Chapter 7

Experiment III: Within-trial stimulus changes

The previous experiments have established that at higher intensities it is the intensity itself which is the major determinant of OFF time. However, the previous experiments have not established which OFF time is most determined by intensity. OFF time may be determined by the intensity received on the previous initiation (i.e., as a consequence of the stimulation) or OFF time may be determined by the intensity on the subsequent initiation (i.e., as an anticipatory effect), or perhaps even as a result of a more general averaging process. If two levels of stimulation were presented in a fixed relationship to each other within a trial, the manner in which a rat distributes OFF time in relation to the different stimulations should reveal whether OFF time occurs as a consequence of stimulation, or in anticipation of stimulation. The experiments reported in this chapter were therefore designed to determine what changes occur in OFF time when either intensity or duration is increased on each third initiation.

If the stimulation was presented so that two short ON durations were followed by one long ON duration (with intensity constant), the present interpretation of the reward/aversion model predicts that OFF time would not change. If the test durations are less than those previously self-selected, aversive effects sufficiently strong to affect behaviour should not be experienced. Also, OFF time inversely reflects the reward effect provided by the intensity of stimulation: at a constant intensity OFF should not change. If intensity was increased on each third initiation, OFF time should change, but it is not clear from the existing model whether it should be the OFF time before the high intensity that should decrease or the OFF time after the high intensity.

If a self-stimulating rat were to adapt its behaviour in the shuttlebox in order to

optimise the total environmental utility available (Baum & Rachlin, 1969; Rachlin, 1980, 1982; Rachlin, Kagel, & Battalio, 1980: Staddon, 1979, 1980), then the degree to which adaptive behaviour could be demonstrated would provide a measure of the degree to which the timing of the initiation and termination responses were under operant control. For example, if the timing of the termination response was elicited by the stimulation, then ON time should not change as a function of the number of trials in which the environmental constraints were applied. Shuttling behaviour of rats when they were first exposed to the stimulus conditions was compared to the same rats' behaviour after considerable experience. The extent and type of changes that occur should be relevant to the reciprocal inhibition model with a lack of change with learning implying support for the respondent interpretation.

7.1 Experiment IIIa: Within-trial changes in duration

7.1.1 Introduction

The duration of stimulation was systematically varied within trials so that one long duration followed two short durations. In addition, two mean levels of stimulus duration were used: firstly, a low condition in which ON times from Durations 1 and 2 in Experiment IIa, and a high condition in which ON times were taken from Durations 3 and 4 (see Table 7). Deutsch et al. (1976, 1980) have claimed that no further increment in reward value occurs after 1–2 secs. Therefore, a behavioural change should only occur in the low condition and not in the high condition.

From the results of Experiment IIa in which an increase in mean ON time resulted in an increase in mean OFF time, it was expected that mean OFF time in the high condition should be greater than mean OFF time in the low condition.

7.1.2 Subjects and apparatus

The nine animals from Experiment IIa were used for this experiment. A minimum of one week was allowed before testing recommenced after the conclusion of Experiment IIa. All current intensities, pulse widths and interpulse intervals were the same as

those used in Experiment IIa. The T-maze/shuttlebox was used in the standard configuration.

7.1.3 Method

Subjects were continued from Experiment IIa under the conditions of stimulus presentation that applied there: a constant ON subgroup (N = 5) and a variable ON subgroup (N = 4), constituted two independent groups. Testing was staggered in the manner described in the General Method.

For each of the nine animals a stimulus control file was constructed which consisted of 30 ON time values (Section 4.3.4). The 30 time values were distributed in a Stimulus Format (SF) of 2:1 in which two short ON times were followed by one long ON time.

The low stimulus duration condition included ON times (in secs) of 0.5, 0.5, and X_1 while the high stimulus duration condition included ON times of X_3, X_3, X_4 (see the code described in the Method section of Experiment IIa, Tables 6 and 7). Each animal completed both conditions, with one-half of the rats completing the low condition before the high condition and the remaining one-half followed the reverse order. The order of presentation of conditions was determined randomly.

The twenty 0.5 or X_3 values in each block of 30 times came from the files used in Experiment IIa. Similarly, the ten X_1 or X_4 values in each block came from the first 10 values of the corresponding control files used in Experiment IIa.

Each combination of control values was presented for one 60-cross trial, three 30-cross trials and one 240-cross trial. The 240-cross trial was the main test trial, the prior trials were essentially training trials. However, the 60-cross trial was also analysed in order to identify any changes in OFF time distribution that might have occurred as a result of training. In each trial that required greater than 30 stimulus control values, each block of 30 values was repeated — so that the 240-cross trial, for instance, was constructed of eight blocks each of 30 values. See Appendix D for times used and presentation format.

Statistical analysis

The variables analysed included mean OFF time ($\overline{Y}$), standard deviation of OFF time (SD_Y) and the four correlation variables Corr (X, Y), Corr (X, Y_{-1}), Corr $D(X, Y)$, and Corr $D(X, Y_{-1})$. OFF times and standard deviations were transformed by Log10

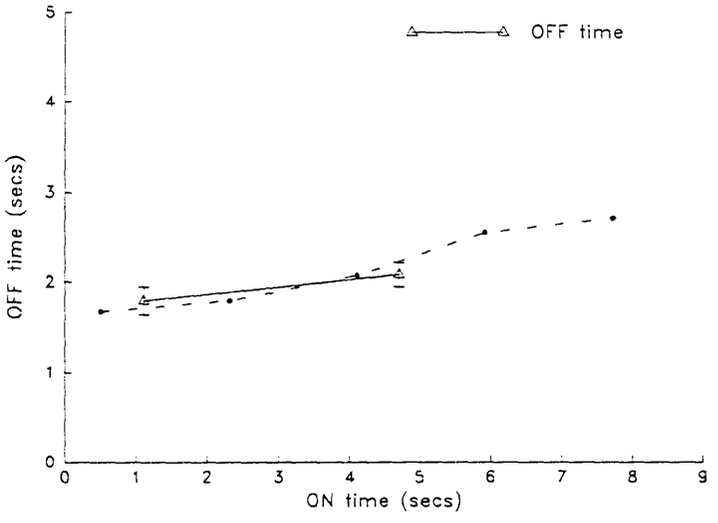


Figure 26: Mean OFF time at two levels of ON time. Dotted line represents the superimposed OFF time data from Figure 20.

before analysis. Correlations were not transformed. All analyses were conducted with the BMDP2V program. Raw data may be found in Tables C.1–C.6 in Appendix C.

7.1.4 Results

Mean OFF time in the high condition was significantly greater than mean OFF time in the low condition ($F = 5.61, df = 1, 7, p < 0.05$ — see Figure 26). No significant differences could be found between the constant ON and variable ON subgroups.

There were also no significant differences between the constant ON and variable ON subgroups on any of the correlation variables. Also, no effects could be found due to trials, and correlations did not differ between the low and high conditions. Note that correlations could be obtained for both the constant ON and variable ON subgroups since even in the former group some variation was introduced by the 2:1 ratio of stimulus presentation.

The overall means for the correlation variables were effectively zero. A significant Group $\times$ Trials interaction was evident throughout all four correlations ($F = 4.67, F = 4.98, F = 4.50. F = 4.21$, for Corr (X, Y), Corr (X, Y_{-1}), Corr $D(X, Y)$, Corr $D(X, Y_{-1})$, respectively, all dfs= 4, 28, all ps< 0.01; see Figure 27).

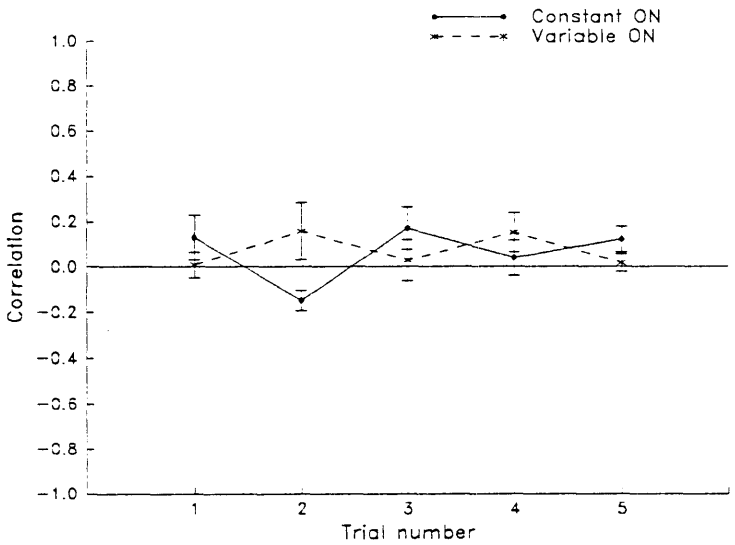


Figure 27: A Group × Trials interaction was significant for all within-trial correlation variables. The figure shows the interaction for Corr $D(X,Y)$.

The mean and variability of OFF time were significantly increased in the 240-cross trial (for mean OFF time: $F = 10.98, df = 4, 28, p < 0.0001$, for SD_Y: $F = 19.49, df = 4, 28. p < 0.0001$). Tukey's HSD test revealed that only the last 240-cross trial was significantly different to the means of the other trials for both OFF time (critical difference= 0.107, mean square error= $0.01218, df = 28$) and SD_Y (critical difference= 0.184, mean square error= $0.03641, df = 28$). The 60-cross trial was not significantly different to the 30-cross trials on either measure.

Individual analyses

Separate individual analyses were conducted on the mean OFF time data in the 60-cross and 240-cross trials for each animal in each condition. The 60 or 240 crosses within each trial were subdivided into 6-cross blocks and subjected to analysis of variance using the BMDP2V program. 10 values for each OFF time were available for the 60-cross trials and 40 values were available for each OFF time in the 240-cross trials. The F-values resulting from these analyses are provided for each animal in Table 10. Figure 28 and Figure 29 give examples of the distributional changes in OFF time as a consequence of the 2:1 presentation of ON time. The OFF time means for the blocks of 6-crosses in each of the trials analysed above may be found in Table 11.

Table 10: F-values calculated for ON time 2:1 trials.

F-values calculated for OFF time when ON time varied systematically within a trial. Calculation based on 6-cross blocks, repeated 10 or 40 times within a trial.

		Condition			
Group	Subject	Low		High	
	(N_c=)	(60)	(240)	(60)	(240)
	(df =)	(5,45)	(5,195)	(5,45)	(5,195)
Constant ON	R03	1.10	1.19	0.81	0.45
	R09	1.79	1.14	1.42	1.32
	R23	4.97*	6.37*	2.20	8.18*
	R41	2.63*	0.02	0.50	0.81
	R49	5.05*	6.12*	5.20*	3.48*
Variable ON	R32	0.23	2.50*	0.20	2.36*
	R33	1.17	3.22*	0.52	1.00
	R55	2.09	1.17	1.02	0.97
	R66	0.80	2.48*	0.68	3.61*

* indicates calculated F-value greater than the tabled F-value at $p = 0.05$.

The corresponding standard deviations may be found in Table C.7, Appendix C. The raw data on which each F-value was calculated may be found in Tables D.1–D.36, Appendix D.

Table 11: OFF time (secs) — ON time presented in 2:1 format.

OFF time for each cross in the 6-cross block when ON time presented in a 2:1 format at two mean levels — either low or high. Y_3 and Y_6 indicate OFF time immediately following the *long* ON time.

