Drug states as modulators of conditioned immobility in a latent discrimination procedure

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Abstract

Midazolam, amphetamine, and flesinoxan were used in four rat experiments to examine the usefulness of a latent Pavlovian discrimination procedure to assess the discriminative-stimulus, or occasion-setting, properties of drugs. Experiment 1 first assessed the unconditioned effect of each of the drugs on the dependent measure used, which was immobility. Relative to saline, midazolam enhanced immobility, whereas flesinoxan, and especially amphetamine decreased it. In each of the Experiments 2–4, subjects received a limited number of training sessions during which they consistently received a footshock in a distinctive box after a drug but not after saline, or vice versa. Subsequently, non-reinforced test sessions were performed in the drug and saline states in both the conditioning box and a novel box. Relative to the saline state, rats previously shocked under midazolam were less mobile in the conditioning box under midazolam, whereas rats previously not shocked under amphetamine or flesinoxan were more mobile under the drug. The remaining animals did not show differential responding. The response profiles were accounted for in terms of the combined operation of an associative, or occasion-setting, effect and a non-associative effect of the drug-induced states.

Keywords: Occasion setting; Pavlovian conditioning; Conditioned immobility; Midazolam; Amphetamine; Flesinoxan

1. Introduction

Using a so-called 'latent discrimination procedure', Davidson et al. (1992) showed that food-deprivation intensity stimuli can acquire modulatory control over an aversively motivated conditioned response. Rats first received discrimination training sessions in a distinctive context in which an electric footshock was delivered or not, depending on the level of food deprivation induced. The animals were shocked when they were under Deprivation Level 1 (e.g. after 24 h of food deprivation) and were not shocked if they were under Deprivation Level 2 (e.g. after 0 h of food deprivation). No more than three shock and three non-shock sessions were given in a semi-random order. At the end of this discrimination training phase, no differential freezing, i.e. a species-specific defense reaction that may be conditioned to contextual cues (e.g. Blanchard et al., 1968; Bolles and Collier, 1976; Fanselow, 1980, 1982), could be observed. All animals showed near-asymptotic freezing, regardless of whether they were under the shocked or under the non-shocked deprivation level. However, differential freezing became apparent in a subsequent test phase which was conducted during extinction. This phase consisted of repeated placement of the subjects in the training box under either Deprivation Level 1 or Level 2, with the shock never being presented. Using this test procedure, more rapid extinction of freezing under the non-shocked deprivation level than under the shocked level could be observed.

These results can be explained in at least two different ways. One explanation is that the training phase resulted in asymptotic responding under both deprivation levels, which reflected a strong context-shock association. In addition, the internal stimuli corresponding with the shock-deprivation level and/or the non-shock deprivation level had become directly associated with the occurrence and the non-occurrence of a shock (direct associations), respectively. The effect of the positive or negative associative strength acquired by the deprivation stimuli only became apparent as the strength of the context-unconditioned stimulus association decreased in the course of testing under extinction, thereby eliminating ceiling effects. During test-

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ing, associative summation of context and the non-shock deprivation cues resulted in a weaker response than did the associative summation of context and shock-deprivation cues.

A second explanation is that the stimuli arising from the deprivation levels modulated the strength of the context-shock association through a process called 'occasion setting' (e.g. Bouton, 1993; Holland, 1985). That is, the deprivation cues were not directly associated with the occurrence or non-occurrence of shock (schematically: shock-deprivation cues → shock, and/or non-shock-deprivation cues → no-shock), but signalled that placement in the training context would be followed by shock (shock-deprivation cues → [context → shock]) or not (non-shock-deprivation cues → [context → no-shock]). This occasion-setting property became apparent in the course of the test sessions as ceiling effects were gradually eliminated.

The present paper addresses two questions. First, is it possible to use a similar latent Pavlovian discrimination procedure in drug-discrimination research? Second, if so, is it possible to differentiate between the different theoretical associative structures underlying discrimination performance?

Concerning the first question, it must be noted that, traditionally, the discriminative-stimulus properties of drugs have been assessed using a two-choice operant task in which a subject has to learn that a reinforcer is only presented after Response 1 (such as a press of Lever 1) when under the influence of the drug, and only after another response, Response 2 (such as a press of Lever 2), when under the influence of saline or another drug (e.g. Colpaert and Slangen, 1982; Slangen, 1991). Typically, tens or even hundreds of discrimination training trials are needed before significant discrimination performance emerges.

Given the length of training required in operant tasks, it may be desirable to search for procedures that enable a faster evaluation of the discriminative properties of drugs. To this end, a classical conditioning procedure was used in the present experiments similar to that described by Davidson et al. (1992). Instead of manipulating food-deprivation levels, different drugs were used that previously have been used successfully in two-lever drug-discrimination experiments. Specifically, the benzodiazepine agonist midazolam, the stimulatory agent amphetamine, and the serotonergic agonist flesinoxan were used. The dose of each drug was determined on the basis of previous drug-discrimination research (e.g. Corrigall et al., 1992; Druhan et al., 1991; Rodgers et al., 1994; Sannerud et al., 1991; Ybema et al., 1994). The aim was to select a dose that would be sufficiently high to obtain reliable discrimination performance and that, at the same time, would minimize any clear direct, unconditioned behavioural effects. Concerning the latter point, it must be noted that one potential problem that may especially occur using the proposed Pavlovian

