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# ACTION OF CHLORDIAZEPOXIDE ON TWO TYPES OF TEMPORAL CONDITIONING IN RATS

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**Summary**—The action of chlordiazepoxide on two kinds of behaviour involving a temporal component is studied. Rats were trained, in an operant conditioning situation to press a lever for food either on a fixed interval schedule of reinforcement or on a schedule of differential reinforcement of low rates of responding. In both cases, a temporal discrimination develops; in the first case, it is *spontaneous*, while it is the condition for reinforcement in the second case. Chlordiazepoxide increases the conditioned activity, as measured by the number of responses, and produces a disruption of timing behaviour. Both effects are more pronounced, and can be observed for smaller doses of the drug, under the fixed interval schedule, where the temporal discrimination *is not* the condition for reinforcement. Results are discussed in terms of drug-behaviour interaction. The muscle relaxant action of chlordiazepoxide is hypothetically suggested as an explanation of its effects on timing behaviour.

## INTRODUCTION

CHLORDIAZEPOXIDE(*) is a now widely used tranquilizer. A number of effects have been described, both experimentally and clinically, including anxiety reducing effects in human patients, taming of aggressive animals, appetite stimulation, muscle relaxation, anticonvulsant action, and others. Animal activity studies gave evidence of its depressive properties (RANDALL, 1961). However, it has been shown in previous experiments, that, unexpectedly enough, chlordiazepoxide increases the rate of conditioned activity in cats (RICHELLE, 1962). The drug had also a disruptive effect on the temporal discrimination generated by the experimental situation used.

The question arises whether this paradoxical effect is specific to cats, or could also be observed in other species, and, secondly, whether it could be obtained in other situations than the one used in our experiment. The aim of the present study is to replicate the experiment with rats and to show the action of the drug on another kind of temporal conditioning.

## METHODS

### (a) *Apparatus*

The experimental cage is a classical Skinner-box, equipped with a small lever and an electromagnetic tap through which a controlled amount of sweet condensed milk is delivered as reinforcement. The cage is isolated in a relatively soundproof compartment, the noise of a ventilator providing for additional masking of the auditory stimuli in the surroundings.

The method of operant conditioning is based on the control of behaviour by its consequences. A reinforcement, here a small amount of food, is made contingent on a well defined response, here pressing the lever.

*Kindly supplied by ROCHE, under the trade name Librium.

The experiment is automatically programmed by means of relays and timers located outside the experimental room. Results are automatically recorded on a cumulative pen recorder and on a series of digital counters.

(b) *Subjects*

Eight white albino rats (Large Wistar strain) were used, the weights of the animals ranging from 150 to 200 g. The rats, about four months old at the beginning of the experiment, were living in individual home cages in the animal room, and spending 1 hr a day, 5 days per week, in the experimental cage. They usually take their food for the day during the experimental session. When not so, they are given a supplement in order to be kept at about eighty five per cents of their ad libitum feeding weight.

Three female rats, numbered 018, 040 and 045, were the subjects in Experiment I. Five rats, numbered 024, 025, 033, 034 and 036, all females except for 025, were the subjects in Experiment II.

(c) *Schedules of reinforcement*

The expression *schedule of reinforcement* refers to the relation between the response and the reinforcement, as operationally defined by the experimenter. Experiments I and II differ with respect to the schedule of reinforcement.

*Experiment I.* The responses are reinforced on a Fixed Interval schedule (interval= 2 min) or F I 2. A reinforcement is delivered following the first response emitted after a 2 min interval has elapsed since the last reinforcement. The animal is free to respond in the interval, though its responses are not reinforced.

The distribution of responses in the interval is recorded by dividing the total interval of 2 min into eight 15-sec periods. By means of a stepper, a given response is recorded by that of eight digital counters which corresponds to the 15 sec period during which it is emitted.

The schedule used in Experiment I is exactly the same as the one used in our previous study with cats.

*Experiment II.* The schedule used here is referred to as Differential Reinforcement of Low Rates of responding, or DRL (FERSTER and SKINNER, 1957). A response is reinforced only if it is emitted after a minimal interval of time has elapsed since the previous response. The interval used is 34 sec. Thus responses spaced by less than 34 sec will never be reinforced. The temporal discrimination is the condition for reinforcement.

(d) *Experimental programme*

*Experiment I.* After the subjects had learned the operant response, they were trained on the F I schedule until their behaviour showed a stable pattern from one day to another. The pharmacological tests were then started. As a rule, no drug was administered unless the behaviour had returned to its baseline value, defined by the total number of responses and their distribution in the interval. The total number of experimental sessions on the F I schedule was about fifty for each animal.