Subject	Condition	(N_c)	ON time SF(2:1)					
			short	*short*	*long*	*short*	*short*	*long*
			Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
R03	Low	(60)	7.22	3.07	4.94	7.87	7.50	3.43
		(240)	3.24	2.99	3.27	2.16	5.20	3.03
	High	(60)	3.20	2.55	3.70	2.98	3.82	4.59
		(240)	4.14	3.87	4.56	3.85	3.49	4.39
R09	Low	(60)	1.45	1.23	1.16	1.23	1.64	1.02
		(240)	1.62	1.72	1.50	1.77	1.62	1.65
	High	(60)	1.80	1.71	1.02	1.94	1.35	1.52
		(240)	2.09	1.86	2.26	1.86	2.04	2.76
R23	Low	*(60)	1.60	0.95	1.60	1.12	1.04	2.12
		*(240)	1.72	1.25	2.30	1.51	1.14	1.85
	High	(60)	1.59	1.86	2.73	2.01	2.43	4.34
		*(240)	1.81	1.89	4.54	1.70	1.96	3.83
R41	Low	*(60)	1.41	1.68	0.87	2.24	1.10	1.23
		(240)	6.12	4.77	6.08	5.95	3.39	3.97
	High	(60)	1.56	2.50	3.19	0.90	1.65	2.11
		(240)	3.04	2.69	2.24	2.39	3.45	2.80
R49	Low	*(60)	2.53	1.01	3.14	1.56	1.50	2.96
		*(240)	1.99	0.97	3.26	1.60	0.99	3.01
	High	*(60)	2.51	4.31	5.24	1.51	4.20	4.68
		*(240)	1.81	4.33	4.25	4.89	3.54	4.82
R32	Low	(60)	1.76	1.60	1.20	1.32	1.06	1.57
		*(240)	1.90	5.33	4.32	2.17	3.92	1.64
	High	(60)	2.54	2.14	1.93	1.55	1.71	2.32
		*(240)	2.76	5.00	7.55	3.36	4.95	8.49
R33	Low	(60)	1.64	1.07	1.54	1.70	1.10	1.36
		*(240)	1.24	1.13	1.73	1.87	1.21	2.31
	High	(60)	1.25	1.65	1.59	1.74	1.66	1.36
		(240)	1.92	1.81	2.09	1.89	1.61	1.73
R55	Low	(60)	0.85	1.11	1.10	0.74	0.87	0.93
		(240)	1.34	1.49	0.86	1.56	1.65	1.72
	High	(60)	1.85	1.12	1.40	1.03	2.10	1.34
		(240)	1.36	2.26	1.81	1.75	2.06	2.76
R66	Low	(60)	1.30	1.32	0.99	1.55	1.26	0.94
		*(240)	1.74	2.17	1.21	1.54	1.63	2.03
	High	(60)	1.28	0.95	1.48	1.54	1.41	1.20
		*(240)	2.23	1.16	1.88	1.97	1.85	1.40

* indicates Trials on which the calculated F-value was greater than the tabled F-value at $p = 0.05$, (i.e., from Table 10).

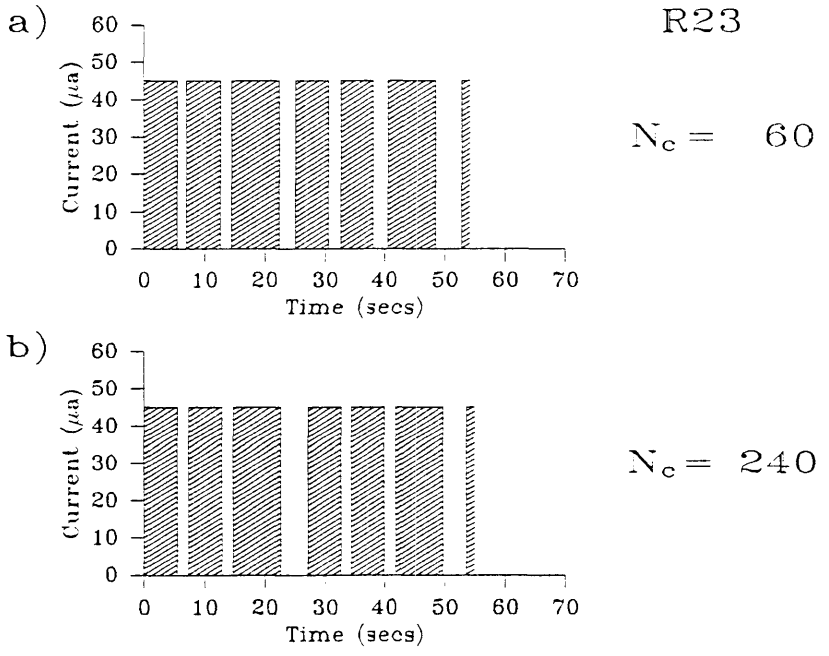


Figure 28: Distribution of OFF time when ON time varied in a 2:1 format. Subject R23 — high condition. Note longer OFF times after each long ON time.
The width of the shaded region indicates ON time, the width of the gaps indicates OFF time. The area of any particular shaded region is directly proportional to the mean charge accepted. Diagram a) indicates initial distribution; diagram b) indicates distribution after repeated exposure. N_c indicates the total number of crosses in the trial. The number of observations for each ON time or OFF time = $N_c \div 6$.

A significant F-value provides an indication of a consistent pattern of responding within the 6-cross block compared to the pattern of responding across the blocks. A large F-value suggests a 'closer look' at the actual times for the block (Table 11). A consistent pattern of OFF responses in relation to the short and long ON times, repeated within the block, should then provide reliable evidence for a consistent behavioural change.

For example, Subjects R23 and R49 showed large F-values and a consistent pattern of responding which showed that long OFF times followed long ON times (Figures 28 and 29). On the other hand, the pattern of responding within the block for Subject R66 did not show a consistent relationship with ON times. A significant F-value was calculated for R66 in both the 240-cross trials, but no consistent pattern in OFF time occurred in relation to the repeated ON times.

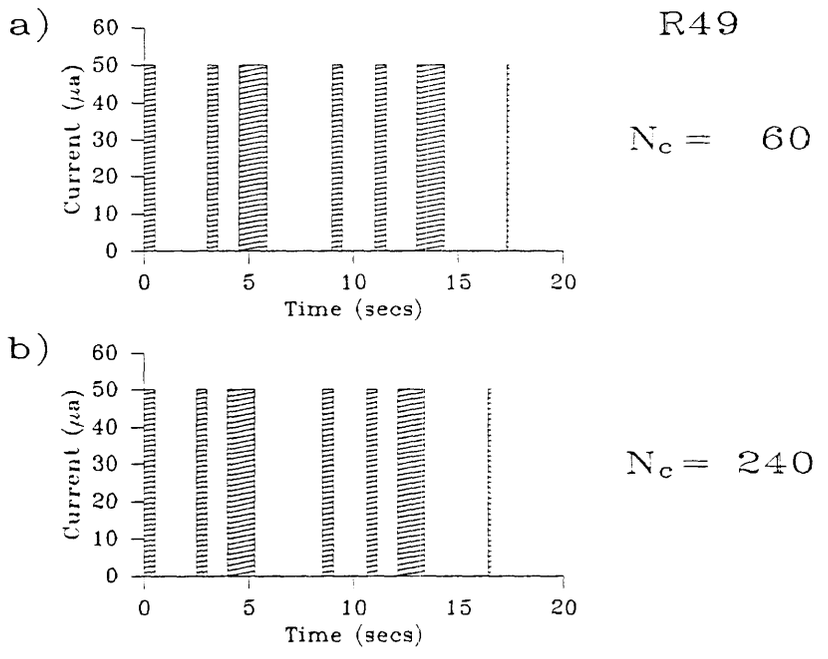


Figure 29: OFF time distribution when duration of stimulation varied in a 2:1 format. Subject R49 — low condition. Note longer OFF times after each long ON time and shorter OFF times before each long ON time.

It should be emphasised that although the calculation of individual F-values provides a legitimate statistic, measuring the ratio of within-block variance to across-block variance, the reference to a particular probability level is intended as a guide only. Tables of F-values and probability levels are compiled on assumptions of independence which may not be correct (however, see Chapter 9). The use of $p = 0.05$ is implied as a convenient, common, cut-off point only. The term 'significant' is therefore used in relation to individual trials, as implying that the calculated F-value was greater than the cut-off point, and not neccessarily to imply a probability of less than 0.05.

The results shown in Table 10 describe a rather inconsistent pattern. Some animals showed no tendency at all to systematically alter OFF time distribution in response to systematically varied ON time (e.g., R03, R09, R55). On the other hand some animals showed a quite consistent pattern of responses (e.g., R23, R32, R49, R66), while still others showed a significant effect in one condition and not the other (R33, R41). There were more significant results with the 240-cross trials (9) than with the 60-cross trial (4). There were more significant F-values in the low condition (8) compared to the high condition (5).

Table 12: Comparison among within-trial correlation variables

Spearman rank order correlations calculated between each F-ratio and each within-trial correlation variable in Experiments IIIa. The absolute value of each within-trial correlation was used.

F-ratio	Correlation variables			
	Corr (X, Y)	Corr (X, Y_{-1})	Corr $D(X, Y)$	Corr $D(X, Y_{-1})$
F_{OFF} (N = 36)	0.510*	0.097	0.616*	0.174

* indicates $p < 0.05$.

7.1.5 Discussion

The significant increase in mean OFF time (from 1.79 to 2.08 secs) as mean ON time was increased (from 1.11 to 4.71 secs) confirms the finding in Experiment IIa. An average increase in stimulus duration (at a fixed, relatively high intensity) results in an average increase in OFF time. This increase was not accompanied by a change in the correlation between ON time and the preceding, or succeeding, OFF time.

In general, where a consistent pattern of responding could be identified (i.e., significant F-value), longer OFF times occurred immediately after the long ON time (e.g., R23 Low (60), R23 Low (240), R23 High (240), R32 High (240), R33 Low (240), R49 Low (60), R49 Low (240), R49 High (60) — see Table 11). The stronger relationship between each ON time and the succeeding OFF time rather than the preceding OFF time can be verified by calculating a Spearman rank-order correlation between each of the correlation variables (regardless of sign) and the F-value (as shown in Table 10). Table 12 indicates that a high F-value is more associated with a higher correlation with the succeeding OFF time than with the preceding OFF time. If the assumption is made that a higher F-value indicates a more consistent pattern of responding, then the immediately following OFF time is the greater determinant of that pattern.

The results also indicate that some qualification is required of the claim by Deutsch et al. (1976) that the reward value of stimulation adapts after 1–2 seconds and durations greater than this are not discriminable. Although more significant changes were found in the low condition, significant differences in behaviour were

still found with durations greater than 2 secs. For example, the most significant changes occurred for rat R23 in the high condition with durations of 5.5 and 8.0 secs. Alternatively, the interpretation of results based on the use of two or three animals (as in Deutsch et al., 1976, 1980) cannot be accepted with confidence because the demonstration of a behavioural effect appears to depend greatly on which animals are included. Moreover, rate of crossing or the magnitude of ON or OFF times are not good predictors of the sensitivity of an animal to stimulus changes.

The results suggest that some SSs can develop a consistent relationship between ON time and adjacent OFF times across conditions and trials (e.g., R23, R49), although other animals show no consistent pattern of responding (e.g., R03, R09). Although not specifically tested, it appeared that a consistent pattern of responding was not predicted by a high rate of crossing (e.g., R09 was a 'good SS', R23 was not), nor by a specific level of ON or OFF time (e.g., R49 mean pre-test ON time was 3.0 secs, R23 was 8.0 secs, while pre-test OFF times were not particularly different 3.34 and 2.75 respectively — Table B.1, Appendix B). The lack of correlation indicates that the longer OFF times do not occur after every long ON time, but are produced by an averaging mechanism that in general produces longer OFF times. In summary, the results suggest that when duration of stimulation is varied in a 2:1 format, it is the subsequent OFF time that is most related to the duration. In general, there was no consistent evidence to implicate the preceding OFF time as associated with ON time (cf., R49 — Figure 29). There were considerable individual differences in sensitivity: animals which did show sensitivity to changes in duration of stimulation, did not show the same sensitivity at both levels of duration. Whether this lack of consistency is due to daily variation in response patterns, or a consequence of experience-related changes has not been determined.

7.2 Experiment IIIb: Within-trial changes in intensity

7.2.1 Introduction

Experiment Ia showed that a 36% increase in mean intensity resulted in a decrease in mean OFF time from 30.02 secs to 2.03 secs. On the other hand, Experiment IIa showed that a 1400% increase in the mean duration of stimulation produced a

slight, but significant, increase in mean OFF time (from 1.67 to 2.71 secs). The latter result was confirmed in Experiment IIIa, where an average increase in duration of 400% resulted in an increase in mean OFF time from 1.79 to 2.08 secs. An increase in intensity therefore has an opposite effect on OFF time when compared to an increase in the duration of stimulation (when intensity was fixed at a relatively high level). Moreover, the extent of the changes in OFF time indicate that intensity of stimulation is by far the greater determinant of OFF time. These considerations predict that when the intensity of stimulation is systematically varied within a trial, rather than duration, stronger evidence for a relationship between ON and OFF time should be found (i.e., more significant F's and higher F-values).

If the stimulus presentation is systematically varied so that intensity is higher on every third initiation, the results of Experiment Ia indicate that mean ON and OFF times should decrease over the trial because of the greater mean intensity. However, the manner in which a rat might distribute OFF times so as to result in an overall reduced mean value is not predictable from existing data. At least three distributional changes in OFF time could occur.

Firstly, the OFF time immediately after each third (i.e., high intensity) stimulation might decrease as a consequence of the stimulation. This would indicate that succeeding OFF times were elicited by intensity, since with experience, a shorter OFF time immediately following a higher intensity does not lead to another high intensity stimulation. The animal has to make a further two crosses before it can again receive higher intensity stimulation. A shorter, succeeding OFF time (for an experienced animal), would therefore give strong support to the hypothesis that OFF time was primarily determined by the intensity of stimulation received during the preceding ON time.

