not directly compare the stimulus parameters used by Atrens (1970) and Valenstein and Myers (1964), the finding of no differences between the 100 Hz group and the 200 Hz group indicates at least some generality in the present results.

Secondly, the anatomical location of electrodes was different in the three studies. Neither of the previous studies used VTM placements. Valenstein and Myers used septal and LH placements while Atrens used a variety of sub-cortical limbic structures which included two septal placements. Valenstein and Myers (1964) used interrupted stimulation to compensate for differences in preferred rate of stimulation

between LH and septal stimulation. They suggested that there may be an important interaction between the rate of stimulation and the site of stimulation and this may be particularly significant for stimulation of the septal region.

The data presented in the Atrens (1970) study indicates that only two of the nine animals show ON and OFF times similar to those found in the present study. Intensity may not have been increased sufficiently in the Atrens' study to demonstrate the increase in total time, or the structures stimulated may not have been as responsive as the VTM.

Considerable statistical differences also existed among the three studies cited. Valenstein and Myers did not compute ANOVAs for their data, apparently relying only on a graphical interpretation. Atrens (1970) did calculate ANOVAs for each of the nine animals and reported that all were nonsignificant. However, no transformations appear to have been used. The present study found that the Log transformation of ON and OFF time data increases F values. Lastly, the ANOVAs for the present study were performed with group data and not separate ANOVAs for each subject. A significant effect for the group data does not imply a significant effect for each subject in the group.

In summary, the results of previous studies may not be directly comparable to the present results for the total time variable. The present results may only be applicable to stimulation of the VTM region with the particular set of stimulus parameters employed in this study. However, the steady rise in TT (or $\overline{P}$) seen in Figure 7 and Figure 8 as intensity was increased suggests that the present results cannot be dismissed as due to chance, and must instead reflect a general trend towards an increased preference for stimulation compared to no stimulation at higher intensities. Poschel (1966) using a 60 Hz sine wave also found an increase in the total time spent on a positive platform.

Charge

The variables of mean charge accepted per cross ($\overline{Q}$) and total charge accepted per trial (TQ) both include intensity in the calculation of the dependent variable and therefore provide measures of the amount of electricity consumed.

The $\overline{Q}$ variable (Figure 8) shows that animals were not attempting to obtain a certain quantity of charge at each initiation. If this were so a horizontal line would result. The shape of the graph and the calculations imply that the $\overline{Q}$ variable is

closely related to ON time. The greater S.E.M. seen with this graph is indicative of the increased variability added by including M11 in the analysis. This animal operated at a higher intensity than the remaining twelve animals.

The results for TQ suggest a strong linear relationship between this variable and intensity. The size of the error bars in relation to the mean also suggest a greater degree of normality with this variable, and the F-value for this variable was higher than any other, despite the fact that no transformations were used.

Correlations

The data for the regression line slope indicate that during a 10-minute trial, self-stimulating rats tend to slow down. Both ON and OFF time tend to increase over a trial. This tendency was more evident at low to moderate levels of intensity and there was also some suggestion that OFF times increased more during a 10-minute trial than did the corresponding ON times (Figure 10). The correlation between each ON time and the succeeding OFF time, and the correlation between each ON time and the preceding OFF time, would tend to show a positive correlation due to the presence of this slowing down. Each OFF time, would, on average, be slightly greater than the preceding ON time and each ON time, would, on average, be slightly greater than the previous one. The increasing linear trend will tend to induce a positive correlation in the data unrelated to the 'true' correlation between the times. For this reason, a first-order differencing process was used to eliminate the positive linear trend in the mean (Box & Jenkins, 1976).

The correlation analysis showed that elimination of the linear trend before the correlations were calculated also eliminated the significant intensity effects found for Corr (X,Y). Also, the overall means for Corr (X,Y) and Corr (X,Y_{-1}) which had been slightly, but significantly positive, became indistinguishable from zero. Thus, when linear trend was accounted for, there was no evidence to suggest a consistent correlation between within-trial ON time and the preceding, or succeeding, OFF time at any level of intensity.

On the other hand, the correlation between mean ON and OFF time declined from a significant positive correlation at the four lowest levels of intensity to near zero at the highest intensity levels. This result emphasises the distinction between calculating correlations from mean values and the calculation of correlations from individual within-trial ON and OFF times. If a correlation between mean ON and

OFF times is reported then it may also be necessary to report from where on the intensity-duration function the correlation was obtained.

The significant positive correlation between mean times at low to moderate intensities may have been due to the fact that at these intensities, the disparity between 'poor' and 'moderate' SSs was quite great. A 'poor' SS tended to spend a long time in both ON and OFF compartments, whereas a 'moderate' SS spent a shorter time in both compartments (even though within-trial times were not related). At higher intensities, all rats were 'good' to 'very good' SSs and the correlation between mean times may have then reflected the same independence between times as was found with within-trial times. Alternatively, duration of stimulation may have a different effect at lower intensities.

5.2 Experiment Ib: Stability of measures

5.2.1 Introduction

The previous experiment clarified the relationship between a number of variables and intensity and amongst the variables themselves. The major result of that experiment established that there was no significant linear relationship between the within-trial ON and OFF times. The present study extends some of these results as well as investigating a methodological problem associated with shuttling behaviour.

Firstly, the possibility remains from Experiment Ia, that a correlation between ON and OFF time might only develop after repeated exposure to the one set of stimulus parameters. In Experiment Ia, the intensity of stimulation was varied each trial in either an ascending, descending, or random sequence. In no case was there an opportunity for correlation to develop in the behaviour if that correlation develops slowly over a number of trials. The present experiment was undertaken primarily to examine the possibility that correlations might develop slowly after increased experience with the one set of stimulus parameters.

The second aim concerns the need to establish some estimate of the stability of the variables discussed in Experiment Ia. It is an empirical question as to how many 10-minute trials are required before ON and OFF time reach a recognisably stable condition. Alternatively, the question might ask what variables remain most stable over a repeated series of observations.

A number of criteria have been cited in the literature for identifying when shuttling

behaviour might be considered stable. Atrens (1970) used a criterion for stability of three consecutive days on which ON time fell within a range of 10% or 5 secs of the mean value (15-minute trials were used). Later studies used 10% or 0.5 secs of the mean (Atrens & Sinden, 1975; Atrens & Von Vietinghoff-Riesch, 1972). Schmitt et al. (1981) allowed a daily two hour training period for two weeks with no specific criterion for stability. Popov et al. (1983) and Mendelson (1969) allowed a specified number of training sessions of 20 to 30 minutes duration each (usually, 3–4). Skelton and Shizgal (1980) describe a requirement that threshold determinations for both ON and OFF time vary by less than 0.2 log units across six successive determinations (140-sec trials). Shizgal and Matthews (1977) continued training until, for three consecutive days, the latency-intensity functions were stable across the 6 blocks of the experimental session (each block contained 8–10 200-sec periods). Montgomery et al. (1981) considered that the standard deviation of the slopes of ON time against the logarithm of the frequency used should be less than or equal to 0.20 on three consecutive sessions.

However, no data was presented in the above studies to justify the use or non-use of a particular stabilisation criterion. The rate at which a particular variable approaches stability, or the degree of variability inherent at the beginning of training have not been explored, or at least reported. in detail. Atrens and Von Vietinghoff-Riesch (1972) did report that 12–30 five minute trials were required to achieve stability of both ON and OFF times. Valenstein and Myers (1964), on the other hand, reported that when naive rats were placed in a test situation at an intensity estimated from similar placements in experienced rats, their behaviour was very similar to the behaviour of the experienced rats as measured by total time with the stimulation ON. The evidence from Valenstein and Myers (1964) suggests that very few trials are needed for a reasonable level of stability, or that different variables show a different degree of stability.

The present experiment was designed to empirically estimate the stability of the variables discussed in Experiment Ia when the same stimulus parameters were used for several trials.

The two groups from Experiment Ia (i.e., the 100 Hz and 200 Hz groups) were continued throughout this experiment maintained at their respective frequencies. Again, differences beween the two groups may not develop when intensity is varied from trial to trial and might only develop after repeated exposure to the one frequency and intensity. A level of intensity was chosen for this experiment at which instability

in response measurement should have been particularly evident.

5.2.2 Subjects and apparatus

The subjects and apparatus were the same as in Experiment Ia. All subjects had been rested for two weeks before testing recommenced. The testing program was staggered.

5.2.3 Method

From Experiment Ia, three rate-curves for each animal were determined. One represented current intensities presented in an ascending sequence, one in a descending sequence, and one in a randomised sequence. These three sequences for each animal were combined into one; from which the higher end of the line segment showing the steepest rise was chosen as the intensity at which a particular animal' s crossing rate was most responsive (again referred to as I_c). The end of the line segment was chosen (rather than the centre) in order to compare behaviour with the behaviour at the same intensity in Experiment Ia (see Table 2).

The six subjects in the 200 Hz group and the seven subjects in the 100 Hz group were tested at their respective frequency and the I_c value for 13 trials. Each trial was 10 minutes. At the 14th trial the frequencies were swapped over, so that the 100 Hz group received the I_c value at 200 Hz and the 200 Hz group received the I_c value at 100 Hz. There was only one 10-minute trial at the swapped frequency.

Statistical analysis

ANOVAs were calculated for each variable described in Experiment Ia using the BMDP2V program. All analyses were conducted on the first 13 trials only and used the same transformations (if any) for each variable as were used in the previous experiment (Section 5.1.4).

Problems with computer recording equipment with the second staggered group (particularly Trials 1 and 13) meant that some values had to be estimated. Because manual records were kept of N_c and $\overline{X}$ and $\overline{Y}$ at the conclusion of each trial, the data for these particular variables were almost always available (although no data were available at all for M23, Trial 13). Those variables which had to be calculated (at some later stage) from the within-trial ON and OFF times had to be estimated. For

the proportion variable, estimates were made from $\overline{X}$ and $\overline{Y}$ (see Results of previous experiment, Section 5.1.5).

However, for standard deviations, regression line slope, Corr (X, Y), Corr (X, Y_{-1}), Corr $D(X, Y)$, and Corr $D(X, Y_{-1})$, missing data was too extensive at Trials 1 and 13 and for M19, so these data were omitted from the analysis. These variables were only calculated for Trials 2 through 12 (i.e., 11 trials) and only included five animals in the 200 Hz group.