Chlordiazepoxide was injected intraperitoneally 1 hr before the experimental session. The solution was 2 mg chlordiazepoxide in 1 ml saline. The following doses were administered to each subject: 1·2 mg, 2, 3, 4, 5, 6, and 7 and 8 mg. These are absolute doses and should be multiplied by five to six to obtain an approximation of doses kg of body weight.

After the standard procedure had been completed with all three animals, rats 040 and 045 were injected with 8 mg of the drug for 22 and 13 consecutive days, respectively. The same experimental procedure was continued during that period.

*Experiment II.* As in Experiment I, the subjects were trained on the particular schedule before the pharmacological tests began. The baseline was simply defined as a regular pattern of behaviour, as it will be seen clearly on the curves below, not necessarily representing the optimal performance that the animal might be able to accomplish, were it run on a few more sessions for training. This provides for a possible modification of behaviour in both directions, plus and minus, under drug action.

The total number of sessions on the DRL schedule was about fifty for Rats 024, 025 and 033, about thirty for Rats 034 and 036. These last two animals died in the course of the experiment, one from an accident when injected, the other from undefined disease.

The conditions of administration of the drug were the same as in Experiment I, except for doses. Plans were made to test doses as high as 13 mg, by steps of 1 mg: Rat 034 died after the dose of 9 mg and Rat 036 after the dose of 7 mg; Rat 024 did not receive the doses of 1·2, 2 and 3 mg, Rat 025 the doses of 3 mg(*).

Moreover, Rats 024 and 025 underwent a series of 14 daily injections of 10 and 11 mg respectively.

## RESULTS

*Experiment I.* Under normal conditions, the Fixed Interval schedule generates a very typical pattern of behaviour, characterized by long pausing after each reinforcement. The subject starts responding towards the end of the interval, thus maximizing its chances of obtaining the reinforcement as soon as it is available. A temporal discrimination is clearly developed, though it is not imposed to the animal as a condition for reinforcement. We will refer to this discrimination as *spontaneous*, meaning that it shows an optimal adjustment of the organism to the conditions of the environment, without these conditions making such an adjustment necessary. A sample of that normal behaviour is given in Fig. 1, curve A, obtained from Rat 045. Similar patterns were recorded for the other two animals. The abscissa is time and corresponds to 1 hr. Responses are cumulated on the ordinate as they are emitted. Each deflection of the pen on the cumulative curve indicates a reinforcement. The pen tracing the horizontal line is deflected when the two minutes delay is completed; it remains in the down position until the reinforced response occurs.

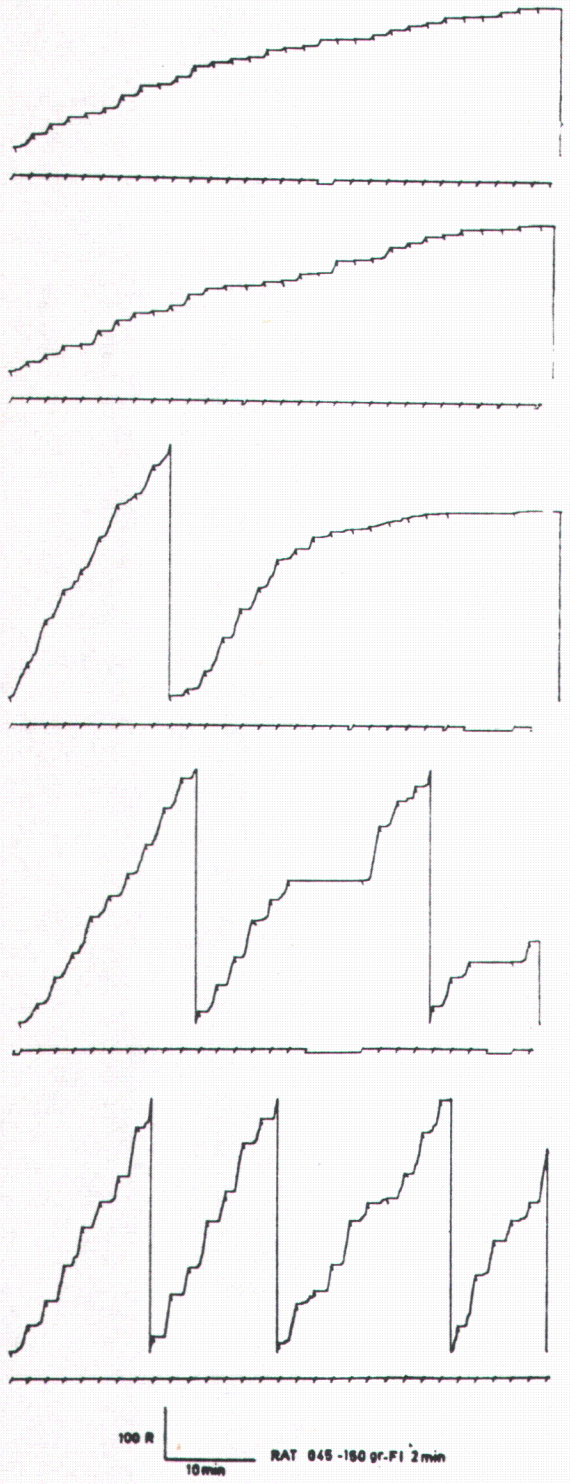
All the subjects return to the pre-drug level of responding, both qualitatively and quantitatively, on the first day following a pharmacological test. This is illustrated by curve B, in Fig. 1.

