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TITRATION SCHEDULE WITH RATS IN A RESTRAINING DEVICE¹

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GHISLAINE DALLEMAGNE AND MARC RICHELLE

UNIVERSITY OF LIÈGE, BELGIUM

In a restraining device, providing a constant localization and easy placement of stimulating electrodes, rats were conditioned to push a front panel in order to reduce the intensity of a shock which otherwise increased regularly every 10 sec. Rapid conditioning was obtained from all subjects. They showed a very stable behavior, with negligible inter- or intra-individual variability throughout very long experimental sessions. The operant nature of the response was demonstrated further in extinction, where no shock was delivered, and in situations where the animal could not change the predetermined intensity of the shock. The interpretation of the obtained threshold was discussed with reference to previous studies on titration schedule. It was suggested that, with the parameters used, the shock intensity at which it was maintained by the rats' behavior, had the value of a warning signal, and that, in this particular case, the titration schedule generated mainly discriminative avoidance behavior rather than escape behavior.

When delivering shock through a grid floor, the experimenter is unable to control with precision the current actually passing through the animal. The position of the subject, the surface of the body exposed to the shock, the kind of tissues in contact with the grid floor, produce unpredictable changes in total resistance. Appropriate shock generators scrambling devices can minimize these disadvantages, but they cannot eliminate the variations due to the differential sensitivity to pain of the various parts of the body, or to the complicated physiological interactions occurring when several parts of the body are stimulated simultaneously. A better control of the stimulus (and not only of the stimulus source) is desirable when the use of shock stimulation is intended to provide a measure of algic sensitivity in normal or drugged animals. The solution is, of course, to deliver the shock through fixed electrodes. For some species, this can be done easily on a subject moving freely in the experimental cage (Azrin, 1959a). Animals such as rats, cats, or monkeys, however, if not restrained will scratch out or bite away their electrodes, or engage in various behaviors that compete with the required response (Weiss and Laties, in press). A number

Azrin, Hopwood, and Powell (1967) reduced the experimental space so as to allow bar pressing while preventing the locomotor activity of the rat. The animal was confined in a triangular cage, its tail protruding from one corner. The electrodes were fastened on the tail in a way that did not eliminate modifications in blood circulation in the tail vessels. Another restraining device for rats was designed by Hall, Clayton, and Mark (1966). It is similar to the one used in the present experiment and makes it possible to fix electrodes easily on the tail of the animal. In addition, it gives access to the head of the subject, and can be used in studies with implanted electrodes. When not much refinement is required, however, a simpler device can be easily built and manipulated. Restraining chairs for monkeys are common and hardly worth mentioning (Weiss and Laties, 1962). Other authors attempted to let the animal move freely in the experimental space and to fix the electrodes very tightly. Kelly and Glusman (1964) implanted subcutaneous electrodes in the flank of cats; subcutaneous wires were run from the stimulation sites to a connector mounted on the skull. Weiss (1967) used a special fastening device to fix electrodes on the rat's tail.

of solutions have been proposed to avoid these difficulties, either by restraining the animal or by fastening the electrodes firmly.

¹Reprints may be obtained from Ghislaine Dallemagne, Université de Liège, Psychologie Expérimentale, 32 Blvd. de la Constitution, Liège, Belgium.

The first aim of the present study was to test a simple restraining device for rats providing for fairly constant localization and easy placement of the electrodes. The device proved satisfactory and was used in pharmacological research on psychotropic and antalgic drugs (Dallemagne, 1970).

A second aim was to develop techniques for simple and rapid conditioning of an operant under aversive control by taking advantage of a respondent motor reaction initially elicited by the aversive stimulus and converting it into an operant. Behavior under aversive control, such as continuous avoidance response or titration schedule, was often difficult to obtain from a reasonable proportion of animals within reasonable time limits. Difficulties in conditioning were reported by several authors (Azrin, 1959b; Weiss and Laties, in press). Frequently, some subjects did not learn and were left aside. For the psychopharmacologist, for example, this selection of subjects, possibly related with emotional pecularities that should not be ignored in analyzing their individual reactivity to drugs, is objectionable. On the other hand, it is time consuming, and there is no argument to reject a shorter procedure.

