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USE OF PROGRESSIVE FIXED-RATIO PROCEDURES IN THE ASSESSMENT OF INTRACRANIAL REINFORCEMENT[1]

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Six rats with electrodes chronically implanted in septal or hypothalamic sites were tested for intracranial reinforcement on a progressive fixed-ratio schedule. Two variations of this schedule were also examined and compared. Functions relating the highest level of stable fixed-ratio responding to a wide range of stimulus currents were, unlike those derived from continuous reinforcement rates, monotonic at all stimulation sites tested. One of the procedures described, in particular, is quite sensitive to intracranial reinforcement parameters, provides a reliable technique for within-session assays of these parameters, and successfully avoids many problems commonly encountered with administration of central stimulation on a continuous reinforcement schedule.

Although still widely employed, continuous reinforcement (CRF) measures of intracranial reinforcement have been criticized on several methodological grounds (Valenstein, 1964). Perhaps the most serious objection is based upon the susceptibility of CRF response rates to reduction by the various motoric side-effects that often accompany intracranial stimulation (ICS). As several studies have illustrated (Hawkins and Pliskoff, 1964; Hodos and Valenstein, 1962; Beer, Hodos, and Matthews, 1964), investigators failing to take account of such influences upon the rate of self-stimulation can be led to erroneous conclusions regarding either the reinforcing properties of high-current stimulation or the relative reinforcement properties of stimulation at various brain sites.

In an effort to minimize the influence of these stimulation side-effects, some investigators have employed either rate-independent measures (Hodos and Valenstein, 1962; Valenstein and Meyers, 1964) or partial reinforcement schedules that serve to space stimulations widely in time (Keesey, 1962; Pliskoff, Wright, and Hawkins, 1965). Another technique that would appear to accomplish this end is the progressive-ratio procedure described by Hodos (1961) and Hodos and Kalman (1963). As originally employed with food reinforcement, this procedure required that an animal emit a progressively increasing number of responses in order to obtain each successive reinforcement. The "terminal ratio", defined as the highest number of responses emitted before a pause of at least 15 min, was found to be sensitive both to variation in the concentration of a liquid reinforcer and the length of deprivation. In a subsequent study, designed to examine the reinforcing properties of long durations of central reinforcement, its sensitivity to ICS parameters was also indicated (Hodos, 1965).

Our early experiences with the progressive-ratio schedule suggested several modifications in Hodos' original procedure, which might better suit it for use with ICS reinforcement. First, the procedure of administering only one reinforcement condition per session, while necessary with food to avoid the confounding effects of progressive satiation, is somewhat inefficient with ICS where satiation effects are not ordinarily encountered. Consequently, a procedure was developed that permitted the successive examination of a series of ICS current conditions, thereby making it possible to obtain a complete current function from an animal within a single test session. Secondly, rather than taking the terminal ratio to be the highest ratio before complete cessation of responding, it is defined in the present study as the highest level of stable fixed-ratio (FR) responding that a given reinforcement condition will maintain. (Elder, Montgomery, and

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Rye (1965) have administered ICS reinforcement on a progressive fixed-ratio schedule that appears similar to one described here. Lack of procedural detail in their report, however, limits comparisons with the present work.) These two modifications resulted in the progressive fixed-ratio procedures utilized in the present study to examine the ICS reinforcement parameter of stimulus current at three different brain sites.

METHOD

Subjects

Six male Sprague-Dawley rats, weighing 220 to 250 g at the beginning of the study, served. Under pentobarbital anesthesia, a twisted bipolar electrode constructed of 0.01-in. diameter stainless steel wire was implanted chronically in the brain of each. The target site was the posterior hypothalamus in two animals, the anterolateral hypothalamus in two others, and the anterior septal region in the final pair. (See Keesey (1964) for details of the operative procedure.) The animals were individually caged and provided with free access to both food and water throughout the experiment.