5.2.4 Results

No significant differences existed between the 100 Hz and 200 Hz groups on any variable. The data for these two groups were therefore averaged to produce 13 observations at each trial. Graphical presentation may be found in Figure 13 to Figure 19 and the data in Tables A.13 to A.24, Appendix A. The overall mean intensity was 21.2 μamps — a value slightly less than level 5 in Experiment Ia. Thus, the I_c value used in this experiment was somewhat higher than that estimated in Experiment Ia (i.e., mainly due to the higher end of the line segment being used rather than the centre). The constant line in each figure represents the value of the particular variable found in Experiment Ia at the same intensity (Table 2) and indicates that after a two week rest period animals began responding at a level very similar to that found in the previous experiment.

The results of the analyses and the graphical presentation indicated that the non-correlation variables fell into two quite distinct categories. Significant changes occurred over the trials with N_c, Log $(\overline{X})$, Log $(\overline{Y})$ and Log $(\overline{Q})$ (N_c: $F = 6.26$; Log$(\overline{X})$: $F = 4.70$; Log$(\overline{Y})$: $F = 4.09$; and Log$(\overline{Q})$: $F = 4.70$, all dfs= 12, 132, all ps< 0.0001). These results confirm the general trends seen in Figures 13– 19, which show that both ON and OFF time tended to increase, while number of crosses declined.

Tukey's HSD post hoc analysis (Roscoe, 1969) revealed that the means for all three variables N_c, $\overline{X}$, and $\overline{Y}$ did not differ significantly over the last 10 trials. The N_c variable had significantly declined by Trial 4 (critical difference = 15.59, error mean square= 149.23, $df = 132$) and the average of the first three trials was significantly higher than the average of the remaining 10. A similar result applied for Log $(\overline{Y})$ in that mean OFF time had significantly increased by Trial 4 (critical difference= 0.167, error mean square= 0.0172, $df = 132$). However, the average of the first three

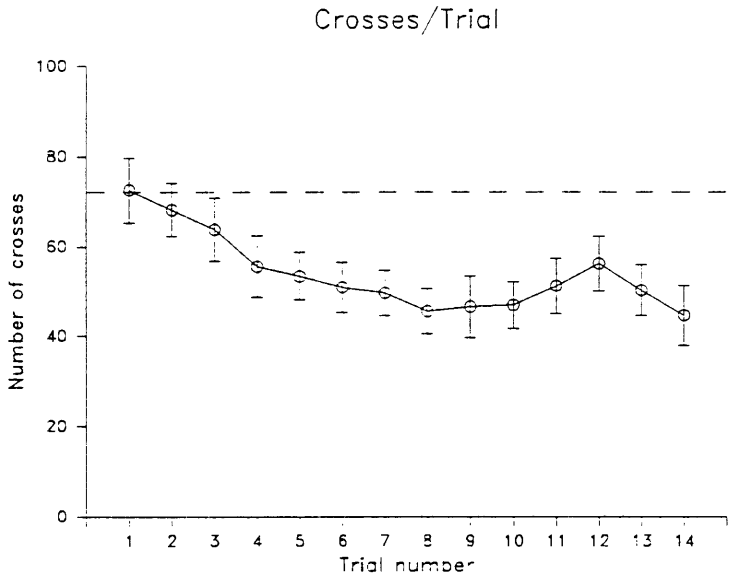

Figure 13: Stability of number of crosses variable over successive observations. Constant line in this figure (and in Figures 14 – 19). indicates the value of the respective variable found in Experiment Ia.

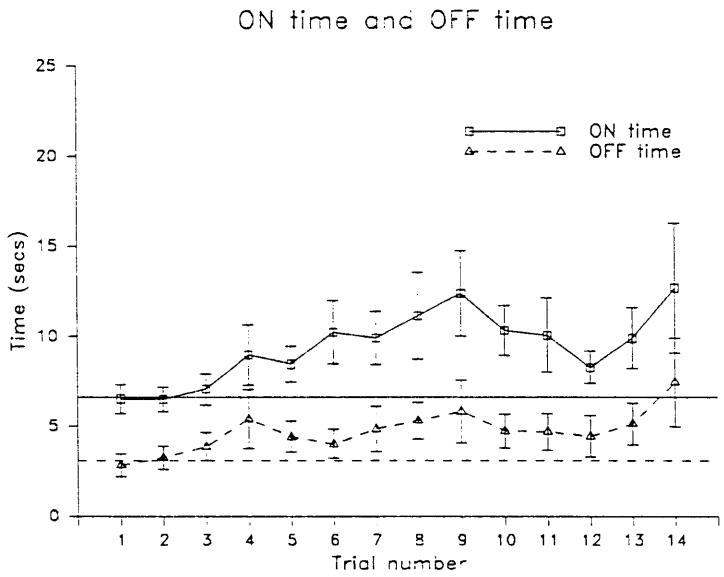

Figure 14: Stability of ON and OFF time measures.

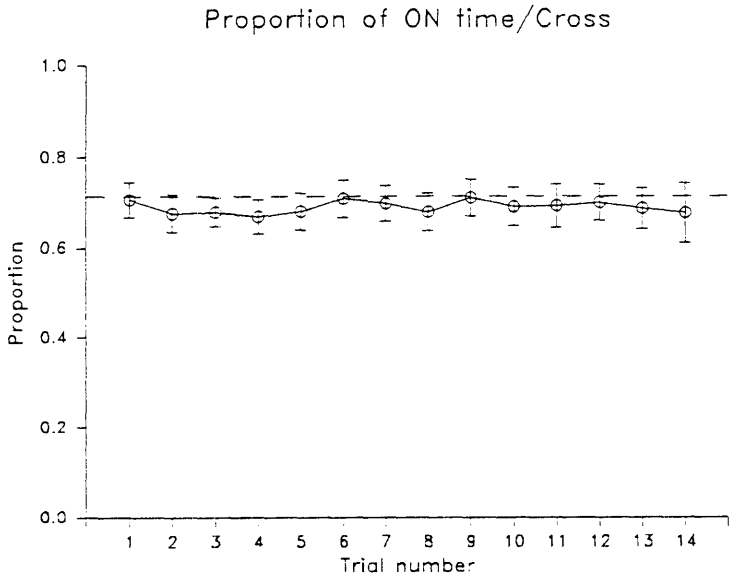

Figure 15: Proportion of each cross time spent with the stimulation ON over successive trials.

trials was not significantly different from the average of the last 10. For Log ($\overline{X}$), a significant increase did not occur until Trial 6 (critical difference = 0.152, error mean square= 0.0142, $df = 132$). Again, the average of the first three trials was not significantly different from the average of the last 10 trials (Figure 14).

The second category of non-correlation variables included the variables: $\overline{P}$, TT, and TQ. There were no significant changes in these variables over the 13 trials. (For Arcsine ($\overline{P}$): $F = 1.15$; Log (TT): $F = 0.59$; TQ : $F = 1.15$ — all dfs= 12, 132, all ps> 0.05).

Analysis of the correlation data revealed no significant changes over the 13 trials, although a significant overall mean was found for Corr (X, Y) and Corr (X, Y_{-1}) (mean = 0.146, $F = 10.82, p < 0.01$: mean = 0.105, $F = 7.40, p < 0.05$, respectively — all dfs= 1, 10). First-order differencing reduced these correlations to values not significantly different from zero (mean = −0.013, $F = 0.05$; mean = −0.017, $F =$ 0.41, respectively — all dfs= 1, 10, all ps> 0.05).

The difference between the overall mean values of Corr (X, Y) and Corr (X, Y_{-1}) was not statistically significant ($t = 1.495, df = 131$, matched t-test). Also at no trial was Corr (X, Y) and Corr (X, Y_{-1}) significantly different. There was also no difference between the overall mean values for Corr $D(X, Y)$ and Corr $D(X, Y_{-1})$ ($t = 0.122, df = 131$).

Trial 14 was the switchover trial: those animals that had been receiving 200 Hz stimulation now received 100 Hz stimulation, and those animals that had been

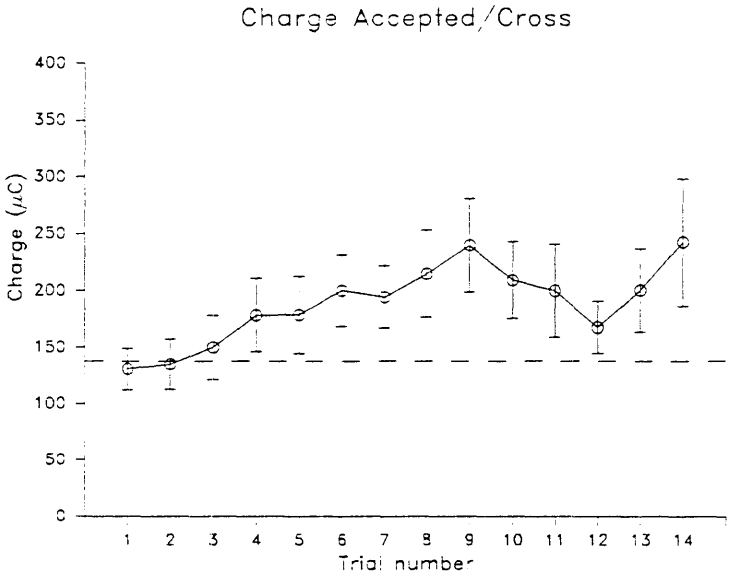

Figure 16: Mean charge accepted per cross over successive trials.

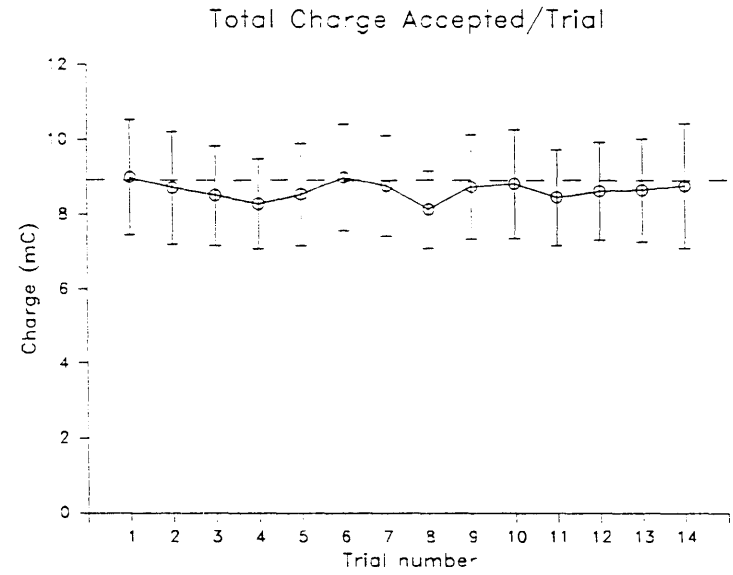

Figure 17: Total charge accepted per 10-minute trial over successive trials.

