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Electroencephalographic and Behavioral Analysis of Drug Effects on an Instrumental Reward Discrimination in Rabbits

By

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With 4 Figures in the Text

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A number of recent papers published primarily in Polish and Russian sources have reported investigations of instrumental learning as well as auditory discrimination (Malinowski 1952; Balinska et al. 1961; Romaniuk 1962) and visual discrimination (Zobkallo 1956) in rabbits. In addition, Zobkallo and Kalinina (1960) have investigated the effects of several drugs (primarily barbiturates) on the performance, by rabbits, of the instrumental discrimination response. In a recent paper Sadowski and Longo (1962) reported the effects of various drugs on the performance of the simple instrumental conditioned response as well as the simultaneously recorded electrical cerebral activity of rabbits. In the present study rabbits were first taught an instrumental ring pulling response. They were then taught an auditory discrimination. EEG activity was recorded during the discrimination training and throughout subsequent tests of the effects of various drugs on discrimination performance. The results, taken together with those of the previous study, provided a comparison of the behavioral and EEG effects of several compounds on the performance of the simple instrumental response and of the discrimination.

Methods and Material

The experiments were conducted using a total of 9 adult rabbits of both sexes. The specific results reported in the present paper are based upon an extended series of investigations performed upon 4 animals, over a period of six months.

A detailed description of the apparatus and of the early stages of the training was presented in the preceding paper (Sadowski and Longo 1962). Briefly, the apparatus consisted of a large wooden box containing

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a ring extending into the wall at one end of the box. When the ring was pulled a piece of food was delivered in a food well in the floor below and to the left of the ring. In the present experiments the training involved the following steps:

1. Instrumental reward training.
2. Electrode implantation.
3. Conditioning of the pulling to the sound of a buzzer.
4. Discrimination training.

1. Instrumental reward training. The animals were first taught to eat from the food well. Then small pieces of the food were fixed to the ring to induce the animal to pull the ring and food was delivered in the food well whenever the ring was pulled. The food was removed from the ring after the animal learned to release the ring as soon as food was delivered. Throughout the several weeks of this training period, as well as subsequent training and testing, the animals were maintained on a 24 hour deprivation schedule. Additional food was given after each day's training session in order to maintain the animals' weights.

2. Epidural electrodes were inserted into the skull at the level of sensorimotor, associative, and optic cortex. Permanent fixation was insured by means of dental acrylic. Training was continued within a few days after the operation and EEG tracings were subsequently registered throughout the sessions. A four channel EEG Grass Model III D was used for the recording.

3. Buzzer conditioning. After the ring pulling response was well established and the electrodes implanted, the rabbits were then trained to pull the ring only during the sound of a 30 second continuous buzzer (CS). Pulls during silent intervals were not rewarded. Thirty to forty trials were given during each session with inter-trial intervals varying from thirty seconds to 3 minutes.

4. Discrimination training. After the animals had learned to pull the ring only at the sound of the buzzer, the training was continued until the response latencies were typically less than 10 seconds for several consecutive days. When this criterion was met, auditory discrimination training was begun. On each day the procedure on $^2/_3$ of the trials was identical to that used previously, *i.e.*, ring pulling at the sound of the continuous buzzer (CS) was rewarded. On the other trials, randomly presented, an intermittent buzzer was presented (DS) and ring pulls were not rewarded. This negative stimulus consisted of a series of 20 short ($^1/_2$ second) signals at 1 second intervals. A total of approximately 45 trials was given on each day. This procedure was continued until the animals learned to pull the ring *only* at the sound of the CS.

Drugs experiments. After the responses to the discrimination were stabilized, the effects of drugs upon the performance of the response was

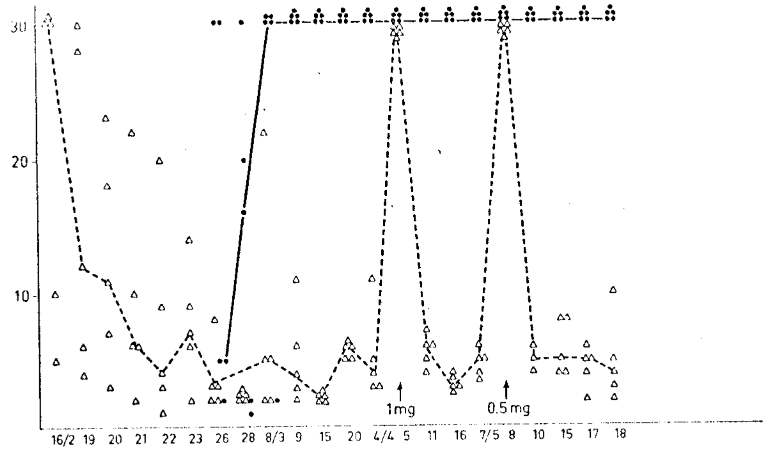

Fig. 1a. Appia (chlorpromazine)