Apparatus

The experimental chamber was a 12 by 12 by 12-in. box housed within a sound-attenuating chamber. On one wall of the experimental box and 3 in. above the grid floor was mounted a Scientific Prototype rat lever. A 1-in. diameter 6-w pilot lamp assembly was centered 3 in. above the bar. Fixed to the ceiling of the outer box was a swivel device that permitted the animal to move freely about the experimental box without tangling or twisting the electrode leads. Conventional operant conditioning apparatus was employed to program the various experimental contingencies and record response data.

The brain stimulus was supplied by a Grass S-4 rectangular pulse generator. The monophasic output of this instrument was converted to a symmetrical biphasic waveform by means of a stimulus pulse reversal unit (Alberts, 1958). Constant-current stimulating conditions were obtained by maintaining a constant voltage displayed on an oscilloscope across a fixed resistor in series with the electrodes. The stimulus parameters of pulse frequency, pulse duration, and train duration were fixed throughout the experiments at 100 pulses per sec, 0.2 msec, and 0.5 sec, respectively. The pulse current was varied throughout the study as indicated in the Procedure section.

Preliminary Observations

In the original progressive-ratio work with food reinforcement (Hodos, 1961) the terminal ratio was defined as the highest ratio run before a pause in responding of 15-min duration. If a series of reinforcement conditions is to be examined within a single session, it is both inefficient and undesirable that responding be completely extinguished before the next reinforcement condition is introduced. To avoid this circumstance, and to increase the reliability of the measure, the terminal ratio was defined in the present study as the highest level of stable FR responding that a given reinforcement condition would maintain. To this end, the data of several pilot animals run under progressively increasing FR conditions provided information concerning the response pattern just before responding ceased. An analysis of these data indicated that as the ratio was increased, the principal change in the response pattern occurred in the pause between each reinforcement and the initiation of the next ratio run. (In a similar vein, Sidman and Stebbins (1954) reported that progressive satiation causes the pause length under fixed-ratio reinforcement conditions to increase, but never fails to alter response rate within a ratio run.) The effects of increasing FR upon this postreinforcement pause are illustrated by the data presented in Table 1 for one pilot animal. Listed under each of the current conditions is the mean pause length obtained during 25 runs at each ratio level. Note that for each pulse current a small increase in mean pause time occurs with each increase in FR until some critical ratio is reached, whereupon a further increase in FR produces a rather large pause-time increment. The ratio at which this sharp pause increase occurs may be seen also to be a function of the pulse current. Closer study of these data indicated that, when the mean pause times jumped to values over 15 sec, the cumulative records indicated a corresponding deterioration in the stability of FR behavior. Fixed-ratio increases beyond this level, ordinarily of only one or two steps, would then consistently result in a very irregular FR response pattern, or, in

Table 1

Pilot data from a single subject (C-356) illustrating the increases in pause length that characteristically occur as the FR level is increased at a given pulse current. Scores represent mean pause times (in sec) for 25 trials.

		Fixed Ratio						
		2:1	4:1	6:1	8:1	10:1	12:1	14:1
	0.20	13.40	20.40	49.44				
	0.30	5.08	8.80	30.56				
Pulse	0.50	3.64	4.28	4.84	9.72	19.92	40.80	
Current	0.70	4.36	4.44	4.76	6.56	6.72	16.92	43.60
	1.00	5.36	6.00	5.24	6.08	6.84	8.00	30.24

some cases, complete cessation of responding. Provided with this information, it was decided to define the terminal ratio for any ICS reinforcement condition as the highest ratio level maintained with an average inter-ratio pause time of less than 15 sec. Conceivably, this 15-sec criterion would not be adequate under all reinforcement conditions, particularly those that generated very high levels of ratio performance. However, at the ratio levels encountered in the present work with ICS reinforcement, it is believed this pause criterion reliably anticipates the breakup of regular FR responding.

