Pharmacology Biochemistry and Behavior, Vol. 64, No. 2, pp. 409–413, 1999

0091-3057/99/$–see front matter

ELSEVIER

PII S0091-3057(99)00088-X

Influence of Training Paradigm on Specificity of Drug Mixture Discriminations

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MARIATHASAN, E. A., I. P. STOLERMAN AND J.-A. W. WHITE. *Influence of training paradigm on specificity of drug mixture discriminations.* PHARMACOL BIOCHEM BEHAV **64**(2) 409–413, 1999.—Generalization to different drugs and drug mixtures has been examined in rats trained to discriminate a mixture of amphetamine (0.4 mg/kg) plus pentobarbitone (10 mg/kg) from saline (AND discrimination, $n = 8$) or to discriminate the same mixture from its component drugs alone (AND-OR discrimination, $n = 9$). The studies used two-lever operant procedures with a tandem variable interval 1-min fixed-ratio 10 schedule of food reinforcement. There was partial generalization to nicotine and midazolam and no generalization to cocaine, caffeine, or ethanol under AND-discrimination conditions and no generalization to any of these drugs in the AND-OR discrimination. Nicotine or midazolam coadministered with the training doses of pentobarbitone and amphetamine, respectively, produced full generalization in the AND discrimination and partial generalization under AND-OR conditions. Cocaine coadministered with pentobarbitone generalized fully under both procedures, but at larger doses in the AND-OR than in the AND discrimination. Mixtures of either nicotine plus midazolam or caffeine plus ethanol produced very marked generalization under AND-discrimination conditions, but were without significant effect in the AND-OR procedure. The results consistently supported the hypothesis that the AND-OR discrimination procedure increases the specificity of discriminations based on drug mixtures. © 1999 Elsevier Science Inc.

Nicotine Midazolam Caffeine Cocaine Amphetamine Pentobarbitone Ethanol Drug discrimination Rats

IN previous studies the discriminative stimulus effects of drug mixtures have been examined to develop a pharmacological approach to polydrug abuse. Stimulus generalization in rats trained to discriminate drug mixtures has now been investigated further to determine how different training paradigms may influence generalizations to novel drugs and drug mixtures. Earlier studies showed that using AND-OR rather than simple AND-discrimination training procedure markedly altered the characteristics of discriminations based upon mixtures of amphetamine plus pentobarbitone (7).

In AND-discrimination procedures, subjects are trained to discriminate a drug mixture from the undrugged state. Previous studies with AND-discrimination procedures examined their basic characteristics with a wide variety of drugs included in the mixtures used for training (5,8,14). In most cases, marked and sometimes complete generalization has been obtained from mixtures to their component drugs given alone (5,8). In some studies where the influence of one of the drugs on the dose–response curve for the second drug has been examined, evidence for modest supraadditive interactions has been reported (7).

Generalizations from the training mixture to other substances given singly has been examined occasionally in rats trained under AND-discrimination procedures. Garcha and Stolerman (3) reported the absence of generalization to either morphine or phencyclidine in rats trained to discriminate a mixture of nicotine plus midazolam, and suggested that using mixtures of drugs for training does not necessarily weaken the pharmacological specificity of discriminations. When a test drug has discriminative effects that resemble those of one training drug, partial or full generalization may occur (5,6,8). Nevertheless, very few studies have examined generalizations from one mixture of drugs to novel mixtures in which either one or both of the components has been changed (called "single substitution" and "dual substitution" tests, respectively). One notable exception is a study in which rats were trained to discriminate a mixture of nicotine plus ethanol from saline (5); these animals generalized fully to a mixture containing amphetamine and pentobarbitone.