discrimination procedure is that, even with relatively low doses, the drugs may still have a measurable unlearned, or non-associative, effect on the response measure used, such as behavioural suppression or activation. Such an effect may confound discrimination performance. However, as will be indicated by the results of the present experiments, the presence of an unlearned effect does not necessarily hinder a correct interpretation of the response profile during discrimination testing. For instance, one can first independently assess the magnitude of any unlearned effect on the dependent measure and use this knowledge when interpreting subsequent discrimination learning and performance. As will be shown, a consistent account of test results is possible regardless of whether one uses a drug that may have sedating effects (midazolam), a drug that may have an activating potential (amphetamine) or a drug that is expected to have less prominent depressing or stimulatory properties (flesinoxan). The different potential unlearned behavioural effects are a further motive for selecting the present drugs.

Regarding the second research question, test sessions were also conducted in a novel and neutral context in each of the Experiments 2–4, to evaluate the associative structure underlying discrimination performance (see below). If no differential responding is seen in this neutral context, the differential conditioned responding observed in the training context (discrimination performance) cannot be based on direct associations between drug states and shock. The associative strength of the drug states is expected to also exert a differential effect on conditioned responding in contexts other than the training context. However, if the drug states were functioning as occasion setters, signalling a specific relationship between the *training* context and shock, no differential conditioned responding should be observed in a box that is distinctively different from the original training box. The drug states simply do not provide any information regarding the relationship between a novel context and shock.

2. Materials and methods

2.1. Subjects

Seventy-two experimentally naive female Wistar rats served as the subjects. Twenty-four were used in Experiment 1, and 16 in each of the Experiments 2–4. The rats had a mean body weight of 221 g (range 181–262 g). The subjects were housed individually and had free access to food and water. They were maintained on a 12-h/12-h light-dark cycle and the experimental manipulations were conducted during the middle portion of the dark phase.

2.2. Apparatus

Four identical boxes were used for training and most of the test sessions. Each box measured 24.5 × 25 × 24 cm

and had a clear Plexiglas front and back wall and aluminium side walls. The lid was clear plastic. The floor was composed of 3-mm stainless-steel rods through which a 0.9-mA, 0.5-s scrambled electric shock could be delivered. Each box was inserted in a sound-attenuating chest. The chest had a clear Plexiglas front wall that enabled monitoring of the behaviour of the rat inside. A ventilation fan provided masking noise. A 24-V, 2.8-W houselight, located 24 cm above the grid floor, illuminated each box. Sessions were recorded by means of a low-light video camera. The camera was placed in front of the boxes in such a manner that four rats could be monitored simultaneously. Pacing of the scoring of the rat's behaviour was aided by a flashing light (see below). This light was monitored by the camera but was not visible to the rats. A second set of boxes, hereafter called 'novel box' or 'context', consisted of two Plexiglas boxes. Each box measured 30 × 24 × 35 cm. The side walls and the floor were green Plexiglas; the front and back wall were clear plastic. These boxes were located in a room that was illuminated by one white fluorescent strip light.

2.3. Drugs

Three drugs were used. The first was midazolam (Roche Nederland). In the first training session of Experiment 2, a dose of 0.32 mg/kg dissolved in saline (0.9% NaCl) was used, whereas on all other midazolam sessions of Experiments 1 and 2, a dose of 0.10 mg/kg was used. The dose was lowered from 0.32 mg/kg to 0.10 mg/kg because the former dose was the first one we used in the present series of experiments and, judging from the rats' spontaneous activity level, clearly had a sedating effect. The second drug was amphetamine (0.5 mg/kg in saline) (RBI Research Biochemicals International). Finally, the third drug used was flesinoxan (Solvay-Duphar). The dose used was 0.3 mg/kg dissolved in saline. All substances were injected subcutaneously in a volume of 2 ml/kg. The injection-box placement interval was 8 min on midazolam sessions, and 13 min on amphetamine and flesinoxan sessions.

2.4. Assessment of unconditioned drug effects on immobility

The purpose of Experiment 1 was to assess whether or not each of the drugs had an unconditioned effect on the response measure used, which was immobility (see section 2.6.). The rats first received a training phase consisting of six sessions. The interval between sessions was approximately 24 h, except for the interval between the third and fourth sessions, which was 72 h (week-end). During each session, the rats were first injected with saline and were placed in the training box 8–13 min thereafter. No events were planned during the first 4 min of each session. After these 4 min, the rats either received a shock or not, according to the following schedule: For one half of the

rats, the order of training sessions was sh, nosh, nosh, sh, sh, nosh, with sh and nosh designating a shock and a no-shock session, respectively, whereas the other half of the rats received the order nosh, sh, sh, nosh, nosh, sh. These sessions were intended to result in substantial levels of immobility on the basis of exactly the same training regimen that was also to be used in each of the drug-discrimination Experiments 2–4. The only difference between the training phase of Experiment 1 on the one hand, and that of each of the Experiments 2–4 on the other was that in the former experiment saline was used on both shock and no-shock sessions, whereas in the latter experiments different internal states were induced on different session types. Hence, the rats in Experiment 1 had no internal cues on the basis of which they could learn to predict the occurrence of shock, whereas those in experiments 2–4 had. The critical tests were performed after training and consisted of 6 two-session test cycles. In the first session of each test cycle, the rats in Group M were injected with midazolam, those in Group A with amphetamine, and those in Group F with flesinoxan. Each of these groups consisted of 8 subjects. Subsequently, the animals were simply placed in the conditioning box for 4 min without any further events being planned. In the second session of each test cycle, the animals received an identical treatment except that all rats were injected with saline instead of with a drug. The question of interest for the rats in each group was whether or not differential immobility would become apparent under each of the drugs vs. saline.