Chlordiazepoxide has two main effects, unequivocally present in all three animals: it induces an increased conditioned activity and a disruption of the temporal discrimination.

The first effect is easily measured by the number of responses emitted. Table I gives the values for each subject, under normal pre-drug and post-drug conditions, and for each dose of chlordiazepoxide. The figures correspond to the mean numbers per reinforcement, i.e., the mean number of responses emitted during one interval of 2 min including

*The reasons for this are accidental and related to a change introduced in the experimental programme. With the intention of keeping our conditions as similar as possible to those used with cats, we started by administering the drug orally, but were not satisfied with the poor control this procedure gave us on this variable.

the reinforced response. Values for normal conditions, pre-drug and post-drug control, are averaged from a minimum of six experimental esssions.




(A) 6-2-62 Pre-drug 287R–30Rf

(B) 14-3-62 Post-drug control 297R–30Rf

(C) 23-2-62 4 mg Chlordiazepoxide 872R–26Rf

(D) 6-3-62 6 mg 1159R–25Rf

(E) 15-3-62 8 mg 1896R–31Rf

FIG. 1. Samples of cumulative curves from Rat 045 on the Fixed Interval schedule of reinforcement. Responses are cumulated on the ordinate as the paper unrolls from left to right. The pen resets automatically to its origin on the ordinate after 500 responses. Oblique pips on the cumulative curves indicate the reinforcements. The pen tracing the horizontal line at the lower part of each graph is kept in the down position when the 2 min interval is over and comes up again as soon as the reinforced response is emitted. R = Responses, Rf = Reinforcements.

TABLE 1. MEAN NUMBERS OF RESPONSES EMITTED PER INTERVAL IN THE FIXED INTERVAL SCHEDULE

| Dose of chlordiazepoxide | Rat 018 | Rat 040 | Rat 045 |
|---|---|---|---|
| Pre-drug control | 18·02 | 7·66 | 11·58 |
| Post-drug control | 17·38 | 6·49 | 12·21 |
| 1·2 mg | 37·05 | 6·17 | 19·70 |
| 2 mg | 27·63 | 9·23 | 24·71 |
| 3 mg | 31·10 | 11·75 | 15·45 |
| 4 mg | 44·14 | 9·69 | 35·08 |
| 5 mg | 37·31 | 13·60 | 28·63 |
| 6 mg | 11·41 | 16·61 | 48·56 |
| 7 mg | 30·55 | 25·61 | 70·58 |
| 8 mg | 28·87 | 31·99 | 65·61 |

Expect for one atypical result in Rat 018 with the dose of 6 mg, we never observed decreased activity under the drug. The relation *amount of activity/doses* is not rigorous, but the general trend is clear. A maximum is reached for different doses by each individual animal. This maximum represents 2·5 times the base line number for Rat 018, 4 times for Rat 040 and 6 times for Rat 045.

These data are illustrated by the examples of cumulative curves obtained from Rat 045 and shown in Fig. 1, C, D and E.

The disruption of the temporal discrimination was measured by computing the proportion of responses emitted in each 15-sec class composing the total interval of 2 min. This proportion is expressed in percentages of the total number of responses in the interval. These results are presented in the form of histograms in Fig. 2. Each histogram corresponds to a given session with the indicated dose for an individual subject. Pre-drug and post-drug controls are averaged from a minimum of six sessions. The higher the proportion of responses in the last parts of the interval (represented by the blocks at the right end of the distribution), the better the temporal discrimination. Obviously, the quality of the discrimination is altered for doses as low as 1·2 mg (Rats 018 and 045) or 2 mg (Rat 040). The disruption does not necessarily reach its maximal value for the highest dose of 8 mg. This might indicate that the animal is able to readjust, at least to some extent, to the environmental conditions in spite of the drug. It might also be interpreted as the behavioural aspect of a simple phenomenon of tolerance.