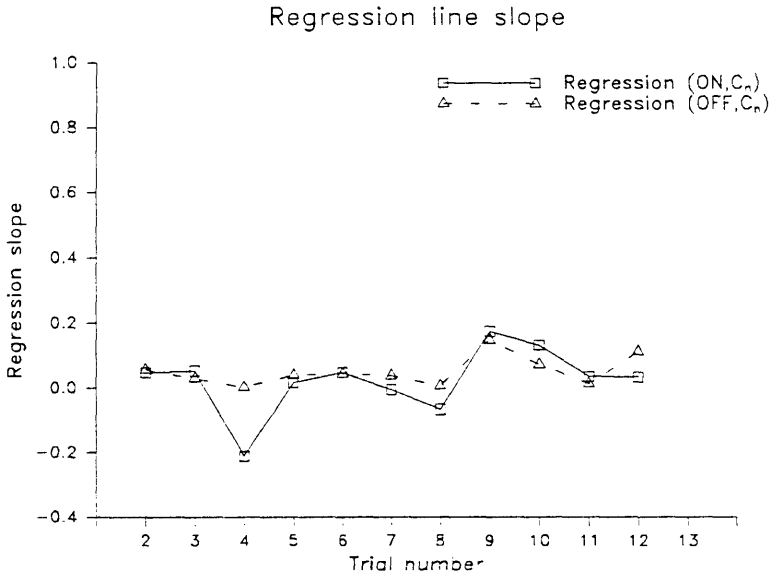

Figure 18: Stability of regression line slope measure. C_n indicates cross number.

receiving 100 Hz stimulation now received 200 Hz stimulation. The switch revealed significant individual differences in the direction of behaviour change when the value for that last trial was compared to the mean value obtained over the previous 10 trials (see Tables A.13, A.14, A.16, and A.18, Appendix A).

With reference to the proportion variable for instance (Table A.18, Appendix A), five animals (four in the 100 Hz group and one in the 200 Hz group) increased the proportion of time allocated to stimulation on the switchover trial. On the other hand, two animals decreased the proportion (one in each group) while the remaining seven showed no significant change (arcsine transformation used, one-sample t-test, rejection criterion = 0.01).

The change seen with rat M01 was quite dramatic. Mean ON times reduced from a steady mean of 5.66 secs to 0.25 secs with a corresponding increase in OFF time from 7.63 to 27.0 secs (Table A.14). Other animals (e.g., M09, M22, M23) increased both ON and OFF times under the new conditions, while still others increased ON time only (e.g., M18). M26 decreased OFF time only, while M05 increased ON time, but decreased OFF time. Only three animals showed no change on at least one of the variables $\overline{X}$, $\overline{Y}$, or $\overline{P}$.

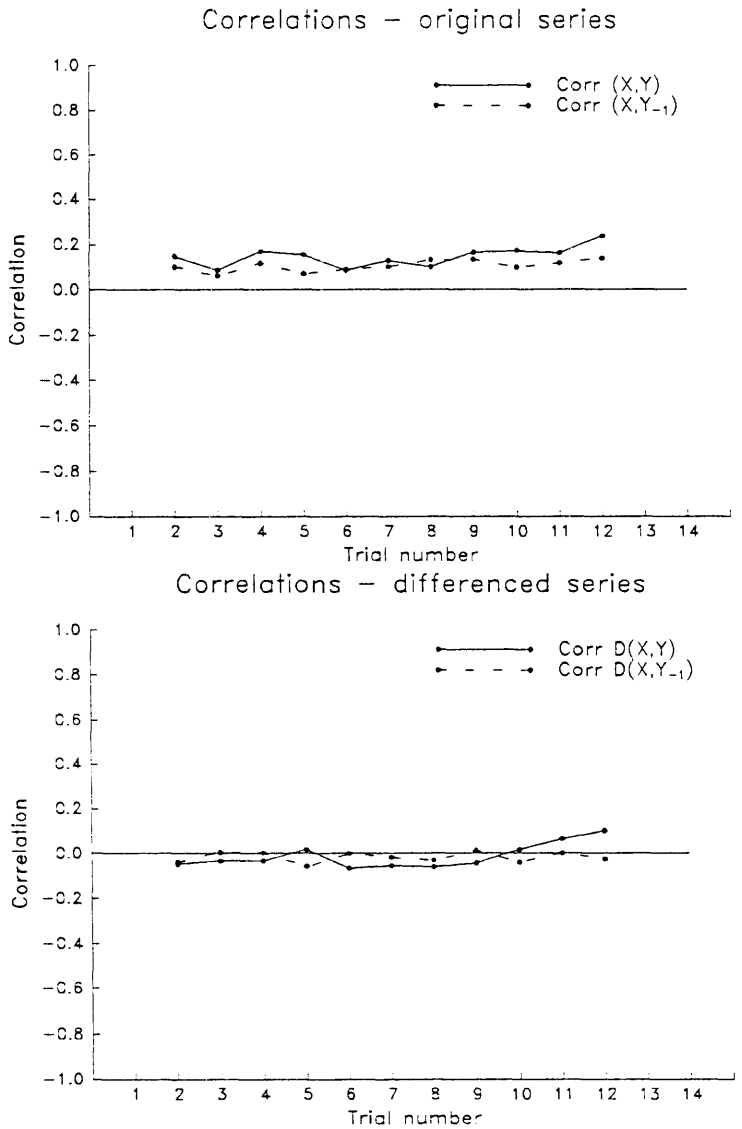

Figure 19: Mean correlation between ON time and succeeding OFF time and ON time and preceding OFF time. For top diagram the original data series were used. For lower diagram, original data series differenced once to remove trend.

5.2.5 Discussion

The correlation between within-trial ON time and the preceding, or succeeding OFF time does not alter as animals gain more experience with a particular set of parameters. There was no evidence for a consistent linear relationship between these times.

There were significant changes in some variables over thirteen 10-minute trials (i.e., N_c, $\overline{X}$, $\overline{Y}$, $\overline{Q}$). However, the variables $\overline{P}$, TT, and TQ showed a remarkable consistency considering the variation in the components that determined their calculation, indicating that animals were maintaining some overall relationship among the component variables while ever intensity was held constant.

The three variables: $\overline{P}$, TT, and TQ. were calculated in different ways and as a consequence represent different properties of the behaviour (Cane, 1961; Ludlow, 1976). The question arises as to which of these variables best represents the parameter being maintained during shuttling behaviour. Total time is unlikely to be a candidate since it suggests no mechanism by which animals could be maintaining total time — without postulating remarkable powers of time estimation. The proportion of time ON per cross measures essentially the same information as total time and it is again unlikely that animals could 'forsee' or estimate time allocation of this order.

TQ on the other hand, measures the amount of electricity consumed rather than merely the amount of time consumed. This variable may reflect a level of neural activation, or autonomic arousal, which is being maintained over an extended period of time during shuttling behaviour. Total charge was also quite normally distributed and produced the highest F-value in Experiment Ia (without transformation).

A constant total charge is maintained over a trial. but because the charge accepted per cross ($\overline{Q}$) varies considerably (both within and across trials), the constant total value is most likely being maintained by an interaction between crossing rate and the charge accepted per cross. As the rate of crossing decreases, ON time increases to a degree that compensates for this decrease. However. it is not clear whether ON time increases first, to be followed by a decrease in rate. or whether rate at first decreases and then ON time increases.

This argument suggests a resemblance to recent research results on the ability of rabbits to maintain a constant level of food intake (Geiselman, Novin, & Kissileff, 1982). These authors have shown by the use of multiple regression techniques, that total food intake is best predicted by an equation that includes both meal frequency

and meal size. In shuttling behaviour, rate of crossing (N_c, or rate of initiation) and charge accepted/initiation ($\overline{Q}$) may interact over some number of crosses to maintain a steady level of total charge.

Stability of measures

The empirical question of how many 10-minute trials are required to reach stability may be answered in at least two ways. Firstly, if the variables $\overline{P}$, TT, or TQ were to be used as measures of shuttling behaviour, then the values of these variables on Trial 13 would be as reliable as those on Trial 1. This result confirms the findings of Valenstein and Myers (1964) with the total time variable.

Secondly, if the primitive variables (i.e., N_c, $\overline{X}$, or $\overline{Y}$) were to be considered, then the results suggest two possible approaches. Of these, one would be to repeat the observations a number of times and to discard the first 3, 4 or 5 observations and use only the remainder. This has been the more usual approach (e.g., Atrens, 1973; Montgomery et al., 1981). However, if the first three, four, or five trials are used then reasonably reliable results would also be achieved. Because this approach offers the practical advantage of taking less time to complete an experimental condition, the use of three to four trials per condition will be employed here. It is important to note that this analysis is only relevant to group results averaged across 13 animals. Also, this experiment was conducted with very experienced animals, which had completed many hours in the shuttlebox during screening, training, determination of rate-intensity functions and all of Experiment Ia. The results do not necessarily apply to all animals or to less well-trained animals. On the other hand, the intensities used in the present study were intensities at which shuttling behaviour should have been particularly sensitive. If measurement instability was to be apparent, it should have been evident at these intensities.

The general decrease in crossing rate and general increase in mean ON and OFF times might be attributable to deterioration of the electrode-tissue interface, such as the buildup of metallic deposits (Gallistel, 1973; Wetzel, Howell, & Bearie, 1969). Although this possibility cannot be entirely discounted, the finding that total charge accepted and total time with the stimulation ON remained constant suggests that little tissue damage has occurred. Valenstein and Myers (1964) produced lesions at self-stimulation sites in the hypothalamic and septal regions and showed that a decrease occurred in the percentage of total time allocated to the positive platform in

a two platform shuttling procedure. The original, pre-lesion percentage of total time on the positive platform could be restored by an increase in intensity (and therefore an increase in the total charge that the rats accepted). Although Valenstein and Myers used interrupted stimulation, their results suggest that tissue damage at the electrode tip is unlikely to account for the general increase in ON time that occurred.