2.5. Assessment of discriminative properties

The training phase for the animals in each of the Experiments 2–4 was identical to that employed in Experiment 1, except that two different internal states were induced in each experiment during shock and no-shock sessions. Specifically, the rats in Group M-sh in Experiment 2 (the number of subjects in this, and all other groups was eight) were shocked under midazolam and not shocked under saline, whereas the subjects in Group S-sh in that experiment were shocked under saline and not under midazolam. Similarly, the rats in Group A-sh in Experiment 3 were shocked under amphetamine and not under saline; the reverse conditions were in effect for the rats in Group S-sh in that experiment. Finally, in Experiment 4, the animals in Group F-sh were shocked under flesinoxan and not shocked under saline, whereas those in Group S-sh again were subject to the reverse contingency. The order of the saline (s) and drug (d) sessions for each rat in each of the Experiments 2–4 was: d, s, s, d, d, s. Subsequently, as in Experiment 1, all animals were tested in the training context in each of 12 sessions according to a simple alternation schedule: The first session was a drug session (thus, midazolam in Experiment 2, amphetamine in Experiment 3, and flesinoxan in Experiment 4), followed by a saline session, and so on.

On the day following the last test session in the training box, one half of the animals in each group was injected with the appropriate drug and was placed in the novel green box several minutes thereafter. They remained in this box for 4 min; no events were scheduled. The remaining rats received the same treatment, except that saline was administered. On the following day, the rats received an identical treatment as on the previous day, except that the alternative substance was used. Thus, the animals that had received the drug now received saline and vice versa. On the day after the last test session in the novel context, the rats in Group S-sh of Experiment 2, and all rats in Experiments 3 and 4, received two standard extinction sessions in the original conditioning box. One half of the animals was under the influence of the drug on the first of these sessions and was under the influence of saline on the second. For the other half, the reverse test order was in effect. The interval between the first and the second test sessions was 72 h. The purpose of these tests was to re-assess conditioned responding in the original training context in each state. This was done because a different pattern of responding was observed during the novel-context tests than during the training-context tests (see below). Re-assessment in the training context could, in case of re-occurrence of the 'old' pattern of responding identical to that observed during the initial training-context tests, assure us that the different pattern of responding in the novel box was due to the change in contextual cues and not to first performing test sessions in the training context.

2.6. Behavioural observation

Paced by a flashing light (1 s on, 4 s off), the rat's behaviour was scored as 'immobile' or 'mobile' once every 5 s (time sampling). A rat was considered to be immobile if no movements could be observed, except those caused by respiration and except for a 'pendulum motion' of the head. Frightened albino rats sometimes show a slow, rhythmical swinging head movement which can easily be distinguished from more abrupt head movements that occur in non-frightened animals. All other behaviour was scored as 'mobile'. Immobility can reflect a freezing response that is conditioned to shock-associated stimuli, such as contextual cues (e.g. Blanchard et al., 1968; Bolles and Collier, 1976; Fanselow, 1982). The present time-sampling procedure is relatively easy and has frequently been used in classical conditioning experiments with a shock as an unconditioned stimulus. Generally, the interobserver reliability using this procedure is high. The dependent measure of each group on each session was expressed in terms of the mean percentage of observations (out of 48) that were scored as immobile. In the present experiments, the reliability of the primary observer's scoring was examined by a second observer who was unaware of group assignment. The second observer re-scored a total of 11 sessions (8832 observations), selected from various

phases of Experiments 1–4. For each of the rats in each of these sessions, the total number of observations scored as immobile was compared with the corresponding immobility score by the primary observer. The mean Pearson's correlation coefficient was $r = 0.94$ (S.E.M. $= 0.01$).

3. Results

3.1. Unconditioned drug effects

The mean percentages of observations scored as immobile for each group during the test sessions in Experiment 1 are presented in Fig. 1. The figure shows that in all 'drug' sessions, the rats in Group M were more immobile than the rats in each of the Groups A and F. Subjects in Group A were less mobile than the animals in Group F on some drug sessions. On test days under saline, there were no consistent differences between the groups. Finally, the rats in Group M were less mobile under midazolam than under saline, whereas the animals in both Group A and Group F were more mobile under their drug state than under saline.