More rarely, studies have used an AND-OR discrimination procedure in which subjects are trained to discriminate between a mixture and either of its component drugs alone

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(7). In this paradigm, one response is reinforced after the drug mixture is administered prior to half of the training sessions, and a second response is reinforced when either of the component drugs is administered prior to other training sessions. In the remaining sessions that occur instead of the saline training sessions in more conventional procedures, each component drug is used for training equally often. Initial studies using mixtures of amphetamine plus pentobarbitone showed that there was no mixture-appropriate responding produced by any dose of the individual drugs, and it was suggested that AND-OR discrimination training may have enhanced pharmacological specificity (7). Experiments with the antagonists mecamylamine and flumazenil on the discrimination of a nicotine plus midazolam mixture supported this idea; the antagonists attenuated the mixture discrimination to a greater extent and at much smaller doses under AND-OR than under AND-discrimination training conditions (9). However, the most direct test of the specificity hypothesis must involve studies of generalization to diverse drugs, and to drug mixtures other than those used to establish the discrimination.

The experiments summarized here test the hypothesis by using discriminations based upon a mixture of (+)-amphetamine plus pentobarbitone. Firstly, generalization from the mixture to the single drugs nicotine, cocaine, caffeine, midazolam, and ethanol has been compared in rats trained under AND- and AND-OR discrimination procedures. Generalization tests have been carried out with one of the drugs changed and with the other drug the same as in the training mixture ("single substitution" tests); thus, the effects of changing the stimulant component from amphetamine to either nicotine or cocaine has been examined with the pentobarbitone component held constant. Similarly, the depressant component was changed to either midazolam or ethanol, while the amphetamine component was held constant. Finally, "dual substitution" tests were carried out with two mixtures: nicotine plus midazolam and caffeine plus ethanol. A more detailed account of these studies has been published elsewhere (10).

METHOD

Animals

Male Lister hooded rats (n = 20; Harlan Olac, Bicester, UK) were housed individually at a temperature of about 21°C with a regular light–dark cycle, and were maintained at 80% of free-feeding weights. Standard two-lever operant chambers (Campden Instruments, London) were used.

Procedures

Training was based on procedures described previously (7). One group of rats was trained according to the AND-discrimination procedure to discriminate a mixture of (+)-amphetamine (0.4 mg/kg SC) plus pentobarbitone (10 mg/kg SC) from saline. A second group of rats was trained under the AND-OR procedure to discriminate the same mixture from either drug administered separately (7). Training took place in daily, 15-min sessions, and the final schedule of food reinforcement was tandem variable interval 1-min fixed-ratio 10. Rats that attained a criterion of 80% accuracy over 10 consecutive sessions were used to test generalization to novel drug doses and mixtures. Generalization data were obtained from extinction tests of 5-min duration. One-factor, repeated-measure analyses of variance were used to examine the data; in cases where there was a significant treatment effect, this was investigated further by Tukey B tests for multiple comparisons (Unistat 4.5 statistical package, Unistat Ltd., London).

Plan of Experiments

In the first group of tests, generalization to a range of doses of nicotine, midazolam, cocaine, caffeine, and ethanol was examined. In the second group of tests (single substitutions), mixtures were tested with one component drug held constant as in training and the type and dose of the other drug was varied. Thus, generalization was tested to mixtures of nicotine or cocaine plus pentobarbitone and to mixtures of midazolam plus amphetamine. In the third group of experiments, generalization tests were carried out in which both drugs tested differed from those used for training ("dual substitution" tests). Thus, generalization was tested to mixtures of nicotine plus midazolam and to mixtures of caffeine plus ethanol.

Drugs

(+)-Amphetamine sulphate, caffeine, cocaine hydrochloride, nicotine bitartrate, midazolam maleate, and pentobarbitone were dissolved in isotonic saline. Injections were given subcutaneously 15 min before sessions except for ethanol, which was administered intraperitoneally as a 15% solution in distilled water 15 min before sessions.