Finally, the lack of a significant difference between animals responding for 100 Hz stimulation compared to those responding for 200 Hz stimulation, indicates that the repetition rate of a continuous waveform does not significantly influence the variables considered. Alternatively, the behaviour produced by a pulse width of 5 msec is not measurably different to behaviour produced by a pulse width of 2.5 msec, when the pulses are constantly alternating to provide continuous (i.e., zero IPI) stimulation. The switchover trial did suggest that many self-stimulating animals could detect the change in frequency, but the response was highly individualistic. Whether this individualism reflected differences in the site of stimulation or in some other factor has not been determined.

5.3 Conclusions from Experiment I

The main results from Experiments Ia and Ib indicate that when within-trial trend in both ON and OFF times is accounted for, there is no significant linear relationship between ON time and the preceding, or succeeding, OFF time at any mid-range intensity. These results confirm then, the early claims made be Atrens and his coworkers (e.g., Atrens, 1970, 1973; Atrens & Becker, 1975; Atrens & Von Vietinghoff-Reisch, 1972) that there is no significant correlation between the ON and OFF time measures. The present results extend and clarify these previous results to include a relatively large number of intensity levels, both preceding and succeeding correlations, and the differenced series as well as the original data. The differenced and untransformed within-trial ON and OFF times are therefore effectively independent.

If the within-trial times are not differenced first then a significant positive correlation may be obtained (but still a low r), which may also be influenced by intensity. Also, if untransformed mean ON and OFF times are correlated, then it may be necessary to specify from where on the intensity-duration function the correlation was obtained. Alternatively, if mean ON and OFF times are required to be independent then a relatively high intensity should be used.

The above conclusions may also only apply to stimulation of the VTM. Atrens

(1973) and Atrens and Becker (1975) found non-significant, but negative, correlations between mean times when hypothalamic sites were stimulated. Also, different selection procedures for what constitutes a self-stimulator in different studies may influence the relationship between ON and OFF time for those animals that are finally selected.

The results of Experiment Ia confirmed the three features that were used to select the reward/aversion model and the reciprocal inhibition model (Chapter 3). These models must now also accommodate the finding that ON time is not consistently related to either the preceding, or succeeding, OFF time at any intensity (at least over the central range of the RIF). Since the present interpretation of the reward/aversion model says that the reason for the shorter ON times at high intensities is that the accumulating aversion reaches a fixed level more rapidly, and that decreased OFF time reflects only the increased reward value provided by the higher intensities, these two effects of intensity must be independent. This conclusion would most easily be accommodated by a two system view of the underlying neural substrate rather than by a single substrate opponent process. Because there was considerable variation in the times, both within and across trials, the variation that did occur must reflect normal, biological, 'white-noise' variation in the parameters of the system. That is, variation in the rate of accumulation, variation in the fixed criterion level, or variation in the magnitude of the reward effect received on initiation.

The decrease in the standard deviations and S.E.M.s also associated with the increase in intensity may be seen as evidence for an increased precision in the timing of ON and OFF responses. This might further be interpreted as evidence for the control of an aversive system (Liebman, 1983; Stein, 1962). However, rats are unlikely to be experiencing significant aversion at these intensities, because any extra aversion that accumulated during longer ON times would be expected to take longer to dissipate and hence produce longer OFF times and a positive correlation. It is more likely that well-trained SSs have learnt to terminate before significant aversion has developed.

For the reciprocal inhibition model with fixed parameters, a constant input of stimulation would be expected to result in a consistent relationship between the cyclic periods of dominance (although no empirical data exists on this at present). To incorporate random variation into the periods of dominance, fixed intensity stimulation of directly stimulated neurons might produce 'white-noise' input to a second stage network (the mean level of the white-noise input may still be determined by intensity). A white-noise input to the second stage network may be sufficient to produce uncorrelated periods of dominance in the output.

The above considerations suggest that duration of stimulation is not a major variable in the determination of OFF time, however, the results do not distinguish between intensity and duration in terms of the mean decrease in OFF time. A correlation between two variables measures the variation in one variable that may be accounted for by the variation in the other. The mean decrease in OFF time could still be due, at least in part, to the decrease in duration that also occurs.

The present results also do not rule out the possibility of a nonlinear relationship between ON and OFF time since the Pearson product-moment statistic is a measure of the degree of linear relationship between two variables. Scatter plots quite effectively rule out the possibility of a curvilinear relationship such as quadratic or cubic, but the remaining possibility of a nonlinear relationship, for instance based on one-dimensional dynamics (e.g., May, 1980, May & Oster, 1976), cannot be dismissed at this stage.

Chapter 6

Experiment II: Fixed durations

The present series of experiments investigates the relationship between ON and OFF time and the relationship among these two variables and the intensity of stimulation: including what relationship exists between the timing of the initiation and termination responses, what factors most significantly determine the timing of these two responses and what mechanisms might be proposed to account for the observed relationships. Experiment I found that although both ON and OFF time significantly decreased as intensity was increased, within-trial correlation between these times was negligible at all levels of intensity. Therefore, the hypothesis that OFF time occurs as a function of the duration over which the stimulation applies was not supported.

The correlation coefficient is an index of the concomitant variation of two variables: it indicates nothing about the magnitude of the variables (Roscoe, 1969). A lack of correlation does not negate the possibility that at least part of the decrease in mean OFF time occurs as a consequence of the decrease in mean ON time. The two experiments reported in this chapter specifically examined the effect of duration of stimulation on the selection of OFF time (Experiment IIa) and the effect of duration of no stimulation on the selection of ON time (Experiment IIb).

6.1 Experiment IIa: Fixed ON time

6.1.1 Introduction

Several studies have reported results in which ON time has been controlled. The typical lever press procedure is of this sort, with ON time usually set at 0.5 secs. Some studies have systematically varied ON time to values considerably beyond this (see

Section 3.1). When stimulus durations were presented on a variable interval schedule (e.g., Keesey, 1964), or a progressive ratio schedule (Hodos, 1965), lever press response rates continued to increase for stimulus durations beyond the durations that had been previously self-selected. Gallistel (1973) has argued that the increase in response rate reported by Keesey (1964) and Hodos (1965) was due to increased drive-like aftereffects of long durations rather than increased reward. Because an increase in response rate, on the same type of schedules, can also be obtained by an increase in intensity, frequency, or pulse width (Atrens, 1970; Hawkins & Pliskoff, 1964; Keesey, 1962, 1964; Wauquier et al., 1972), an increase in train duration should, at least, have a similar neurophysiological effect as the others. This suggests that an increase in duration of stimulation provides an increment in reward value over and above that provided by intensity of stimulation.

As a consequence, an increase in ON time should lead to a decrease in OFF time. This conclusion is also supported by the study of Atrens et al. (1983), which showed that under partial reinforcement schedules, initiation rates were low for short ON times (approximately 50% of maximum at 2.5 sec), increased rapidly to a maximum at 5–10 secs and then declined again for ON times greater than 10 secs. Although the preferred duration was not indicated in that particular experiment, the preferred duration data that was presented for a previous experiment (and which contained many of the same animals), indicated a mean preferred ON time of 4.6 secs. Thus, from a minimum ON time to an ON time at or just beyond the preferred duration, initiation rates increased (i.e., OFF time decreased).

In summary, because both ON and OFF time decrease as the intensity of stimulation is increased, the decrease in OFF time might result from the decrease in the duration of stimulation that also occurs. However, some evidence indicates that over the range from one sec to about the preferred duration a decrease in ON time might be accompanied by increased OFF time. The effect of a decrease in duration might therefore be opposing the effect of an intensity increase.

Because no study has examined the effect of an increased duration of stimulation on OFF time when shuttling behaviour is under CRF, the present experiment was designed. ON time was controlled from a minimum value of 0.5 sec (i.e., a time commonly used in lever press studies) to a value beyond each animal's preferred duration, in five equally spaced steps. The number of crosses and intensity were fixed throughout so that mean charge obtained on each initiation and total charge available during a trial were also fixed for any particular test duration. The use

of a fixed number of crosses also requires all animals to cover approximately the same distance in the shuttlebox and hence expend a similar amount of physical energy. Because of the constant intensity, any change in OFF time would indicate a dependence of OFF time on duration of stimulation only.

A relatively high constant intensity was chosen for these two experiments because Experiment Ia showed that the proportion of time ON increases as intensity is increased. If duration of stimulation was to have a significant effect on OFF time, it should be more evident at higher intensities.

Predictability of initiation and termination

A second factor which was examined in this and the following experiment was the predictability of stimulus initiation and termination. Some studies have reported a significant effect for the variable presentation of an ICSS stimulus (e.g., Mason, Laferrière, & Milner, 1983; Steiner et al., 1969). For example, Steiner et al. (1969) found that rats terminated with a shorter latency from a regularly patterned stimulus than from a variably patterned stimulus. This may indicate that the factor of predictability of stimulation is most significant for a rat's selection of ON time (i.e., Experiment IIb). However, Mason et al. (1983) found that rats reinstated a period of self-initiated lever pressing with a shorter latency after the previous, experimenter-administered, stimulation had been presented on a variable interval schedule compared to when the stimulation had been presented on a fixed interval schedule. This may indicate that initiation latencies (i.e., OFF time) should be shorter when the duration of stimulation is presented variably compared to when constant durations are used.

The factor of predictability was compared in the present experiment by randomly allocating rats to either a constant ON subgroup or a variable ON subgroup. The level of variability in ON time was set at 7% of the mean. This is less than the natural variability in a free-shuttling situation which at Level 6 in Experiment Ia was 46% (see Tables A.3 and A.5, Appendix A). However, a 7% value was chosen so as to reduce the chance of overlap between adjoining test durations to a negligible value. This meant that 0.5 secs variable was little different to 0.5 secs constant, so that a significant difference between the subgroups would be most reflected in an ON time $\times$ Group interaction. A variable ON time also allowed the calculation of within-trial correlations in order to compare with the results of Experiment I.

Finally, the analysis used to assess the change in behaviour included an analysis

of the variablity of within-trial OFF time as well as an analysis of the mean level of OFF time. The variability of behaviour has been argued to be a valid measure of reinforcement (e.g., Rachlin, 1982; Staddon & Simmelhag, 1971). The arguments for this interpretation of reinforcement will not be considered in detail, it is noted only that according to this interpretation, a decrease in the variability of behaviour indicates an environment of higher utility (i.e., greater reinforcement value).

6.1.2 Subjects and apparatus

The subjects for this experiment were randomly drawn from a pool of 32 rats defined as self-stimulators by the criterion that each rat complete 20 crosses in less than five minutes at two consecutive intensities, without obvious interference from motor artifact (see General Method, for more details). One exception to this was made. Rat R01 completed the 20-cross blocks in approximately six minutes. However, the consistent and deliberate initiation and termination responses (without artifact problems) prompted the inclusion of this animal in the available pool of SSs. All the SSs had participated in a previous experiment.