A Group × Drug (saline vs. no saline, i.e. midazolam, amphetamine or flesinoxan) × Cycle analysis of variance (ANOVA), with Group as a between-subject factor and Drug and Cycle as within-subject factors, was performed on the test data. The analysis revealed a significant main effect of Drug ($F(1,21) = 7.70$, $P < 0.05$) and Cycle ($F(5,105) = 52.33$, $P < 0.001$). The Group × Drug, and Group × Drug × Cycle interactions were also significant ($F(2,21) = 21.86$, $P < 0.001$, and $F(10,105) = 2.79$, $P <$
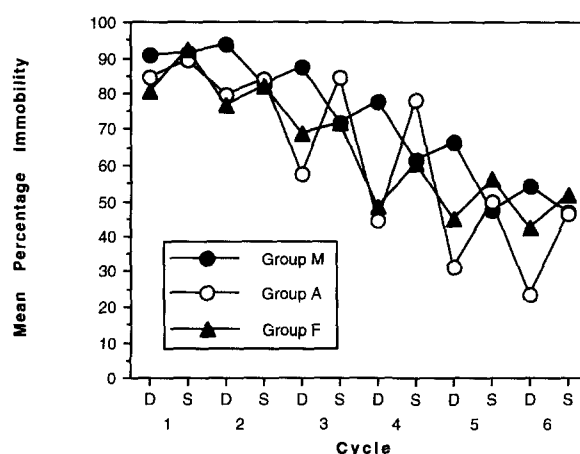

Fig. 1. Mean percentage of observations scored as immobile during the test sessions of Experiment 1, which were conducted in the conditioning box after the administration of a drug (D) or saline (S). In Group M, each test session under the drug state was performed after a midazolam injection, whereas in Group A and Group F, these tests were conducted under amphetamine and flesinoxan, respectively. All animals previously had received an equal number of shock-reinforced and shock-non-reinforced exposures to the conditioning box. Each of these exposures had taken place under saline.

0.01, respectively). The interaction between Group and Drug was examined by simple main effect analyses (Winer, 1971). The interaction reflects the fact that, across cycles, groups differed in mobility on drug days ($F(2,24) = 3.55$, $P < 0.05$), but not on saline days ($F < 1$). On drug days, the rats in Group M were less mobile than the rats in each of the other two groups (Newman-Keuls, $P < 0.05$), which did not differ (Ps > 0.05). The three-term interaction was examined further by means of simple interaction analyses (Winer, 1971). These revealed that the Group × Drug interaction was significant on Cycles 3–6 (Fs$(2,111) > 6.48$, Ps < 0.01), but not on the first two cycles (Fs < 2.38, Ps > 0.05). Furthermore, Groups did not differ on all drug days; differences between groups were significant on each of the drug days of the last four cycles (Fs$(2,49) > 3.29$, Ps < 0.05), but not on the first two cycles (Fs < 1). On each of the drug days of Cycles 3–6, the rats in Group M were less mobile than those in each of the Groups A and F (Newman-Keuls, Ps < 0.01), and the subjects in Group A were more mobile than those in Group F (Newman-Keuls, Ps < 0.05).

3.2. Discriminative properties

The left side of Fig. 2 presents the mean number of observations that were scored as immobile for each group during each of the test sessions in the conditioning box performed in Experiment 2 (midazolam vs. saline).

The figure shows that Group M-sh consistently was more immobile when tested under midazolam than under saline. Group S-sh did not show a consistent difference in mobility between the different drug conditions. Furthermore, groups primarily differed on midazolam sessions

and not on saline sessions. A Group (2) × Cycle (i.e. pair of two sessions: 6) × Drug (2) repeated measures ANOVA was performed using the data depicted in Fig. 2. The analysis revealed significant main effects of Cycle ($F(5,70) = 21.90$, $P < 0.001$) and Drug, $F(1,14) = 22.14$, $P < 0.001$). The interactions between Group and Drug ($F(1,14) = 19.49$, $P < 0.001$), Cycle and Drug ($F(5,70) = 2.76$, $P < 0.05$), and Group, Cycle, and Drug ($F(5,70) = 2.44$, $P < 0.05$) were also reliable. Subsequent simple interaction analyses revealed that there was a significant interaction between Group and Drug on each of Cycles 4–6 (Fs$(1,69) > 11.39$, Ps < 0.01). The Group × Drug interaction on each of these cycles reflects the fact that the animals in Group M-sh were more immobile under midazolam than under saline (Fs$(1,14) > 11.14$, Ps < 0.01), whereas for the animals in Group S-sh, there was no difference in mobility under midazolam vs. saline (Fs$(1,14) < 2.53$, $P > 0.05$). Furthermore, in each of these cycles, the animals in Group M-sh were more immobile than those in Group S-sh when tested under midazolam (Fs$(1,14) > 7.78$, Ps < 0.05), but not when tested under saline (Fs < 1).