Rats 040 and 045 remained at the same level of performance, both qualitatively and quantitatively, during the whole period of chronic administration of 8 mg per day. This part of the experiment, which is only exploratory, shows also that the behavioural action of chlordiazepoxide never lasts as long as 24 hr: when no drug is injected prior to the experimental session, or when saline solution only is injected, the behaviour returns to its normal baseline.

*Experiment II.* The best information regarding the results of Experiment II may be obtained from Figs. 3 and 4, taken as representative examples (Rat 025 and 034). Particularly, the graph corresponding to the first animal performance has been selected because the effects of the drug are especially conclusive. The curves can be read in the same manner as those in Fig. 1. The pen tracing the horizontal line is deflected and kept in the down position whenever the 34 sec delay has elapsed without a response. The reinforced response brings it back to its original position, recycling the timer.

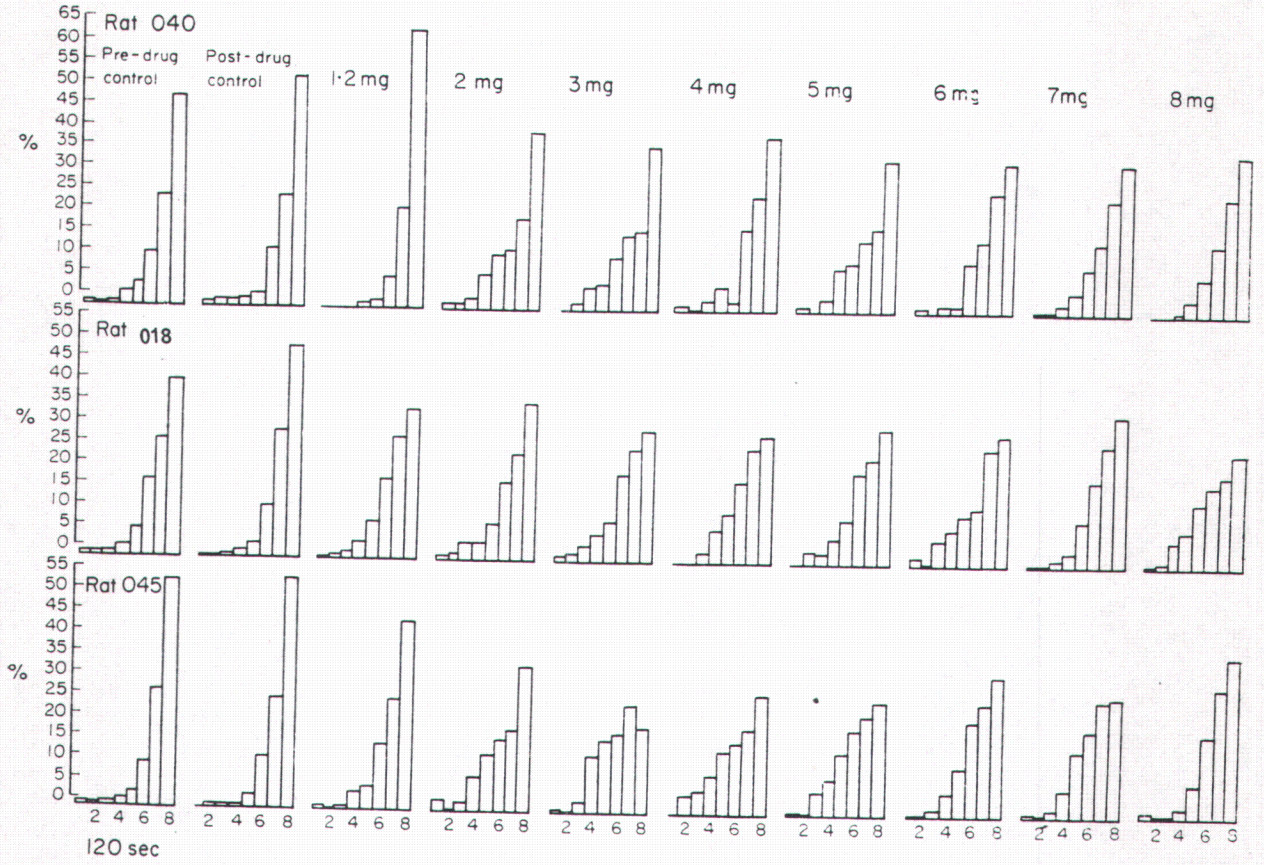
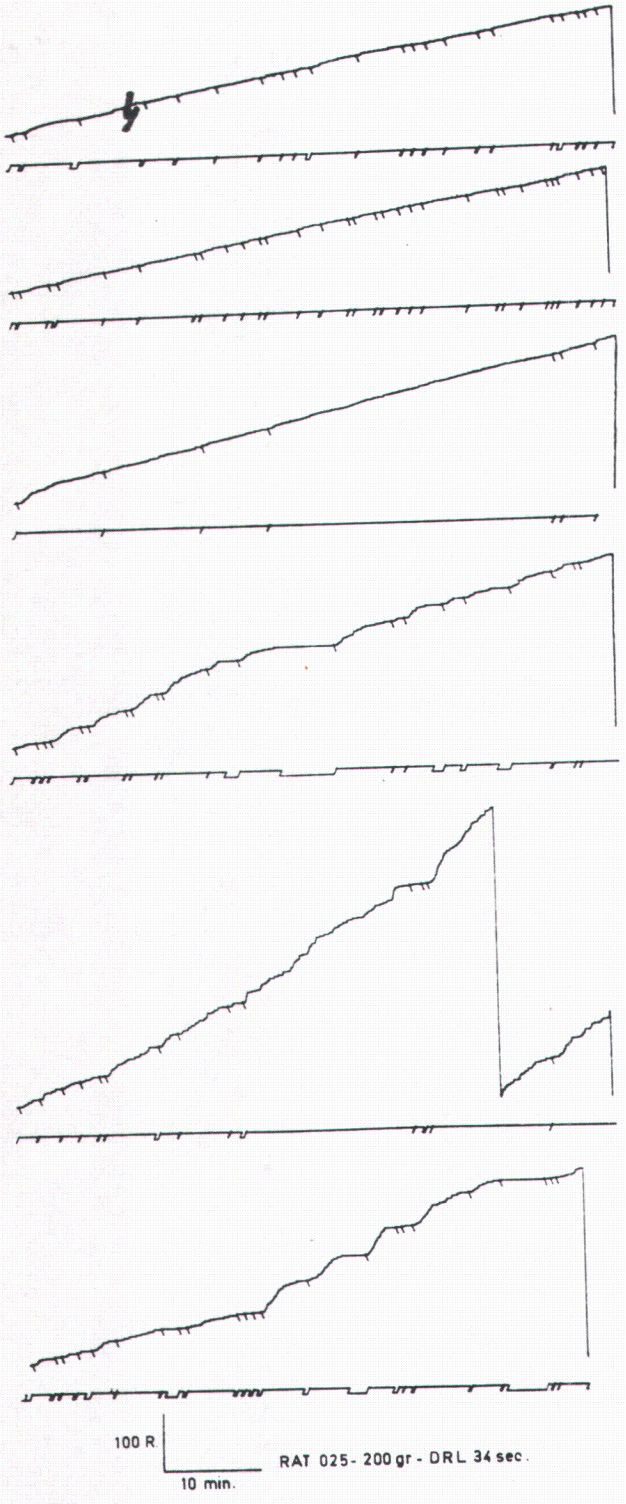