6.1.3 Method

The selection, training and testing procedures followed in this experiment and the following experiment were the same. These procedures are described in detail here as they pertain to both experiments, however, the introduction, results and discussion for Experiment IIb are considered separately.

Training of subjects

In order for subjects to gain experience with the particular stimulus conditions (i.e., controlled ON time or controlled OFF time), and to assess the effect of the stimulus conditions on alley running, an initial experiment was conducted. This experiment required the completion of 50 20-cross trials presented in two blocks of 10 crosses, with one alley run per block of 10 crosses. The 50 trials were composed of 4 free-shuttling trials, followed by 46 trials in which either ON time or OFF time was controlled (this experiment is not reported in further detail).

The times chosen for the stimulus control values (i.e., those used in the 46 trials) were the 10 times self-selected by each animal in the first block of crosses on the fourth

trial (see General Method for description of 'stimulus control values', Section 4.3.4). In other words, for the ON time controlled group, 46 20-cross trials were conducted in which ON time was fixed at the values each rat had previously self-selected on the fourth, free-shuttling trial (this group is referred to as the Fixed ON group). Exactly the same procedure was followed with the OFF time controlled group (this group is referred to as the Fixed OFF group).

In addition, approximately one-half the animals in the Fixed ON group had ON times set at a constant ON time equal to the mean of those times that had been previously self-selected (referred to as the constant ON subgroup). The remaining one-half of the animals retained the ON times that had been previously self-selected (referred to as the variable ON subgroup). Again, exactly the same procedure was followed with the Fixed OFF group.

At the completion of this training stage of the experiment, 8 animals in the Fixed ON group (5 in the constant ON subgroup and 3 in the variable ON subgroup) had received a minimum of 920 stimulations in which the duration of stimulation, or ON time, remained unchanged. Similarly, 7 animals in the Fixed OFF group (4 in the constant OFF subgroup and 3 in the variable OFF subgroup) had received a minimum of 920 stimulations in which the duration of no stimulation, or OFF time, remained unchanged. These animals will be referred to as 'experienced' animals.

At the conclusion of the 50 trials, a further five animals were randomly assigned to the Fixed ON group and Fixed OFF group to increase the numbers in each group to 10 (five in each subgroup). The five animals came from a free-shuttling situation that had formed a control group during the training trials. These five animals will be referred to as 'naive' animals. For the five additional animals the ON or OFF time control values were taken from the first block of 10 crosses on the last free-shuttling trial.

The intensities chosen for each animal represented a relatively high intensity. Pulse width and interpulse interval were 3 msec and 4 msec respectively. All stimulus parameters remained unchanged throughout the training trials. A minimum of one week elapsed from the completion of the training trials to the start of Experiments IIa and IIb.

'Warm-up' and testing procedure

The 'naive' animals were given three 60-cross trials under their respective control conditions (i.e., two SSs with ON time controlled, three SSs with OFF time controlled). The 10 stimulus control values were repeated six times.

All of the 10 animals in each major group (i.e., Fixed ON group and Fixed OFF group) then completed a further six 30-cross trials. The first three of these trials were used to 'warm-up' the experienced animals after more than a week without testing (as described in the General Method), and to provide further training for the 'naive' animals. For the sake of reference, these three 30-cross trials will be referred to as 'warm-up' trials.

The second three 30-cross trials were the same as the first three except that for animals in the variable subgroups the variable values were no longer those previously chosen by the animal. The mean value was retained but distributed instead as a normal variable with a standard deviation set at 7% of that mean value.

The self-selected control values were found to vary quite considerably for a particular animal, and from animal to animal, with occasional extreme values that would have overlapped substantially with neighbouring test durations to be used in the main part of the experiment. Therefore, a uniform variablity was introduced for all animals and at all test durations. The 7% figure was chosen so as to make highly unlikely the chance of overlap between one test duration and the next. These three 30-cross trials were referred to as 'pre-test' trials and allowed comparisons to be made between the major groups and subgroups with regard to mean ON and OFF times before testing actually began.

For the Fixed ON group, the 30 normally distributed control values were calculated for each rat at each test duration. However, because all the Fixed OFF group used the same OFF times at each test duration, the one set of normally distributed OFF values were used for all animals in this group. For the Fixed ON group, five test durations were calculated for each animal and presented in a randomised block design. The minimum test duration was 0.5 seconds and the fourth duration was the mean ON time used before testing began. The second and third durations were calculated to divide equally the interval between 0.5 and the previously preferred duration (X_p). The fifth duration was the mean value extended by an amount equal to the previous divisions (Table 6).

Each test duration was presented for four trials: three 30-cross trials and a fourth

If X_p = preferred mean ON time for a particular animal, then the times (in seconds) used were:

Table 6: Calculation of fixed durations
ON time (secs)

X_1	X_2	X_3	X_4	X_5
0.5	$\frac{(X_p-0.5)}{3}+0.5$	$\frac{2(X_p-0.5)}{3}+0.5$	X_p	$\frac{4(X_p-0.5)}{3}+0.5$

For the Fixed OFF group the same OFF times were used for all animals and were designed to parallel the range of mean ON times used for the Fixed ON group experiment. The durations (in seconds) used were:

OFF time (secs)

Y_1	Y_2	Y_3	Y_4	Y_5
0.5	1.5	3.0	5.0	8.0

60-cross trial. Every animal therefore experienced 150 stimulations at each test duration before moving to the next value. Table 7 provides a summary of the experimental procedure.

Statistical analysis

The data for OFF time means and standard deviations were transformed by the Log10 transformation before analysis. Analysis of variance was then performed on the transformed data using the BMDP2V program. Repeated measures on two dimensions (labelled 'Duration' (5) and 'Trials' (4)) and one independent dimension (labelled 'Group' — i.e., either variable or constant subgroup) were analysed. One animal in the variable subgroup (R57) did not complete the procedure due to major threshold changes (see Chapter 8). The analysis was therefore based on the data from nine animals. The raw data may be found in Tables B.1–B.8, Appendix B.

6.1.4 Results

Comparison between Fixed ON and Fixed OFF groups

No differences existed between the Fixed ON group and Fixed OFF group with regard to mean ON and OFF times in the pre-test (or warm-up) trials (see Table

B.1–B.2, Appendix B). Considering the pre-test trials only; for OFF time, $t = -0.725$ ($df = 17, p > 0.05$, two-sample t-test with the three trials averaged for each rat), and, for ON time, $t = 1.638$ ($df = 17, p > 0.05$).

Within each major group, the variable and constant subgroups did not differ significantly from each other on either measure. Mean ON times for the variable OFF subgroup compared to the constant OFF subgroup were substantially, though not significantly, higher (5.19 and 2.34 secs respectively, $t = -2.044, df = 8, p > 0.05$).

Mean charge accepted per cross averaged across the three pre-test trials for all 19 animals (using 100 Hz, 3 msec positive and negative phases) was 179.4 μC. Proportion of time ON was 0.63. Mean ON time was 4.79 secs and mean OFF time was 2.96 secs. Values from Experiment Ia at Levels 6 and 7 (using 100 Hz, 5 msec positive and negative phases) were for ON time: 6.00 and 5.84 secs, and for OFF time: 2.20 and 2.03 secs (Table 2). Mean charge accepted per cross at Levels 6 and 7 was 134.3 and 139.9 μC and proportions were 0.73 and 0.74. Although the times recorded in the present experiment were comparable to Levels 6 and 7 in Experiment I, the measure of microcoulombs accepted was greater and the proportion of time ON was lower.

It is not clear why rats in the present experiment accepted a greater mean charge at a similar level of behaviour. It is possible that differences in the dimensions of the T-maze/shuttlebox compared to the standard shuttlebox, or differences in the waveform used in both experiments may account for the discrepancy. However, 3 msec pulse-widths should have been more efficient in delivering a particular quantity of charge than 5 msec pulse widths (Barry et al., 1974; Section 2.1). The low proportion of time ON may be due to several factors, including the different dimensions (particularly the larger OFF side), the inclusion of one or two animals with particularly low proportions, or the possibility that proportion of time ON may be lower when one or the other of ON time or OFF time is fixed.

Table 7: Experimental design II

Mean ON times for Fixed ON group and mean OFF times for Fixed OFF group. These times were the independent variables in the following experiments.

Fixed ON group:-

Subgroup	Subj.	μa	Pre-test	ON time duration (secs)				
Constant ON	R03	75	8.3	0.5	3.1	5.7	8.3	10.9
	R09	55	3.7	0.5	1.6	2.7	3.7	4.8
	R23	45	8.0	0.5	3.0	5.5	8.0	10.4
	R41	32	11.3	0.5	4.1	7.7	11.3	14.9
	R49	50	3.0	0.5	1.3	2.1	3.0	3.8
Variable ON	R32	60	8.2	0.5	3.1	5.6	8.2	10.8
	R33	40	4.3	0.5	1.8	3.0	4.3	5.5
	R55	23	1.4	0.5	0.8	1.1	1.4	1.7
	R66	90	5.1	0.5	2.0	3.6	5.1	6.6
Overall means =		*52.2*	*5.92*	*0.50*	*2.31*	*4.11*	*5.92*	*7.71*

Fixed OFF group:-

Subgroup	Subj.	μa	Pre-test	OFF time duration (secs)				
Constant OFF	R01	48	15.1	0.5	1.5	3.0	5.0	8.0
	R06	90	0.9	0.5	1.5	3.0	5.0	8.0
	R12	55	4.4	0.5	1.5	3.0	5.0	8.0
	R13	42	1.3	0.5	1.5	3.0	5.0	8.0
	R24	38	1.8	0.5	1.5	3.0	5.0	8.0
Variable OFF	R18	90	2.4	0.5	1.5	3.0	5.0	8.0
	R28	42	1.5	0.5	1.5	3.0	5.0	8.0
	R29	48	2.0	0.5	1.5	3.0	5.0	8.0
	R64	190	2.3	0.5	1.5	3.0	5.0	8.0
	R69	55	2.8	0.5	1.5	3.0	5.0	8.0
Overall means =		*69.8*	*3.45*	*0.50*	*1.50*	*3.00*	*5.00*	*8.00*

— ON times were calculated individually on the basis of each rat's preferred duration (X_p) and the minimum ON time of 0.5 secs (see Method, Section 6).

— OFF times were the same for all subjects. They were designed to cover a similar range of durations as those used for the Fixed ON group.