Figs. 1a—d. Summarizes graphically the development of the CS and DS in four animals over the entire period of the training. Dates of the trial are indicated in the abscissa. The ordinate indicates the delay in seconds between the onset of the stimulus and the pulling of the ring. The individual marks (dots: DS; triangles: CS) indicate the response latencies of 5 randomly selected trials. The lines connect the medians of the response latencies: *CS* interrupted lines, *DS* continuous lines. Examples of the effect of various drugs are also given

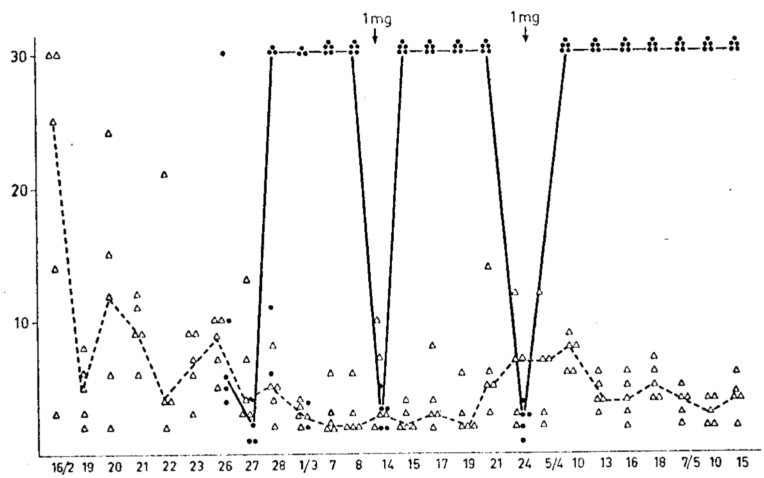

Fig. 1b. Brutus (amphetamine)

then studied. The following drugs were used: chlorpromazine, lysergic acid diethylamide, d-amphetamine, scopolamine, and eserine. In each testing session the animals were first given several control discrimination trials prior to the drug administration. Then the drug was injected intravenously and testing was continued, after approximately 5 minutes, for a period lasting from 1 to 2 and $^1/_2$ hours. In each animal, several experiments using only saline were conducted in order to exclude possible effects of the injection procedure on the animals' behavior.

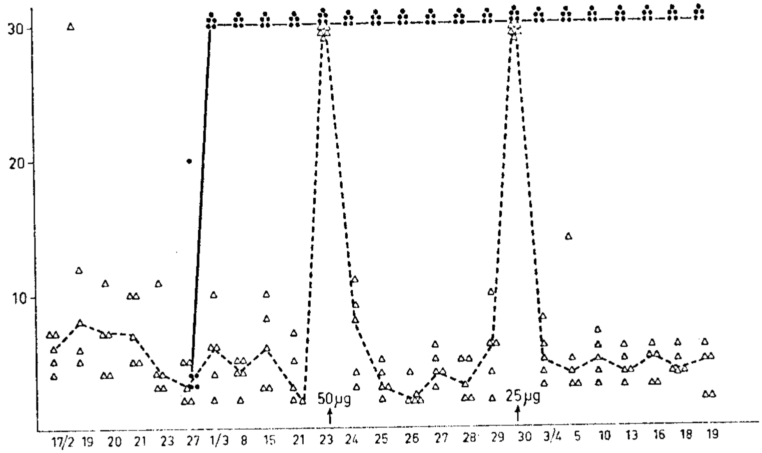

Fig. 1c. Cesare (LSD)