Pretraining: Day 1 to 12

In the first pretraining session each animal was trained to bar-press for electrical stimulation. Care was taken at this stage to select a current level for each rat that maintained slow but regular responding with the stimulation delivered on a continuous reinforcement schedule. When these conditions were established, an FR 2 schedule was introduced at the same current level and the animal permitted to run until a regular pattern of responding was evident in the cumulative record. If the FR 2 responding could not be maintained, the current was increased until it did stabilize. The ratio requirement was then raised to FR 3 and the same procedure repeated. In this manner, the FR conditions continued to be increased until a level was reached at which responding could not be maintained despite further current increases. The session was terminated at this point. Starting again with an FR 1 schedule and the low-current condition in the next session, this procedure was repeated daily throughout a 12-day pretraining period.

During the pretraining period, the range of effective current values was noted for each animal. Six to eight levels were selected from this range; the lowest current was one capable of maintaining only CRF or low-ratio responding and the highest came from among those currents that maintained responding at its upper FR limits. The remaining four to six current conditions were chosen so as to sample representative points between these two extremes.

Experimental Procedure 1

In this procedure the entire current series was examined within a daily test session. Starting at FR 1, the lowest current level was presented for a 2-min warmup period. Then, 25 reinforced responses were permitted provided they could be completed before the elapsed postreinforcement pause time exceeded 375 sec (*i.e.*, an average pause interval of 15 sec). If these conditions were met, the ratio requirement was raised to FR 3. Again a 2-min warmup period was employed, first, for the purpose of acquainting the animal with the new conditions, and, secondly, to minimize the influence on responding of the preceding conditions. Following this, responding was permitted under these conditions until either 25 ratio runs were reinforced or the accumulated time between each reinforcement and the second response of the next ratio run exceeded 375 sec. If this time criterion was met, the ratio level was raised to FR 5 and the same testing procedure repeated. Were the pause.time to exceed the criterion value, however, the next current value in the series was introduced and the ratio condition, including the warmup period, rerun. Ordinarily, responding would be brought within the pause criterion by the current increase. In such a circumstance, the ratio conditions would be increased, as before, in steps of two responses until this time limit was again exceeded. In a few cases, the introduction of the next higher current level failed to restore responding to within the 375 sec of pause time. In such cases, the ratio requirement was then lowered in steps of two until this criterion was met. Following the above procedure, a terminal ratio value was obtained for each of the current levels in a daily test session. The entire procedure was repeated daily for a total of five sessions.

Experimental Procedure 2

In the second stage of the experiment, only one current condition was studied within a daily session. Five or six current levels were selected for each animal and administered in daily sessions, first in an ascending and then a descending order. Within each daily test session the subject self-stimulated at a single current level, starting with FR 1 and moving through a progressive FR series in steps of two responses. As before, a 2-min warmup period preceded the accumulation of the pause time at each ratio level. Also as before, sessions were terminated whenever the average pause between ratio runs exceeded the criterion value of 15 sec (*i.e.*, a total of 375 sec for 25 ratio runs). This procedure was then repeated on the following day with the next current value in the series. After one ascending and one descending run through the current levels, this stage of the experimentation was terminated.

RESULTS

Procedure 1

Table 2 shows the results from a single test session of Rat C-382 selected to illustrate the procedures employed for presenting experimental conditions and recording response data. The numbers in each column represent the *Mean* pause time in seconds (time between the last reinforced response and, except for the FR 1 condition, the second response of the next ratio run) over the 25 ratios

Table 2

Data from a single test session (Rat C-382) selected to illustrate the effect of both ratio level and stimulus current on the mean pause time between ratio runs. A detailed explanation is provided in the text.