These considerations led us to develop the technique presented here, derived from the procedure known as titration schedule with a shock stimulus first described by Weiss and Laties (1958).

METHOD

Subjects

Thirteen albino rats of both sexes were used. Their weight at the beginning of the experiment was about 180 g. They lived in individual home cages and had free access to water and food.

Apparatus

Experimental cage. Figure 1 shows the restraining device used as the experimental cage. The rat was put into a Plexiglas cylinder, 8.4 in. (21 cm) in length and 2.75 in. (7 cm) internal diameter. These dimensions made it impossible for the animal to turn around. Holes were drilled through the top part of the cylinder to provide ventilation. Long sessions were possible, provided that excessive sudation was prevented by adequate ventilation and temperature control (20° C max). Other holes

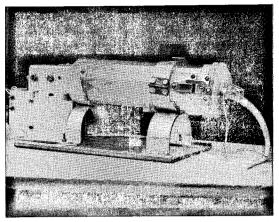


Fig. 1. Picture of the restraining device for rats, used for precise control of the shock stimulus in titration schedule. On the left, the response-level mechanism, enclosed; on the right, the electrode holder fixed on the tail.

in the bottom part, at the back, allowed the elimination of urine. In the front wall of the cylinder, a circular aperture 1.2 in. (3 cm) in diameter was filled with the response lever, an iron disc mounted on a horizontal rod (see Fig. 2, A, c). When the rat pushed the disc (Fig. 2, A, a) with its nose, the end of the rod operated a microswitch (Fig. 2, A, d). An adjustable spring brought the lever back to the rest position (Fig. 2, A, b). At the other end of the cylinder was a Plexiglas lid, in which a radial section had been cut so that the tail of the animal could be inserted easily (see Fig. 2, B). When the rat had been placed in the cylinder, the lid was fastened with suitcase clips. The electrical stimulus was delivered through silver thread electrodes (length: 2 cm. diameter: 1.5 mm). These were fixed on the proximal part of the tail by an electrode holder shown in Fig. 2, C and D. It was built in a Plexiglas block (the dimensions are indicated on the figure). A hole was drilled centrally, with a diameter slightly larger than the diameter of the rat's tail in its proximal part. The block was cut in two halves, attached together by a small hinge. The central hole was given a conical rather than cylindrical shape, in order to fit perfectly the rat's tail, and was walled by a sheet of soft rubber to avoid hard contact with the skin. A simple locking device was built with a screw-nut and a threaded rod. The two silver electrodes were curved in U shape and set on each half of the electrode holder, as shown in Fig. 2, D, a.

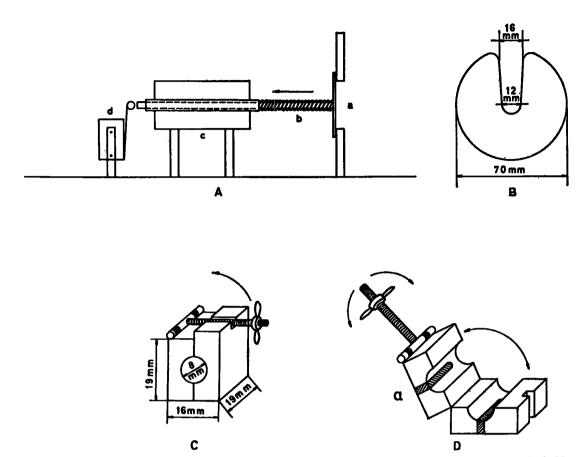


Fig. 2. Drawing of the response-lever mechanism (A); the back door of the chamber (B); the electrode holder closed (C) and open, with the electrodes shown (D). Small letters are referred to in the text.