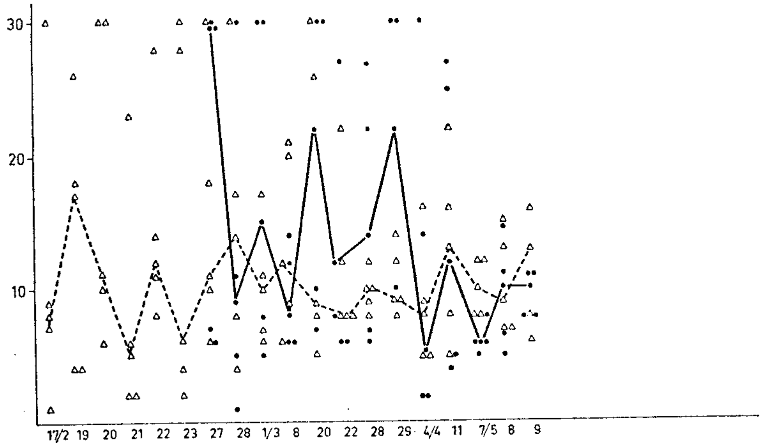

Fig. 1d. Diaz

Results

Behavioral and EEG patterns during instrumental reward and discrimination training

The results obtained during the first three steps were similar to those described previously (Sadowski and Longo 1962).

During the first several sessions the animals adapted to the testing cage and learned to eat from the food well. An additional 5 to 10 sessions were required for the subjects to learn the pulling response. When first presented, the CS inhibited the pulling response. Then, after an adaptation period, the response reappeared. At this stage of training, the non-rewarded pulls during the silent intervals were both frequent and vigor-

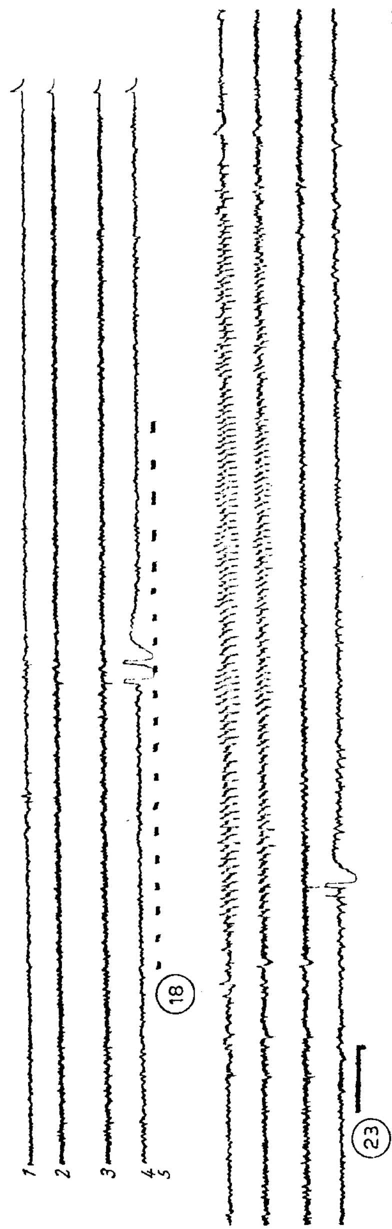

Fig. 2. Electroencephalographic patterns during the instrumental conditioned response (CS) and the differential stimulus (DS). During both CS (18th trial) and DS (23rd trial) the tracing is desynchronized. During the eating period "masticatory" waves appear in the anterior cortex leads. Brutus, 28/2/1962. Leads: *1* L anterior — R anterior sensorimotor cortex; *2* L anterior — L posterior sensorimotor cortex; *3* L posterior — R posterior sensorimotor cortex; *4* L optic — R optic cortex; *5* The heavy lines indicate the sound of the buzzer. The vertical mark indicates the rabbit response of pulling the ring. The number of the presentation is written below the line. Calibration: 100 μV 2 sec

ous. After 5 or 6 sessions the animals gradually learned to pull only at the sound of the CS.

In the initial stages of the discrimination training the DS provoked orientating, or in some cases a fear response, during which no pulling occurred. No disturbances were observed in the response of the animals to the CS. After a few presentations, the subjects begun to pull the ring at the sound of either CS or DS. When the discrimination was learned, the animals typically retired to the rear of the cage at the sound of the DS. The performance latencies to the CS and the discrimination learning process are illustrated in the early portions of the curves in Fig. 1. From the figure it can be seen that learning of the discrimination was quite rapid in three of the animals, while one animal (Diaz) never completely learned the discrimination.