		Current (Ma)							
		0.20	*0.30*	*0.40*	*0.60*	*1.00*	*1.50*	*2.00*	*2.50*
	1-1	118.1							
		↓							
	3-1	(20) →	194.3						
			↓						
	5-1		(16) →	134.0					
				↓					
	7-1			174.6					
				↓					
	9-1			(18) →	109.9				
					↓				
	11-1				131.2				
					↓				
	13-1				172.8				
Ratio					↓				
	15-1				170.0				
					↓				
	17-1				265.3				
					↓				
	19-1				(24) →	164.2			
						↓			
	21-1					188.9			
						↓			
	23-1					223.1			
						↓			
	25-1					334.1	372.7 →	366.7	353.3
						↓	↑	↓	↑
	27-1					(24) →	(24)	(23) →	(24)

meeting the criterion for stable FR responding. Numbers in parentheses indicate the ratio runs completed before the point at which the accumulated pause time exceeded the criterion total (375 sec). By following down any one current column in this table, one can see the progressive increase in the pause time as the ratio requirement increases. Likewise, whenever the pause time exceeds the criterion value, it is possible to observe the effect of a current increase upon responding at a given ratio by looking to the next column on the right. An advantage of compiling the data in this manner is that the form of the complete current function is readily apparent. In this particular case, the terminal ratio increased with current to an asymptotic level that is reached by 1.0 ma. Note the flow of the arrows during the final determinations between 1.00 and 2.50 ma.

In analyzing the data, the terminal ratio for each current condition was defined as the highest level of ratio performance maintained within the time criterion. The current functions seen in Fig. 1 in the row headed Procedure 1 show both the mean and range of the terminal ratios obtained over five daily test sessions for each animal. Of special interest in these data is the observation that all functions, regardless of stimulation site, appear to be generally monotonic in form. Comparison of the different reinforcement sites also offers several points of interest. Septal reinforcement, for example, is associated with a graded current function that compares quite favorably with those obtained with hypothalamic stimulation. Finally, mention should be made of the reliability of the terminal ratio at each current level. In several cases (C-387, C-383), as seen in Fig. 1, the terminal ratio determined for a given current level did not display any variation across the five test days. More common was the case in which the five terminal ratios varied from their mean by one or two ratio steps. Only in rare cases did the variability exceed these limits.

Procedure 2

In this procedure only one current condition was examined per session, starting each day at an FR 1 level and proceeding in FR increments of two responses in the manner previously described until the average interratio pause time exceeded 15 sec. Figure 2 presents the cumulative records of animal C-384 (receiving posterior hypothalamic stimulation) selected to illustrate the characteristics of FR responding under these conditions. These data demonstrate more clearly than those from Procedure 1 the critical role of the current in determining the length of the pause after a ratio run. It is also possible in some cases to see the pattern of increasing variability in the postreinforcement pause length as the