A second possibility is that the OFF time preceding each high intensity stimulation might decrease as a result of an 'anticipation' or 'expectancy' effect (Stein, 1964). Decreased OFF time immediately preceding each high intensity stimulation would suggest that OFF time was not determined by the intensity of stimulation received during the previous ON time. Instead animals must be able to retain the memory of the high intensity stimulation through two lower intensity stimulations. Two periods of lower intensity stimulation do not disrupt the memory of, and anticipation for, a period of high intensity stimulation at each third initiation.

A third possibility is that a change might occur between 'consequence' and 'anticipation' as a result of increased experience or learning. Such a change would reflect

the ability of the rat to adapt its behaviour to the overall stimulus contingencies (e.g., Baum & Rachlin, 1969; Rachlin, 1982; Staddon, 1979). Evidence of a change in distribution with increased experience would support the idea that some qualitative comparison was being made between the reward values obtained on different initiations.

If no consistent relationship between within-trial ON and OFF times could be found, a strong averaging process must occur in the neural substrate underlying ICSS that averages over several stimulus inputs regardless of temporary changes in intensity.

One further condition was included in the experimental design. This was a comparison between increased intensity and increased pulse width so that the charge/pulse available at each third initiation was kept constant. This comparison was introduced because even though an increased charge delivered as a result of increased pulse amplitude has been found to be more effective in modifying ICSS behaviour than the same increased charge delivered as a result of increased pulse width (Section 2.1), it is possible that pulse width has a different, or stronger, effect on the relationship between ON time and the preceding, or succeeding, OFF time.

7.2.2 Subjects and apparatus

Subjects were 8 self-stimulators from the R-Series. The subjects had been rested for at least one week before testing recommenced and the testing was staggered in the manner described in the General Method. The experiments to be described here were conducted before any of the experiments that required the control of ON time or OFF time (i.e., Chapter 6, Experiment IIIa). This eliminated possible confounding in a free-shuttling situation that might have been occurred if animals had previously experienced controlled durations. The apparatus was the T-maze/shuttlebox in the configuration of the standard shuttlebox.

7.2.3 Method

The 8 animals were randomly assigned from a pool of 13 SSs and were given three to four 'warm-up' trials, of variable length (approximately 20–30 crosses), during which current intensities for each animal were adjusted to find a value at which a moderately-low rate of shuttling could be maintained (called a low intensity). Pulse

width and interpulse interval were 3 msec and 4 msec respectively. Table 14 summarises the design of the experiment.

The test value for current intensity was determined from the low intensity and was calculated to be 1/3 greater than the low intensity (referred to as the high intensity). The high intensity was fairly strong for most animals (Levels 6-7, Experiment Ia).

The pulse width test value was 4 msec (i.e., both positive and negative phases were 4 msec) with an interpulse interval of 2 msec. This meant that the increase in electrical charge contained in each pulse (i.e., from 3 msec to 4 msec), was the same as the increase produced by increasing the intensity by 1/3. The interpulse interval was shortened to 2 msec to maintain the rate of presentation of pulses at 100 Hz.

All animals were given three 30-cross trials at the low intensity and three 30-cross trials at the high intensity (referred to as the before- trials). The six trials were presented in a random order for each animal. After completion of the six before-trials, one-half of the animals in each group received the pulse width test condition before the intensity test condition. The remaining one-half received the intensity test condition before the pulse width condition. The order of presentation was determined randomly. Finally, all animals repeated the six trials at the low and high intensities in the same order as at the beginning of the experiment (referred to as the after-trials)

The test trials of either intensity or pulse width were conducted over six trials. The first was a 60-cross trial, then four 30-cross trials and finally either a 120-cross trial or a 240-cross trial. The 120-cross trial was for the pulse width condition, while the 240-cross trial was for the intensity condition.

The six before- and after- trials were intended to examine three possible sources of error. Three hypotheses concerning the means of the four conditions: low (before), high (before), low (after), and high (after), were to be tested in the manner indicated in Table 14. These comparisons are orthogonal and allow the use of Fisher's LSD post hoc test (Roscoe, 1969).

Table 13: Experimental design III

Number of crosses (N_c) per trial in the following experiments.

	Condition	Trials					
μ_1:	*Low intensity*	30	30	30			
μ_2:	*High intensity*	30	30	30			
	Pulse width 2:1	60†	30	30	30	30	120†
	Intensity 2:1	60†	30	30	30	30	240†
μ_3:	*Low intensity*	30	30	30			
μ_4:	*High intensity*	30	30	30			

† indicates trials on which individual analyses conducted.

Hypotheses to be tested concerning the above μ's :-

$$H_1 : \tfrac{1}{2}(\mu_1 - \mu_3) = \tfrac{1}{2}(\mu_2 + \mu_4)$$

$$H_2 : \mu_1 = \mu_3$$

$$H_3 : \mu_2 = \mu_4$$

Intensities used in the following experiments (μa). All pulse width/interpulse interval dimensions were 3/4 msec, except for the ***Pulse width*** condition which was 4/2 msec.

	Intensity		
Subject	Low	Low	High
[1]R12	42.0	42.0	56.0
[1]R16	75.0	75.0	100.0
[2]R18	83.0	83.0	110.0
[2]R24	34.0	34.0	45.0
[2]R48	51.0	51.0	68.0
[2]R49	42.0	42.0	56.0
[1]R58	98.0	98.0	130.0
[1]R60	66.0	66.0	88.0

Order for test trials:

[1] indicates Pulse width $\longmapsto$ Intensity

[2] indicates Intensity $\longmapsto$ Pulse width.

The first of these hypotheses tested whether the mean for the high intensity trials (both before- and after-) was equal to the mean of the low intensity trials. A significant result would verify that an increase in mean intensity did decrease both mean ON time and mean OFF time. The second hypothesis tested whether the before- and after- low intensity means were equal, and the third hypothesis tested whether the before- and after- high intensity means were equal. A significant result for either of the last two hypotheses would indicate that a significant change had occurred across the duration of the experiment in the average response of the animals. Measurement instability may be a problem with shuttling behaviour (Liebman, 1983; Experiment Ib).

Statistical analysis

The variables mean ON time ($\overline{X}$), mean OFF time ($\overline{Y}$), standard deviation of ON time (SD_X), and standard deviation of OFF time (SD_Y) were logarithmically transformed before analysis of variance. All post hoc comparisons were also made on the transformed means. Correlation data was not transformed before analysis of variance. The raw data for the 24 trials making up this experiment may be found in Tables C.8–C.15 in Appendix C.

7.2.4 Results

Before- and after- Trials

ANOVA results indicated significant differences among the four before- and after-conditions for the four variables $\overline{X}$, SD_X, $\overline{Y}$, and SD_Y. ($\overline{X}$: $F = 9.01, p < 0.001$; SD_X: $F = 5.58, p < 0.01$; $\overline{Y}$: $F = 8.67, p < 0.001$; SD_Y: $F = 8.96, p < 0.001$; all df's= 3, 21. See Figure 30). There were no significant trials effects nor Trials $\times$ Condition interactions.

The results of the Fisher LSD comparisons for the three hypotheses H_1, H_2, H_3 confirmed that high intensity trials, on average, produced lower mean values than did the low intensity trials. Furthermore, the average values for both low intensity trials and high intensity trials were not significantly different after the test trials compared to before the test trials. This indicates that no significant change in responsivity occurred across the duration of the experiment. Mean ON time, mean OFF time, and the standard deviations of both were relatively stable over the duration of the experiment (see Table 14 for summary).

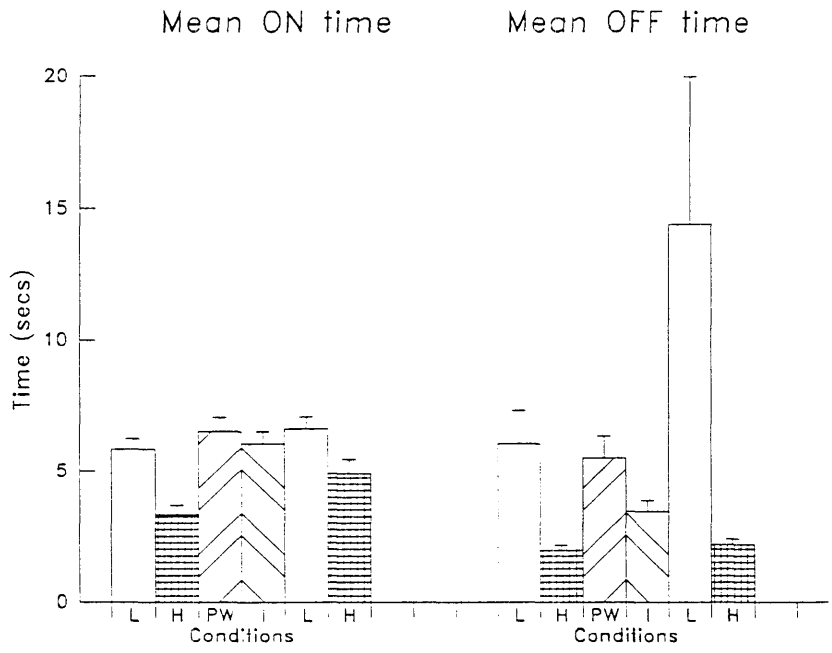


Figure 30: Mean ON and OFF time changes across experimental conditions. Abbreviations: L = low intensity condition, H = high intensity condition, PW = pulse width 2:1 test condition, I = intensity 2:1 test condition.

Table 14: Results of orthogonal comparisons

Comparisons based on the first six trials and the last six trials. $p < 0.05$ was used. See Table 13 and text for more explanation.

	Hypotheses		
Variable	H_1	H_2	H_3
$\overline{X}$	S	NS	NS
SD_x	S	NS	NS
$\overline{Y}$	S	NS	NS
SD_y	S	NS	NS

S $\Rightarrow$ Significant NS $\Rightarrow$ Not significant.

Analysis of the correlation data revealed no significant effects, although a significant overall correlation existed for Corr (X, Y): mean $= 0.155$; $F = 7.63, df = 1, 7, p < 0.05$, and for Corr (X, Y_{-1}): mean $= 0.114$; $F = 14.02, df = 1, 7, p < 0.01$, which were removed by first-order differencing.

2:1 test trials

The change from a 2:1 format of pulse width presentation to a 2:1 format of intensity presentation had no significant effect on mean ON time ($F = 0.12, df = 1, 7, p > 0.05$), nor on the standard deviation of ON time. Mean OFF time was significantly decreased by the 2:1 intensity condition ($F = 14.71, df = 1, 7, p < 0.01$), as were the standard deviations of OFF time ($F = 7.81, df = 1, 7, p < 0.05$). A significant trials effect also occurred with OFF time ($F = 2.94, df = 5, 35, p < 0.05$).

The correlation between ON time and the succeeding OFF time (i.e., Corr (X, Y)), was significantly higher in the 2:1 pulse width condition than in the 2:1 intensity condition (means= 0.196 and -0.033, respectively: $F = 5.71, df = 1, 7, p < 0.05$). The overall mean correlation for Corr (X, Y_{-1}) was significant (mean= 0.101, $F = 7.61, df = 1, 7, p < 0.05$). There were no significant effects in the differenced data.

The four trials indicated in Table 13 were individually analysed for 6-cross blocks repeated either 10, 20 or 40 times within each trial. Due to experimenter error some stimulus control files were not constructed correctly. Moreover, the errors were not uncovered until some time after the experiment was completed and the trials could not be repeated. Three trials out of the 32 were not based on the intended number of 2:1 blocks — the relevant trials are indicated in Table 15 along with the number of formatted blocks that were usable. ON time, OFF time, and standard deviations for the 6-cross blocks for these particular trials were based on only the usable number of blocks. However, trial means, standard deviations, and correlations provided in Tables C.8–C.15, and used for the group analysis included the completed number of crosses as they provided the best estimates available for these variables.

Individual data

The results of the individual analyses (Table 15) revealed significant behavioural changes as a consequence of the increase in intensity on each third initiation. However, a closer inspection of the times allocated by each animal (see Tables 16 and 17) showed that

individual response patterns varied considerably in relation to the high intensity stimulation.

Means for ON and OFF time for each cross in the block are shown in Tables 16–17. Standard deviations may be found in Tables C.16–C.17, Appendix C. Figures 31–34 depict particular results. The raw data for each trial and on which the calculated F-value was based can be found in Tables E.1–E.32, Appendix E.

Standard Errors of the Mean were not included in the figures in order to keep them uncluttered. Note that in Figures 31–34, the duration of ON time or OFF time is represented by the width of the columns and the width of the gaps (respectively). The area of the shaded region is proportional to the mean charge accepted.