The middle panel of Fig. 2 shows that each group in Experiment 2 was more immobile under midazolam than under saline when the rats were tested in the novel context. The factor Test Order had no significant effect and neither did it reliably interact with any other factor. The same was true using all other test data of this and the following experiments. Hence, this factor was excluded from further analyses in all experiments. A Group × Drug ANOVA using the immobility scores obtained in the novel box only revealed a significant Drug effect ($F(1,14) = 11.39$, $P < 0.01$). The main effect of Group, and the Group × Drug
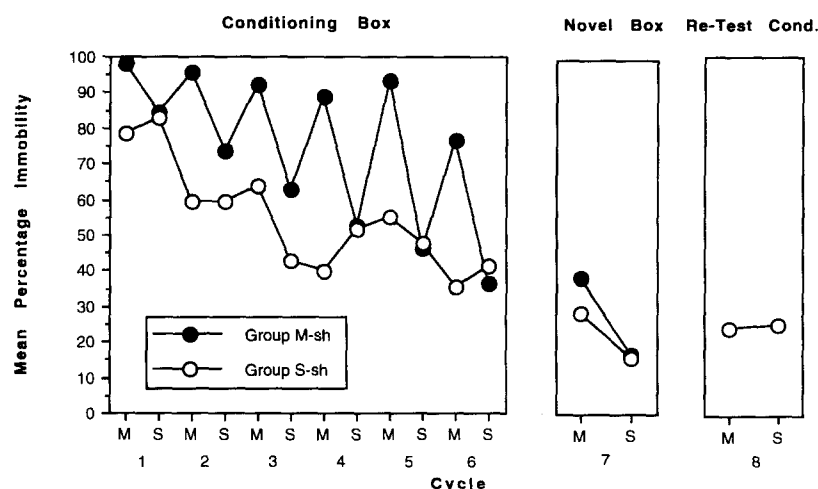

Fig. 2. Left: Mean percentage of observations scored as immobile for each group during the extinction test sessions in Experiment 2 under midazolam (M) and saline (S). Each of the test sessions was performed in the conditioning box. During a previous training phase, the rats in Group M-sh consistently had received a shock after placement in that box when injected with midazolam and no shock when injected with saline. The rats in Group S-sh had previously been shocked under saline and not shocked under midazolam. Middle and right portion: Mean percentage of observations scored as immobile in Experiment 2 during the midazolam and saline test sessions performed in a novel box and the original conditioning box after the novel-box tests, respectively.

interaction were not significant ($Fs < 1$). Thus, in both groups, midazolam evoked higher immobility levels than saline when the animals were in a novel box.

Finally, the right panel of Fig. 2 displays the immobility levels obtained for Group S-sh during the re-test in the conditioning context of Experiment 2. Different immobility levels could not be detected, as had also been the case during the previous test sessions in that context. A one-factor ANOVA with Drug as within-subject factor did not reveal a significant effect ($F(1,7) < 1$). Thus, in Group S-sh, a significant effect of midazolam vs. saline only emerged in the novel context; in the conditioning box, the different internal states had no effect on mobility.

The left panel of Fig. 3 presents the mean immobility levels for each group during each test session in the conditioning box in Experiment 3 (amphetamine vs. saline). The figure shows that Group S-sh systematically showed less immobility under amphetamine than under saline. Instead, Group A-sh consistently showed more immobility under amphetamine than under saline, although differential responding was far less prominent than was the case in Group S-sh.

A Group × Cycle × Drug repeated measures ANOVA on these data revealed a significant main effect of Cycle ($F(5,70) = 15.45$ $P < 0.001$) and of Drug ($F(1,14) = 10.63$, $P < 0.01$). In addition, the following interactions were also reliable: Group × Drug ($F(1,14) = 30.45$, $P < 0.001$), Cycle × Drug ($F(5,70) = 4.87$, $P < 0.001$), and Group × Cycle × Drug ($F(5,70) = 3.00$, $P < 0.05$). The Group × Drug interaction reflects the fact that under amphetamine, Group S-sh was less immobile than Group A-sh ($F(1,20) = 16.06$, $P < 0.01$), but not under saline ($F < 1$). Furthermore, Group S-sh was less immobile un-

der amphetamine than under saline ($F(1,14) = 38.53$, $P < 0.001$), whereas in Group A-sh, the internal state had no significant effect on mobility ($F(1,14) = 2.55$, $P > 0.05$). Finally, the three-term interaction was caused by the fact that Group S-sh differed from Group A-sh on the fifth test under saline, where the former group was more immobile than the latter ($F(1,46) = 4.59$, $P < 0.05$), but not on each of the other saline tests.

The middle portion of Fig. 3 shows the mean immobility score of the two groups during the tests in the novel box in Experiment 3. The figure suggests that the animals in both groups were more immobile under saline than under amphetamine. A Group × Drug ANOVA on the novel-box test data indeed only revealed a significant main drug effect ($F(1,14) = 6.81$, $P < 0.05$, other $Fs < 1.10$, $Ps > 0.30$).