— The 'variable' condition was defined as thirty values randomly selected from a 'normal' distribution with mean as shown above and standard deviation equal to 7% $\times$ the mean.

Fixed ON group

As the duration of stimulation was increased from 0.5 secs to a value beyond each animal's previously self-selected duration, mean OFF time increased significantly ($F = 5.10, df = 4, 28, p < 0.01$). Tukey's HSD test revealed that Durations 1 and 4, 1 and 5, and 2 and 5 were significantly different (critical difference = 0.1356, mean square error = 0.03939, $df = 28$). See Table 8 and Figure 20.

The variability of OFF time behaviour also increased significantly (i.e., for standard deviations, SD_X: $F = 5.33, df = 4, 28, p < 0.01$). No other main effect, nor interaction was significant at the 0.05 level (see Table 8).

Mean OFF time determined from the pre-test scores (see Figure 20) and from the fourth ON time level (i.e., for equal ON times) were not significantly different ($t = 1.654, df = 26, p > 0.05$, matched t-tests). Only the first three 30-cross trials at the fourth ON time level were used for this calculation (to make the comparison with the three, 30-cross pre-test trials equivalent).

Correlations

No significant main effects nor interactions could be found with the correlation variables. The overall mean correlation was not significantly different to zero. The correlation data for the variable ON condition shows no evidence to suggest a consistent relationship between within-trial ON times and preceding, or succeeding, OFF times. This result is in accord with the results of Experiment I in which the even greater natural variability in ON time did not result in a significant correlation with OFF time at any intensity level.

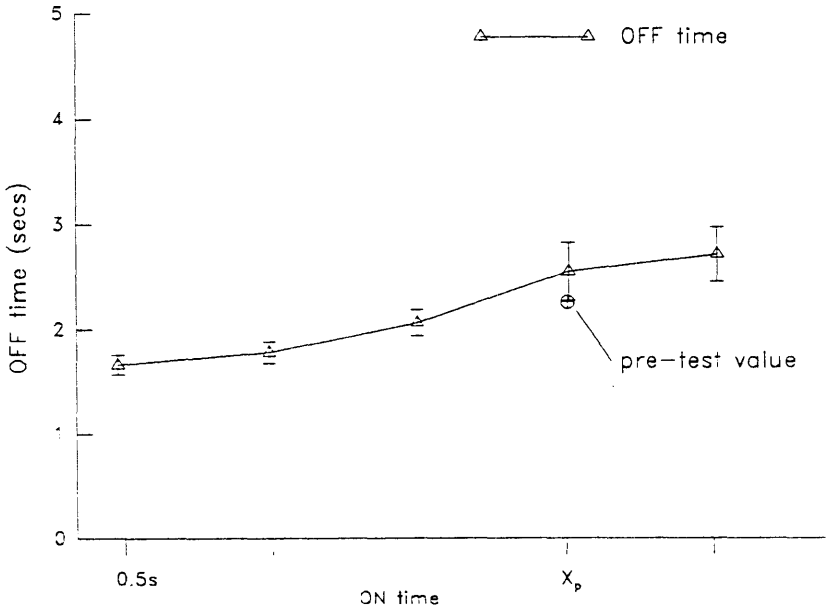

Figure 20: Mean OFF time as a function of duration of stimulation. The single data point indicates mean OFF time calculated from the pre-test scores. The difference between the pre-test OFF time and the OFF time obtained at the equivalent test duration (i.e., directly above the 'pre-test value') was not significant. X_p represents the preferred duration for each animal.

Table 8: Summary of analysis of variance on OFF time data

Mean OFF time data for Fixed ON group. Log 10 transformation used.

Source	Sum of squares	*df*	Mean square	*F*	Tail prob.
Group	0.84232	1	0.84232	3.50	0.1035
Error	1.68393	7	0.24056		
Duration	0.80327	4	0.20082	5.10**	0.0032
DG	0.04090	4	0.01023	0.26	0.9013
Error	1.10278	28	0.03939		
Trials	0.19696	3	0.06565	2.90	0.0589
TG	0.01563	3	0.00521	0.23	0.8743
Error	0.47518	21	0.02263		
DT	0.21908	12	0.01826	1.32	0.2254
DTG	0.13269	12	0.01106	0.80	0.6527
Error	1.16598	84	0.01388		

OFF time standard deviation data for Fixed ON group. Log 10 transformation used.

Source	Sum of squares	*df*	Mean square	*F*	Tail prob.
Group	1.26063	1	1.26063	2.02	0.1983
Error	4.36925	7	0.62418		
Duration	1.32622	4	0.33155	5.33**	0.0026
DG	0.25362	4	0.06341	1.02	0.4147
Error	1.74273	28	0.06224		
Trials	0.54580	3	0.18193	2.48	0.0888
TG	0.12496	3	0.04165	0.57	0.6418
Error	1.53835	21	0.07325		
DT	0.23659	12	0.01972	0.41	0.9571
DTG	0.44696	12	0.03725	0.77	0.6799
Error	4.06756	84	0.04842		

** indicates $p < 0.01$.

6.1.5 Discussion

The results indicate that an increase in duration of stimulation significantly increases both the central location, and variability, of the OFF time measure. Animals tended to spend a longer period of time with the stimulation OFF as a consequence of the stimulation being ON for longer periods and the variablity of the selected OFF time also increased.

The significant increase in OFF time contrasts with the changes seen when intensity was increased. Experiment I showed that as intensity was increased (by 36%), OFF times decreased substantially (from 30.02 secs to 2.03 secs). The present results show that an increase in the duration of stimulation (approximately 1400%) actually results in a slight, but significant, increase in OFF time (from 1.67 secs to 2.71 secs). The extent of the changes suggest that duration of stimulation is not a major determinant of OFF time; the intensity of stimulation has a considerably greater role. However, because shorter ON times tend to lead to shorter OFF times, part of the decrease in OFF time could be due to the decreased duration that is also received at higher intensities.

The results of the present experiment pertain to one relatively high intensity level. At lower intensities an increase in duration might have a more pronounced or even different effect on OFF time. A consequence of the relatively high intensities, was that the self-selected OFF times were quite low and may have been near the lower behavioural limit. If no change in OFF time had occurred, then it may have been argued that 'floor' effects prevented a decrease. However, because an increase in OFF time occurred, 'floor' effects are unlikely to be significant.

Because of the inherent relationship between the mean and standard deviation previously observed with the ON and OFF time measures (see Experiment I), the significant increase in variability found in the current experiment, would, most likely, be secondary to the significant increase in mean OFF time. This interpretation is also supported by the fact that no differences could be found between the constant ON subgroup compared to the variable ON subgroup (nor a significant Duration $\times$ Group interaction). If OFF time was dependent on ON time, a greater OFF time variability would be expected under the variable ON condition than under the constant ON condition.

6.2 Experiment IIb: Fixed OFF time

6.2.1 Introduction

Experiment Ia showed that both ON and OFF time decrease as intensity of stimulation is increased. Although counter-intuitive, it is possible that the decreases observed with the ON time variable were partly due to the decreases observed with the OFF time variable. More generally, the role the period of no stimulation might play in the determination of ON time has not been sufficiently examined.

Experiments that have controlled OFF time have usually been referred to as 'escape' procedures, in which an animal has control over the termination response only and therefore determines, or selects, ON time (e.g., Atrens & Becker, 1975; Beyra, 1976; De Witte & Bruyer, 1980; Frutiger, 1986; Hoebel, 1976).

De Witte and Bruyer (1980) used a situation in which an animal was required to press a lever to gain a period of no stimulation from a stimulus that was delivered automatically. The CRF self-stimulation rate was 'played back' to the animal which could gain a one second 'time-out' by pressing the lever. Frutiger (1986) described a similar situation in which 0.5 sec trains of biphasic pulse pairs were delivered automatically once per second until a lever was pressed. Depression of the lever resulted in a 5 sec time-out. Steiner et al. (1969) tested escape behaviour at two durations of OFF time and found reduced escape latencies when OFF time was reduced from 20 secs to 4 secs (4 secs was the self-selected time-out).

For a number of reasons existing data concerning the relationship between ON and OFF time, when OFF time is varied in a systematic way, cannot be confidently applied to the present situation. For example, latency to escape (ON time) obtained in the shuttlebox does not correlate significantly with escape latency obtained in a single lever task when the same animals are used in both tasks (Atrens & Becker, 1975). Also, rates reported for 'switch-off' or 'escape' behaviour tend to be quite low in comparison to OFF time determined from shuttling. For example, Frutiger (1986) reported mean rates of 0.36–2.8 responses/minute. De Witte and Bruyer (1980) reported approximately two switch-offs per minute, whereas the average rate of termination at Level 6 in Experiment Ia was about eight per minute.

The factor of predictability was examined in the present study because Steiner et al. (1969) and Mason et al. (1983) have found significant differences for variable, compared to constant, stimulus presentation. Steiner et al. found that rats escaped

more readily from regularly patterned stimulation than from a variable, pre-recorded rate. This may predict shorter ON times when the onset of stimulation occurs regularly. Again, major differences exist between the study of Steiner et al. (1969) and the present study, including Steiner et al.'s use of a regular pattern of 0.5 sec bursts as the regular stimulus compared to the present use of a constant duration.

The present experiment varied OFF time from 0.5 seconds to 8.0 secs in either a constant OFF condition or a variable OFF condition. Because the design of the experiment was intended to parallel the design of the Fixed ON experiment, the OFF times were calculated to cover a similar range of values as those calculated for ON time and, therefore, did not bear any relationship to each rat's preferred value of OFF time.

A similar argument applies in this experiment, as in the previous experiment, if differences between the variable OFF and constant OFF subgroups were to be found. At 0.5 sec the variable OFF and constant OFF values were virtually identical: it was not until greater mean values were used did the calculations allow meaningful variation (e.g., even 3 standard deviations only produces values of 0.6 and 0.4 at a mean OFF time of 0.5 sec). Therefore, a significant difference between the two groups would be most evident in a significant interaction term, with the data very similar at 0.5 sec and only diverging at higher OFF durations.

Finally, the standard deviation of ON time was also examined in the analysis. The variability in the timing of OFF responses (i.e., standard deviation of ON time) might be a more sensitive measure of behavioural change than the mean latency to respond (Rachlin, 1982; Staddon & Simmelhag, 1971).

6.2.2 Subjects and apparatus

The subjects and apparatus used in this experiment, and the training programme adopted to ensure that rats were highly experienced with controlled OFF time conditions have been described in detail in the relevant sections of the previous experiment (Section 6.1.2).