Table 15: F-values calculated for intensity 2:1 trials

F-values calculated for individual trials when intensity or pulse width presented in a 2:1 format.

		Condition			
	Subject	Pulse width		Intensity	
	(N_c=)	(60)	(120)	(60)	(240)
	(df =)	(5,45)	(5,95)	(5,45)	(5,195)
a)	– ON time –				
	R12	4.73*	1.82	12.49*	66.48*
	R16	2.24	1.97	14.36*	[2]7.76*
	R18	0.72	0.75	4.82*	25.45*
	R24	0.61	2.92*	1.22	1.67
	R48	5.08*	[2]0.84	[1]68.64*	29.09*
	R49	1.20	5.85*	28.85*	69.05*
	R58	1.59	0.58	4.40*	25.93*
	R60	0.25	0.75	1.81	45.68*
b)	– OFF time –				
	R12	3.37*	1.64	6.55*	17.17*
	R16	2.48*	1.53	2.51*	[2]0.43
	R18	1.02	2.61*	0.45	6.93*
	R24	1.81	1.22	3.75*	0.72
	R48	2.36	[2]3.77*	[1]10.44*	5.63*
	R49	0.72	0.90	3.39*	7.57*
	R58	1.77	3.01*	1.74	8.93*
	R60	1.22	0.62	3.00*	18.58*

* indicates the calculated F-value greater than the tabled F-value at $p = 0.05$.

Some Trials based on less than the specified N_c value:

[1] indicates based on 30 crosses only (df = 5,20)

[2] indicates based on 60 crosses only (df = 5,45).

Table 16: ON time (secs) — intensity 2:1 trials

Within-trial mean ON time when intensity or pulse width presented in a 2:1 format.

Subject	Condition	(N_c)	Format (2:1)					
			low	*low*	*high*	*low*	*low*	*high*
			X_1	X_2	X_3	X_4	X_5	X_6
R12	Pulse width	*(60)	9.04	8.20	5.19	10.80	11.26	5.08
		(120)	5.83	5.58	4.72	5.78	5.66	4.94
	Intensity	*(60)	11.90	8.74	5.27	10.47	8.72	5.29
		*(240)	8.99	7.70	4.42	8.77	7.94	4.52
R16	Pulse width	(60)	7.46	6.96	5.43	12.39	9.33	6.04
		(120)	9.68	11.49	13.49	13.04	13.10	13.09
	Intensity	*(60)	7.66	6.90	13.76	5.80	7.99	13.72
		*(240)	6.35	7.12	17.09	5.71	10.23	8.51
R18	Pulse width	(60)	5.65	4.77	4.63	4.74	4.90	4.72
		(120)	6.79	5.87	6.67	6.45	7.29	6.20
	Intensity	*(60)	6.26	6.21	9.59	5.82	4.92	9.33
		*(240)	6.06	7.32	10.64	6.52	9.31	11.18
R24	Pulse width	(60)	3.42	3.86	4.13	3.44	3.93	4.63
		*(120)	9.79	12.97	11.24	11.70	9.27	13.32
	Intensity	(60)	7.40	5.21	5.55	8.95	5.43	5.25
		(240)	6.81	6.19	6.31	6.37	6.18	6.06
R48	Pulse width	*(60)	10.22	11.97	9.12	11.24	13.16	9.75
		(120)	9.46	8.18	9.68	9.30	10.66	10.69
	Intensity	*(60)	5.54	4.24	1.12	5.60	4.86	1.20
		*(240)	6.15	5.25	3.43	6.05	5.49	3.35
R49	Pulse width	(60)	6.48	6.17	6.92	6.35	4.92	7.29
		*(120)	2.95	3.24	2.19	3.29	2.73	2.20
	Intensity	*(60)	10.67	11.64	3.92	9.50	10.93	3.83
		*(240)	4.78	4.59	2.23	4.98	4.51	2.25
R58	Pulse width	(60)	7.91	4.82	4.58	6.83	6.20	6.54
		(120)	7.87	8.52	7.52	8.35	8.24	6.72
	Intensity	*(60)	6.96	4.16	4.42	6.71	3.61	4.24
		*(240)	4.62	3.26	3.49	4.98	2.97	3.31
R60	Pulse width	(60)	3.92	3.65	3.41	3.18	4.57	4.28
		(120)	2.35	2.56	2.71	2.30	2.21	2.70
	Intensity	(60)	4.93	5.75	3.34	8.07	6.51	3.30
		*(240)	2.43	2.25	1.39	2.48	2.41	1.46

* indicates the calculated F-value > the tabled F-value at $p = 0.05$, (i.e., from Table 15).

Table 17: OFF time (secs) — intensity 2:1 trials.

Within-trial mean OFF time when intensity or pulse width test condition presented in a 2:1 format. For example, Y_3 and Y_6 represent the OFF time immediately following each *high* pulse-charge stimulation.

Subject	Condition	(N_c)	Format (2:1)					
			low	*low*	*high*	*low*	*low*	*high*
			Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
R12	Pulse width	*(60)	43.01	28.62	16.55	24.50	48.89	21.79
		(120)	13.14	15.58	10.87	12.80	14.51	12.89
	Intensity	*(60)	16.82	16.96	6.67	17.59	19.81	7.79
		*(240)	7.02	6.06	10.47	6.57	5.94	9.83
R16	Pulse width	*(60)	4.00	4.52	2.77	6.69	3.15	3.48
		(120)	15.50	13.46	19.82	17.69	17.62	12.39
	Intensity	*(60)	4.64	3.33	4.90	5.25	21.19	9.99
		(240)	9.28	2.39	6.00	4.15	7.23	6.06
R18	Pulse width	(60)	1.99	3.21	1.65	2.81	2.84	2.10
		*(120)	3.91	4.52	2.72	3.87	4.45	3.40
	Intensity	(60)	1.52	1.56	1.41	1.97	1.85	1.28
		*(240)	1.83	3.44	1.84	1.57	3.00	1.90
R24	Pulse width	(60)	1.95	3.46	3.14	3.32	2.30	2.65
		(120)	9.56	7.79	8.97	10.06	21.08	5.66
	Intensity	*(60)	2.37	3.41	2.24	3.16	3.34	2.37
		(240)	5.96	6.40	5.55	6.78	5.71	5.46
R48	Pulse width	(60)	1.38	1.43	1.48	1.34	1.70	1.50
		(120)	1.45	1.48	1.44	1.45	1.93	1.43
	Intensity	*(60)	0.94	1.00	1.30	0.94	1.02	1.22
		*(240)	1.25	1.34	1.36	1.20	1.26	1.46
R49	Pulse width	(60)	14.14	11.55	14.29	17.92	9.97	16.56
		(120)	8.77	9.12	7.85	8.84	8.20	7.41
	Intensity	*(60)	7.02	10.95	6.07	8.04	8.06	5.74
		*(240)	7.02	8.00	6.02	7.48	8.78	6.41
R58	Pulse width	(60)	0.79	0.82	0.97	0.85	0.77	0.82
		*(120)	1.24	2.03	1.26	1.34	1.45	1.10
	Intensity	(60)	0.98	1.40	0.74	1.00	1.15	0.75
		*(240)	0.81	0.80	0.95	0.84	0.88	1.04
R60	Pulse width	(60)	2.40	2.05	1.67	2.18	2.43	2.81
		(120)	1.53	1.57	1.48	1.59	1.46	1.44
	Intensity	*(60)	1.49	1.69	1.32	1.76	2.09	1.42
		*(240)	2.20	2.02	3.27	1.91	1.78	3.30

* indicates the calculated F-value > the tabled F-value at $p = 0.05$, (i.e., from Table 15).

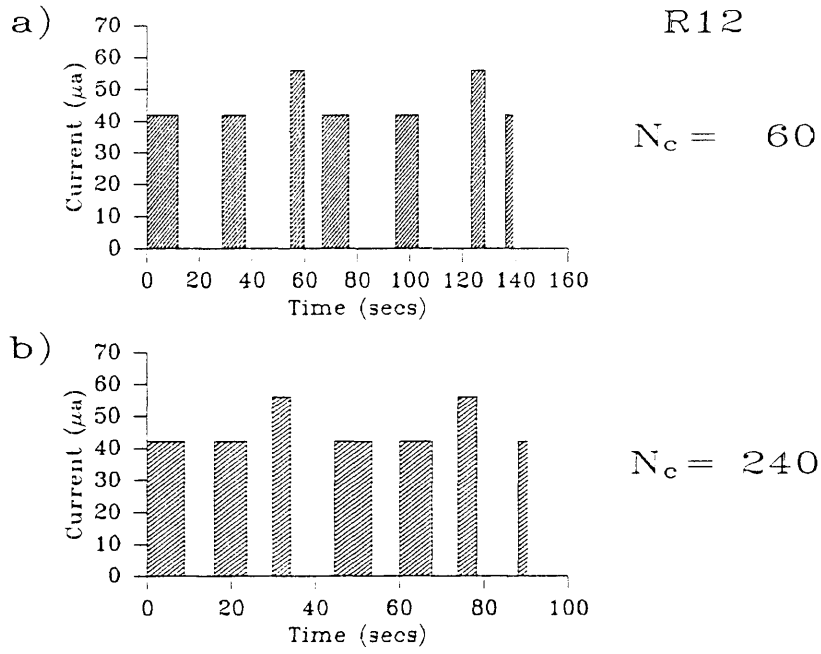


Figure 31: ON and OFF time distribution when the intensity of stimulation varied in a 2:1 format. Subject R12. Note shift in distribution of OFF time before and after high intensity with more experience.

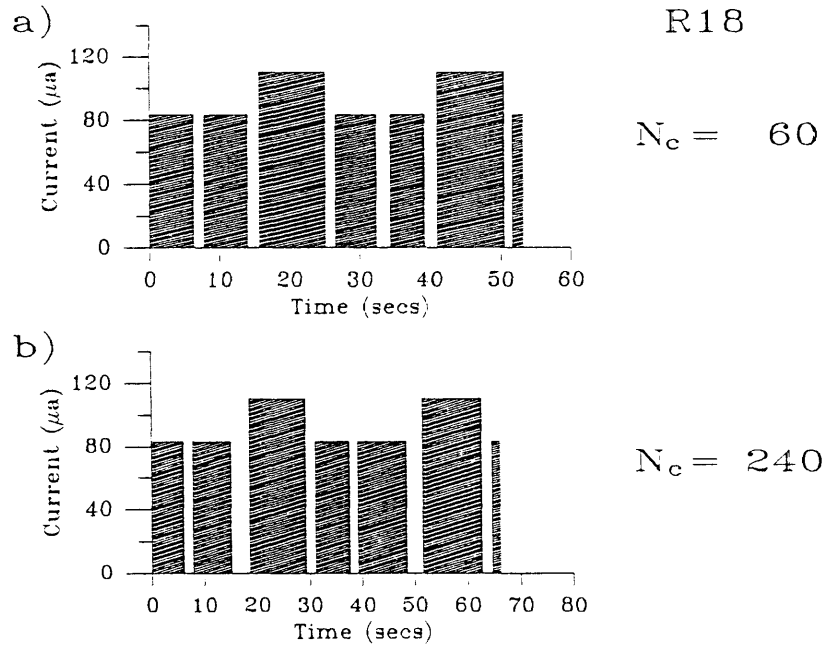


Figure 32: Intensity of stimulation varied in a 2:1 format. Subject R18. Long OFF time before high intensity. Long ON time when high intensity prevailed.

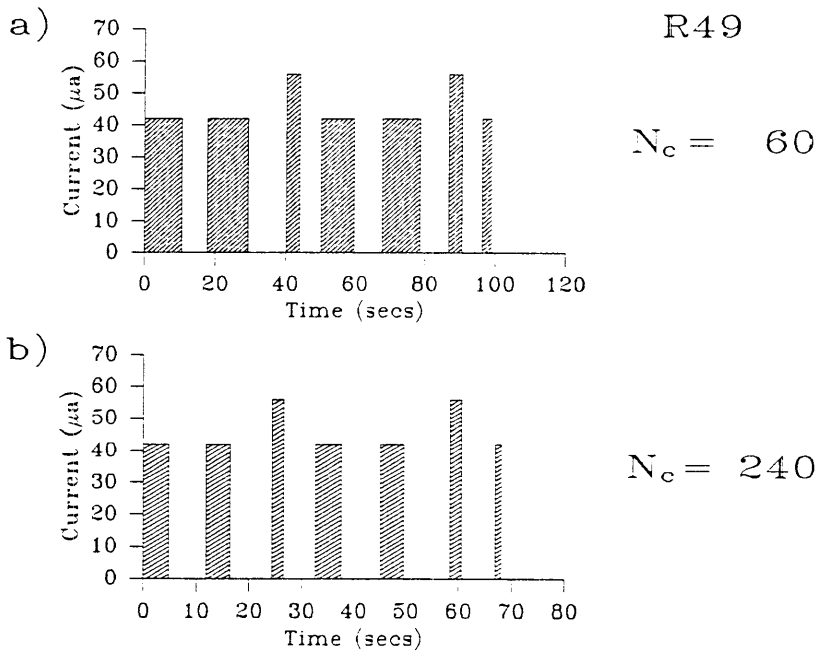


Figure 33: Intensity of stimulation varied in a 2:1 format. Subject R49. Long OFF time before each high intensity period but short ON time when high intensity prevailed (cf., Figure 32).

