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Isodirectional Conditioning Effects of d-Amphetamine and Pentobarbital on Schedule-Controlled Operant Behavior in Pigeons

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WATANABE, S. *Isodirectional conditioning effects of d-amphetamine and pentobarbital on schedule-controlled operant behavior in pigeons.* PHARMACOL BIOCHEM BEHAV **36**(1) 157–161, 1990.—Pigeons were trained to peck a key on a multi FR30–FI3′ schedule. Presession injections of d-amphetamine (2 mg/kg) and pentobarbital (7.5 mg/kg, 10 mg/kg) were paired with presentation of a red light on the ceiling of the operant chamber. After the five pairings separated by two saline sessions in which a white light was lit, the red light was presented without drug injection. The red light came to cause an isodirectional (drug-like) effect on operant behavior. When the red light was paired with drug injections and operant behavior was prevented from occurring, the light did not acquire isodirectional conditioned effect. Thus, responding in the presence of the drug effect is necessary to establish the conditioning.

Conditioning Operant behavior Amphetamine Pentobarbital

REPEATED administration of a drug can lead to a loss or an enhancement of its initial effect. The former is tolerance and the latter sensitization. These phenomena result from two factors; a change of the pharmacological effect itself and environmental factors associated with the repeated administration. Repeated drug administration in an environment can be considered to be a respondent (classical) conditioning procedure in which the environment is a CS (conditioned stimulus) and the drug is a US (unconditioned stimulus). Therefore, drug effects in chronic administration should be a summation of pharmacological effects of the drug (unconditioned response) and effects elicited by an environmental CS (conditioned response). In fact, a CS alone can produce drug-like effects after repeated administration. For example, after several administrations of amphetamine, certain aspects of the environment in which amphetamine was given came to have drug-like (isodirectional) effects on body temperature (7), motor activity (1,11) and operant behavior (9). Such effects were also obtained with CS associated with cocaine injections (2).

However, CSs associated with drug injections have also been found to produce conditioned opposite directional effects instead of isodirectional effects. For example, conditioned hyperthermia was observed after repeated administration of drugs which originally caused hypothermia, such as ethanol (13,14) or pentobarbital (4). Conditioned hypothermia and hyperalgesia were observed after repeated morphine injection (16,18). Such opposite directional effects have been considered to be a compensatory response which reduces the drug effect.

Using tolerance to morphine analgesia, some aspects of respondent conditioning such as latent inhibition (17,19), blocking (5), overshadowing (5,20) and sensory preconditioning (6) have also been examined. However, little information is available about conditioning of drug effects on operant behavior. In the following experiments, respondent conditioning of drug effect on schedule-controlled operant behavior was examined with pigeons, and the role of emission of the operant behavior in drug-induced state was also examined.

EXPERIMENT I

As described in the introduction, stimulants have caused isodirectional conditioned effect in autonomic responses. In Experiment I, respondent conditioning of the effects of d-amphetamine on schedule-controlled behavior was examined to clarify whether the isodirectional conditioning occurred also in operant behavior.

METHOD

Subjects

Five pigeons (*Columba livia*) were used. They had history of operant conditioning but not of pharmacological experiments. The birds were maintained at 80 percent of their free-feeding weights throughout the experiment.

Apparatus

The experimental chamber was an operant chamber for the pigeon (30 × 30 × 30 cm) with a single key. The diameter of the

key was 3 cm and line stimuli were presented on it by an in-line microprojector. The line stimuli were horizontal or vertical illuminated lines of 2 mm wide on a dark field. Red and white miniature lamps (DC 24 V) were fixed on the ceiling of the chamber. Luminance of the lamps was 1 lux. There was continuous fan noise during the experiment. The experiment was arranged by a computer system.