The right portion of Fig. 3 depicts the level of immobility on the re-test cycle in the conditioning box, performed after the novel-box test in Experiment 3. A Group × Drug ANOVA on these data revealed a significant main drug effect ($F(1,14) = 11.10$, $P < 0.01$), as well as a significant interaction between Group and Drug ($F(1,14) = 6.79$, $P < 0.05$). This interaction was caused by the animals in Group S-sh showing less immobility under amphetamine than under saline ($F(1,14) = 17.63$, $P < 0.01$), whereas the rats in Group A-sh did not show differential immobility under the different states ($F < 1$). These results indicate that the same pattern of immobility was present in the conditioning box as had been present in that box during the sessions prior to the test in the novel box. Therefore, the context change was responsible for the change in the pattern of mobility seen in Group A-sh in the novel vs. conditioning box.
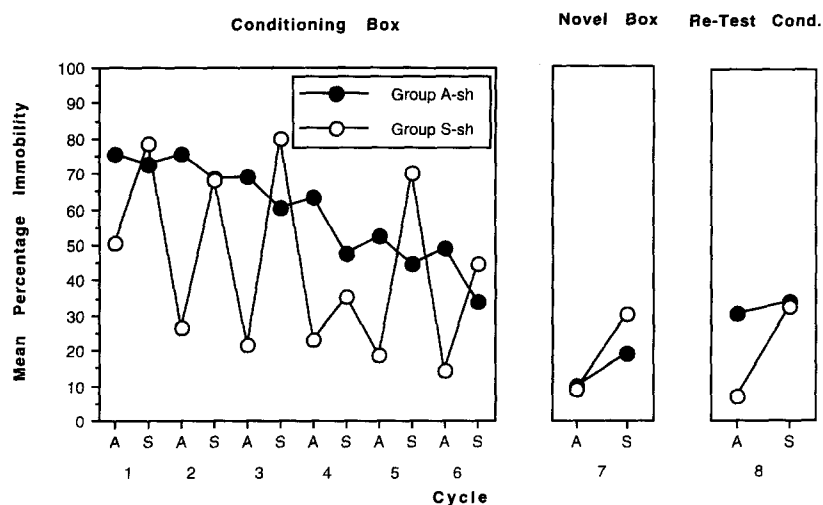

Fig. 3. Left: Mean percentage of observations defined as immobile for each group during the test sessions of Experiment 3. Sessions were performed in the conditioning box under amphetamine (A) and saline (S). During discrimination training, Group A-sh consistently had received shocks under amphetamine and no shocks under saline, whereas for Group S-sh these conditions were reversed. Middle: Mean percentage of observations scored as immobile for each group during the test sessions of Experiment 3 that were conducted under amphetamine and saline in a novel box. Right: Level of immobility during the last test cycle in the conditioning box, performed after the novel-box test sessions.

The results of the first test phase in the training context performed in Experiment 4 (flesinoxan vs. saline) are depicted on the left side of Fig. 4. It shows the mean percentage of observations that were scored as immobile for each of the two groups on each of the test sessions in the conditioning box. As can be seen, in the course of the test sessions, the animals in Group S-sh consistently were less immobile under flesinoxan than under saline, whereas in Group F-sh, there was only a gradual decrease of immobility without a consistent difference between flesinoxan and saline sessions.

A Group × Cycle × Drug repeated measures ANOVA using the immobility percentages from Fig. 4 revealed significant main effects of Cycle ($F(5,70) = 30.41$, $P < 0.001$) and Drug ($F(1,14) = 18.30$, $P < 0.01$). The Group × Drug interaction was also significant ($F(1,14) = 23.34$, $P < 0.01$) and is caused by the rats in Group S-sh showing more immobility under saline than under flesinoxan ($F(1,14) = 41.49$, $P < 0.001$), whereas the animals in Group F-sh did not have different immobility scores in saline vs. flesinoxan test sessions ($F < 1$).

The middle panel of Fig. 4 shows the immobility scores for the groups for each session in the novel box in Experiment 4. A Group × Drug ANOVA on the appropriate data only revealed a significant effect of Group ($F(1,14) = 12.11$, $P < 0.01$, other $Fs(1,14) < 3.25$, $Ps > 0.09$). Thus, in a novel test context, neither group showed different, state-dependent immobility.

Finally, the right panel of Fig. 4 shows the results of Experiment 4 of the test sessions which were performed in the conditioning box after the tests in the novel box. A Group × Drug ANOVA did not reveal any significant main or interaction effects ($Fs < 2.38$, $Ps > 0.15$).

4. Discussion

The results of Experiment 1 showed that midazolam, amphetamine, and flesinoxan have an unlearned, or performance, effect on conditioned immobility. Relative to a saline condition, under the conditions of our first experiment, midazolam increased the level of immobility, whereas flesinoxan and, especially, amphetamine reduced it. These effects must be incorporated in an interpretation of the results of the following experiments, Experiments 2–4, in which the drug states were used as potential discriminative stimuli.

Only one of the two groups included in each of the Experiments 2–4 demonstrated a statistically significant discrimination performance. Specifically, Group M-sh in Experiment 1, and Group S-sh in each of the Experiments 3 and 4 responded different in their shocked vs. non-shocked internal state, whereas Group S-sh in Experiment 2, Group A-sh in Experiment 3, and Group F-sh in Experiment 4 did not. This pattern of results can be most parsimoniously accounted for by referring to the joint operation of two different processes. The first is the above-mentioned and established unlearned (performance) effect of each of the drugs. The second process either worked for or against this unlearned drug effect and was based on the discrimination training regimen through which the internal drug cues acquired a discriminative potential. Somehow, the internal drug cues came to predict the occurrence or non-occurrence of shock in the conditioning box (for a discussion of the exact nature of the underlying associative structure, see below). This potential only became visible after partial extinction of the strong association between training context and shock that was apparent
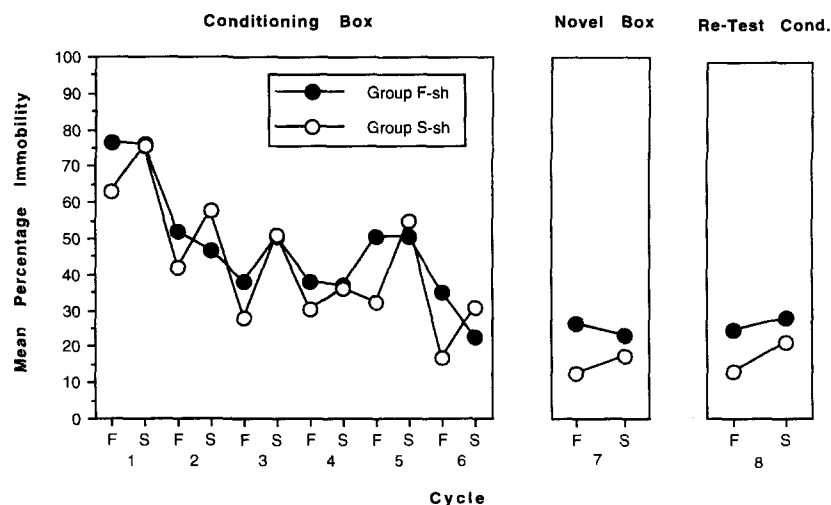