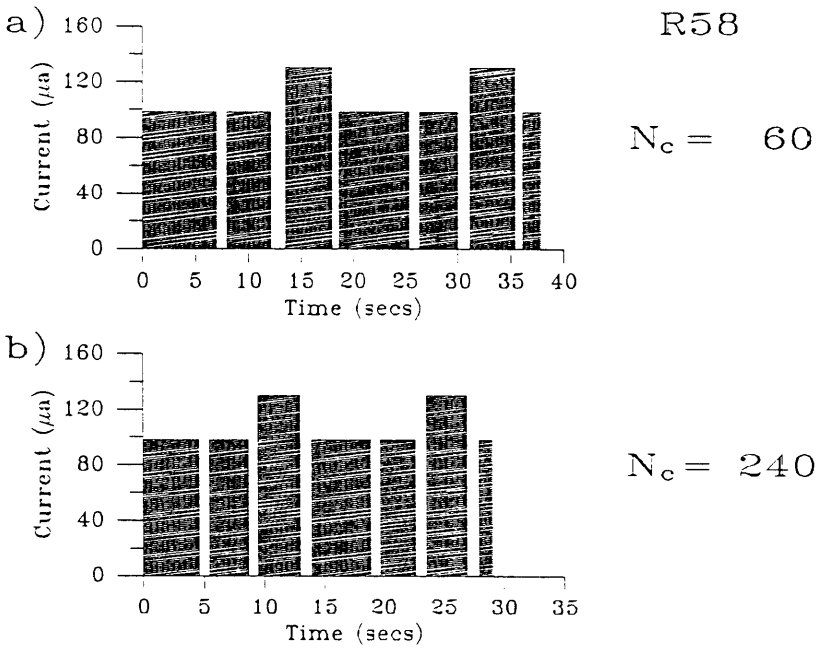


Figure 34: Intensity of stimulation varied in a 2:1 format. Subject R58. Note pattern of ON time changes. First ON period after a high intensity period longer than second ON period (i.e., at same intensity).

7.2.5 Discussion

Gross indicators of behaviour (i.e., mean ON and OFF times) confirmed that low intensity, in general, produced longer ON and OFF times both before and after the central trials of interest and high intensity produced shorter ON and OFF times (Figure 30 and Table 14).

ON time changes

The individual analyses indicated an even greater range of response patterns than was found in Experiment IIIa. This is highlighted in the Figures provided (Figures 31–34). In general, ON times decreased on high intensity crosses (as expected), but one animal showed increased ON times when the high intensity stimulation prevailed (R18, Figure 32).

The lack of a consistent relationship between ON time and the preceding, or succeeding, OFF time as a result of increasing intensity or pulse width is supported by the lack of a significant rank order correlation between each F-value and each within-trial correlation (regardless of sign — see Table 18). When duration was systematically varied, it was the correlation with the succeeding OFF time that was most related to a consistent behavioural change. However, when intensity or pulse width were varied, no consistent behavioural changes occurred across animals.

OFF time changes

OFF time distributions also showed a marked individuality. In general, animals showed longer OFF times after high intensity stimulation (e.g., R12 Intensity (240), R48 Intensity (60) and (240), R58 Intensity (240), R60 Intensity (240)). Other animals showed shorter OFF times after the high stimulation (e.g., R12 Pulse width (60), R18 Pulse width (120), R49 Intensity (60) and (240)).

Some animals showed longer OFF times before the high intensity (e.g., R18 Pulse width (120) and Intensity (240)). The longer OFF times for R18 before each high intensity may have been related to the increased ON times under high intensity (see Figure 34). However, R49 also showed increased OFF times before the high intensity stimulation (Intensity (60) and Intensity (240)), but decreased ON times (see Figure 33).

Table 18: Comparison among within-trial correlation variables

Spearman rank order correlations calculated between each F-ratio and each within-trial correlation variable in Experiments IIIb. The absolute value of each within-trial correlation was used.

	F-ratio		Correlation variables		
N		Corr (X,Y)	Corr (X,Y_{-1})	Corr $D(X,Y)$	Corr $D(X,Y_{-1})$
32	F_{ON}	0.085	-0.081	0.214	-0.076
32	F_{OFF}	0.098	-0.239	0.237	0.211

None of the above correlations was significant at the $p = 0.05$ level.

Learning changes

Of the four animals that showed significant effects in the OFF time data of both the 60- and 240-cross intensity trials, R12 and R60 showed a change in distribution with more experience. For the 60-cross trial both animals showed shorter OFF times after each high intensity, but in the 240-cross trial both showed longer OFF times after each high intensity. Of the other two animals (R48 and R49), R48 showed longer OFF times on both occasions, and R49 showed shorter OFF times on both occasions.

In summary, changes in ON and OFF time distribution as a consequence of systematic within-trial changes in intensity did not indicate a consistent pattern. This may have been due to slight differences in electrode location, or to different levels of experience with the format of stimulation, or due to overriding arousal or forcement effects (Atrens, 1984). At least one animal (R24) showed only weak response patterns in the intensity trials, yet this animal was considered a 'good SS' in that crossing rates were high, times regular, and motor artifact negligible. The sensitivity of animals to changes in stimulus parameters may not be predicted by rate of responding or by mean ON and OFF times.

Different animals may require a different number of trials to learn the schedule and hence to show a consistent pattern of responding. If the intensity trials had been continued further, more animals might have developed a particular pattern of responses (such as long OFFs after each high intensity). However, increased experience with the presentation format did not lead to a greater number of significant

F-values in the intensity condition. Four animals completed the pulse width condition before the intensity condition, while four followed the reverse order (Table 13). The four animals that experienced 2:1 intensity first produced 12 significant F-values in the intensity condition, while the four animals that experienced the 2:1 intensity last produced 13 significant F-values in the intensity condition. Overall, 2:1 intensity produced 25 significant F-values whereas 2:1 pulse width produced only 9 such values, which confirms that pulse amplitude is a greater determinant of ON and OFF time than pulse width. There were as many significant changes with ON time as OFF time (17).

Finally, the data do indicate that the relationship between ON and OFF time in shuttling behaviour may be manipulated by within-trial changes in intensity or pulse width. This complements the findings of Experiment IIIa in which it was also found that within-trial changes in the duration of stimulation could modify the relationship between these times. The relationship is not a simple one-to-one relationship and is subject to change because of increased experience or because of juxtaposing different parametric combinations.

7.3 Conclusions from Experiment III

Experiment IIIa showed that although as a group mean OFF time increased as the duration of stimulation was increased, animals do not develop consistent patterns of responding in relation to the durations. However, in general, the most frequent response was longer OFF times after longer ON times.

Experiment IIIb also found that marked differences occurred when pulse width or intensity were systematically varied within a trial. Although most animals decreased ON time when high intensity prevailed, R18 increased ON time. In general, the most frequent response was to increase the OFF time immediately following each high intensity initiation and to decrease the preceding OFF time. An increase in the duration of stimulation has a more consistent effect on the following OFF time than either an increase in intensity or pulse width.

Response patterns in shuttling behaviour can differ considerably as a result of within-trial changes in stimulus parameters but the direction of change is not predicted by rate of responding or magnitude of mean ON time or mean OFF time. Brain structures other than the VTM may show a greater sensitivity to within-trial changes in intensity. Figure 35 gives an example of the behavioural changes that

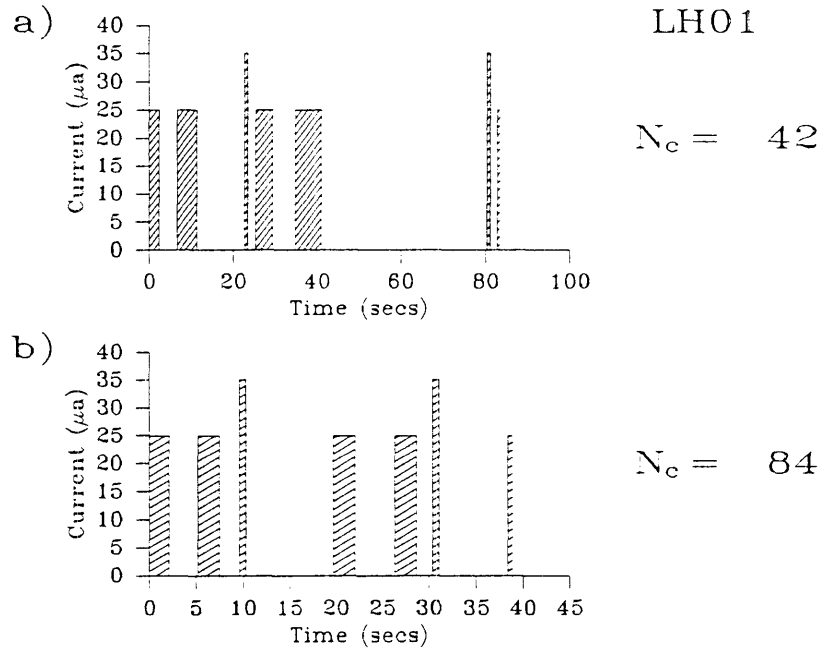


Figure 35: The change in distribution of OFF time after greater experience was quite marked for an animal with an electrode implanted in the lateral hypothalamus.

occurred when intensity was increased on each third initiation for an animal with an electrode in the lateral hypothalamus. The changes that occurred in OFF time were clear.

The marked differences in response patterns observed in Experiment IIIa, and IIIb suggest that any model proposed to account for ICSS shuttling behaviour must be capable of considerable flexibility. Evidence was found that could be interpreted to support both of the models being considered here.

When the duration of stimulation was increased on each third initiation, and when there was evidence for a consistent response pattern, it was the change in the succeeding OFF time that determined that pattern. In general, the tendency was for longer OFF times to occur after longer ON times. This pattern might be explained by the time required for aversion to dissipate or for the neural substrate to recover from adaptation. Because the times used in Experiment IIIa were all less than or equal to the duration of stimulation previously selected by each animal (at the same intensity), significant aversion should not be experienced. Therefore, the more likely explanation is that the longer OFF times represent the time required for some recovery to occur in the neural substrate. It is still possible, however, that the longer OFF times represent a period of time required for recovery from excessive

autonomic arousal.

Both the reward/aversion model and the RI model would predict that more significant behavioural effects should occur in the high stimulation condition than in the low duration condition, because of greater aversion or greater adaptation. Instead, more changes occurred in the short duration condition. This might support to some extent the argument of Deutsch et al. (1980), that durations beyond 1 to 2 secs are not discriminable, However, because at least some changes occurred in the long condition, stimulation beyond 1 to 2 secs can still affect shuttling behaviour.

Evidence for a significant element of elicitation in the determination of ON time may be found by referring to the general lack of change in ON time with further training. Six animals showed a significant response pattern in both the initial exposure trial and in the final trial when intensity was systematically varied (i.e., R12, R16, R18, R48, R49, R58). In all cases the relative duration of ON time in the high intensity condition compared to the low intensity conditions remained very similar. For example, the data for R58 showed a long ON time on the first of the two low intensity conditions in the initial exposure trial and in the final trial (Figure 34). If the timing of the termination response was under operant control (i.e., maintained by its consequences), and if each high intensity stimulation can be considered more rewarding than each low intensity, an experienced animal might be expected to complete the low intensity initiations more rapidly, in order to gain access to the more rewarding high intensity initiation. However, the relative durations of ON time on the low intensity and high intensity initiations generally remained unchanged.

Self-stimulating rats do appear to have a greater degree of control over the initiation response. If OFF time was solely a function of the intensity of stimulation, short OFF times should have followed each high intensity stimulation invariably, and long OFF times preceded each high intensity stimulation (since elicited by the preceding low intensity stimulation). In some cases this distribution actually reversed, indicating that the timing of the initiation response is under a higher degree of operant control (Fibiger, 1978).