Behavioral Procedure

Preliminary training. Because the birds had not been used for experiments for months, they were first trained on continuous reinforcement and then FR training before training on multi FR30-FI3′ began. The training began with the horizontal line presentation. The 30th response in the presence of the horizontal line was reinforced, and the stimulus on the key was changed to the vertical line. If the bird did not emit 30 pecks for the horizontal line within 3 min, the stimulus on the key was also changed to the vertical line without reinforcement. When the vertical line appeared on the key, the first response after 3 min was reinforced and the stimulus was changed again to the horizontal line and the FR30 schedule became effective. If the bird did not respond for 2 min after passage of 3 min from the start of the FI, the stimulus was changed to the horizontal line without reinforcement. One daily training session consisted of ten presentations of each stimulus. During this training the white ceiling lamp was lit. This training continued until the birds showed a steady schedule-controlled behavior under both schedule components on visual inspection of cumulative records.

Criterion of steady state was 1) getting 10 reinforcements on each schedule, 2) responding without pause on FR schedule, and 3) a scallop pattern of responding on FI schedule.

Habituation

Saline was injected 10 min before start of daily training. The behavioral procedure was identical to that in the preliminary training sessions except that a red lamp was lit as a ceiling light. This training was repeated until the birds showed no disturbance of responding by the red lamp.

Conditioning

After the habituation phase, the subjects were injected with d-amphetamine or saline 10 min prior to the daily behavioral sessions. The CS (the red ceiling light) was lit when d-amphetamine was injected, whereas a white ceiling lamp was lit when saline injected. d-Amphetamine was injected on every third day, and the sequence of saline-saline-drug was repeated five times.

Test

The birds were injected with saline 10 min before the start of behavioral training and during the behavioral training the red lamp (CS) was lit.

Pharmacological Treatment

d-Amphetamine was dissolved in physiological saline and injected into a breast muscle in a volume of 1.0 ml/kg body weight. Dose was 2 mg/kg in terms of salt.

RESULTS

The subjects were divided into two groups according to effects of d-amphetamine on FI schedule-controlled behavior during the conditioning phase. As shown in Fig. 1 the drug increased response rate in three birds but decreased in other two. Two-way ANOVA (group × session) of the relative change of response rate gives a significant difference between two groups, $F(1,15)=6.86$, $p<0.01$, but no significant effect of sessions, $F(4)=0.26$, or interaction, $F(4)=0.20$.

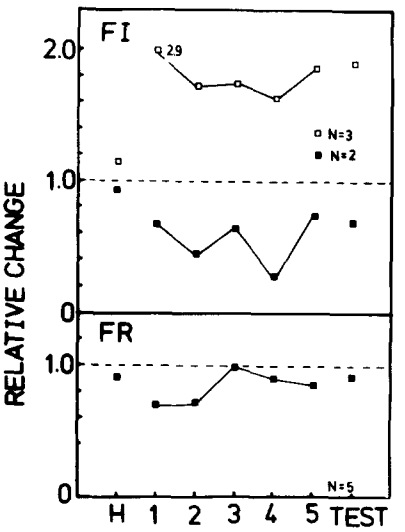

FIG. 1. Relative change of response rate in FI and FR components. The change is calculated by dividing the number of response in each session by that in a session immediate before the session. H indicates the final session of habituation and TEST the session with CS without d-amphetamine. Pairing of CS and the drug was repeated five times. Three subjects showed increase in responding for FI component and two subjects showed decrease.

Mean response rate in the FI component in the five saline sessions just before the drug sessions during conditioning was 24.6 respones/min, ranging from 10.8/min to 32.4/min for the increased rate group, whereas the rate for two birds in the rate-decreasing group was 80/min and 57.6/min respectively. According to a two-way ANOVA of response rate (group × session), in the saline sessions there was a statistically significant difference in response rate between the increased rate birds and the decreased rate birds, $F(1,15)=15.0$, $p<0.05$, but no effects of sessions, $F(4)=0.57$, or interaction, $F(4)=0.87$.

Therefore, the birds with high rate responding under the FI schedule showed decreased responding by d-amphetamine injection, and those with low rate responding in the baseline sessions showed increased responding in the drug sessions.

For the birds which showed the increasing effect of d-amphetamine, the CS followed by saline injection (test session) produced increased responding (Fig. 1). Correlated one-tailed t-test gave a significant difference between the habituation and the test, $t(3)=7.45$, $p<0.005$. On the other hand, the CS in the test session decreased response rate in the subjects which showed decreased rate during the conditioning phase, $t(2)=3.09$, $p<0.05$.