They could be removed at will, as required for repair or cleaning. In order to minimize polarization and time damage, the electrodes were periodically chloridized by electrolysis through an NaCl solution.²

Electric stimulation and control units. A shock generator delivered a dc current³ ranging from 0 to 2 mA. The whole scale was divided in 50 equal steps of 0.04 mA, corresponding to the 50 points of a two-way stepper,

In the absence of responses, an electronic timer operated the stepper, causing an increase (0.04 mA) of the shock level every 10 sec. Every response operated the stepper in the other direction, reducing the stimulus intensity by one step. The shock was interrupted for 0.5 sec every second by an eccentric mounted on a synchronous motor. This proved satisfactory, in preliminary experiments, to prevent damage to the skin of the animal at high intensities and habituation at low intensities.

³dc current was chosen for purely incidental reasons: the Trub-Tauber recorder available for this experiment was designed for dc.

each of which closed the shock circuit through a different calibrated resistance. From the high-voltage dc source (800 v), the current passed through one of the 50 possible resistances, ranging from 21.980 K ohms to 420 K ohms, to the stimulating electrodes. As the impedance between the electrodes was always smaller (varying from 12 to 20 K ohms) than the calibrated resistance in the circuit, the current passing through the electrodes at a given step was fairly constant.

^aIn further experiments, we used a much simpler type of electrode placement. Taking advantage of the impossibility for the rat to get rid of anything attached to its tail in this situation, we turned two lead strips (50 mm in length, 3 to 4 mm in width) around the tail of the subject, 10 mm apart. A thin layer of foam rubber previously dipped in a conducting solution (two-thirds saline solution, one-third glycerine) was put between each electrode and the skin.

Every push-response was converted into a short pulse so that prolonged movements forward were no more effective in reducing the shock than brief, discrete pushes.

All experimental operations were automatically controlled by relay circuits. The schedule allowed alternation of shock periods (S^D) and timeout periods (S^A). Shock periods were associated with an auditory stimulus (the noise of the synchronous motor used to interrupt the shock every second) and with a visual stimulus (a green light above the cylinder).

Recording. The shock intensity actually passing through the rat's tail was recorded continuously on a Trub-Tauber paper recorder during the shock periods (S^D). The maximal excursion of the recording pen corresponded to the higher end of the shock-intensity scale, i.e., 2 mA. The electrodes were connected in series with the amperemetric recorder. Throughout the experiment, all responses and the alternation of shock periods and timeout were recorded on the two channels of an event recorder. In addition, two digital counters were used to sum all responses and responses emitted in timeout periods respectively.

Procedure

The experimental program was designed to test:

Acquisition of the response. Eight rats were trained on the titration schedule. Experimental sessions took place three or four times per week, and lasted 1 to 2 hr. Shock periods and timeout periods, 3 to 4 min each, alternated throughout the sessions. Training extended over five to six sessions.

Stabilization. The same subjects were run in the same situation for 10 sessions, lasting 75 min each. These sessions were used to draw the individual results.

Extinction. The same subjects were run for one to two sessions on an extinction procedure, after the stabilization. The situation was identical to the one used in the preceding phases, except that the shock was disconnected after 45 to 60 min, always at the start of a "shock period". The exteroceptive discriminative stimuli associated with the shock period were still present. Extinction sessions lasted a minimum of 2 hr.

Controls. In order to test whether other aspects of the experimental situation, besides the shock, were responsible for the responses recorded, some of which might be initially or continue to be of a respondent nature, the following controls were used: (1) a naïve rat was put into the experimental cylinder for 5 hr. without electrodes on the tail. We wanted to test the effect of the restraining device on the general motor activity, which could result in disc-pushing responses. All other conditions were as above. (2) The same rat was put into the experimental cage for 2.75 hr with electrodes but no shock was delivered. (3) Three naïve rats were placed in the apparatus for two or three sessions of 60 min each. They were given, according to the same schedule used with conditioned rats, a non-contingent shock of 0.2 mA. Responses had no effect on the shock level. (4) One naïve rat was placed in a similar situation except that the shock level was 0.3 mA and the session lasted 6.5 hr. (5) The same animal was given a shock of 2 mA in one session which extended over a period of 5 hr.