A reward/aversion model might be supported by the finding that one animal increased ON time when high intensity prevailed (R18). Because juxtaposing two low intensity stimulations with one high intensity stimulation should elevate the reward value of the high intensity (i.e., positive contrast), a greater accumulation of aversion would be required to outweigh the increased reward effect and the termination response would be delayed.

In conclusion, the findings from within-trial stimulus changes must remain tentative until further research can determine some of the factors which led to the high degree of individual variability. Because of this the results described above did not distinguish clearly between the two models being considered. Atrens et al. (1983) and Liebman (1983) have suggested that both aversion and adaptation may determine the duration of ON time depending on electrode location. Perhaps the most parsimonious interpretation of the present results is to suggest that variations in electrode locus, although still within the ventral tegmental area, explain the different response patterns.

Chapter 8

Histological results

8.1 Histology

The histological analysis revealed that the majority of electrode tips for animals defined as positive for ICSS were within the boundaries of the VTM (Pellegrino & Cushman, 1967). Conversely, the electrode tips for those animals defined as ICSS-negative were, generally, outside this region (see Figures 36 to 38). The electrode locations were found to be in good agreement with other reported data for this brain region (e.g., Anzelark, Arbuthnott, Christie, & Crow, 1973; Corbett & Wise, 1980; Crow, 1971, 1972; Miliaressis & Cardo, 1973; Shizgal et al., 1980).

Overall, the electrodes were located too far toward the caudal part of the VTM (particularly the R-Series), and this may account only a 50 % success rate. Corbett and Wise (1980) found that ICSS was not as easily obtainable from the more caudal aspects of the VTM. The coordinates employed in the present study were AP = −4.0 to −4.3 and were based on the reports of Miliaressis et al. (1975) and Miliaressis and Cardo (1973). More accurate placements might have been obtained with A-P = −3.7 to −4.1 mm (with incisor bar = −3 mm).

Placements just outside the VTM boundaries have been shown to be capable of supporting vigorous ICSS (Anzelark et al., 1973; Corbett & Wise, 1980; Crow, 1971, 1972; Miliaressis & Cardo, 1973). Anzelark et al. (1973), Corbett and Wise (1980), and Miliaressis and Cardo (1973) report positive ICSS from the region just dorsal to the interpeduncular nucleus (IP). The electrode locations for rats R12 and R29 were in this region. Corbett and Wise reported that they did not obtain ICSS from sites clearly within the IP. The present study, in general, agreed with this, although four sites (M08, M23, R69, R64) appeared to impinge at least partly, on the IP. At least

one positive site was located at the very base of the IP (R64). Miliaressis and Cardo (1973) report one electrode tip in a similar location. Montgomery et al. (1981) report one electrode tip in the IP, and Anzelark et al. (1973) report "a few cases" in the IP.

Corbett and Wise (1980), and the present study, found positive ICSS sites in the region directly caudal to the VTM (i.e., AP = −3.8). However, the lack of a further caudal extension to the ICSS-positive substrate (i.e., AP < −3.8), was evident in the present study and agrees with the conclusions of Corbett and Wise. The electrode for R12 (Plate 2; Figure 37) was more caudal than the electrode sites for the other self-stimulators. However, this site was also just dorsal to the IP and hence in a position similar to those reported by Miliaressis and Cardo (1973).

Photomicrographs (Plates 1 to 5) provide direct evidence for the verification of the electrode locus in a number of animals. Note that these are arranged in alphanumeric order (for ease of subject reference) and not in terms of anterior-posterior coordinates. In general, the more anterior sections are indicated by a wider separation of the substantia nigra pars reticulata (SN) and the IP, and often by the lack of an IP altogether. The IP was often left behind during brain removal (Konig & Klippel, 1970, Figures 46-49, show similar results). Also to be noted, is that although all electrodes were implanted in the same side of the brain, whether the block of tissue was sectioned from the anterior face or from the posterior face determined the side showing the electrode tract. Some photomicrographs show electrodes on the left side of the brain while others show the electrode on the right side, but they were all as shown in Figures 36– 38.

All photomicrographs except the last two (R50 and R52) were from rats defined as self-stimulators. R50 shows an electrode going through to the base of the brain, while the electrode for R52 appeared to be in, or very near, the VTM, but this animal did not respond positively to stimulation. Histology was not available for rats M11, M21 (ICSS-positive) and M06, R05, R15, R35, R39, R45, R63 (ICSS-negative).

The indication of small, discrete regions of stimulation in Figures 36 to 38 may not accurately represent the rather large, bipolar electrodes used in the present study. 254 μm bipolar electrodes separated by 0.5 mm cover a minimum distance of 1 mm. The whole AP extent of the VTM in Pellegrino and Cushman's atlas covers only about 1 mm. Also, biphasic pulses may alternately stimulate different populations of neurons (Gallistel, 1973: Steiner, Bodnar, Nelson, Ackermann, & Ellman, 1978: Wetzel et al., 1969). Therefore, the effective area of stimulation may be larger than indicated. The locations indicated in the figures were determined from the section

showing the deepest electrode penetration and estimated the centre of the two poles of the bipolar electrode.

Abbreviations adopted from Pellegrino and Cushman (1967).

In Plates 1-5 and Figures 36- 38:

ip = interpeduncular nucleus
lm = medial lemniscus
pm = mamillary peduncle
sn = substantia nigra

Other abbreviations in Figures 36- 38:

DTV = decussation of Forel
HP = tractus habenulo-interpeduncularis
PC = cerebral peduncle
VTN = Tsai's ventral tegmental nucleus (VTM)

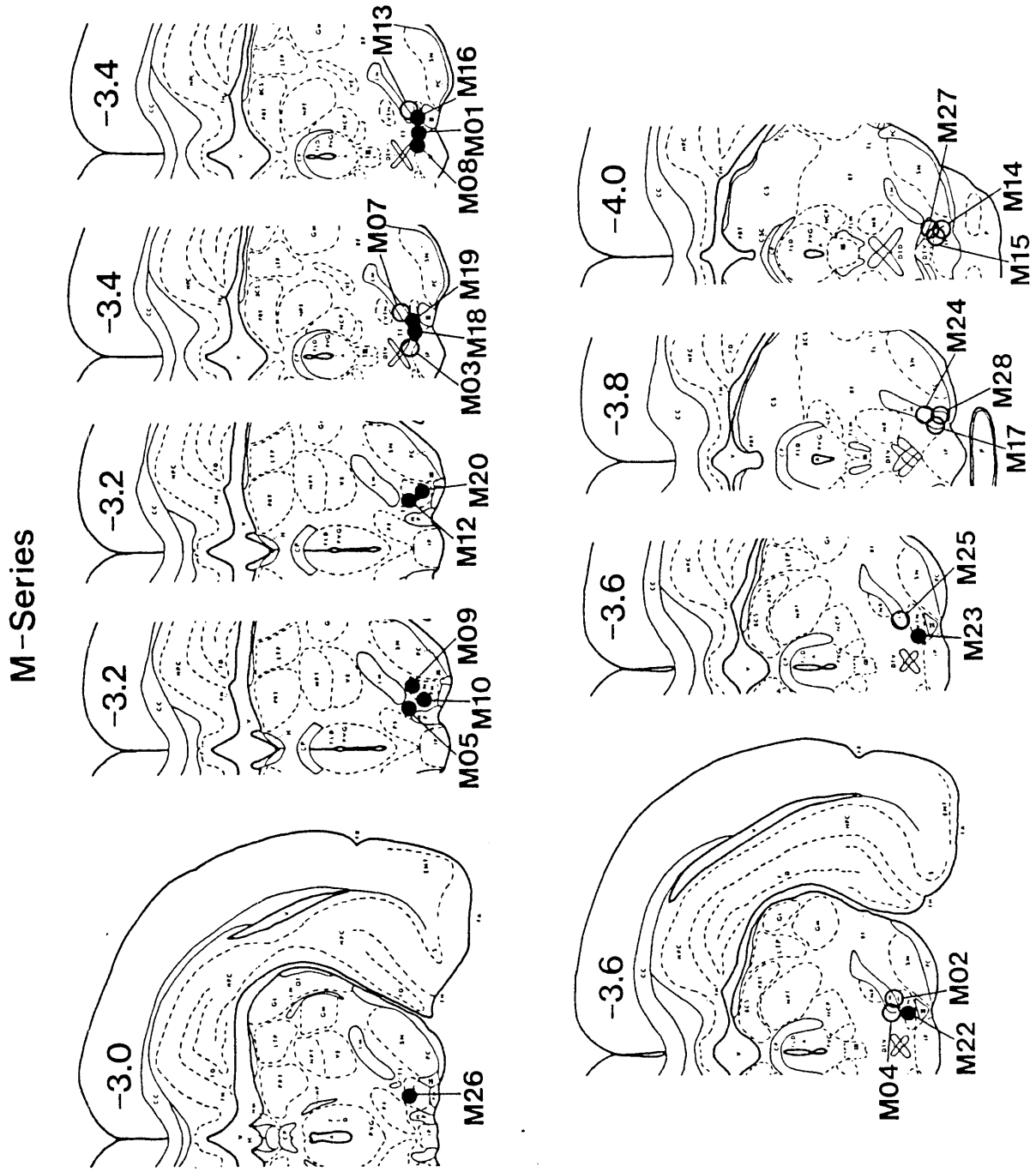


Figure 36: Electrode locations for M-Series animals. Filled circles ICSS-positive. Open circles ICSS-negative.

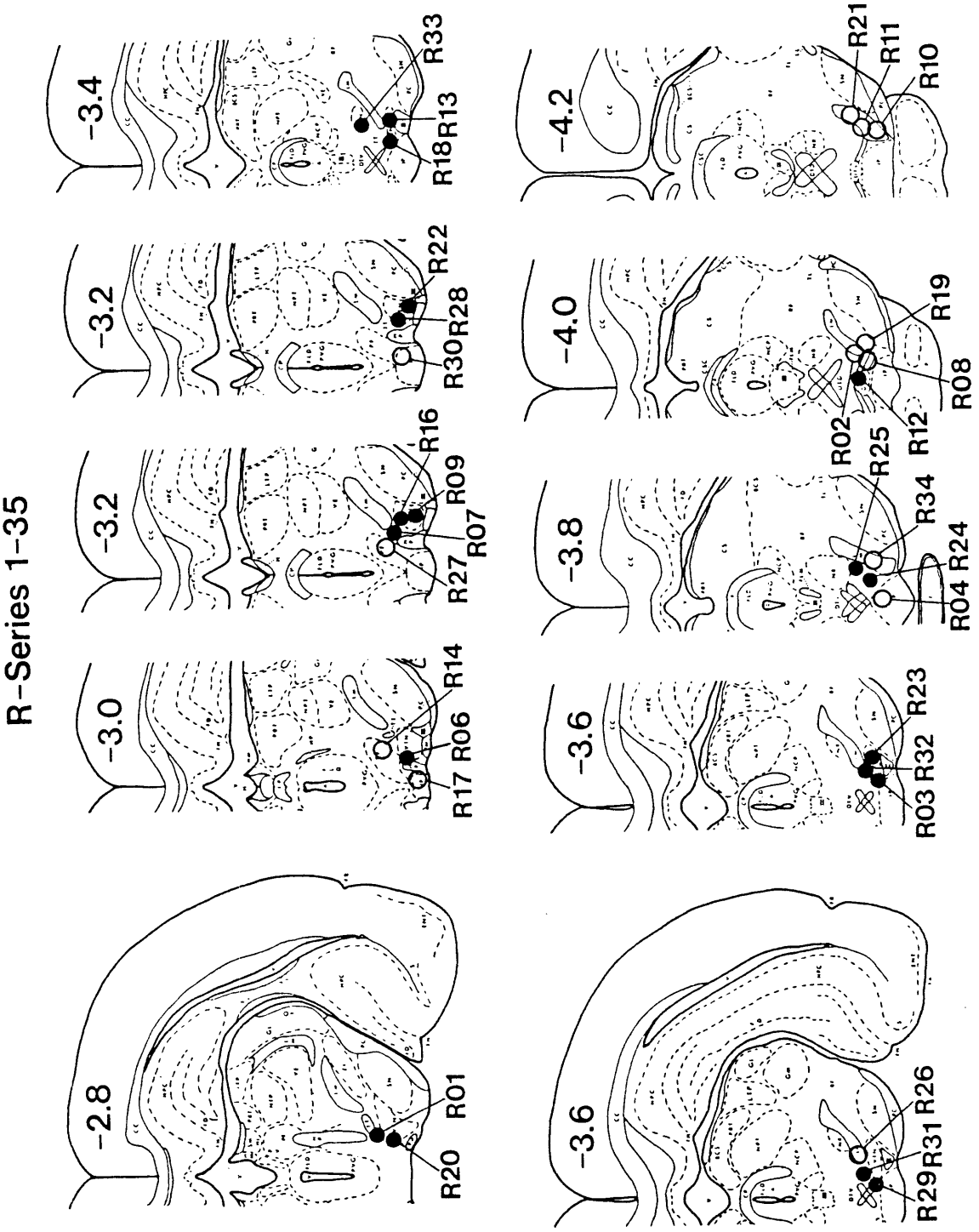


Figure 37: Electrode locations for R-Series animals R01–R35. Filled circles ICSS-positive. Open circles ICSS-negative.