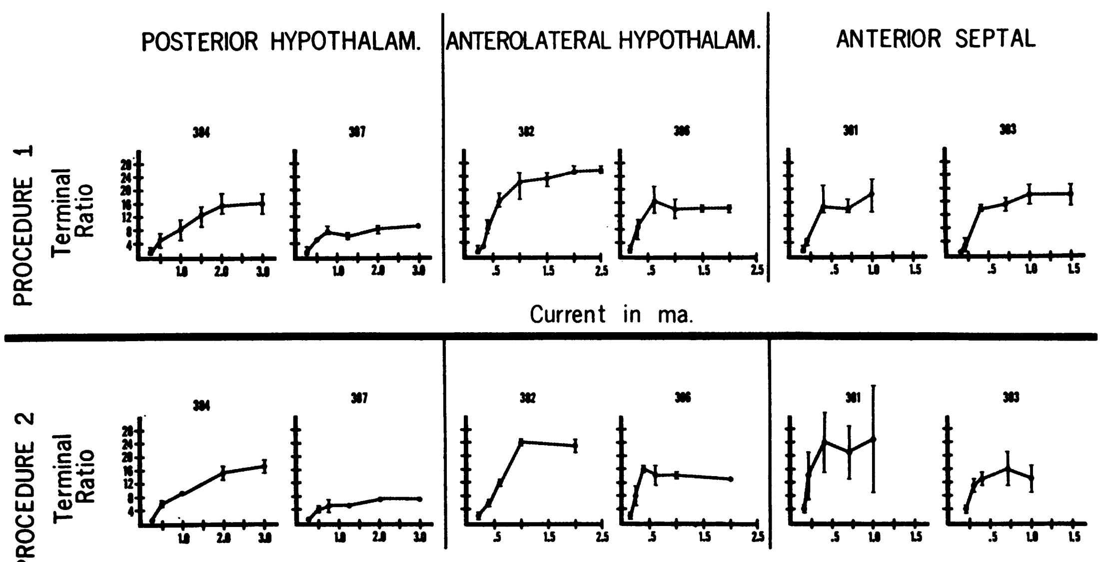

Fig. 1. Influence of ICS current level on the terminal ratio attained under two progressive-ratio test procedures.

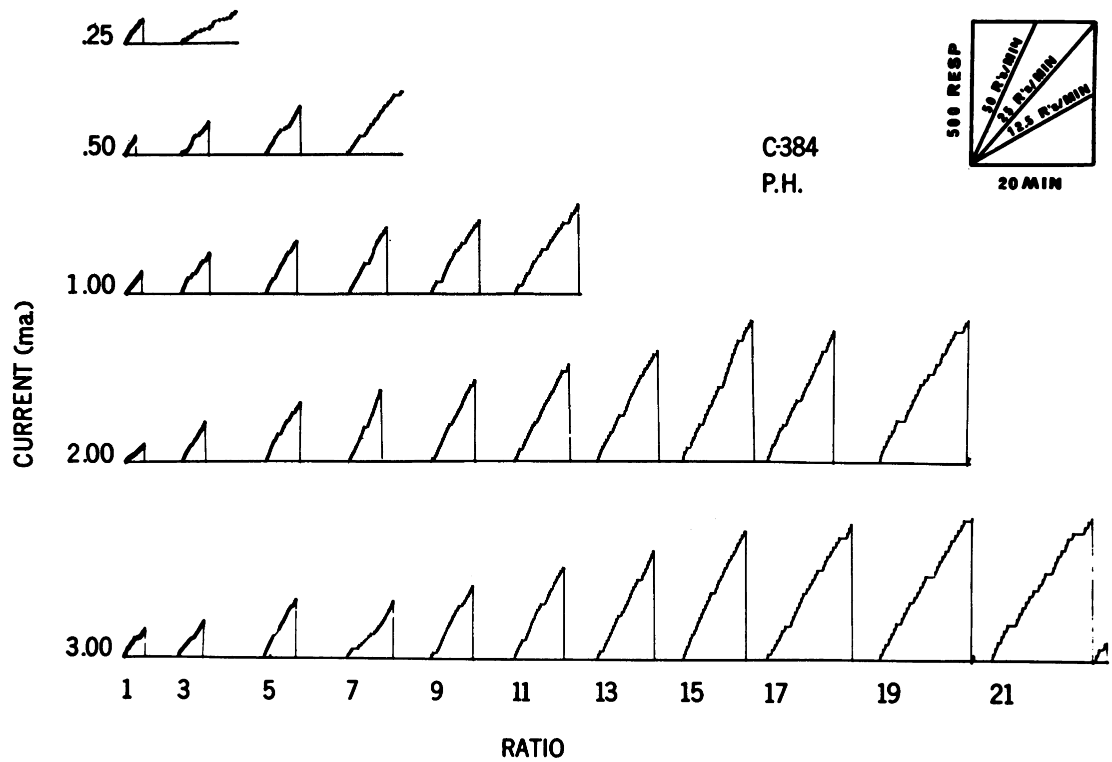

Fig. 2. Sample cumulative records illustrating the influence of ICS current on the pause time between ratio runs.

terminal ratio is approached at each current level.