Analysis of Results

The shock intensity tolerated by the animal was expressed as the median value of a distribution of discrete measures obtained in the following way. The continuous records were analyzed with a transparent grid—the ordinate intensity scale, 0 to 2 mA, subdivided in 20 steps, 0.1 mA each, the time abscissa divided in 20-sec intervals by thin lines—allowed us to measure the level of intensity throughout the session. The median and semi-interquartile ranges were taken from these points. The first 500 sec of the session were omitted. In a session starting with the shock at the minimal intensity, a rat that would not respond at all would receive a 2-mA shock after 500 sec, the median value for this period being half-way on the scale. It would be misleading to include these data in expressing the threshold of tolerance to the shock in a given session. This point was especially important when the technique was applied to drug research, where responses are eventually completely suppressed, and the maximal level of intensity is accepted by the animal.

In addition to this measure, the number of responses per minute during the shock periods and during the timeout periods were calculated for each animal. Means and standard deviations were calculated from stable sessions.

RESULTS

Conditioning

From the beginning of the first session, the rats produced responses. Evidently, these were unconditioned reactions to the electric shock: they were respondent rather than operant in nature. The electric stimulus induced forward lunges. As we did not know the differential sensibility of the rats to shock intensity, and consequently ignored whether or not, at the various points of the scale, a decrement of 0.04 mA was a noticeable difference, we reduced the shock intensity by three or four steps at once when the rat pushed the disc. This was intended to facilitate the relation between the response and its consequences and thus help transform the initially respondent reaction into an operant.

Table 1
The First Six Sessions, Rat 365

Dates	R/min in S ^D	R/min in S ^Δ	Median Thresh- old in mA	Semi-Inter- Quartiles Ranges in mA
14/0/66	1.7	0	0.705	0.437
16/9/66	6.2	1	0.114	0.051
21/9/66	5.7	1	0.135	0.050
22/9/66	15.2	1.8	0.072	0.042
26/9/66	5.4	0.8	0.190	0.072
28/9/66	9.6	0.4	0.065	0.032

Table 1 gives the results, session by session, for the first six sessions for Rat 365. The respondent reactions obtained in the beginning were given in irregular bursts. They were due to the electric stimulation, and not to the restraining device itself, since they did not appear during the timeout: generally, there were no responses in S^Δ during the first session. These respondent reactions, moreover, being irregularly distributed in time, did not suffice to maintain the shock at the level at which it would be maintained later in conditioning. They were concomitant to emotional reactions: squealing, defecating, tremor, etc.

As the experiment progressed, responding became more regular and more efficient, the shock intensity rarely increasing by more than one or two steps before a response occurred. Responses were produced in bursts of four to five, or one at a time, and were systematically performed with the nose or with the forepaws.

In some cases, bursts of responses during S^Δ appeared not in the first session but in the second or third, generally in the beginning of the session. The evolution of behavior throughout the first six sessions in terms of responses per minute during S^D and S^Δ is shown, for each animal, in Table 2. For all subjects, the number of responses during S^D increased. The number of responses during S^Δ remained generally low, in most cases close to zero per minute. Rat 368 was an exception, showing frequent bursts of responses during

Table 2

Rat #		First Session	Second Session	Third Session	Fourth Session	Fifth Session	Sixth Session
361	R in SD	3.3 0	4.8 0	5.9 1.1	7.2 0.1	7.6 0.3	10.9 0.8
362	R in SD SA	4.4 0	4.9 0.1	5.9 1.9	7.2 1.0	7.7 4.7	12.1 2.5
363	R in SD SA	4.1 2.6	6.6 1.3	8.1 0. 3	8.9 1.5	13.5 1.5	14.1 3
365	R in $_{S^{\Delta}}^{S^{D}}$	1.7 0	6.2 1	5.7 1	15.2 1.8	5.4 0.8	9.6 0.4
366	R in $\frac{S^D}{S^\Delta}$	3.1 0.3	5.1 0.4	6.5 0	5.6 1.2	7.3 1.8	6.3 0.4
368	R in SD SA	5.4 0.5	7.3 1.5	9.5 1	17.4 6.8	14 9.7	10.6 8.3
369	R in SD SA	3.4 0	4.1 0	4.6 0	7.1 0.1	7.3 1.0	11.4 1.9
370	R in SD	5.2 1.5	4.0 0.1	3. 4 0	7.3 0	4.8 0	8.2 0.1