FIG. 2. Distribution of responses in the interval under the 2 min Fixed-Interval schedule of reinforcement, for Rats 040, 018 and 045. Each histogram is divided into eight blocks, corresponding to eight fifteen seconds periods. The height of a block corresponds to the proportion of responses emitted during that 15 sec period, expressed as a percentage of the total number of responses. The larger percentage of responses massed toward the end of the interval, i.e., in the block at the right end of a histogram, the better the *spontaneous* temporal discrimination. Results with drug are for one session with the indicated dose of chlordiazepoxide. Pre-drug and post-drug controls are averaged from a minimum of six sessions.

Under normal conditions, the DRL schedule generates a very regular pattern of responding as shown by curves A in Fig. 3 and 4. Timing is not always accurate, so that a certain proportion of responses only are reinforced. However, the pause between responses is never considerably long as compared to the required delay: as can be seen, the pen is kept in down position for a generally negligible length of time.

During sessions without drug, the animals return to the pre-drug behaviour (see curves B in Figs. 3 and 4). In some subjects, timing behaviour appears somewhat improved, resulting in a larger proportion of reinforced responses (Rat 025). It should be remembered that the pharmacological tests were started when the subjects had not necessarily reached their best performance on the schedule. Longer training is probably responsible for the improvement observed in post-drug controls, rather than some hypothetical after-effect of the drug.

The action of chlordiazepoxide is qualitatively similar, if not quantitatively, to that observed in Experiment I, i.e. increased conditioned activity and disruption of the temporal discrimination.




