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BRIEF COMMUNICATION

The Discriminability of Aspirin in Arthritic and Nonarthritic Rats

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WEISSMAN, A. *The discriminability of aspirin in arthritic and nonarthritic rats.* PHARMAC. BIOCHEM. BEHAV. 5(5) 583–586, 1976. – Aspirin, 56 mg/kg IP, was shown to be mildly, but significantly discriminable from saline in a group of 12 nonarthritic rats exposed to a 2-lever fixed ratio 10 drug discrimination protocol for 56 trials. In a concurrently-tested group of 12 rats made arthritic by injection of *Mycobacterium butyricum* into a hind paw, the discrimination of aspirin from saline was enhanced. The results exemplify how drug discriminability may vary depending on the pathological state of the subjects exposed to drug-discrimination training.

Aspirin Drug discrimination Adjuvant arthritis Operant behavior

SEVERAL systemically-administered drugs are known to serve as discriminative stimuli in appropriately trained laboratory animals. This subject has not only been frequently reviewed [e.g., 2], but is the focus of active current research in many laboratories. For the most part, research to date has been concerned with identifying those drugs that generalize to a discriminated drug, and the specificity of a given drug cue.

Among the many potential offshoots of this research area is the problem of determining states of an organism that favor or disfavor the acquisition of discriminative properties by a drug. Although formal studies have not been addressed to this point in humans, it seems evident that a human opiate addict should be more readily able to discriminate naloxone than a nonaddict and that a human with headache or arthritis should be more able to discriminate aspirin than a nonsufferer. The present study was undertaken as an attempt to demonstrate whether a poorly discriminated drug is better discriminated by rats discriminated by rats subjected to pathological conditions relieved by that drug. Specifically, it was addressed to the determination of whether or not aspirin can serve as a discriminable stimulus, and whether acquisition of an aspirin vs saline discrimination develops more rapidly in arthritic than in normal rats.

METHOD

Animals

Twenty-four individually housed adult male albino Sprague-Dawley rats from Charles River Laboratories weighing about 220 g on arrival were used. They received liquid food reinforcement during the conditioning procedures described below, and sufficient supplemental Purina rat chow after each session and on weekends to maintain their weights at about 250 g throughout the experiment. Water was available ad lib in home cages.

Apparatus

Training and testing were accomplished in 6 identical isolated commercial operant chambers (Foringer type) programmed by electromechanical circuitry. The important working parts of each chamber were two Foringer levers mounted on each side of a motor-driven dipper, and a houselight which remained on during the 15 min session. Reinforcement consisted of 3 sec presentation of 0.2 cc of Carnation Slender, a commercial liquid diet food consisting of sucrose-sweetened skim milk with protein, vitamins and minerals added, diluted 1:1 with water.

Procedure

The procedure was a slight modification of the 2-bar fixed ratio 10 (FR 10) drug-discrimination protocol first described by Colpaert *et al.* [3] in their studies of fentanyl discrimination.

Following food deprivation to a sustained 250 g weight, each rat was shaped to bar press, first on a continuous reinforcement CRF) schedule on each lever, and then on an FR schedule that was rapidly escalated to FR 10 on each lever. These steps were accomplished with a single lever in the cage, for 2 sessions on the left side, and then for 2 additional sessions on the right side, of the dipper.

Drug training was then begun, using the protocol of Colpaert *et al.* [3]. Depending on whether the rat received aspirin or saline (see below for details of treatment),

reinforcement was programmed exclusively on either the left or the right lever on the FR 10 schedule. Only responses on the left lever were reinforced after drug administration, and only responses on the right lever after saline administration. Rats were tested 5 days each week under the alternating drug sequences used by Colpaert *et al.* [3]: aspirin-saline-saline-aspirin-aspirin and saline-aspirin-aspirin-saline-saline. To avoid the possibility that the correct lever for rats previously tested in the chambers could serve as a cue, a very real possibility based on results of pilot studies, the sequence of aspirin or saline treatment on any day was alternated as the 4 subgroups of 6 rats were tested. After each session of 15 min, rats were returned to their home cages and fed a quantity of Purina lab chow equal to the difference between their body weights, determined prior to drug treatment, and 250 g. On weekends rats were fed 10–12 g/day of pelleted lab chow, which maintained their weight reasonably constant at about 250 g.

In summary, other than the drug treatments employed, the prime modifications of the Colpaert *et al.* [3] training procedure used in this study were the type of operant chambers used, the reinforcing stimulus, and the method of depriving rats.