Throughout the tests the EEG remained consistently desynchronized. No differences were found in the tracing registered during the response to the CS and DS (Fig. 2). In the later periods of some sessions, when the animals ceased performing and lay down in the rear of the cage, high voltage slow waves appeared in the tracings and spindles were recorded from the anterior leads. Rhythmic high voltage (200—500 μV) sharp waves, synchronous with chewing movements, typically appeared in the tracing from the anterior cortex and, less frequently, from the posterior cortex, while the animals were eating the food. These waves, originally described by ECTORS (1936) and referred to as "ondes masticatrices", are a real electrical phenomenon and not artifacts produced by movements of the animal. An extensive discussion of these waves can be found in previous papers (LONGO 1960; SADOWSKI and LONGO 1962). In the text, these waves will be referred to as "masticatory" waves. When the animals finished eating, these "masticatory" waves disappeared and the tracing again became desynchronized.

Effect of amphetamine on the discrimination

Amphetamine was administered in doses varying from 0.5—2 mg/kg in four animals for a total of 8 experiments.

Within a few minutes after the administration of amphetamine, the rabbit started to hop around the cage in circling movements. Defecation was usually observed. During this hyperactive period the force of the pulling was greatly diminished. In the peak of the motor effect of the drug, which usually occurred during the first 15 minutes, all responding was completely inhibited. Evidence of disorientation was indicated by other symptoms including searching of the food well during the sound of the buzzer without any prior pulling as well as pulling in the absence of either CS or DS.

The most characteristic effect of this compound was that of the break down of the discrimination. In all cases, during some period following the injection, the animals pulled the ring at the sound of either the CS or DS. This effect was not simply due to a shortening of the response latency and a consequent inability of the animal to discriminate between the onset of the CS and the DS. In fact, most often the pullings were initiated after a latent period of 10—12 seconds. In addition, the animal frequently searched the food well after first having responded to the DS.

In one animal (Diaz), which normally performed the exercise rather poorly and typically ceased responding after 20 stimuli, the administration of amphetamine increased the number of responses to approximately 55 per session.

The effect of the drug on cerebral electrical activity consisted of a desynchronization of the tracing, which continued uninterruptedly throughout the test.

Lysergic acid diethylamide (LSD)

LSD was administered in doses varying from 0.01 to 0.05 mg/kg in three animals in a total of 8 experiments. Of the drugs investigated, LSD was found to be the most effective in completely inhibiting the responses.