(A) 31-1-62 Pre-drug 188R–22Rf

(B) 16-3-62 Post-drug control 171R–31Rf

(C) 6-2-62 1·2 mg Chlordiazepoxide 246R–7Rf

(D) 9-2-62 4 mg 297R–21Rf

(E) 6-3-62 11 mg 634R–14Rt

(F) 14-3-62 13 mg 314R–23Rf

FIG. 3. Samples of cumulative curves from Rat 025 on the schedule of Differential Reinforcement of Low Rates. See Fig 1 for key to reading. The fixed pen tracing the horizontal line at the lower part of each graph is kept in the down position whenever the 34 sec delay has elapsed; the reinforced response brings it back to its original position.

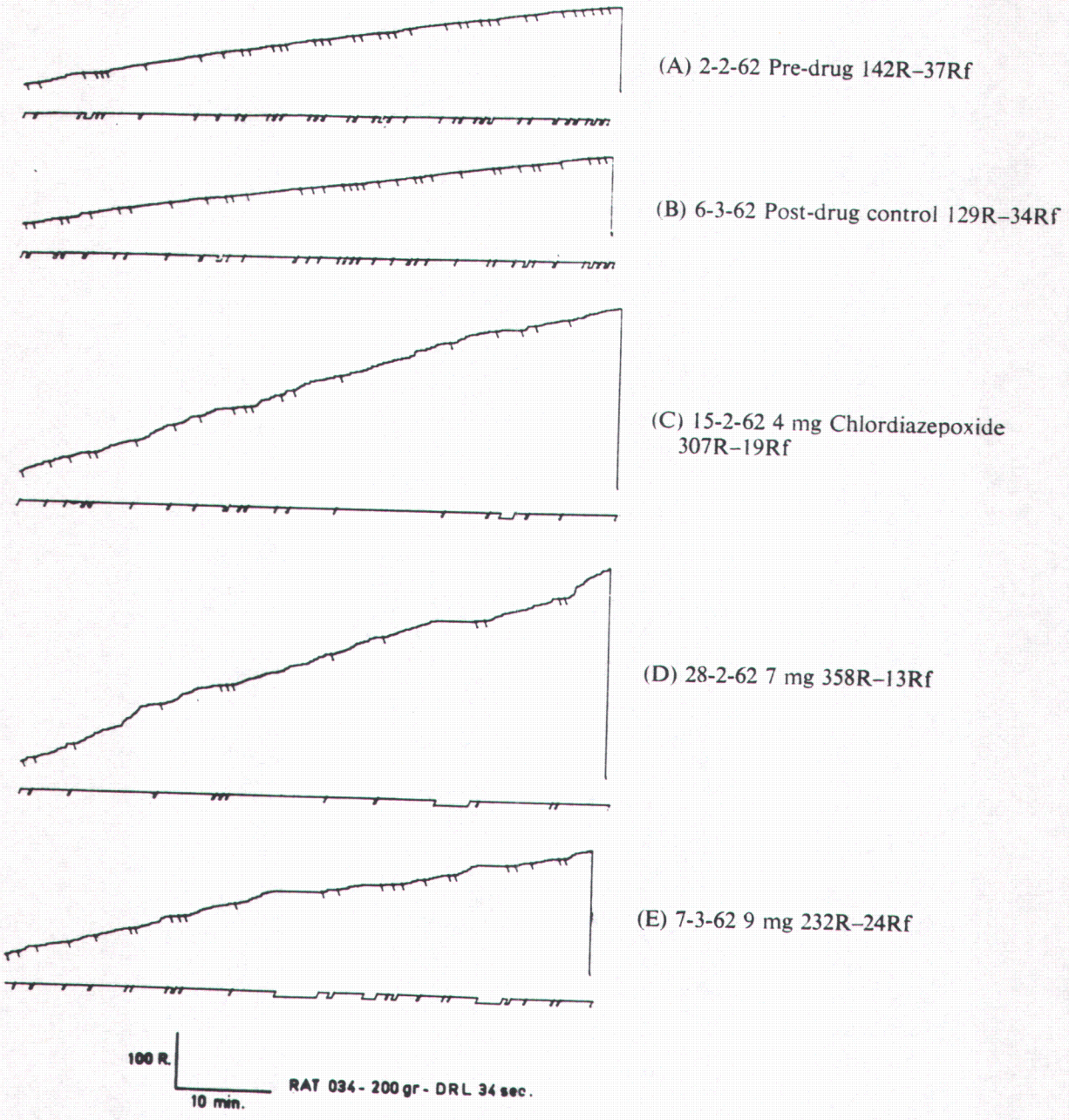



FIG. 4. Samples of cumulative curves from Rat 034 on the schedule of Differential Reinforcement of Low Rates. See Figs 1 and 3 for key to reading.