Fig. 4. Left: Mean percentage of observations scored as immobile for each group during the test sessions of Experiment 4. Sessions were performed in the conditioning box under flesinoxan (F) and saline (S). Group F-sh previously had been shocked after placement in the box after administration of flesinoxan, and not shocked after an injection with saline. The reverse relationship between induced internal state and the occurrence of shock in the box had been in effect for Group S-sh. Middle and right: Mean percentage of observations scored as immobile for each group during test sessions of Experiment 4 that were performed in a novel box and, subsequently, again in the conditioning box under flesinoxan and saline, respectively.

in each experiment in the first test session after training (elimination of ceiling effects). Accordingly, in Group M-sh of Experiment 2, midazolam directly suppressed mobility because of its unconditioned effect and also had become a discriminative signal for shock, which further reduced mobility. Likewise, for the rats in Group S-sh in Experiments 3 and 4, the internal cues present during drug sessions (amphetamine and flesinoxan, for Experiments 3 and 4, respectively) caused an unconditioned reduction of immobility and also had become discriminative stimuli for the absence of an otherwise expected shock, which further decreased the immobility level in the drug state, relative to the immobility level in the saline state. However, for the rats in Group S-sh of Experiment 2, the unconditioned immobility-enhancing effect of midazolam was counteracted or masked by the acquisition of discriminative properties by the midazolam cues (signals for the absence of shock), or vice versa. Similarly, for each of the groups of Experiments 3 and 4 that had received a shock under the drug but not under saline (Group A-sh and Group F-sh), the unconditioned mobility-enhancing drug effect was masked by discrimination learning, or vice versa.

At least three different theoretical views concerning the nature of the associative process that underlies discrimination learning can be proposed. First, the stimuli arising from the administration of the different drugs during training may have become directly associated with the presence or absence shock. However, the results of the test sessions in the novel box in each of the Experiments 2–4 are at variance with this explanation. If direct drug state-(no-)shock associations were responsible for test performance in the conditioning context, one would expect an equal pattern of immobility in the novel context and the conditioning context. However, such a result was not obtained. In the novel box, if anything, only an effect was observed in each of the groups that corresponded with the unconditioned effect on mobility of the specific drug under investigation, as was demonstrated in Experiment 1.

A second explanation rests on the assumption that conditioning context X in combination with internal state Y yields one configural, or unique, cue XY that directly becomes associated with the presence or absence of shock. One manner of conceiving this principle is that context X is perceived as a different context under different internal states and that rats come to associate context X under state Y (configuration XY) with shock, and context X under a different state, state Z (configuration XZ), with the absence of shock (see Pearce, 1987, for a formal configural learning model). Combined with the notion of an unlearned effect of each of the drugs, this principle could explain the results. Although such an explanation cannot be ruled out on the basis of the present results, it is inconsistent with previous findings that conditioned responding after simple aversive conditioning using an electric footshock (i.e. in the absence of a discrimination procedure of some sort) generally is not specific to the

conditioning context (Bouton, 1993). Thus, a stimulus conditioned in one context generally is perceived as the same stimulus, and evokes a conditioned response, when subsequently tested in another context, and the strength of this response does not differ from that seen in the original conditioning context. In our experiments too, conditioning on training Day 1 under one internal state yielded conditioned immobility when the animal was brought in a different state on training Day 2.

A third explanation rests on the notion of contextual occasion setting. Accordingly, rats learned to 'disambiguate' the current 'meaning' of the conditioning box in terms of the future occurrence/non-occurrence of a shock by using their internal state as a contextual stimulus signalling the relationship between box and shock. Specifically, the drug state either signalled or reminded the animal of the presence (positive occasion setting) or absence (negative occasion setting) of a box-shock association, and, in combination with its non-associative effect, determined the level of immobility under the drug state. An occasion-setting explanation is also in line with the test results in the novel box. Hence, the drug cues did not provide any information concerning the relationship between the novel context and shock/no-shock. Consequently, occasion setting was eliminated in each group, with the result that only the unconditioned drug effect could affect performance. Furthermore, the temporal arrangement between the stimuli is also consistent with the notion of occasion setting. Exposure to the target stimulus, viz. the conditioning box, was 'embedded' in exposure to interoceptive stimuli. To the extent that in the present experiments the internal cues function like external stimuli do, this temporal arrangement is conducive to occasion setting instead of simple associative learning (Holland, 1986).