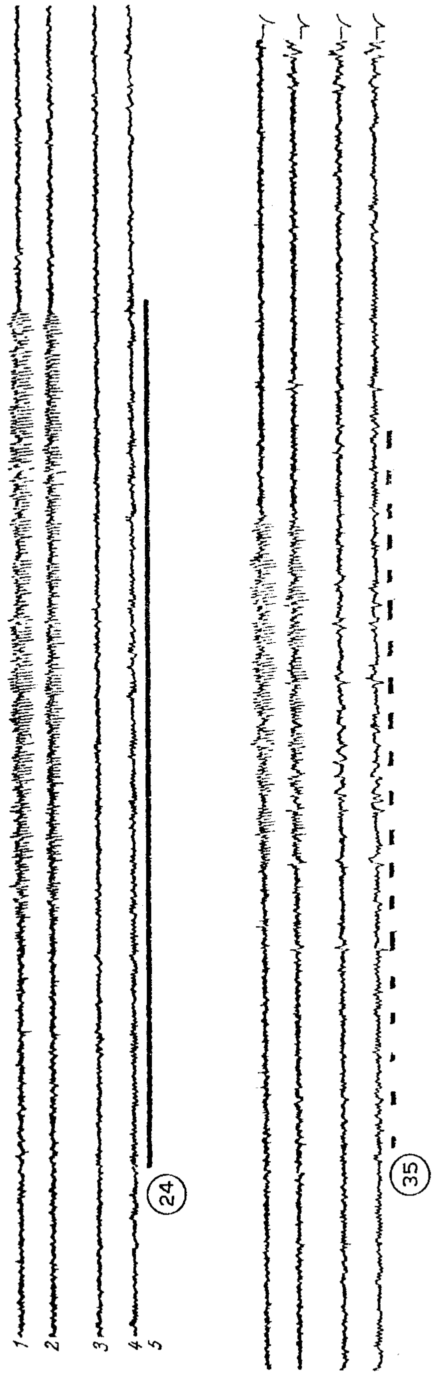

Fig. 3. Tracing registered 40 minutes after the administration of LSD 0.025 mg/kg. During both the CS and DS the appearance is noticed of waves at 5—6 c/sec. These waves are concomitant with rapid movements of the jaw. Ring pulling response after CS is blocked. Cesare, 29/3/1962. Leads: *1* L anterior — R anterior sensorimotor cortex; *2* L anterior — L posterior sensorimotor cortex; *3* L posterior — R posterior sensorimotor cortex; *4* L optic — R optic cortex; *5* The heavy lines at 24 (CS) and 35 (DS) indicate the sound of the buzzer. Calibration 100 μV 2 sec

In fact, in two out of the three animals, the drug in a dose of only 0.01 mg/kg blocked the instrumental responding to both stimuli for a period of approximately 25 minutes. This blocking was accompanied by other behavioral disturbances such as decreased locomotion and slight ataxia. The animal seemed to be completely disoriented and repeatedly looked for food in the absence of any signal. More severe disturbances of behavior were observed with higher doses. These alterations did not consist of a motor excitation, as was found with amphetamine, but rather of impaired movements, due apparently to the motor deficit of the forelegs. A most characteristic response was backward locomotion and rythmic continuous shaking of the head. At the sound of either signal the rabbit remained immobile or, in some cases, retired to the rear of the cage. In one case, after 0.025 mg/kg, there was observed a "disinhibition", in the sense that the DS was followed by the pulling of the ring. After the high doses a completely normal response was not observed for a period of a least two hours.

EEG modifications appeared with doses as low as 0.01 mg/kg, but were more pronounced with higher doses. The modification consisted of a decrease in voltage of all leads, resulting in an extremely "flat" tracing. An unusual picture, which was observed with two animals, is illustrated in Fig. 3. During both types of signals, waves of a frequency of 7—8 c/sec appeared in the anterior leads with a topographical distribution similar to that of the "masticatory" waves. A careful observation of the animals suggested that this EEG picture was associated with tremors of the jaw muscles. This phenomenon typically appeared no sooner than 10 min after the injection, in a period during which all instrumental responses were blocked. Usually the waves ceased with the termination of the signals.

Chlorpromazine

Twelve experiments were performed in four animals, using doses from 0.5—1 mg/kg of the drug. The effect of the lower dose was variable; in some cases responses were completely

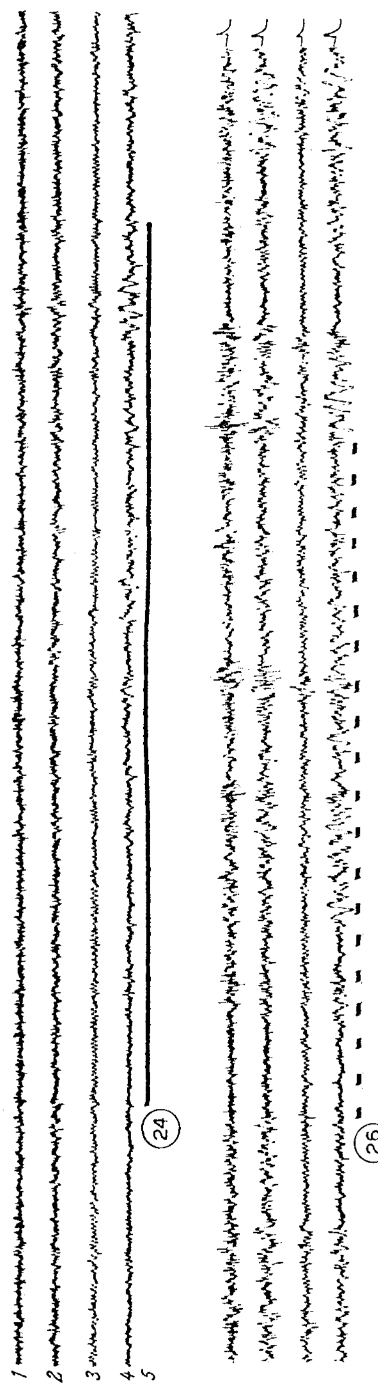

Fig. 4. Tracing registered one hour after the administration of chlorpromazine 0.5 mg/kg. During the CS the tracing is almost completely desynchronized, but the response is still absent. During the DS spindles and slow waves appear. Appia, 3/5/1962. Leads: *1* L anterior — R anterior sensorimotor cortex; *2* L anterior — L posterior sensorimotor cortex; *3* L posterior sensorimotor — L optic cortex; *4* L optic — R optic cortex; *5* The heavy lines indicate at 24 (CS) and 26 (DS) the sound of the buzzer. Calibration 100 μV 2 sec