6.2.3 Method

The durations used in this experiment, and how these were determined is described in the Method section of the previous experiment. Also see Table 7 for a summary of the experimental design. All analysis methods were the same.

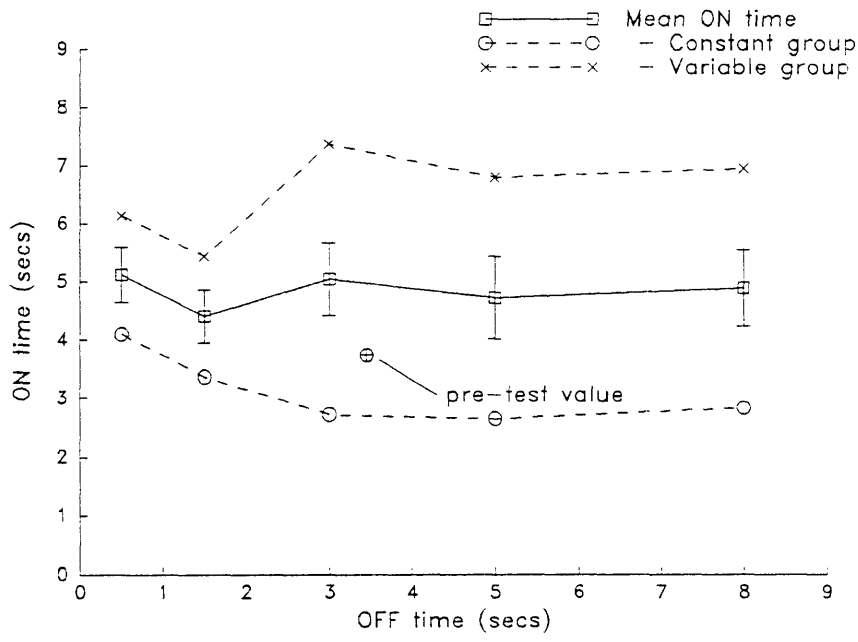

Figure 21: ON time as a function of OFF time. The dotted lines indicate the means for each of the subgroups – as there was a significant difference between the constant OFF and variable OFF subgroups. The solid line represents the combined data ± S. E. M. The single data point indicates mean ON time calculated from the pre-test scores. The difference between the pre-test ON time and the (interpolated) ON time on the solid line was significant.

The main independent variable of interest (i.e., OFF time) has been referred to as 'Duration' in the ANOVA. The two subgroups: constant OFF and variable OFF, were referred to as 'Group' in the analysis. The four trials at each level were referred to as 'Trials' (see Table 9). The raw data may be found in Tables B.9–B.14, Appendix B.

6.2.4 Results

Mean ON time

OFF time had no significant effect on mean ON time over the values tested (Figure 21). A significant effect for trials and a group difference between the constant OFF and variable OFF subgroups were found (Table 9). There were no significant interactions.

Because the pre-test OFF time scores for rat R01 were markedly different to the other animals in this group, the test responses for this animal might also be different. Repeating the analysis with R01 omitted eliminated the significant group effect ($F = 4.46, df = 1, 7, p > 0.05$). However, no other shift in the results of the analysis occurred.

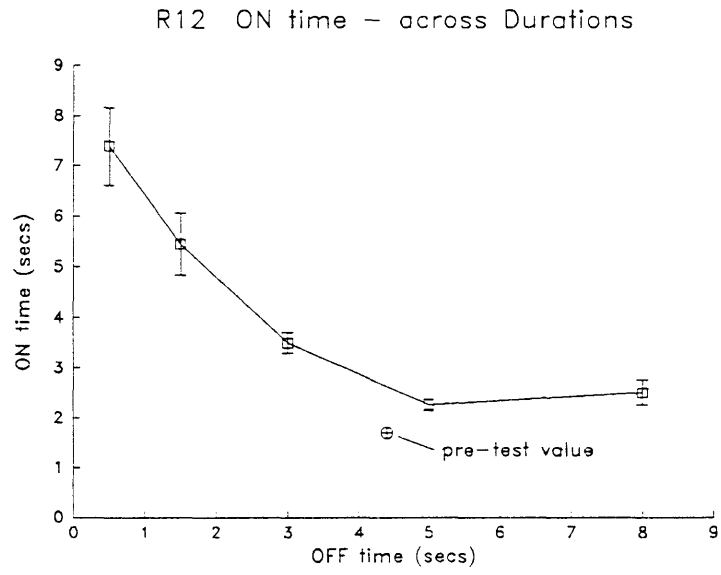

Figure 22: ON time as a function of OFF time for Subject R12 (data averaged across all four trials).

Separate analyses on the first three trials and on the last trial only (because of the significant trials effect), revealed a significant group effect for the first three trials only ($F = 7.22, df = 1, 8, p < 0.05$). No significant differences were found for the single 60-cross trial between groups ($F = 3.48, df = 1, 8, p > 0.05$), nor across duration ($F = 0.26, df = 4, 32, p > 0.05$), nor D $\times$ G interaction ($F = 1.92, df = 4, 32, p > 0.05$).

The general increase in mean ON time between the pre-test scores and the (interpolated) values from the first three trials at Durations 3 and 4 (i.e., to correspond to a mean OFF time = 3.45 sec) was significant ($t = 2.311, df = 29, p < 0.05$, matched t-test; Figure 21).

The analysis of mean ON time as a function of OFF time indicated that no significant effect could be attributed to this variable, but some animals did show consistent duration-related effects (Figure 22). All animals, in all trials, continued to initiate the very first cross suggesting that the stimulation did not lose its reward value despite the fact that the next 29 or 59 stimulations were not under the animal's control.

Table 9: Summary of analysis of variance on ON time data

Mean ON time data for Fixed OFF group. Log 10 transformation used.

Source	Sum of squares	*df*	Mean square	*F*	Tail prob.
Group	4.32373	1	4.32373	6.40*	0.0353
Error	5.40801	8	0.67600		
Duration	0.20716	4	0.05179	0.77	0.5529
DG	0.45650	4	0.11413	1.70	0.1752
Error	2.15295	32	0.06728		
Trials	0.40056	3	0.13352	5.72**	0.0042
TG	0.03790	3	0.01263	0.54	0.6585
Error	0.55983	24	0.02333		
DT	0.12210	12	0.01018	1.04	0.4215
DTG	0.12661	12	0.01055	1.08	0.3889
Error	0.94138	96	0.00981		

ON time standard deviation data for Fixed OFF group. Log 10 transformation used.

Source	Sum of squares	*df*	Mean square	*F*	Tail prob.
Group	7.50478	1	7.50478	6.26*	0.0369
Error	9.59744	8	1.19968		
Duration	1.12973	4	0.28243	2.74*	0.0454
DG	1.60534	4	0.40134	3.90*	0.0109
Error	3.29250	32	0.10289		
Trials	0.97115	3	0.32372	6.18**	0.0029
TG	0.05883	3	0.01961	0.37	0.7724
Error	1.25806	24	0.05242		
DT	0.25707	12	0.02142	0.85	0.6041
DTG	0.53450	12	0.04454	1.76	0.0665
Error	2.43291	96	0.02534		

* indicates $p < 0.05$. ** indicates $p < 0.01$.

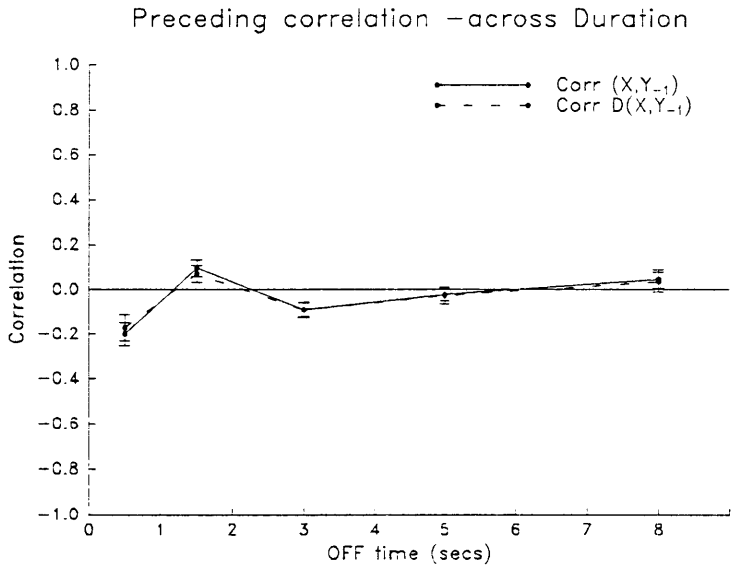

Figure 23: For the Fixed OFF group, the correlation between each ON time and the preceding OFF time was significantly altered by OFF time duration. Only the first and second data points were significantly different (Tukey's HSD test).

Correlations

The analysis of the correlation data for the variable OFF subgroup revealed a significant duration effect for the correlation between each ON time and the preceding OFF time for both the original series and the differenced series (Figure 23). For the differenced series (Corr $D(X, Y_{-1})$: $F = 3.27. df = 4, 16, p < 0.05$), Tukey's HSD test revealed a significant difference between the 0.5 sec duration and the 1.5 sec duration only (critical difference= 0.2325, mean square error= $0.05768, df = 16$).

A significant trials effect was found for the correlation between each ON time and the succeeding OFF time for both the raw data and the differenced series (Figure 24). Considering the differenced series only (Corr $D(X, Y)$: $F = 4.87, df = 3, 12, p < 0.05$), Tukey's HSD test found a significant difference between Trials 1 and 3 only (critical difference= 0.1446, mean square error= $0.02964, df = 12$). The overall mean correlation was not significantly different to zero.