Drug administration. Throughout training, rats received either normal (0.9%) saline or aspirin suspended in normal saline (56 mg/kg at a volume of 5 ml/kg). Injections were made intraperitoneally 1 hr prior to exposure of the rat to the operant chamber.

After 14 days of training, adjuvant arthritis was produced in 12 randomly chosen rats by a close approximation of the procedure of Walz *et al.* [7], which consisted of a single intradermal injection of 0.75 mg of *Mycobacterium butyricum* (Difco Laboratories, Detroit, MI), suspended in paraffin oil, into the right hindpaw. The physiological progress of adjuvant arthritis after this treatment has been described in detail [7]. The remaining 12 rats were untreated. Thus, beginning on day 15 of training, the 24 rats were subdivided into 2 groups of 12, an arthritic and a nonarthritic group. Otherwise, dosing and training procedures continued identically for both groups.

Statistical treatment. Choice and response rate data were coalesced in blocks of 7 trials, as shown in the Results section. For individual rats, blocks of 7 trials always consisted of 4 trials under one treatment condition (saline or aspirin) and 3 trials under the other condition. Nonparametric statistical tests performed [5] are identified in the Results section.

RESULTS

Acquisition of the Aspirin-Saline Discrimination

On average, the nonarthritic rats consistently chose the correct lever for about 60% of the last 4 blocks of 7 trials (Fig. 1). The combined choice percentage data from the 12 individual rats were significantly greater than the 50% level to be expected from random choices on blocks 5 through 8 and blocks 7 through 8 ($p<0.05$; binomial test) and signify that aspirin, given under the conditions of this experiment, was discriminated from saline, although poorly. The poor choice performance on block 4 has no apparant explanation. Table 1 shows that before the 56-trial experiment was completed, 7 rats in the nonarthritic group reached a criterion of 8 correct choices out of 10 successive trials, and that 4 reached a criterion of 9 correct choices out of 10. No rats in this group chose the correct lever for 10 successive trials.

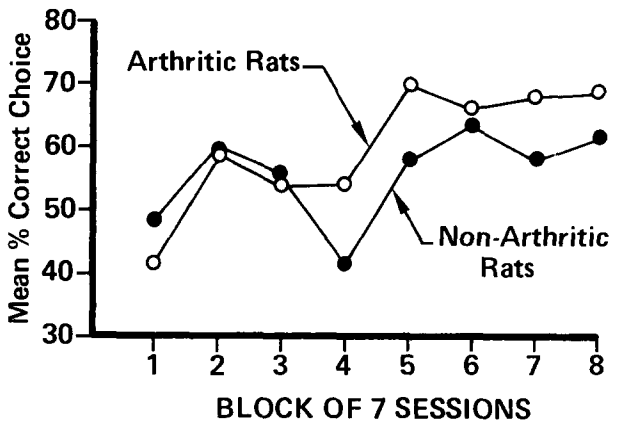

FIG. 1. Mean percent correct lever choice of arthritic and nonarthritic groups as a function of trials in blocks of 7. N equalled 12 in each group until reduced by mortalities (see Table 1). Note that arthritic rats, on average, consistently chose the correct lever more frequently than did nonarthritic rats blocks 5 through 8, but that both groups exceeded change (50%) lever selection on these trials.

The arthritic rats reached a greater level of accuracy than the nonarthritic rats, as shown by the fact that their mean percent correct choice consistently reached about 70% during blocks 5 through 8 (Fig. 1). The individual choice data were significantly higher than the 50% to be expected from random lever selection during blocks 5 through 8 and 7 through 8 ($p<0.01$; binomial test). The choice level was also significantly higher than the correct choice percentage of nonarthritic rats on blocks 5 through 8 ($p<0.05$; Mann-Whitney U-test). Even this difference probably understates the true difference between the arthritic and nonarthritic rats, since the 2 mortalities in the arthritic group (Table 1) occurred in good rats that rapidly reached 8/10 and 9/10 correct choice criteria.

More rats in the arthritic than the nonarthritic group reached the criteria of 8, 9 or 10 correct responses out of 10 successive trials, as shown in Table 1. The difference between the numbers of rats from each group reaching the 10 out of 10 criterion (0/12 vs 5/12) was significant ($p<0.02$; Fisher exact probabilities test).