RESULTS

Generalization Tests With Single Drugs

In the AND-discrimination group, midazolam and nicotine produced no more than partial generalization at the largest doses tested (Table 1). In the AND-OR discrimination group none of the drugs tested increased mixture-appropriate responding significantly at any dose. With regard to total numbers of responses, midazolam, nicotine, and cocaine reduced the rate of responding in the AND-discrimination group, whereas caffeine and ethanol were without significant effect (Table 1). A larger (0.75 g/kg) dose of ethanol suppressed responding so much that discriminative effects could not be assessed. In AND-OR discrimination, the baseline rate of responding was lower than in AND-discrimination and none of the drugs tested affected this rate significantly.

Generalization Tests With One Drug in the Mixture Changed

In these single-substitution experiments, a novel drug was substituted for one of the drugs in the training mixture; the second drug in the test mixtures was the same as in training. In the AND-discrimination group 0.4 mg/kg of amphetamine and 10 mg/kg of pentobarbitone produced 42.7% and 61.1% mixture-appropriate responding, respectively. Table 2 shows that when the same dose of pentobarbitone was given as a mixture with of nicotine, full generalization was obtained. These effects of mixtures of nicotine (0.1–0.4 mg/kg) plus pentobarbitone were near maximal, regardless of the dose of nicotine. When 1.0–10.0 mg/kg of cocaine was substituted for the amphetamine in the training mixture, there was again full generalization to the training mixture at all doses. Finally, full generalization was also seen when the training dose of amphetamine was administered in mixtures with 0.05–0.2 mg/kg doses of midazolam. These results show that it was possible to obtain full generalization when either a range of doses of nicotine or cocaine was substituted for the amphetamine in the training mixture, and when a range of doses of midazolam was substituted for the pentobarbitone.

TABLE 1

EXTINCTION TESTS IN RATS TRAINED TO DISCRIMINATE A MIXTURE OF (+)-AMPHETAMINE (0.4 mg/kg) PLUS PENTOBARBITONE (10 mg/kg) UNDER AND-AND AND-OR DISCRIMINATION PROCEDURES (n = 7–8)

	Mixture Responses, %		Total Responses	
Test drug	AND	AND-OR	AND	AND-OR
Saline	3.8 ± 1.9	15.1 ± 3.3	462 ± 50	168 ± 25
Amphetamine 0.4 mg/kg + pentobarbitone 10 mg/kg	97.4 ± 1.7*	90.6 ± 5.2*	288 ± 54	326 ± 47
Nicotine 0.4 mg/kg	50.9 ± 9.9*	11.4 ± 4.5	30 ± 8*	77 ± 18
Midazolam 0.2 mg/kg	36.7 ± 8.6*	30.2 ± 7.9	117 ± 33*	85 ± 17
Cocaine 10 mg/kg	21.2 ± 12.4	8.5 ± 3.3	144 ± 59*	222 ± 39
Caffeine 30 mg/kg	21.4 ± 7.9	21.1 ± 2.7	288 ± 35	111 ± 16
Ethanol 0.5 g/kg	14.8 ± 9.3	14.2 ± 4.0	223 ± 77	90 ± 15

Drugs were tested at several doses but results are shown only for the largest doses tested of each drug at which discriminative effects could be assessed (means ± SEM). Significant ($p < 0.01$) differences from saline data are shown (*).

In the AND-OR discrimination group, neither amphetamine nor pentobarbitone given separately increased mixture-appropriate responding above the control level. When the same dose of pentobarbitone was given as a mixture with different doses of nicotine, the maximum effect was partial generalization (Table 2). When cocaine was substituted for the amphetamine in the training mixture, full generalization was obtained, but only when the dose of cocaine was 10 mg/kg. Finally, when the training dose of amphetamine was administered in mixtures with different doses of midazolam, only partial generalization was seen. These results show that in the AND-OR procedure it was possible to obtain full generalization when one dose of cocaine was substituted for the amphetamine in the training mixture, but no dose of nicotine substituted fully for amphetamine and no dose of midazolam substituted fully for pentobarbitone.