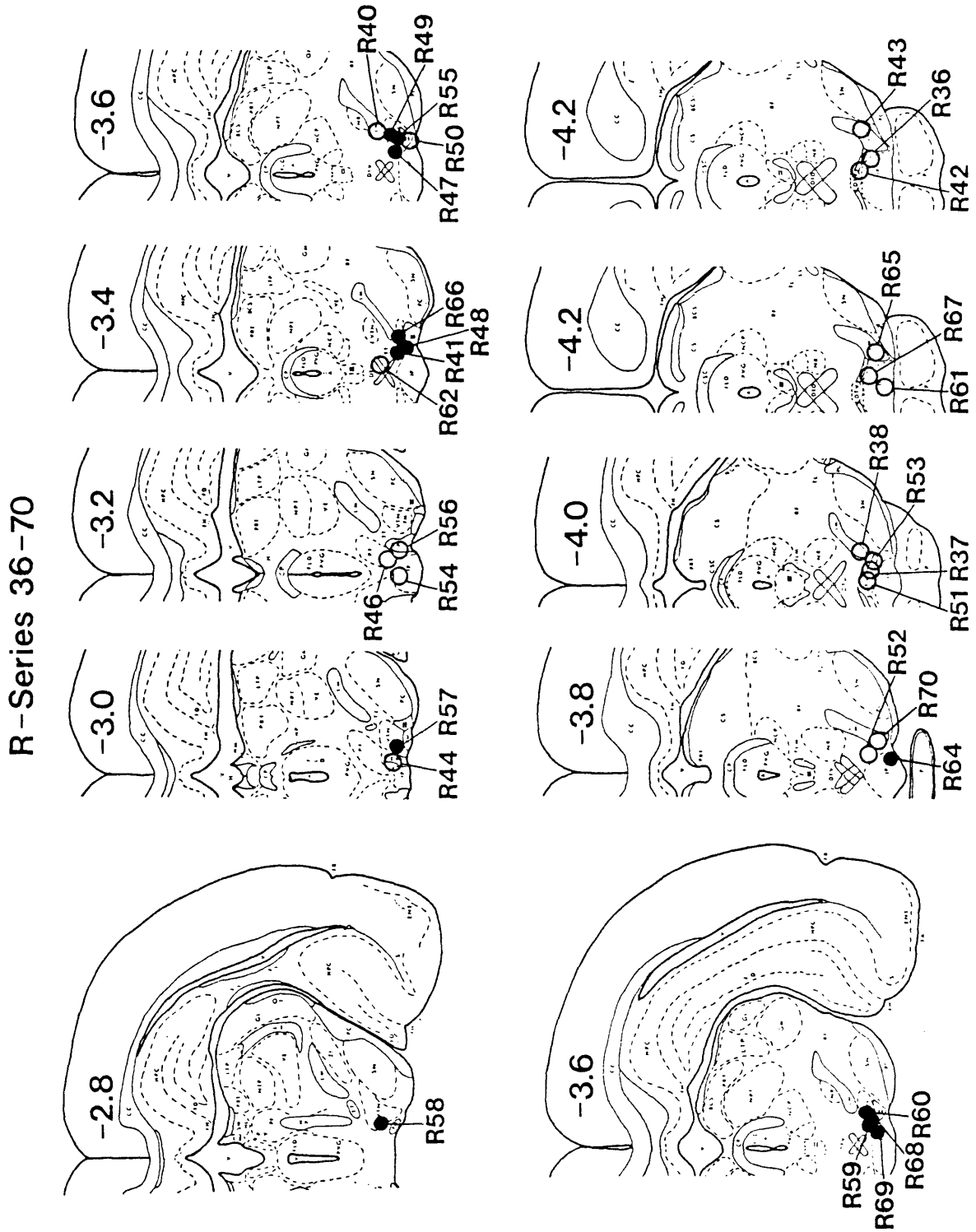


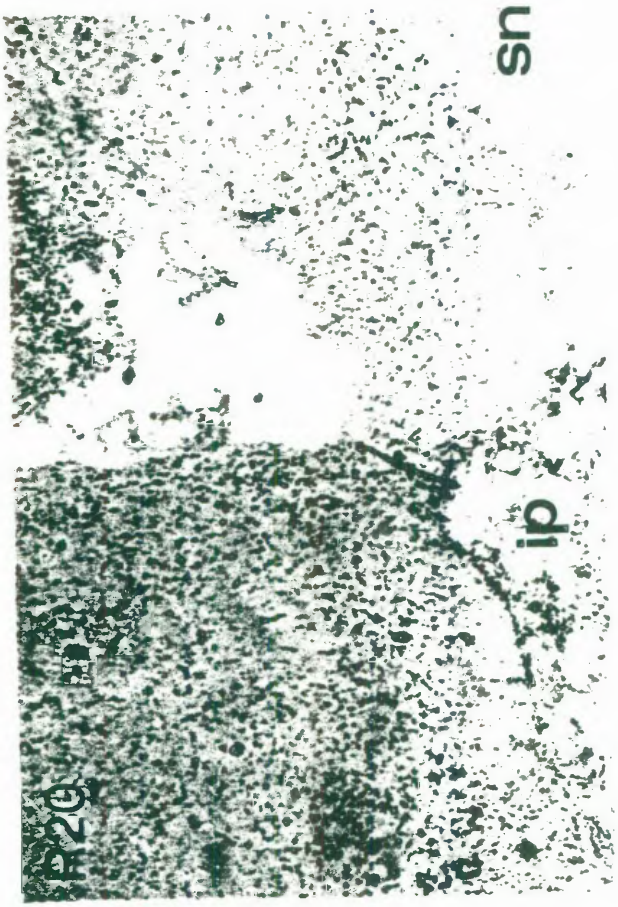
Figure 38: Electrode locations for R-Series animals R36–R70. Filled circles ICSS-positive. Open circles ICSS-negative.