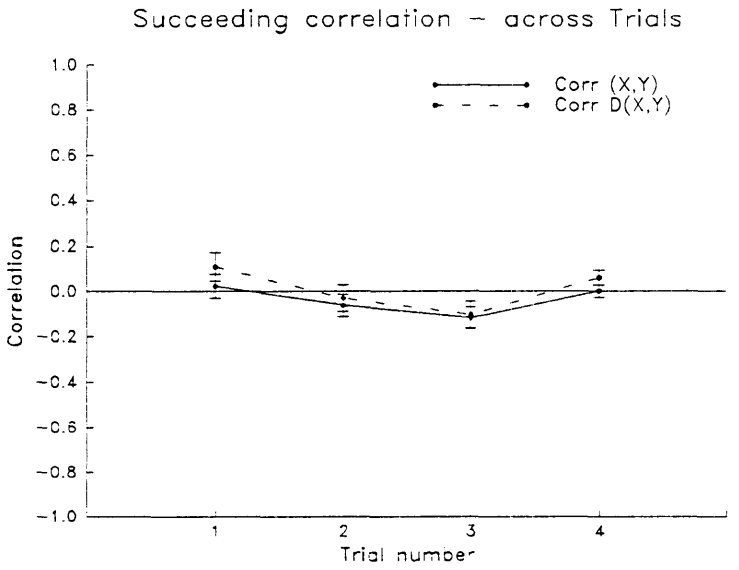

Figure 24: For the Fixed OFF group, the correlation between each ON time and the succeeding OFF time showed a significant trials effect. Only the first and third data points were significantly different (Tukey's HSD test).

Standard deviation of ON time

The ANOVA results (Table 9) revealed a significant effect for the three variables of interest (i.e., duration, group, and trials). The variability of ON time significantly increased as OFF time was increased, with the variable subgroup showing a higher level of variability than the constant OFF subgroup. Variability also increased with trials and a significant Duration $\times$ Group interaction occurred.

The data was therefore reanalysed in an attempt to clarify where these differences lay. Because of the significant trials effect in the original analysis, the data was reanalysed separately for the first three (30-cross) trials and for the last (60-cross) trial. The data for the first three trials indicated a remaining significant effect between the groups ($F = 7.96, df = 1, 8, p < 0.05$), a significant effect for OFF time ($F = 3.12, df = 4, 32, p < 0.05$) and a significant D $\times$ G interaction ($F = 2.73, df = 4, 32. p < 0.05$). The first three trials did not differ significantly ($F = 0.82, df = 2, 16. p > 0.05$).

The remaining (two-way) analysis on the last trial at each level of OFF time revealed a significant D $\times$ G interaction only ($F = 4.04, df = 4, 32, p < 0.01$). This result is depicted graphically in Figure 25. All animals were included in this analysis

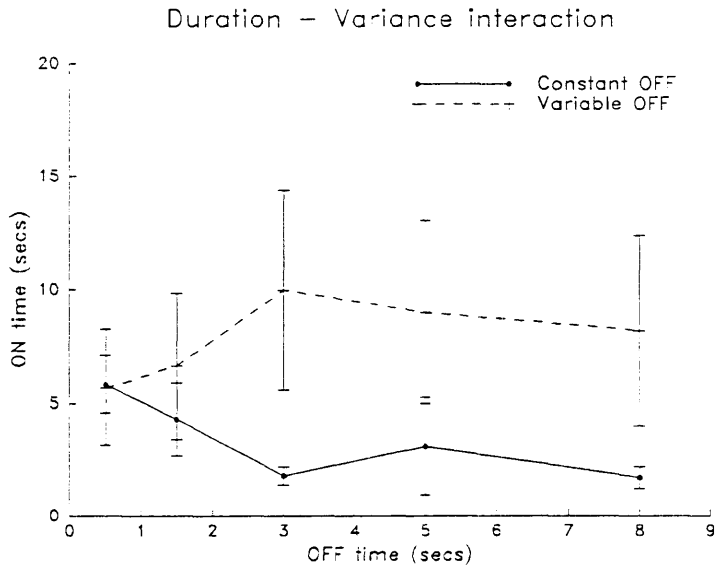

Figure 25: A significant Duration × Group interaction was evident in the standard deviation data for the last trial for the Fixed OFF group.

and no significant differences existed between the two groups as a result of OFF time only.

6.2.5 Discussion

The results indicate that an increase in the period of time between stimulations has no effect on the selected ON time. Significant differences were found between the variable OFF condition and the constant OFF condition which showed that ON times were significantly higher for the variable OFF subgroup than for the constant OFF subgroup. Because ON times for the pre-test were not significantly different between the subgroups, lack of predictability in stimulus initiation, may lead in some way to greater ON times. A lack of predictability of stimulation onset might interfere with the mechanism responsible for the timing of the termination response.

Steiner et al. (1969) had found that a decrease in the selected duration of stimulation occurred when OFF time was decreased from 20 secs to 4 secs. The present study found no effect for OFF time on the selection of ON time. Steiner et al. also found that rats escaped more readily from a regular pattern of stimulation than from a variable pattern. The present study confirmed this to some extent by finding that

rats escaped more readily (shorter ON times) from a regular onset of stimulation.

Although, as a group, no significant effects on mean ON time could be attributed to an increase in mean OFF time, some animals did show a consistent duration-dependent change. Rat R12 showed a steady decline in ON time from approximately 7.4 secs to 2.5 secs (Figure 22). Observation of this animal suggested a high degree of 'helplessness' at short OFF times. It had appeared to have 'given up' on how to terminate the stimulation — until the OFF time reached a duration similar to that which had maintained prior to the experiment. At that point the animal had regained a more normal composure and would terminate the stimulation regularly.

The possibility exists that the differences between the variable OFF and constant OFF subgroups may simply have been due to the chance allocation of two animals with atypical response patterns, to the variable subgroup. ON times for animals R18 and R28 were markedly higher and more variable than the remaining three animals (see Tables B.9 and B.10). Pre-test ON time for the variable subgroup was more than twice that of the constant group (i.e., 5.19 and 2.34 secs), but was not significantly different ($t = -2.044, df = 8, 0.10 > p > 0.05$). However, the difference may have been accentuated sufficiently during the experiment to explain the later differences. It seems unlikely that the small variation introduced into OFF time would explain the observed mean differences, when the major variable (i.e., OFF time) had no effect whatsoever. As explained previously, a significant effect for the group condition would be most evident in a significant interaction term, but this was not apparent in the mean ON time data.

With regard to the analysis of standard deviations, it was found that the variability in the latency of OFF responses was sensitive to the effects of duration, groups, and trials (Table 9). A significant Duration $\times$ Group interaction was also found. The changes that occurred in the variability of ON time were not exactly the same as the changes that occurred in the mean level of ON time (cf., Experiment IIa). This indicates that these two measures of behaviour (i.e., mean latency to respond and the variability in the latency to respond) are not as strongly linked for OFF responding as they are for ON responding.

The data for the SD_X variable did follow the predictions made with regard to differences between the variable OFF and constant OFF groups which stated that differences between the groups should have been most apparent in the Duration $\times$ Group interaction. If the last 60-cross trial only is considered (since at this trial, animals should have been most experienced with the particular test duration), then

it was apparent that ON time standard deviation was approximately equal for both groups at 0.5 sec OFF time but diverged at longer durations. The less variability in responding in the constant OFF group suggests that a predictable onset of stimulation, when that stimulation is at a relatively high level, may be a more reinforcing situation than is a variable onset.

These differences could not have been due to order effects in the presentation of the OFF time durations, since these were randomised for each animal and counterbalanced across each group (the 0.5 sec condition occurred as the second condition for R18, and the third condition for R28). The results are also unlikely to be due to the presence of experienced versus 'naive' animals since an approximately equal number were in each group (R18 had received a minimum of 1100 stimulations at these conditions and intensity before the experiment began — i.e., 'experienced'; R28 had received at least 360 stimulations prior to the experiment — i.e., 'naive').

Instead the results suggest that variability in OFF time can significantly alter the variability of ON time. The finding of significant differences in the correlation between ON time and the preceding OFF time over the durations tested, suggests that variability in OFF time, or perhaps more importantly, the variability in the time of onset of stimulation, may have an effect on the variability of the ON time which immediately follows. The timing of the ON response may be more crucial to the maintenance of total charge than the timing of the OFF response. Further investigation is required of the significance of OFF time variability in shuttling behaviour.

6.3 Conclusions from Experiment II

The principal results from the preceding experiments were that an increase in ON time produced a small, but significant, increase in OFF time, but a similar increase in OFF time had no significant effect on ON time. The increase in OFF time that occurred when the duration of stimulation was increased by an average of 1400% was minor when compared to the change that occurred when intensity was increased by an average of 36% (Experiment Ia).

Because shorter ON times tend to lead to shorter OFF times, the decrease in OFF time that occurs as intensity is increased may be partly accounted for by the decrease in duration that also occurs. However, the principal determinant of OFF time at higher intensities is the amplitude of the stimulating pulses and not the duration of stimulation. One possible explanation for the increase in OFF time might be that

at longer durations rats simply wandered further from the ON beam and therefore took somewhat longer to reinitiate once the stimulation had terminated. However, if this were the case, the same finding should have been made when OFF time was controlled.

These results provide some difficulty for both the reward/aversion model and the reciprocal inhibition model. The durations used in Experiment IIa were from 0.5 sec to just beyond each animal's previously self-selected duration. At these durations no significant aversive effects should have been experienced, because well-trained animals normally terminate well before this point. Perhaps the duration of stimulation has an effect on OFF time apart from the aversive effect that occurs at much longer durations (Atrens et al., 1983). For example, some general increase in arousal or autonomic activity may occur (without necessarily being aversive) which requires a somewhat longer OFF time in order to dissipate (Ángyán, 1976; Sadowski, 1976). However, the increase in OFF time as duration of stimulation is increased is more likely to represent an adaptation effect. Perhaps the initial reward effect begins to adapt from the moment of initiation while aversion begins to accumulate (Atrens et al., 1983). Whether the adaptation occurs in the directly stimulated substrate or after the stimulation has been transformed into reward (Deutsch et al., 1980) is still not clear.

For the reciprocal inhibition model, if there were two mutually inhibiting systems for which the respective periods of dominance were reflected in ON and OFF time, then the same effect should occur whether ON or OFF time is controlled. As a minimum, the findings indicate that the system responsible for OFF time cannot be represented in exactly the same way as the system responsible for ON time. This is probably due in part to no direct stimulation being received during OFF time. OFF time might represent feedback or reverberatory stimulation of several kinds, or from several sources, and the processes that underlie this type of stimulation are unlikely to be exactly the same as the processes that underlie the direct effects of electrical stimulation. However, the RI model may still be used as an approximation to the processes underlying shuttling behaviour because the difference between the effects of controlling ON time or OFF time were not great. Another approach might be to investigate different coupling arrangements between the two neurons or between the two neurons and a third neuron. For example, a third neuron might receive excitatory input from neuron A but have inhibitory connections with neuron B. The effect of different coupling arrangements is

difficult to predict in configurations like the RI model (and usually require a simulation approach), but a third neuron might gate, or in some way modify, the inhibition in one direction.