blocked, in others the animals responded to all auditory stimulation while in others no effect was observed. With 1 mg/kg the effect in every case except one was that of a complete blocking of responses. A slight ataxia was observed during the peak effect of the drug. The duration of the blocking varied from thirty minutes to one hour, and was associated with synchronization of the tracing. For a period following the peak effect of the drug the performance of the instrumental response returned, accompanied by a desynchronized tracing, while the DS provoked a return of the spindling (Fig. 4). In the cases in which the animals responded to both CS and DS the tracings were continuously desynchronized.

Scopolamine

Scopolamine was injected in doses varying from 0.025 to 0.05 mg/kg in three animals for a total of 10 experiments. The lower dose, after a period of 5—10 minutes, completely blocked the responses. This blocking lasted for a period of 15 to 30 minutes, and was followed by a second period in which the animals pulled the ring at the sound of both the CS and DS. Then the performance gradually returned to the pre-drug level.

The dose of 0.05 mg/kg also provoked a complete blocking after a short latent period. In one animal this complete blocking was preceded by a brief period in which the animal responded during both the CS and DS. These changes were accompanied by an increase of spontaneous activity without any motor impairment.

The EEG changes consisted of the appearance of spindles and slow waves, even with the lower dose. As was observed with chlorpromazine, following the peak effect, when the tracing returned to the usual desynchronization the DS frequently elicited spindling. In two cases, eserine (0.05 mg/kg), administered during the peak effect of the higher dose of scopolamine, produced an immediate recovery of the discriminative response and a concomitant EEG activation.

Discussion

Findings of this study indicated that the animals differed both in rate of learning of the auditory discrimination as well as the level performance reached. One animal learned the instrumental ring pulling response but never completely mastered the discrimination. These performance differences may have been due as much to differences in emotionality as to differences in learning ability, since the animals were quite emotional during the early stages of discrimination training, and the learning of the appropriate response to the two auditory signals occurred only after the animals adapted to the DS.

In the previous study (SADOWSKI and LONGO 1962) EEG activation was observed both before and during the performance of the simple instrumental conditioned response. In the present experiments, differences in EEG pattern were not observed during or after the application of CS or DS. In fact, all throughout the trials the EEG tracings were constantly desynchronized.

A number of studies have reported the onset of slow waves following the negative stimulus during discrimination training. The present findings of a continuous desynchronization are consistent with those of ROWLAND and GLUCK (1960) in which under food motivation slow waves were not observed following DS. The same authors (GLUCK and ROWLAND 1959) however, found slow waves to appear after the differentiated stimulus when aversive stimulation was used as a source of motivation. The differential motivational states induced by aversive and reward conditioning situations thus seem to produce a corresponding difference in EEG pattern. An understanding of this difference would require a careful analysis of the physiological states induced by these diverse procedures. Differences in EEG reactions to CS and DS were observed only during the recovery period following the peak effect of the two "synchronizing" drugs scopolamine and chlorpromazine.

In the present study, both EEG pattern and performance of the learned responses were modified by the drugs investigated in doses lower than those found to be effective in the previous investigations in which the animals were required to make only the simple instrumental ring pulling response. For example, doses of 0.2 to 1.0 mg/kg of chlorpromazine, as well as 0.05 to 0.1 mg/kg of LSD and 0.05 mg/kg of scopolamine were previously found to have no disrupting effects on the instrumental response. In the present study 1.0 mg/kg of chlorpromazine consistently blocked all responding, while LSD was effective in doses as low as 0.025 mg/kg. Scopolamine also blocked the responses and produced EEG synchronization in doses lower than those previously found to have no effect.