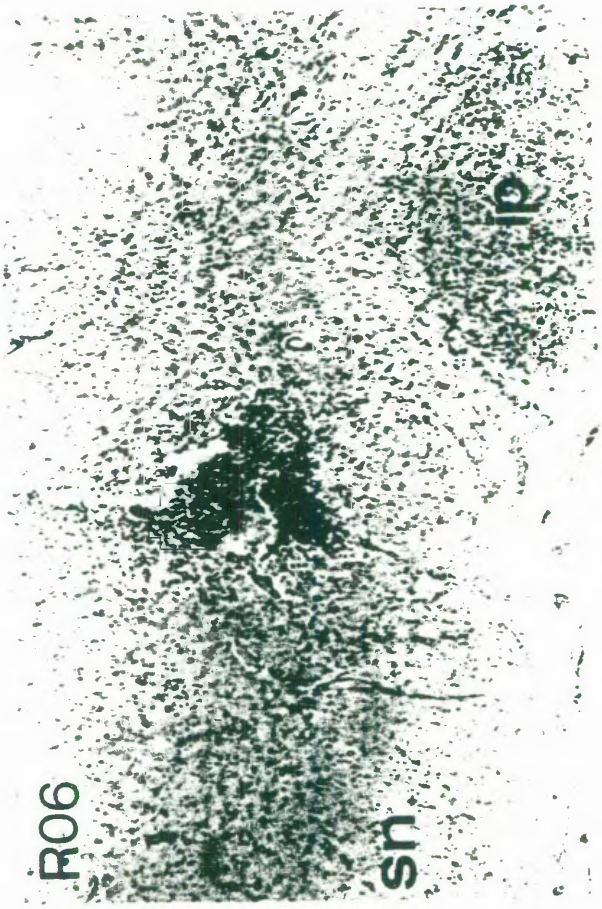

dl

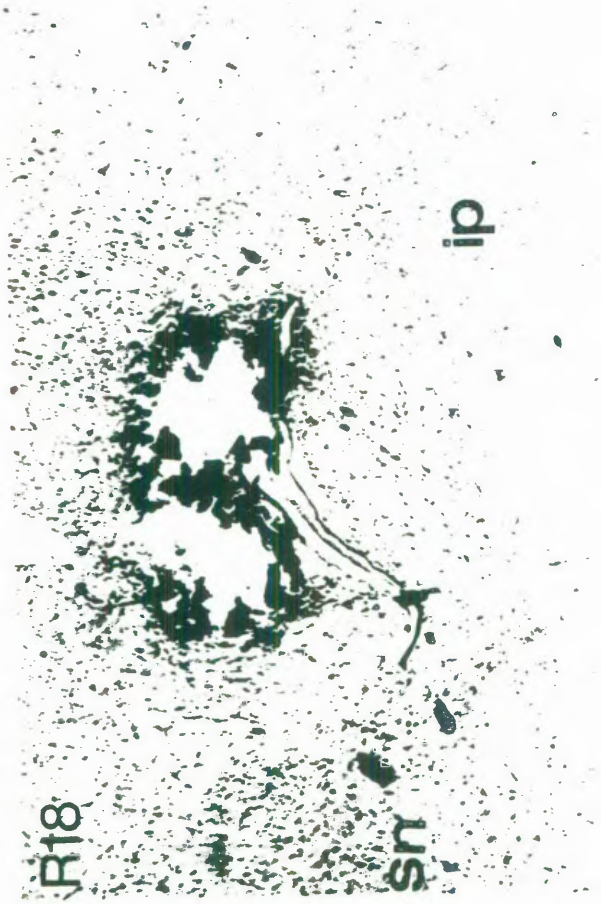

dl

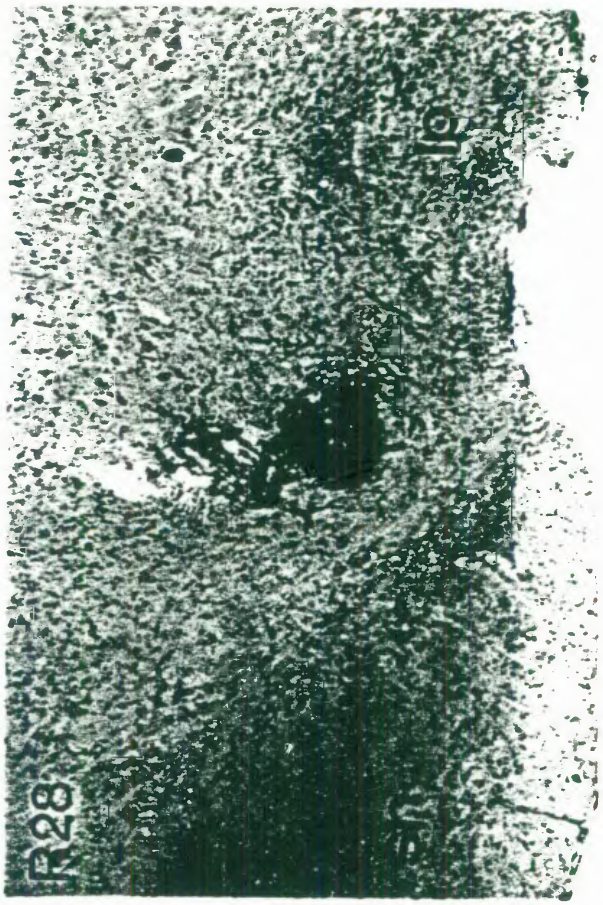

us
dl

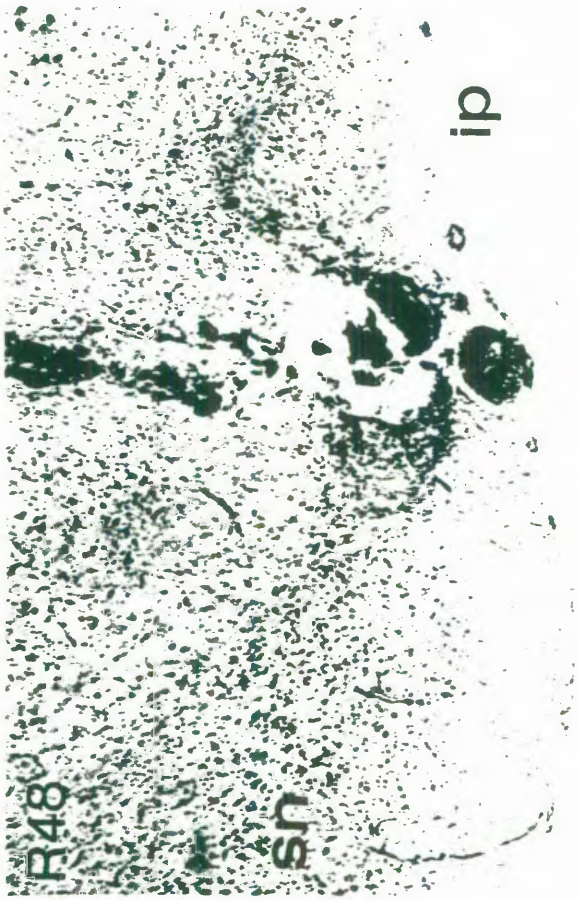

R12
lm


R20
ip
sn


R06
ip
sn


R18
ip
sn


R28
ip

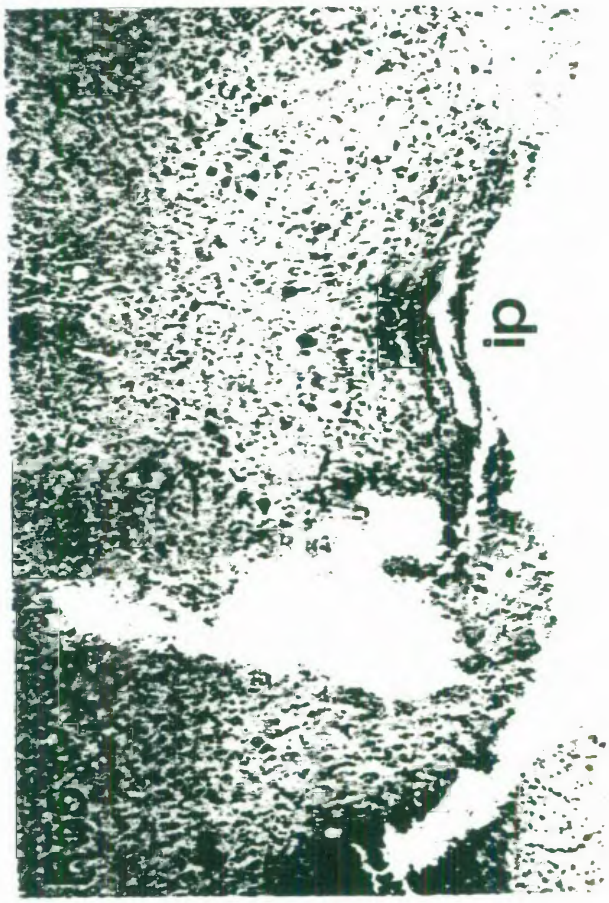

R48
sn
ip


R24

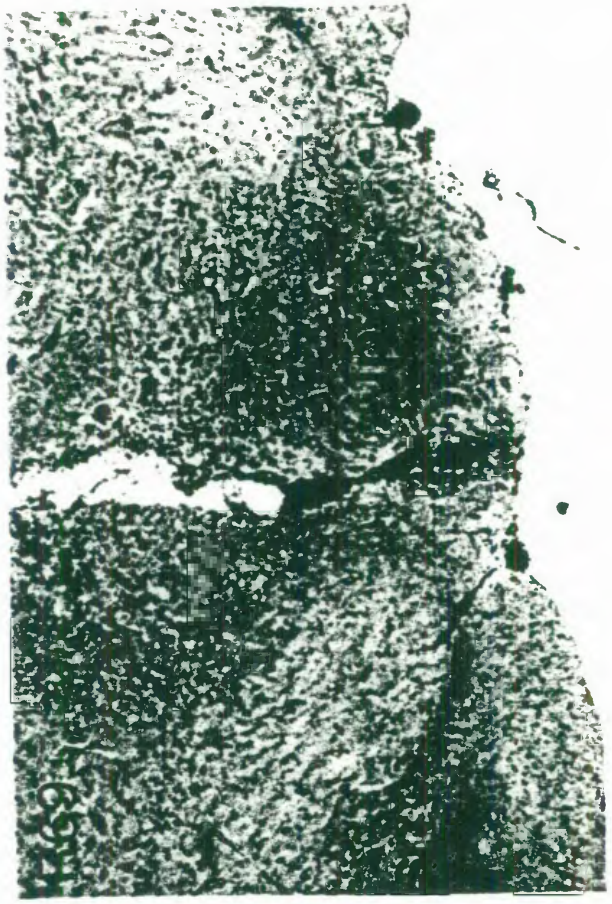

ip

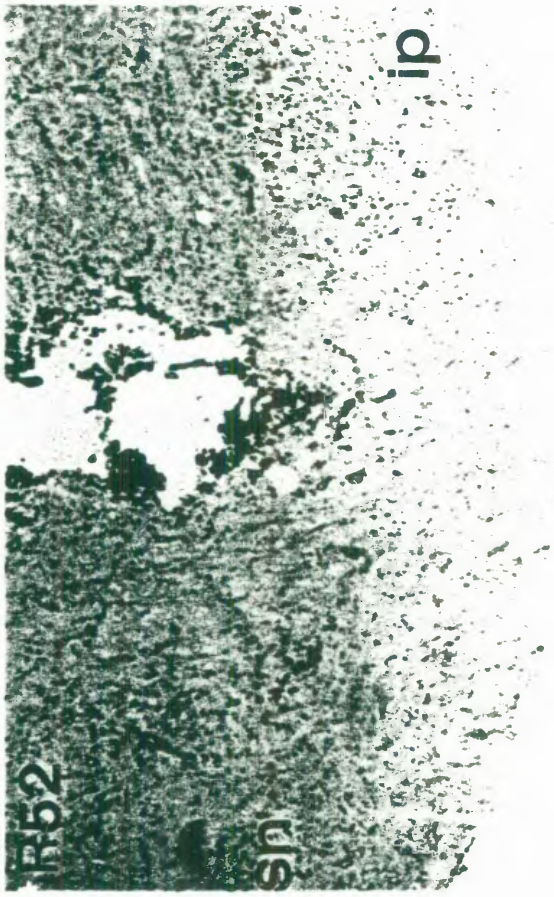

sn
ip


ip
RGC


ip
RGC


pm
RGC


R52
sp
ip


R52
sp
ip


R64
sp
ip


R50
ip

8.2 Electrode orientation

The R-Series animals were divided into two groups with regard to the orientation of the bipolar electrode. Approximately one-half were implanted with the two poles of the electrode oriented in a medio-lateral (M-L) direction, and one-half with the two poles oriented in the antero-posterior (A-P) direction. This was in response to a report by Szabo and Milner (1973) that significant differences in lever press response rates could be obtained from stimulation of the VTM and the posterior hypothalamus when this factor was examined. Szabo and Milner found that 60 Hz sine wave stimulation was more effective in stimulating the reward system when the tips of the bipolar electrode were oriented in the M-L direction than in the A-P direction. Long periods of hyperpolarisation (8.3 msec for 60 Hz sine wave) at one pole should block transmission of impulses generated by the depolarising current at the other pole if the same axons pass under both poles of the electrode. The results suggested that axons in the VTM and the posterior hypothalamus conducted impulses in the A-P direction.

The present study employed 3 msec hyperpolarising and depolarising phases and found no difference between animals in the M-L group and animals in the A-P group in terms of ICSS success rate or the current intensities employed in the experiments.

ICSS success rate

Seventy animals were implanted with electrodes in the R-Series of operations. Thirty-two of these were defined as self-stimulators. The factor of electrode orientation was of no significance in the determination of ICSS success rate ($\chi^2 = 0.032, df = 1$).

Table 19: ICSS success rate.

Number of implanted rats defined as positive or negative for ICSS when electrode tips oriented antero-posteriorly (A-P) or when oriented medio-laterally (M-L).

	ICSS		
	+	−	**Total**
A-P	15	17	32
M-L	17	21	38
Total	32	38	70

Selected current intensities

Twenty-three of the thirty-two animals defined as self-stimulators were used in the experiments reported in Chapters 2 and 3. Table 20 lists the electrode orientation for each of the 23 animals. Four animals were used in the experiments reported in both chapters.

Table 20: Subject electrode orientation.

List of subjects with electrodes oriented antero-posteriorly or medio-laterally.

A-P	M-L
R01, R03, R16, R24	R06, R09, R12, R13
R28, R29, R41, R60	R18, R23, R32, R49
R64, R69	R48, R49, R55, R58
	R66

With regard to the intensities used in Experiments IIa and IIb (Table 7), 11 animals had electrodes oriented in the M-L direction and 8 had electrodes oriented in the A-P direction. Mean current intensities were not significantly different ($t = -0.449, df = 17$, two-sample t-test).

8.3 Threshold changes

The photomicrographs give evidence of the extent of damage around the electrode tip, which may account for the gradual changes in threshold (or measurement instability) observed throughout the experiments and which has been reported by others (e.g., Liebman, 1983). ICSS threshold increases were defined as being evident whenever a persistent increase in both mean ON and OFF time occurred despite all parameters of the test situation (e.g., stimulus parameters, apparatus, testing regime) remaining unaltered.

The threshold changes observed fell into three categories. These may be described as gradual short term changes, gradual long term changes, and abrupt, irreversible, changes. Each will be discussed briefly.

Short term changes

In general, ON and OFF times increased across the period of a 10-minute trial. Also, trials in which a large number of crosses were required (e.g., 240) often resulted in a significant increase in the mean and variability of selected times. Short term threshold changes were measured by the regression line slope in Experiment Ia and Ib (e.g., Figures 10, 18, Table 3). The effects of the short term threshold increase could be removed statistically by first-order differencing. Mean ON and OFF times could be restored by an increase in intensity, or, by allowing at least a two hour rest period between test trials.

Long term changes

Gradual long term changes may be described as changes that occurred over the period of a few days to several weeks and may also be referred to as changes in baseline. Significant increases in threshold were found in Experiment Ib and Experiment IIb. Experiments II and III included specific controls in the experimental design to monitor and measure the degree of threshold change that occurred across the duration of the experiment.

These changes could be reversed by an increase in intensity. Rest periods of the order of one to two weeks could also help restore the original response levels.

Abrupt changes

Occasionally, very abrupt increases in threshold were observed. These were particularly evident with the R-Series animals and tended to occur in animals which had undergone an extensive amount of testing, were often quite dramatic, and were not reversible by increases in intensity.

For example, rat R06 had been shuttling rapidly for 74 crosses (i.e., ON and OFF times less than about 3 secs), when on the next initiation, it started a vigorous circling behaviour that may have persisted indefinitely without forced termination. The animal no longer would, (or could), terminate the stimulation. Increases or decreases in intensity, pulse width or frequency would not restore the self-stimulation behaviour. An increase in intensity served only to increase the vigour of the circling.

Not all changes were as abrupt as that observed with R06. R47 developed a pattern of 10-15 rapid crosses (less than 3 secs) at the start of a trial and then, within the next two to three crosses, ON times would increase to greater than 100

secs. An increase in intensity would delay the onset of the shift somewhat, but not entirely. Most of the animals showing these abrupt changes would still initiate the stimulation if forced OFF or when placed in the OFF side initially, but there appeared to be an increased degree of 'hesitation' or 'reluctance' to initiate.

The abrupt behavioural changes were somewhat similar to changes seen when an electrode had been dislodged. However, in no case was there any evidence to suggest that this had happened. A very small movement might have occurred at the electrode tip but this was not apparent at a gross observational level. The animals that showed the very abrupt changes included: R06, R09, R48, R49. Those animals showing a more delayed, or more gradual, but still irreversible, change included: R03, R16, R47, R57. When these changes occurred, the animal could not be used for further experimentation and data was discarded.

8.3.1 Electrode materials

The electrodes used in the present study were stainless steel. Evidence from Bollinger and Gerall (1971) indicates that threshold changes can be eliminated to a significant degree by the use of platinum electrodes rather than stainless steel. Similar claims have been made by Wetzel et al. (1969) and Gallistel (1973). The evidence from the present study suggests that procedures that can minimise threshold changes should be adopted and hence platinum electrodes should be an improvement on the techniques reported here. Although a significant increase in ON and OFF time occurred in Experiment Ib, there was no accompanying increase in the total charge accepted by the animals, nor a decrease in the total time with the stimulation ON. This suggests, at least, that tissue damage or metallic deposits around the electrode tip is not the sole cause of the observed baseline changes. 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 paradigm. The original, pre-lesion percentage of total time on the positive platform, could be restored by an increase in intensity (i.e., an increase in the total charge that the rats accepted). Because total time ON and total charge accepted did not change in Experiment Ib, tissue damage at the electrode tip is unlikely to account for the general increase in ON time that occurred.