Effects of Aspirin and Adjuvant on Response Rates

Mean response rate data for the nonarthritic rats (Fig. 2A) show that response rates increased during the first 4 blocks of 7 trials from about 400–450 to about 650–700 total responses during the 15 min session. Consistently over 90% of these responses were on the correct lever as subjects learned to discriminate not only the drug state, but also the response source of the first reinforcement. Throughout the experiment, mean FR 10 response rate under the aspirin condition was less than under the saline condition in the nonarthritic group.

In the arthritic rats, as is evident from a comparison of Fig. 2A and 2B, mean response rates following the saline vehicle were consistently lower than in the nonarthritic rats, hovering around 600 responses per 15 min session until the last block of 7 trials. Such a depressed level of responding could be expected from rats with seriously

TABLE 1

DATA ON TRIALS-TO-CRITERION AND MORTALITY AFTER TRAINING ON INTRAPERITONEAL ASPIRIN VS SALINE, AND ON LEVER SELECTION AFTER ORAL ASPIRIN IN INDIVIDUAL RATS

	Trials-to-Criterion*			Day of	Lever choice
	8/10	9/10	10/10	Death	After Oral Aspirin†
Non-arthritic Rats					
Rat #61	56	>56	>56		A
62	>56	>56	>56		A
63	>56	>56	>56		S
64	>56	>56	>56		A
65	>56	>56	>56		S
66	36	37	>56		A
79	33	34	>56	(58)	—
80	37	38	>54	54	—
81	>56	>56	>56		A
82	56	>56	>56		S
83	43	>56	>56		S
84	43	44	>56		A
Totals	7/12‡	4/12‡	0/12§	1/12	6/10 A
Arthritic Rats					
Rat #67	34	>56	56		A
68	28	34	46		A
69	35	40	43		A
70	48	>56	>56		A
71	21	>56	>56		A
72	30	31	32	35	—
73	39	45	46		A
74	>56	>56	>56		S
75	>56	>56	>56		A
76	32	34	>44	44	—
77	48	49	56		A
78	41	43	44		S
Totals	10/12	7/12	5/12	2/12	8/10 A

*Trials until a rat reached the criterion of 8, 9 or 10 correct lever choices on 10 consecutive trials.
†Lever selection on a trial given 3 days after the termination of acquisition. On this trial rats were given aspirin, 100 mg/kg orally, 1 hr prior to testing, and the lever on which each rat first completed 10 responses was ascertained. A signifies aspirin (left) lever; S signifies saline (right) lever.
‡$p>0.05$ vs arthritic rats: Fisher exact probabilities test.
§$p>0.02$ vs arthritic rats: Fisher exact probabilities test.

inflamed paws, as were apparent on inspection. In the arthritic rats, unlike the nonarthritic rats, however, aspirin did not depress response rates as compared with response rates under the saline condition.

Effect of a Single Probe with Oral Aspirin

Following the accumulation of the data reported above, two additional training trials were run. On the following session all surviving rats (10 in each group) were administered aspirin, 100 mg/kg orally, 1 hr prior to the test session. Results of this single generalization probe are shown at the right of Table 1. It can be seen that 6/10 nonarthritic rats and 8/10 arthritic rats chose the aspirin lever (that is, first accumulated 10 responses on the aspirin lever), suggesting that systemic, rather than local effects of aspirin were the basis for its discrimination.

DISCUSSION

Aspirin has not previously been studied for its ability to serve as a cue in drug discrimination experiments. The present study demonstrates that intraperitoneal aspirin can be discriminated from saline in nonarthritic rats during a course of 56 trials. The discrimination formed, while statistically significant, was not robust. On average, nonarthritic rats chose the correct lever about 60% of the last 28 sessions, and while 7 out of 12 rats met a criterion of 8 correct choices during 10 consecutive trials, only 4 of the 12 rats met a 9 out of 10 criterion, and none of the 12 rats met a 10 out of 10 criterion. By way of contrast, Colpaert and his colleagues, using a very similar protocol to the present one, have shown that rats discriminate fentanyl, 0.04 mg/kg sc [3] and apomorphine, 0.16 mg/kg sc [4] to a 10/10 criterion in fewer than 40 trials. Elevating the dosage of intraperitoneal aspirin in this type of experiment is unlikely to yield better discriminability, since pilot studies undertaken in this laboratory have shown appreciable mortality to result from repeated intraperitoneal administration of aspirin at 100 or 75 mg/kg under a protocol similar to the present one. Even under the 56 mg/kg dose presently used, 2 rats died by 58 trials after the onset of aspirin treatment, given 2 or 3 times weekly. The present study strongly suggests that aspirin becomes more discriminable in rats made arthritic by an intradermal