There was no overlapping case between the two groups in response in the test phase. Thus, the direction of CS effects agreed with the direction of effects of US (drug).

Relative changes in the pause in FI component are shown in Fig. 2. The pause was defined as the time from the start of the component to the occurrence of the first pecking response. The increased rate group showed shortened pauses during conditioning and also in test, and the decreased rate group had prolonged pause during both conditioning and test. The results of two-way ANOVA

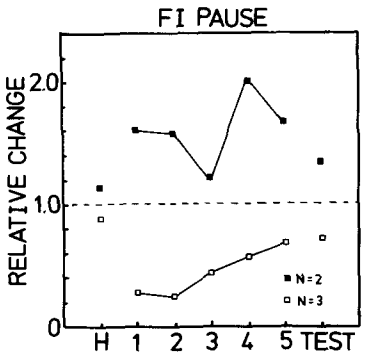

FIG. 2. Relative change of pause in FI component. The change is calculated by a similar method to the method described in Fig. 1.

of the relative change of the pauses during the five conditioning sessions disclosed that there was a significant difference between the two groups, $F(1,15)=49.1$, $p<0.05$, but no significant effect of the sessions, $F(4)=1.4$, or interaction, $F(4)=0.58$. And there was no overlapping case between the groups in the test session.

Injection of d-amphetamine did not have clear effects on FR-controlled behavior. There was no significant difference in the relative rate in the FR component between the two groups [two-way ANOVA, $F(1,15)=0.61$]. The effects of sessions and interaction were not significant either, [$F(4)=0.61$, and $F(4)=0.10$, respectively]. Effects of CS on FR rate in the test session did not differ from those in the habituation session, nor from those in the conditioning sessions.

EXPERIMENT II

Experiment I clearly demonstrated isodirectional conditioning of amphetamine effects on operant behavior. In Experiment II conditioning with a depressant was studied using pentobarbital. Before and after procedure of behavioral tolerance has shown that emission of behavior in presence of a drug effect is critical in order to develop tolerance [for example, (3)]. In Experiment II the role of the occurrence of operant behavior in the presence of drug was examined in conditioning paradigm.

METHOD

Subjects

Eight experimentally naive pigeons (*Columba livia*) were used. They were maintained at about 80 percent of their free-feeding weights.

Apparatus

The experimental chamber was similar to that used in Experiment I.

Procedure

The preliminary training and CS habituation was exactly the same as that for Experiment I. That is, the birds were trained on mult FR30-FI3′. After habituation to CS (a red ceiling lamp in the chamber), the birds were divided into two groups of four. The conditioning group was injected with pentobarbital 10 min before the start of training on every third daily session. When the drug was injected, the chamber light was red, whereas it was white on the other days when saline was injected. This treatment (three-day cycle of saline-saline-drug) was repeated five times. The control group received the same pharmacological treatments. But when these birds were injected with the drug, they were put in the operant chamber with the key covered for 30 min. During this 30 min no schedule was in effect and the red ceiling lamp was lit. On the saline sessions the white lamp was lit and mult FI-FR was effective.

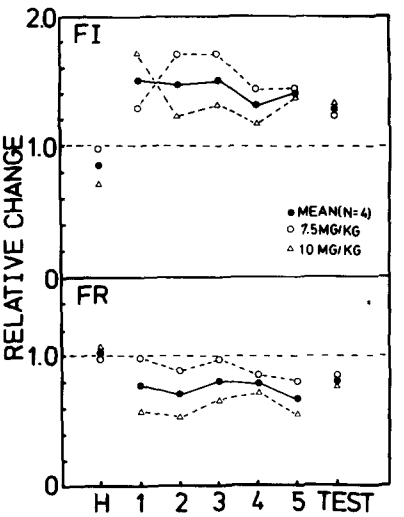

FIG. 3. Relative change of response of Conditioning Group in FI and FR components. H and TEST indicate the final session of habituation and the session with CS without pentobarbital respectively. Pairing of CS and the drug was repeated five times before the test session.