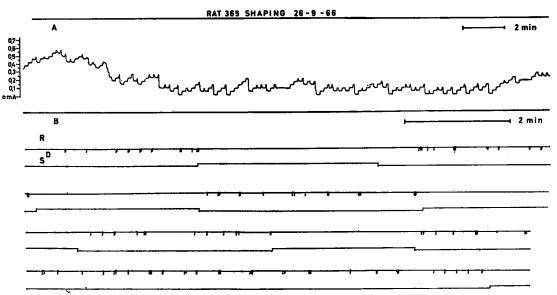


Fig. 3. Records from Rat 365 during shaping session. A: continuous record of shock intensity during S^D periods. B: two-channel recording of responses (R, first channel) and shock and no-shock periods (S^D, second channel); the first channel pen is deflected downward when a response is given; the second channel pen is deflected during shock periods (some of them were accidentally longer than 3 to 4 min). Successive parts of R-S^D paired records are ordered from top to bottom. The records are from a fragment of the session starting 30 min after the rat was put in the restraining device.

S^a. These bursts are easily differentiated in the records from the regularly spaced responses during S^b. Figure 3 gives a fragment of the

fifth session for one rat. It is clear from this record that responses were related to the shock increments.

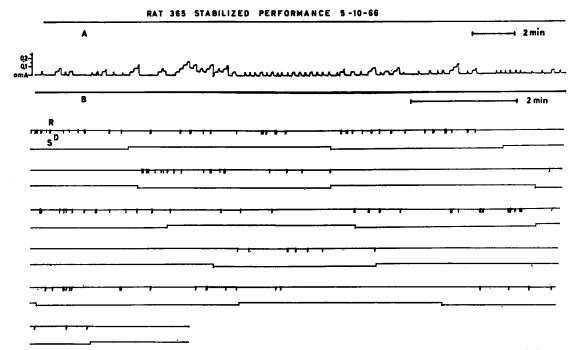


Fig. 4. Stabilized performance of Rat 365. Fragment of records starting 18 min after the beginning of the session. A: shock intensity during S^D periods. B: responses (R) and shock-no shock periods (S^D) (see Fig. 3 for details).

Stabilization

After a maximum of five or six sessions, the shock level was efficiently controlled by all eight animals, which maintained it around its lowest value; the mean for the group was 0.069 mA, with an average number of responses per minute of 11.42 during shock periods and of 2.82 during S4. Figure 4 shows a fragment of the record for Rat 365 and illustrates how regularly the shock was maintained around 0.1 mA. Responses during Sa were rare, except in the first So period. Table 3 gives for each animal the mean number of responses per minute during SD and SA, the intensity level averaged from the 10 median values obtained in the 10 stabilization sessions, the corresponding semi-interquartile ranges. The very small inter- and intra-individual variability in shock level will be noted. The clearcut contrast in responding between S^D and S[∆] proved that responding was for the larger part due to the shock. That it was an operant, at this stage, rather than a respondent, was evidenced by the fact that the shock was maintained at a very low level that could not by itself produce such an unconditioned activity. This point was confirmed in the control (see below).

Table 3

Rat #	Mean of R/min in S ^D	Mean of R/min inS ^Δ	Mean of Threshold in mA	Mean of Semi-Inter- Quartile Ranges in mA
361	8.77	0.71	0.071	0.040
362	14.02	4.36	0.058	0.029
363	11.81	1.49	0.061	0.031
365	11.14	1.37	0.079	0.052
368	16.15	7.9	0.058	0.029
369	8.96	1.68	0.075	0.047
· 370	9.27	1.33	0.073	0.043

Extinction

When the shock was disconnected after 45 to 60 min, regular responding extinguished. In one case, the extinction was immediate. This indicated that, for this animal at least, the shock was perceived, and had the function of a discriminative, if not of an aversive, stimulus. In other cases, two or three S^D periods without shock were necessary before extinction occurred. Generally, a few responses were emitted at the beginning of S^D periods, probably triggered by the discriminative stimuli (light, noise of the motor). Three rats showed increased responding in S^D during extinction. An example of behavior during extinction is shown in Fig. 5.