The results of the ascending and descending test series were combined to give a mean terminal ratio value for each current level studied. These data are represented graphically in the bottom row of Fig. 1, labeled Procedure 2. It is apparent that the data from the posterior and anterolateral hypothalamic animals closely resemble those obtained from Procedure 1. The septal-implanted rats show certain differences with the two procedures, however. Both of these animals showed tonic-clonic seizures during those Procedure 2 periods when high-current ICS was administered at relatively low fixed ratios. Seizures were elicited on three of the four high-intensity test sessions in the case of C-383, and in two of these sessions with C-381. Terminal ratios obtained from sessions in which convulsions were observed were markedly smaller. As a consequence, the function for C-383 is nonmonotonic under this procedure. In the second septal-implant (C-381), seizures occurred only during the ascending series of current presentations. In addition, this animal showed a marked improvement in bar-pressing technique during this procedure (learning to use both paws to "flutter" the bar) so that the combination of convulsive effects and training effects appears to result in a current-response function that does not differ significantly from the corresponding function from Procedure 1 in shape, but shows considerably more variability.

Histological Results

Subjects were sacrificed at the end of Procedure 2, exsanguinated, and perfused with 10% formalin. Forty-micron frozen sections were taken and the tissue stained with cresyl violet. The locus of each electrode tip was determined and represented on one of the König and Klippel (1963) plates seen in Fig. 3.

The electrode termination in the two anterolateral subjects (C-382, C-386) is seen to have been at the border of the medial forebrain bundle and the anterior hypothalamic nucleus. Both septal electrodes (C-381, C-383) were located in the anterior region of the lateral septal nucleus at the boundary of the anterior hippocampus. The electrode tips in the two

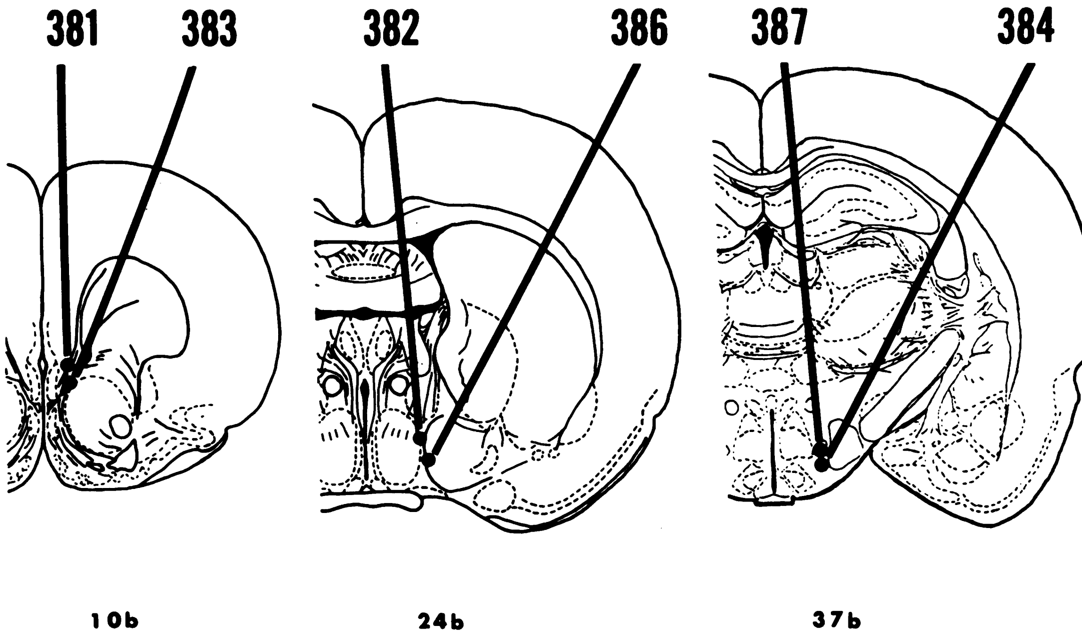

Fig. 3. Site of electrode termination in the six experimental subjects. The number below each section identifies the plate of the König and Klippel (1963) rat stereotaxic atlas from which the present drawings were made.

posterior hypothalamic animals (C-384, C-387) terminated at the border of the medial forebrain bundle and the posterior parts of the ventromedial nucleus.