Generalization Tests With Both Drugs in the Mixture Changed

In these dual substitution experiments, two novel drugs were substituted for the drugs in the training mixture. In the AND-discrimination group increasing doses of a mixture of nicotine plus midazolam (with the dose ratio held constant at 2:1) increased mixture-appropriate responding to a maximum of 74% (Table 3). Increasing doses of caffeine coadministered with ethanol also increased mixture-appropriate responding, in this case to a maximum of 52.9%. In the AND-OR discrimination group, mixtures of nicotine plus midazolam did not increase mixture-appropriate responding significantly. Similarly, increasing doses of caffeine coadministered with ethanol were without significant effect. In the AND-discrimination procedure, the mixtures of nicotine plus midazolam and of caffeine plus ethanol reduced response rates in comparison with rates after administration of saline (Table 3). In contrast, in rats trained under the AND-OR discrimination procedure none of the novel mixtures altered response rates significantly, although this should be seen in the context of the lower response rate after saline than after the mixture used for training.

DISCUSSION

The present study provides evidence for marked differences between the AND and AND-OR discrimination procedure with respect to generalization to novel drugs and drug

TABLE 2

RESULTS OF "SINGLE SUBSTITUTION" AND CONTROL TESTS IN RATS TRAINED TO DISCRIMINATE A MIXTURE OF (+)-AMPHETAMINE (0.4 mg/kg) PLUS PENTOBARBITONE (10 mg/kg) UNDER AND-AND AND-OR DISCRIMINATION PROCEDURES (n = 7–8)

	Mixture Responses, %		Total Responses	
Test drug	AND	AND-OR	AND	AND-OR
Saline	3.3 ± 2.3	10.3 ± 3.8	462 ± 50	168 ± 24
Amphetamine 0.4 mg/kg plus pentobarbitone 10 mg/kg	97.9 ± 2.0*	88.2 ± 7.0*	288 ± 54	326 ± 47
Nicotine 0.4 mg/kg plus pentobarbitone 10 mg/kg	84.8 ± 6.5*	62.2 ± 13.9*	241 ± 53*	100 ± 31
Cocaine 10 mg/kg plus pentobarbitone 10 mg/kg	98.1 ± 1.3*	83.8 ± 13.7*	283 ± 67	429 ± 42
Amphetamine 0.4 mg/kg plus midazolam 0.2 mg/kg	94.8 ± 3.6*	45.0 ± 12.0*	50± 22*	273 ± 38

Other details as for Table 1.

TABLE 3

RESULTS OF "DUAL SUBSTITUTION" AND CONTROL TESTS IN RATS TRAINED TO DISCRIMINATE A MIXTURE OF (+)-AMPHETAMINE (0.4 mg/kg) PLUS PENTOBARBITONE (10 mg/kg) UNDER AND-AND AND-OR DISCRIMINATION PROCEDURES

	Mixture Responses, %		Total Responses	
Test Drug	AND	AND-OR	AND	AND-OR
Saline	4.1 ± 1.6	16.8 ± 3.5	471 ± 52	211 ± 26
Amphetamine 0.4 mg/kg plus pentobarbitone 10 mg/kg	98.1 ± 1.1*	90.1 ± 4.6*	329 ± 34	315 ± 38
Nicotine 0.4 mg/kg plus midazolam 0.2 mg/kg	74.0 ± 7.6*	34.8 ± 9.8	167 ± 26*	235 ± 51
Caffeine 20 mg/kg plus ethanol 0.5 g/kg	52.9 ± 13.4*	18.0 ± 8.9	136 ± 34*	96 ± 27

Other details as for Table 1.

mixtures. The findings consistently support the hypothesis (7,9) that AND-OR discrimination training enhances pharmacological specificity.