Since different animals were used as subjects in the previous and present experiments it is possible that the difference in the effect of low doses in the two studies was due to variations in the particular rabbits used as subjects. It is also possible, however, that the effect was due to the difference in complexity in the two problems used. This latter interpretation is consistent with the findings of other investigators (POLIDORO 1961) that the sensitivity of the behavioral test to drugs effects depends upon the complexity of the problem.

A related observation was that of an increased sensitivity of the EEG of the animal performing the discrimination to the effect of drugs. This was noticed particularly with scopolamine and chlorpromazine, which, together with the blocking of the responses, produced EEG synchronization in doses (respectively 0.025 mg/kg and 1.0 mg/kg) previously found to have no effect. It seems that the EEG sensitivity to drugs may also depend upon the complexity of the animal's problem. In view of the potential significance of this observation, this problem would seem to warrant further investigation.

An analysis of the differential effect of the drugs investigated on the performance of the discrimination response indicates that the different compounds affect performance and EEG activity in quite different ways. Of the drugs investigated, amphetamine was the only one found to reliably interfere with the discrimination. In fact, at a certain dosage level, *all animals* responded by pulling the ring at the sound of either CS or DS. With the other compounds, blocking of this type was observed only occasionally either preceding or following a period of complete unresponsiveness. Observation of the animals as well as analysis of the response latencies suggest that amphetamine impairs the discrimination by interfering with the animals' ability to inhibit their response to the DS.

The effect of amphetamine on the animal which was only able to perform the simple instrumental response was similar to that obtained in the previous investigation: the performance was enhanced rather than impaired by the drug. Although the animals were highly active, their behavior was not disorganized in a manner observed with LSD. This compound, which was the most active of the drugs investigated, produced a general loss of the ability of the animals to recognize the significance of the critical stimuli in the test. Although they remained generally mobile, the organized performance of the instrumental response and discrimination were completely disrupted. In a recent report on the effect of LSD on auditory discrimination in cats, KEY (1961) concluded that this drug interfered with the discrimination by decreasing the subject's ability to distinguish between significant and insignificant stimuli. The more complete disorganization found in the present study may be due to a difference in technique, species, or dose level. An additional observation regarding LSD, the twitching of the jaw accompanied by the sharp high voltage waves in the EEG (Fig. 3), deserves some comment. Since the phenomenon was observed during the auditory signals, it is possible that was a residual of the response blocked by the drug.

In view of the interest in understanding the basis of the tranquillizing effects of chlorpromazine, most behavioral studies on this drug have used techniques requiring the subjects to escape or avoid noxious stimuli. In general, the findings of these investigations have indicated that chlorpromazine interferes with the ability of subjects to avoid a noxious stimulus without affecting other abilities (DINSMOOR and LYON 1961).

The present findings indicate that the drug may also affect performance based on *approach* motivation, if the requirements of the situation are complex.

The effect of scopolamine was similar to that of chlorpromazine in that the EEG was synchronized and performance disrupted. However, these changes with scopolamine were accompanied by an increase in motor activity rather than a decrease as was found with chlorpromazine. In a previous study (SADOWSKI and LONGO 1962) both the EEG and behavioral effects of scopolamine were counteracted by injections of eserine. Thus these findings provide additional evidence that the effects of cholinergic compounds can be observed on both EEG *and* behavior when the behavioral tests required a reasonably complex type of response.

Summary

This paper reports an investigation of the effect of several drugs on the performance of an instrumental reward discrimination as well as on the cerebral electrical activity of freely moving rabbits. Four adult rabbits bearing chronically implanted cortical electrodes were first trained to pull a ring at the sound of a buzzer for a food reward. The animals were later trained to discriminate between a continuous buzzer (which was followed by a reward) and an intermittent buzzer (which was not followed by a reward).

No differences were observed in the EEG responses to positive and negative stimuli; the EEG was at all times desynchronized.

The effect of several drugs upon different components of the instrumental discrimination responses and EEG activity was then studied. The drugs investigated tended to fall in three general classes:

1. Amphetamine, which blocked the discrimination without affecting the instrumental response. The EEG in these cases was continuously desynchronized.

2. Chlorpromazine and scopolamine, which blocked concomitantly the discrimination and the instrumental response. During the block of the response the EEG was synchronized.

3. LSD, which blocked concomitantly the discrimination and instrumental response. During the block of the response the EEG showed no signs of synchronization.

The significance of these findings are discussed.

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