DISCUSSION

The present results for the hypothalamic-implanted animals indicate no major differences between the two progressive-ratio procedures. Whether the entire current series was administered within a single session (Procedure 1), or a single current was tested each day (Procedure 2), the resulting current functions have essentially a montonic form. Such a result suggests that neither the longer session length nor the successive current presentations of Procedure 1 have an important bearing upon the terminal level of ratio performance. This is significant in evaluating the present procedures since reports exist of both satiation (Olds, 1958) and behavioral contrast (Williams, 1965) effects with ICS reinforcement. If it can be assumed that the influence of such factors is minimal in Procedure 1, there are several reasons for preferring it to Procedure 2 for subsequent work. First there are the practical advantages of being able to test a number of stimulus conditions within a single test session. Secondly, the combination of frequent stimulations and high current levels, a situation prevailing under the CRF and low FR conditions at the start of a high-current Procedure 2 test session, can lead to undesirable consequences. The most serious of these are behavioral seizures, whose aftereffects, the data from the septal animals suggest, may persist throughout an entire test session. As previously noted, seizures were observed in C-383 on three of the four Procedure 2 test days involving high-current levels. In these three sessions the resulting terminal ratio was at least several steps below that regularly found in Procedure 1, or under that found in Procedure 2 sessions not marked by seizures. The result was a non-monotonic current function with this procedure. In contrast, under Procedure 1, where the animal rarely encountered high currents until ratio conditions were such as to guarantee that stimulations would be well spaced in time, only one instance of seizure was noted for this animal. The resulting current function was monotonic in form in spite of the fact that the current series included a value 50% higher than any used in the second procedure.

It would appear from the present data that these progressive ratio procedures, particularly the Procedure I modification, are capable of satisfying the major objections to CRF rate measures of ICS reinforcement. There is little evidence to indicate that the post-stimulation effects, which often limit self-stimulation rates, impose any similar restrictions on the terminal ratio measure. In the present work, an animal was permitted to continue responding for stimulation until the average pause length between ratio runs exceeded 15 sec. This means that the animal could meet the criterion for stable FR responding by self-administering no more than four stimulations per minute, a rate below the limit that motoric side-effects of high-intensity stimulation might normally impose. Of further significance is the correspondence of the present data with those obtained by others employing intermittent reinforcement schedules. The finding of a monotonic current function appears to be characteristic of most such ICS studies (Keesey, 1962; Hawkins and Pliskoff, 1964; Beer *et al.*, 1964).

Comparisons among the data from the different brain sites add further to the arguments against the use of reinforcement schedules that lead to high-stimulation densities at high-current levels. The data from the septal animals are of particular interest in this regard since it is generally reported that the CRF rate for septal stimulation is uniformly low across a wide range of current conditions, resulting in a "square" current function (Olds, Travis, and Schwing, 1960). The current functions from septal animals using the progressive-ratio techniques are evidently not only graded in shape, but reveal levels of ratio performance that compare quite favorably with those found with hypothalamic placements. This finding is consistent with Hodos and Valenstein's observation (1962) that animals in choice situations often preferred septal to hypothalamic stimulation, even though the latter site yielded the higher rates of self-stimulation.

As with any of the techniques described for assessing ICS reinforcement effects, there are several features of our modification of the progressive-ratio procedures that may limit its general applicability. One is the rather prolonged period of pretraining necessary to stabilize the ratio performance before testing can begin. Even after a long pretraining period, an animal may suddenly develop a more efficient means of responding. After a number of sessions, during which quite regular data was received from one animal, it learned to respond at quite high rates by placing one paw over and the other under the lever. The consequence was an immediate displacement upward in the current function of several ratio steps. In spite of such drawbacks, however, the sensitivity of the measure, as well as the feasibility of examining high currents with only minimal interference from stimulation side-effects, may warrant its use in many circumstances.

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