One further important result is that the groups in each of Experiments 2–4 did not significantly differ when tested under saline. In terms of a contextual occasion-setting explanation, this means that saline did not acquire either positive (in the S-sh groups) or negative (in the other, drug-shock groups) occasion-setting properties. The absence of modulation of conditioned responding by the saline cues may be caused by the fact that a saline state is hardly noticeable (see also Rescorla, 1991). Furthermore, negative occasion setters have been shown to lose their inhibitory potential when presented alone without the target stimulus (Holland and Gory, 1986; Maes and Vossen, 1994). In our experiments, the saline state, which is comparable to the normal, daily state, was present on all occasions, except when a specific drug was administered. Consequently, many 'presentations' of the state corresponding with the saline state occurred in the absence of the stimuli provided by the conditioning box and may have eliminated any negative occasion-setting potentials that this state initially could have acquired.

Aside from a direct effect on mobility level, each of the

drugs used, in principle, could have had other direct pharmacological effects, for instance on pain threshold and anxiety. It is important to note that the present experiments do not provide evidence for a significant contribution of such pharmacological effects to the response profile observed during testing. For instance, a clear pharmacological effect of a drug in terms of a lowering of the pain threshold would imply that the rats that were shocked under such a drug would have received a shock that effectively was more intense than that received by rats shocked under saline. This, in turn should be reflected in higher overall levels of immobility during testing under extinction in the drug-shocked group than in the saline-shocked group. This should be true regardless of the state present during testing (drug or saline). However, no such pattern was found. Between-group differences only emerged on test sessions under the drug and not on sessions under saline.

Concerning the question of the usefulness of the present discrimination procedure in relation to the commonly used operant drug-discrimination procedures, it must be noted that the major advantage of the present procedure is the speed with which one can demonstrate discrimination learning. Using the current procedure, discrimination learning was already visible within 10 days: 6 training days with a total of only three shocks, followed by 2 test sessions in each state. Furthermore, pre-training manipulations were not necessary.

The present results suggest that discrimination learning can already have taken place before one can observe reliable discrimination performance during the training phase itself. Discrimination learning after a limited number of training sessions may be hidden or latent and may be revealed in subsequent tests during extinction of the response. At present it is not clear whether the same also holds for discrimination learning using operant procedures.

One drawback of the current procedure is its susceptibility to unconditioned drug effects. It must be noted that the results of traditional two-lever drug-discrimination experiments are probably less confounded by unconditioned drug effects of the sort referred to in this paper if discrimination performance is expressed in terms of a choice measure. However, as demonstrated, possible unconditioned drug effects can be assessed first and used in the subsequent interpretation of test results. To facilitate data collection with respect to possible unlearned effects, one could use a design in which all animals, both drug-shock and saline-shock, are subjected to a training schedule in which the internal state is not the same for each animal in a given session (as in the present Experiments 2–4, all animals were trained under the drug in the first session, under saline on the second, etc.), but in which the shock/no-shock condition is the same for each animal in a given session. This would have as an effect that in each training session, one half of the rats would be in the drug state, and the other in the saline state. Such a design would enable between-group comparisons regarding the effect of the drug on the response measure in early stages of discrimination learning (unconditioned drug effects).

In standard drug-discrimination procedures, successful discrimination learning may be followed by generalization tests examining discrimination performance under different doses of the specific drug under investigation, or under different drugs. It is important to note that the present procedure also enables generalization tests to be performed. As can be seen from the test results obtained in the training context, discrimination performance remained intact even after repeated test cycles. Consequently, there is enough 'room' to conduct various generalization tests after the short training phase.

Another discrimination procedure that usually yields a rapid demonstration of discrimination learning is the so-called 'taste-aversion paradigm'. Here, a drug is first administered to a thirsty subject which is then allowed to consume a flavoured solution. Following consumption of the solution (the 'target' of discrimination learning), the animal is injected with an ill-making substance. After administration of a placebo, the subjects is not made ill after drinking the flavoured solution. Using this procedure, the animal withholds consumption of the flavoured solution when in the drug state, but not when in the no-drug state, after no more than a few pairings of the drug and the toxin (e.g. Lucki, 1988; Martin et al., 1990; Mastropaolo et al., 1989).

The current discrimination procedure has important characteristics in common with the taste-aversion procedure, such as speed of reliable discrimination learning and performance, and susceptibility to possible unconditioned drug effects. The results of the present experiments, then, indicate that rapid discrimination learning is not restricted to a taste-aversion paradigm, but can also be obtained using a Pavlovian discrimination procedure with shock as the unconditioned stimulus and an external context as the target. Further research might even reveal that discrimination learning with the same drugs as used in the present experiments can be mastered after just a single shock in a training context under one state, followed by one or two extinction sessions under another state (see also Bouton et al., 1990). Furthermore, future research should establish whether or not rapid discrimination learning is also possible using appetitive Pavlovian procedures.

Acknowledgements

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