The increase of activity is evidenced by the number of responses presented in Table II. It is far less dramatic than in Experiment I. The maximum number of responses represents 1·5 to 3·4 times the control numbers, according to the subject being considered. The critical dose inducing this effect unequivocally is usually higher than for animals trained on the FI Schedule. The interpretation of these results will be discussed in the last section of this paper.

An increased number of responses, compared with the regular rate observed under normal conditions, goes together with an impairment of timing behaviour. If more responses are emitted within the one hour session, they are likely to be spaced by too short intervals to have the possibility of being reinforced.

TABLE 2. NUMBER OF RESPONSES EMITTED DURING THE ONE HOUR SESSION IN THE DRL SCHEDULE, UNDER CONTROL AND DIFFERENT DOSAGE CONDITIONS. IN BRACKETS: NUMBER OF REINFORCEMENTS

| Rat | 024 | 025 | 033 | 034 | 036 |
|---|---|---|---|---|---|
| Dose | | | | | |
| Pre-drug control | 125 (30) | 188 (22) | 128 (34) | 142 (37) | 205 (20) |
| Post-drug control | 145 (41) | 171 (31) | 129 (39) | 129 (34) | 193 (21) |
| 1·2 mg | — | 246 ( 7) | 132 (34) | 170 (23) | — |
| 2 mg | — | — | 180 (20) | 246 (15) | 302 (13) |
| 3 mg | — | — | 169 (25) | 201 (21) | 231 (18) |
| 4 mg | 119 (34) | 297 (21) | 167 (19) | 307 (19) | 199 (17) |
| 5 mg | 176 (47) | 293 ( 6) | 172 (14) | 222 (21) | 294 (12) |
| 6 mg | 120 (26) | 247 (15) | 196 (15) | 331 (17) | 315 (18) |
| 7 mg | 140 (28) | 249 (15) | 271 ( 8) | 358 (13) | 109 (34) |
| 8 mg | 105 (47) | 365 (14) | 252 (17) | 341 (19) | — |
| 9 mg | 102 (30) | 317 (11) | 241 (23) | 232 (24) | — |
| 10 mg | 320 (16) | 506 (11) | 171 (28) | — | — |
| 11 mg | 232 (21) | 634 (14) | 313 ( 8) | — | — |
| 12 mg | 221 (21) | 622 (10) | 188 (27) | — | — |
| 13 mg | 213 (23) | 314 (23) | 252 (16) | — | — |

The shortening of interresponse time is already observable with small doses of the drug and results in fewer reinforcements. However, the rate of responding is still fairly regular, as it is evidenced by the smoothness of curve C in Fig. 3. With higher doses, the subjects respond more erratically, fast bursts of responses alternating with unusually long pauses. Curves E and D in Figs. 3 and 4 illustrate this effect. The slope is irregular, with very steep portions indicating high rates. It should be observed that such irregular pattern of responding, particularly evident for high doses, can produce more reinforcements. These are clearly not due to a better timing, but to the fact that any response following a long pause will necessarily be reinforced. This effect could be avoided by using a DRL schedule with limited hold in which the reinforcement is available for only a short period after the delay is completed.

The modifications of operant behaviour in Rats 024 and 025 persisted through the series of chronic administration of chlordiazepoxide. The same observations were made as in Experiment I as to the recovery of predrug performance when nothing or saline only was injected.

## DISCUSSION

The present study confirms the results previously obtained with cats in the same experimental conditions as those used in Experiment I (RICHELLE, 1962). The only difference is in the way of administration of the drug: the cats were given chlordiazepoxide per os. Increased activity and disruption of the temporal discrimination were found in both cats and rats. However, rats always returned to the normal behaviour in the first control session following by 24 hr a session with drug. For cats, three to fifteen daily sessions were necessary for the recovery of a normal behaviour after administration of high doses—corresponding to 4 and 8—mg in the rat study if expressed proportionally to the animal's body weight. This difference might be related to a difference in the rate of metabolization. Rats were found to metabolize chlordiazepoxide significantly faster than men or dogs (SCHWARTZ and KOECHLIN, 1961), but data are lacking for a comparison with cats in this respect. At this point, a purely behavioural explanation is still tenable: for some unknown reasons, the baseline behaviour, once disrupted, is to be *reacquired* by cats, while the behaviourai effects of the drug would not outlast its pharmacological effects in rats.