RAT 365 EXTINCTION 13-10-66

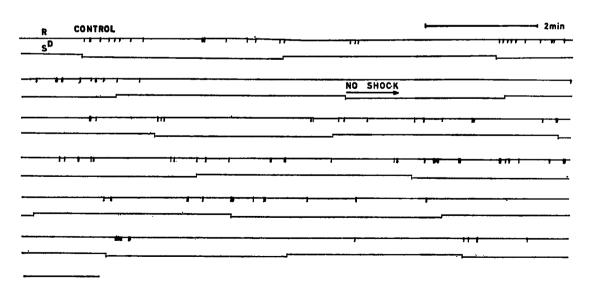


Fig. 5. Record from Rat 365 in extinction. The session was started with the usual alternation of shock and no-shock periods. The shock was disconnected (all other stimuli being unchanged from the place marked "No shock"). Successive segments of two-channel records ordered from top to bottom. R: responses; S^D: shock and no-shock periods (see Fig. 3 for details).

Controls

Effect of the experimental situation, minus electrodes and shock. Naïve Rat 480, placed in the cylinder for 5 hr without electrodes, showed a general motor activity resulting in a few responses in the beginning of the experiment. No more than 10 responses occurred after the first five min.

Effect of the experimental situation minus shock. The same rat, maintained for 165 min in the cylinder with the electrodes on the tail, produced an average of 2.2 responses per minute (S^D and S^A periods confounded), coming in fast bursts at long intervals; periods as long as 30 min without responses were observed. As the session progressed, the bursts were more and more widely spaced and, at the end of the session, the rat seemed half asleep. This observation indicated that the electrode holder, though responsible for a few responses, did not produce the rate observed with shock.

Non-contingent shock, 0.2 mA. Three naïve rats (342, 343, 344) were put in the experimental cage for two or three sessions of 60 min each. Whatever their behavior, they received non-contingent shocks maintained at the constant intensity of 0.2 mA. S^D and S^D periods were alternated. Bursts of fast responding were observed in the beginning of the session, but their frequency decreased within the first hour and they eventually disappeared. The

average number of responses per minute during shock was 4.1 for Rat 343; 11.2 for Rat 344, and 1.5 for Rat 342. The relatively high value obtained for Rat 344 did not mean that the responses would be adequately distributed as operant: bursts were very fast and rather long, but spaced in time; moreover, on the second of three sessions, this average of responses per minute dropped to 3.7 (vs 15.2 and 13.7 on the other two sessions).

Non-contingent shocks, 0.3 mA. As a further test of the non-contingent shock, Rat 481 was run during a prolonged session lasting 6.5 hr with a non-contingent shock of 0.3 mA during S^D. This value was selected by the experimenters by being aversive for rats. Figure 6 shows the last 4 hr of the record. For 144 min, the rat produced an average of 8.5 responses per minute during S^D and no responses during S^A. During the next 246 min, the responses dropped to 1.9 per minute. In very long phases at the end of the session, extinction was complete.

Non-contingent shock, 2 mA. Most convincing as to the character of respondent reactions to shock were the results of the last control made on the same rat (481). A procedure similar to that described above was used, the shock level being maintained at 2 mA during one 5-hr session. Respondent reactions were numerous in the first part of the session, averaging 22.6 responses per minute for S^D periods.

RAT 481, NON-CONTINGENT SHOCK: .0,3 mA.