The findings from tests with single drugs in the AND-discrimination procedure can be related to previous work on discriminations supported by the individual drugs in the mixtures. Thus, rats trained to discriminate (+)-amphetamine generalise partially to nicotine (12,13); the partial generalization to nicotine may, therefore, be associated with the amphetamine component of the training mixture. Conversely, the partial generalization to midazolam is probably associated with the pentobarbitone in the training mixture because barbiturates can generalize to midazolam in rats (4). The lack of generalization from the amphetamine-barbiturate training mixture to caffeine is compatible with observations that amphetamines and barbiturates rarely generalize with it (1,11). Similarly, ethanol generalizes incompletely with barbiturates (2). However, the failure to see generalization to cocaine was unexpected because amphetamine and cocaine typically exhibit cross-generalization (15). The absence of generalization under AND-OR discrimination conditions to any of the single drugs is consistent with previous reports that the effects of the mixture are fully dissociated from those of its component drugs by this procedure (7).

Under AND-discrimination conditions, the relatively strong patterns of generalization in the single substitution tests carried out where only one component of the drug mixture was changed are also compatible with previous findings. In these studies, all three drug mixtures studied produced full generalization, perhaps reflecting two facts. The first is that the novel drugs administered in these studies have discriminative effects resembling to varying extents those of one of the training drugs (1,2,4,11,12,15). The second fact is that there was, as expected, a strong response to the unchanged component drugs of the training mixture; therefore, identity of effect between a novel drug and the component of the training mixture for which it is substituted may not be required for generalization to occur.

In the AND-OR discrimination condition, there was only partial generalization to mixtures of amphetamine plus midazolam or of nicotine plus pentobarbitone. As noted before, each of these mixtures produced full generalization over precisely the same range of doses in the AND-discrimination procedure. Mixtures of cocaine plus pentobarbitone yielded full generalization in the AND-OR procedure at only the largest of three doses, each of which produced full generalization in the AND-discrimination. Overall, it seems clear that with the AND-OR discrimination procedure, generalization occurs less readily to mixtures when even one of their component drugs is not identical with a drug used for training. This observations support the hypothesis of enhanced pharmacological specificity under AND-OR conditions.

As described above, none of the constituent drugs (nicotine, caffeine, midazolam, and caffeine) used for the dual substitution tests produced more than partial generalization when administered separately (Table 1); it was not surprising that binary mixtures containing these drugs produced only partial generalization in the dual substitution tests (Table 3). Most striking was the finding that no generalization at all was seen when these mixtures were tested under AND-OR discrimination conditions. This suggests that the AND-OR procedure is considerably more specific than the AND discrimination.

In summary, several lines of evidence suggest that AND-OR training increases specificity: first, with this procedure, there is no generalization from the mixture to any dose of its constituent drugs given separately (7). When novel drugs are tested singly (Table 1), generalization is absent. Next, when novel drugs are tested in both single substitution tests (Table 2) and dual substitution tests (Table 3), generalization is substantially attenuated. Finally, the effectiveness and potency of specific antagonists is increased (9). For comparisons of abused mixtures for similarity with respect to their stimulus properties, the enhanced specificity of the mixture discrimination may be a significant advantage. In the situation where a novel abused mixture is tested for generalization in rats trained with a standard mixture (i.e., a dual substitution test), the AND-discrimination procedure is likely to result in full generalization if only one of the substituted novel drugs is identical in effect to either drug in the training mixture (8); such a result might reasonably be considered as a "false positive" in pharmacological terms. Even one identical and one inert drug can produce such a result. In contrast, with the AND-OR discrimination, the effects of both drugs must be reproduced for full generalization to occur. It seems unlikely that any single drug would have such a property. It may also be the case that full generalization in the AND-OR procedure can be obtained only if the substitute drugs reproduced not only the effects of each individual training drug, but also produced the same novel stimulus condition that might be associated with a nonadditive interaction between them.

ACKNOWLEDGEMENTS

This research was supported by NIDA Grant DA 05543.

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