Test

Every subject was injected with saline 10 min before the start of the session and the red lamp was lit during the session.

Pharmacological Procedure

Sodium pentobarbital (Somnopentil; Pitman Moore) was dissolved in physiological saline and injected into breast muscle in a volume of 1.0 ml/kg body weight. For two birds in each group the dose was 7.5 mg/kg and the dose was 10 mg/kg for other birds.

RESULTS

Figure 3 showed performance of the conditioning group in the last session of the habituation phase, the conditioning sessions, and the test session. Response rate in each session was expressed as a ratio to the response rate in the last saline session just before the treatment sessions. Mean response rate in the saline sessions was 30.5/min for FI and 174/min for FR. In the habituation phase the CS had a slight decreasing effect on responding under the FI schedule and no effects on that under the FR schedule. Injection of pentobarbital resulted in increased responding under FI schedule but in decreased responding under the FR schedule. The increase in responding on FI schedule was stronger after the 7.5 mg/kg injection than after the 10 mg/kg injection, whereas the 10 mg/kg injection produced larger decreases in responding under FR schedule than did the 7.5 mg/kg injection.

In the test session, the CS alone increased responding under FI and decreased responding under FR. Thus, the CS acquired isodirectional conditioned effects on both FI and FR schedule-controlled behavior. There was a statistically significant difference

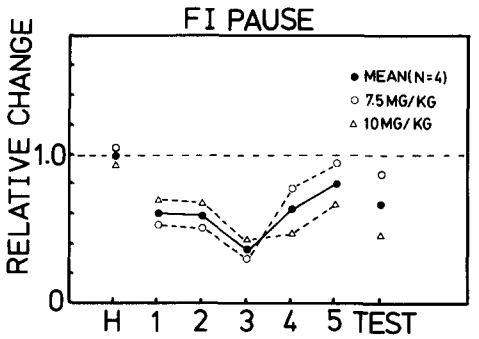

FIG. 4. Relative change of pause in FI component obtained from Conditioning Group.

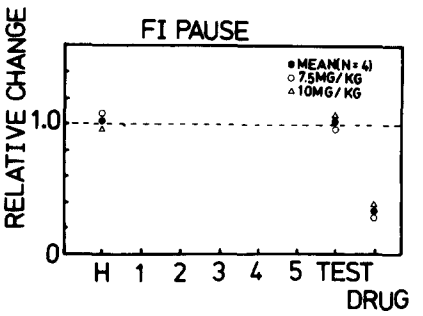

FIG. 6. Relative change of pause in FI component obtained from Control Group.

in response rate in FI component between the habituation and the test [correlated two-tailed t-test, $t(3)=3.50$, $p<0.05$], but not between the last session of conditioning and the test, $t(3)=0.45$. For FR-controlled behavior, however, there was a small difference between the habituation and the test, $t(3)=2.63$, $p<0.10$, and no significant difference between the last session of conditioning and the test, $t(3)=1.44$.

Figure 4 shows change of pause in the FI component. The change was expressed as change relative to the pause obtained from the last session before the treatment sessions. Pentobarbital injection during conditioning shortened the pause, and the CS alone seemed to result in a shortened pause in the test session. There was no significant difference test, $t(3)=1.10$, however, the pause in the test session differed from that in the habituation with a small significance, $t(3)=2.00$, $p<0.25$.

Figures 5 and 6 present results from the Control group. After five sessions of pairing of CS and pentobarbital injection, CS did not acquire any effects upon schedule-controlled behavior. Effect of CS in the test habituation session [$t(3)=1.42$, for response rate under FI schedule, $t(3)=0.93$ for FR schedule, and $t(3)=0.09$ for pause in FI schedule].

Comparison of results of the test session in both groups produced a significant difference in response rate under the FI schedule [uncorrelated two-tailed t-test, $t(6)=2.45$, $p<0.05$], but almost no significant difference in rate under the FR schedule, or in the length of pause in FI component, $t(6)=1.03$ and 1.67 respectively, $p<0.25$.