The comparison of the effect of chlordiazepoxide in the FI and in the DRL schedule makes clear two points. First, the increased activity and the disruption of the temporal discrimination are not specifically bound to the FI conditions, since they are also present in the DRL program. Secondly, the increase of activity appears very different in the two experiments, both in terms of degree, as shown by the number of responses and in terms of the dose which produces a clear-cut modification. It will be remembered that, under the DRL schedule, sufficiently large spacing of responses is the condition for reinforcement. One might expect the performance in such conditions to be more resistant to the action of the drug than the performance in the FI program, where the number of reinforcements is unrelated to the rate of responding. By responding at a higher rate in the DRL schedule, the animal practically puts itself in a situation where no reinforcement may be obtained as long as the high rate is maintained. This is equivalent to experimental extinction, in which the probability of responding is progressively reduced to zero as a consequence of withdrawing the reinforcement. Thus the increased activity leads to temporary extinction, which leads itself to decreased activity, which in turn makes reinforcement more probable. Contrariwise, there is no unfavourable counterpart to increased activity in the FI schedule.

The comparison between the two experimental programs provides a very typical instance of drug-behaviour interaction (SIDMAN, 1959). It is irrelevant to qualify a psychotropic drug as a depressant or a stimulant of activity unless the situational context in which activity has been measured is specified. In our study the modifications of activity induced by the drug are clearly a function of the schedule of reinforcement. Still more striking is the opposition between the effects observed in the two experiments described above and studies which showed the depressant action of chlordiazepoxide on general activity (FROMMEL *et al.*, 1960). The conditioned operant behaviour is modified by the drug in a direction and to an extent which are fully unpredictable from the modifications observed in the general behaviour of the animals. With larger doses (generally above 8 mg), the subjects are somnolent, show marked muscular hypotony, can be handled like animals unable to manifest any aggressive reaction; they keep sitting quietly in the cage and show a seriously impaired coordination when they move. All these observations are in the line of a depressing effect on activity. But once in the experimental cage, as was seen, the picture is different.

If the notion of activity has any usefulness at all in characterizing a tranquilizing drug, which kind of activity, general or conditioned, is to be given more attention? And what is the relevance of each kind to human behaviour?

Our results leave important problems open to further investigations, in order to explain the paradoxical action of chlordiazepoxide. The main question is whether the increased activity and the disruption of the temporal discrimination are causally related, and, if so, which of these two aspects is the cause of the other. One might suggest to interpret, hypothetically, the disruption of timing behaviour as being the primary effect, with the increased activity being the consequence. The muscle relaxant action of chlordiazepoxide would be reponsible for the impairment of the temporal discrimination. This interpretation is based on the hypothesis that timing behaviour is related to proprioceptive cues from muscular tonus. A first experimental step toward the solution of this problem would be a comparison between the action of chlordiazepoxide on two schedules of reinforcement, one involving a temporal regulation of behaviour, the other providing for a measure of activity alone.

**Résumé**—L'action du chlordiazepoxide a été étudiée sur deux types de comportement impliquant une régulation temporelle. Dans une situation de conditionnement operant, des rats ont été entraînés à presser un levier pour obtenir de la nourriture soit selon un programme de renforcement à intervalle fixe, soit selon un programme de renforcement des débits de réponses lents. La discrimination temporelle qui se développe est *spontanée* dans le premier cas; elle est la condition du renforcement dans le second. Le chlordiazepoxide provoque un accroissement de l'activité conditionnée et une détérioration de la discrimination temporelle. Ces effets sont nettement plus marqués et surviennent pour des doses plus faibles, dans le type de comportement où la discrimination n'est pas la condition du renforcement. Les résultats sont interprétés en termes d'*interaction médicament—comportement*. Le rôle de l'action hypotonique du chlordiazepoxide est invoqué, à titre d'hypothèse, comme une explication possible de son effet sur les régulations temporelles.

**Zusammenfassung**—Die Wirkung von Chlordiazepoxyd auf zwei Verhaltensformen, die eine Zeitkomponente beinhalten, wird untersucht. Ratten wurden unter einer Bedingungssituation trainiert, einen Hebel für Nahrung zu drücken: Einmal in einem Verstärkungsschema mit festgeleten Zeitintervallen, zum andern in einem differentiellen Verstärkungsschema mit niedriger Reizbeantwortungsfrequenz. In beiden Fällen entwickelt sich eine zeitliche Diskriminierung. Chlordiazepoxyd vermehrt die bedingte Aktivität, gemessen an der Zahl der Reizantworten und verursacht eine Unterbrechung des zeitlichen Verhaltens. Beide Effekte sind unter dem festgelegten Intervallschema ausgeprägter und mit kleineren Medikamentgaben zu beobachten; die zeitliche Diskriminierung ist nicht die Bedingung für die Verstärkung. Die Ergebnisse werden unter dem Gesichtspunkt Arzneimittel/Verhalten-Wechselwirkung diskutiert. Die muskelrelaxierende Wirkung von Chlordiazepoxyd wird hypothetisch als eine Erklärung der Wirkung auf das zeitliche Verhalten vorgeschlagen.

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