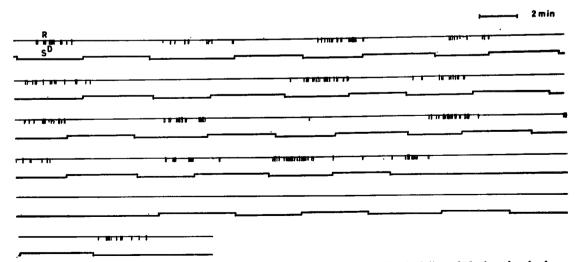


Fig. 6. Control of unconditioned responses to an inescapable shock of 0.3 mA delivered during the shock periods. Record from Rat 481. Numerous responses in the beginning of the session eventually disappear during the last shock periods. This does not occur in the titration schedule (see Fig. 3 for key to reading).

For the rest of the session, this value dropped to 8.2. No response was given throughout this session during S^A. It is clear from these data that respondent reactions, even to high intensities of shock, do not persist indefinitely as respondent. The last part of the record showed entire periods in which the shock was delivered to the animal but did not elicit responding.

DISCUSSION

From the purely technical point of view, the device used in this experiment solved the problem of locating electrodes with fairly good precision for electrical nociceptive stimulation in rats. The device is, in some aspects, analogous to the one described by Hall et al. (1966). It has more limited applications, since the head of the animal is not accessible for brain studies and food reinforcement could not be delivered. However, it recommended itself, when none of these possibilities was required, for easy manipulation of the animals, rapidity of conditioning, and simple and cheap design.

Conditioning on a titration schedule was easily and rapidly obtained for all subjects tested. Performances were very stable, showing little intra- and inter-individual variability.

Problems arose as to the respondent and/or operant characters of the response, and as to the significance of the intensity threshold maintained by the animal. In its initial stage. the response was undoubtedly part of a respondent reaction to the electric shock delivered to the tail, producing forward movements of the animal. This was clear from the fact that responses occurred immediately at the onset of the shock in the titration schedule and occurred in control animals submitted to inescapable shock of constant intensity. The restraining device was responsible for a small part of this respondent activity but it was easily recognizable because it was not correlated with shock periods. How is the shock level tolerated by the animal in this experiment to be interpreted? Does it correspond to the intensity value where the shock becomes aversive, or is it a discriminative stimulus warning the animal that aversive values are forthcoming, or is it most of the time below the absolute threshold? In other words, did we generate escape behavior, discriminated avoidance, or pure avoidance behavior? Similar problems were met by other experimenters.

Weiss and Laties (1963, in press) pointed out that the type of behavior generated by their titration schedule was largely dependent upon temporal parameters. The inter-increment interval was proved critical: the shorter the inter-increment interval, the greater the proportion of time spent at higher shock levels. With a 10-sec interval (the value used in the present experiment), a large part of the responses were presumably avoidance responses. Reducing the interval to 2 sec, or making the shock increment contingent upon a number of responses (FR) produced a higher threshold, presumably maintained by escape behavior.

The behavior obtained from the present subjects, if it cannot be considered as true escape behavior, was certainly not pure avoidance. Were it so, one would expect that the auditory and visual stimuli associated with shock periods would suffice to maintain it, at least for some time, when the shock was disconnected. The rapid and in some cases immediate extinction when the shock was no longer delivered, demonstrated its decisive role in keeping the behavior in strength. These results are similar to those obtained with monkeys by Weitzman and Ross (1962).

It can be suggested that the shock level maintained by the present animals, except for occasional increases reaching aversive values and producing escape responses, functions as a discriminative stimulus. This was shown to be the case in the experiment by Boren and Malis (1961) on monkeys. Using intracerebral aversive stimulation, they also observed very low shock levels not likely to reflect real pain threshold, despite the fact that the inter-increment interval was only 1 sec. Introducing an FR component did increase the shock level, as in the Weiss and Laties experiments. By limiting the range of intensities and keeping it below the presumably aversive level, they obtained extinction of the conditioned behavior.

This confirmed that the low shock was effective in maintaining responses only if it signalled the aversive stimulus of increased shock. Whether this hypothesis holds true in the present situation will be decided after the completion of ongoing complementary investigations. The interpretation of pharmacological effects, for the study of which the technique was developed, is dependent, of course, upon

a clear understanding of the behavior involved.

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