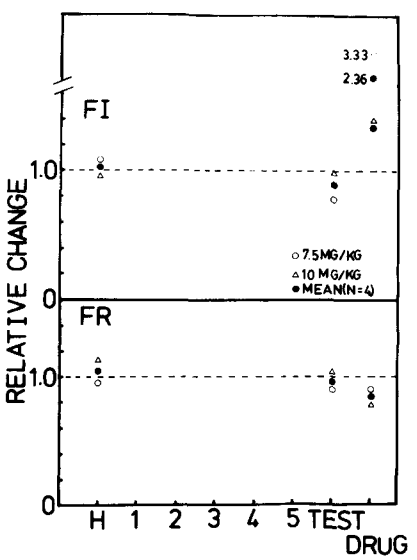

FIG. 5. Relative change of response in Control Group. There was no behavioral training in intoxicated state during the conditioning sessions. Test means CS without pentobarbital and DRUG indicates performance in intoxicated state.

DISCUSSION

The present results demonstrated 1) that establishment of isodirectional conditioning of drug effect upon schedule-controlled operant behavior by both stimulant (amphetamine) and depressant (pentobarbital) drugs and 2) that the conditioning effect requires responding in the presence of the drug.

In most of the published experiments CS associated with the drug came to have drug-like actions on autonomic responses [for example, (7,8)]. Thus, these behavioral results with d-amphetamine agree with previous results. Furthermore, CS decreased responding in the subjects for whom the drug (US) decreased their responding, and increased responding in those for whom the drug increased their responding. CS exactly mimicked the direction of the drug action. The length of pause in FI schedule-controlled behavior was prolonged by the CS alone when US (the drug) had prolonged the pause. Thus, the CS acquired a drug-like effect on pattern of responding too.

The birds which showed decreasing effect of d-amphetamine responded with high rate in the habituation phase, whereas those which showed increasing effect by the drug responded less often in the habituation phase. Thus, direction of the drug action depended on responding rate in the baseline session (rate-dependency) was observed.

CS associated with pentobarbital has been shown to result in compensatory autonomic responses (4,12). But the present results clearly showed not opposite directional, but isodirectional conditioned effects. Analysis of the pause in FI schedule-controlled behavior showed that the CS-induced behavior pattern was similar to that induced by the US. Unfortunately, there has been no other report on conditioning of depressants' effect upon operant behavior. Conditioning of drug effects may occur in some aspects of drug actions but not in other aspects. For example, Goldberg and Shuster (10) observed that a CS associated with nalorphine injection to morphine-dependent monkeys caused suppression of responding on FR schedule, vomiting and salivation but did not cause change in respiration or body temperature. Thus, the CS mimicked some aspect of the drug but not all of it. Because the present experiment failed to show clear conditioning of the drug effects on FR-controlled behavior, occurrence of conditioning might depend on the type of schedules. But the drug effects on FR-controlled behavior was weak in the conditioning phase. Thus,

conditioned response was not clearly obtained probably because of weak unconditioned response (the drug effect).

In the present experiment, responding under the drugged condition had an essential role in developing conditioning. In other words, pairing of the CS with emission of operant behavior in the presence of the drug is necessary to make the CS acquire the drug-like action and pairing of CS with drug injection was not enough to establish conditioning. Using so-called before/after design, it has been reported that drug injection before behavioral training developed behavioral tolerance but that the injection after the behavioral training did not [for example, (3,15)]. These results agree with the present results. But Glowa and Barrett (9) found response suppression by the CS associated with postsession injection of amphetamine in pigeon's FI-controlled behavior.

Finally, neither tolerance nor sensitization was observed during the conditioning phase of the present experiment. Tolerance and sensitization have been developed usually within a few repeated administrations, however, the drug injection was administered every third day in the present experiment. Such spaced administration might have disturbed development of tolerance or sensitization. But one important implication is that isodirectional conditioning of the drug effect was obtained even though tolerance or sensitization was not observed. In other words, conditioning and tolerance or sensitization are not identical processes even though they share common features.

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