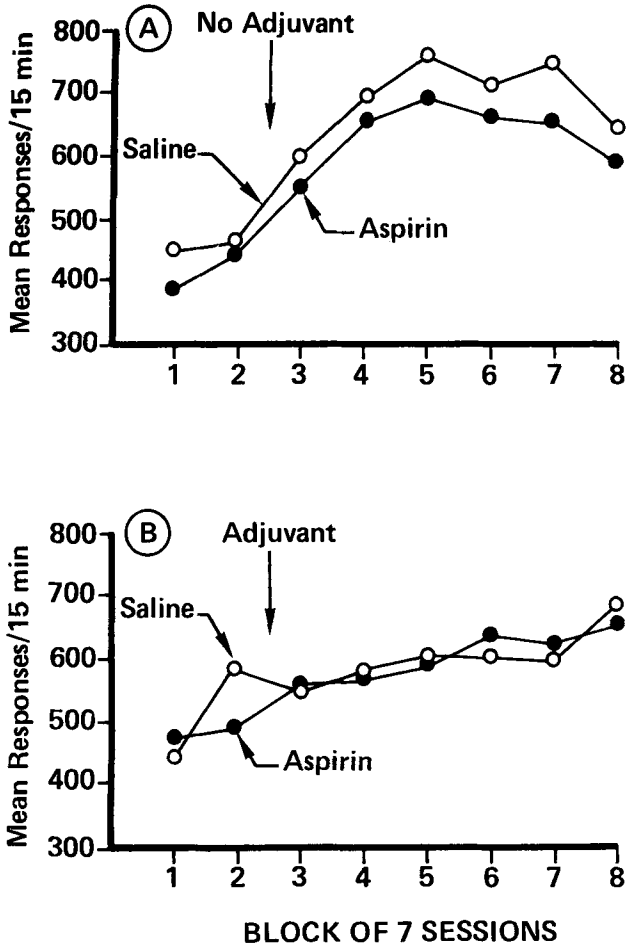

FIG. 2. A. Mean response rates (responses/15 min) of nonarthritic rats under saline and aspirin conditions as a function of trials in blocks of 7. N equalled 12 until reduced by mortality (see Table 1). Note that response rate under the aspirin condition is consistently less than response rate under the saline condition. B. As above, except for arthritic rats. Note that response rate under aspirin and saline conditions were virtually identical.

injection of *Mycobacterium butyricum* into a hind paw than it is in nonarthritic rats. Consistently more rats from the arthritic group reached the criteria of 8, 9 or 10 correct responses out of 10 consecutive trials, and the difference in numbers of rats from arthritic versus nonarthritic groups reaching the 10 out of 10 criterion was significant. Furthermore, arthritic rats, on average, made consistently more correct choices (about 70%) during the final 28 trials of the experiment than did nonarthritic rats.

Paw volume measurements were not made in the present study; however, the rats injected with the adjuvant showed obvious edema, first in the injected paw, and beginning in about a week in the contralateral and front paws. The rats were obviously irritable on handling. When given in the diet from 14 to 28 days after administration of *M. butyricum*, aspirin has been shown to reduce joint diameters at doses of 149 mg/kg and above [1]. Its ED_{50} was 440 mg/kg, where ED_{50} is expressed as the dose that very mardedly reduces joint diameters in 50% of the rats [1]. Aspirin at oral dietary doses as low as 81.3 mg/kg/day has also been shown to significantly reduce paw volumes of rats made arthritic by injection of *M. tuberculosis* [6]. These studies are relevant to the present study in showing that aspirin is effective in alleviating this type of adjuvant arthritis, but the differing protocols (route and chronicity of treatment) make direct extrapolation of conclusions difficult.

The present study exemplifies that drug discriminability depends on the state of the organism being tested. In this example aspirin is better discriminated in rats made arthritic than in nonarthritic rats. Additional unpublished studies in this laboratory, unsurprisingly, have shown that naloxone is highly discriminable in rats made chronically dependent on morphine, and that haloperidol is far better discriminated in rats treated chronically with amphetamine than in nondrugged rats. The designation of a drug as indiscriminable (or for that matter, discriminable) requires description of the animals in which it was tested.

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