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Aversive Stimulus Properties of Scopolamine[1]

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MACMAHON, S. W., N. M. BLAMPIED AND R. N. HUGHES. *Aversive stimulus properties of scopolamine.* PHARMAC. BIOCHEM. BEHAV. 15(3) 389–392, 1981.—The drug state produced in rats by intraperitoneal injections of scopolamine hydrobromide (1.2 mg/kg) was treated as a putative aversive US. This US was paired with a distinctive spatial location in a shuttle box for 6 of 12 daily sessions by confining the subject to one side following scopolamine and to the other side following saline (6 sessions). Two groups of 8 subjects each received zero and 20 min post-injection delays respectively. Following zero delay, but not 20 min delay, subjects avoided the side associated with scopolamine in drug-free, free choice tests. This is evidence that the immediate post-injection drug state induced by scopolamine is aversive.

Scopolamine Aversive drug state Location avoidance Rats

THE question of how one determines whether or not a drug state is aversive to a rat is not easily answered. Four procedures have been developed as attempts to assess the aversive stimulus properties of drugs. Three of the four employ a respondent conditioning paradigm, in which the drug injection is treated as a putative aversive unconditioned stimulus (US). In one or more conditioning trials the drug state is paired with a conditioned stimulus (CS). Then in subsequent drug-free tests, the behavioral effects of the CS alone are assessed, and compared with the effects known to be produced by pairing the CS with an established aversive US such as electric shock. One procedure uses the conditioned taste aversion technique in which a distinctive flavor CS is paired with drug injection [3]. If subsequent consumption and preference tests show the consumption of the flavor is suppressed and/or avoided, it is inferred that the drug state is aversive.

A second method uses the conditioned suppression method [5,6]. If the CS is shown to disrupt an on-going operant response (e.g., bar-pressing) the drug state may be inferred to be aversive. However, conditioned suppression may be produced by USs which are not conventionally regarded as aversive [2,5] a finding which limits inferences from this procedure about the aversive nature of the drug state [6].

The third respondent conditioning procedure is derived from recent investigations of the positively reinforcing effects of drugs [19, 24, 26, 28]. When this procedure is used to assess the aversive stimulus properties of drugs, the drug state is paired with confinement in one side of a two compartment shuttle box. If in subsequent drug-free, free-choice tests the subjects avoid the side paired with the drug state, it is inferred that the drug state was aversive.

A further group of methods involves instrumental conditioning. The injection of the drug follows an instrumental response. If the response rate subsequently increases it is inferred that the drug state is positively reinforcing [31], while if the response declines in frequency, the drug is inferred to be a punishing event [7].

The present research investigated the aversive stimulus properties of the anticholinergic drug scopolamine hydrobromide. Conditioned taste aversions are produced by scopolamine [3,22] and by scopolamine methyl-nitrate [4, 21, 23], and these drugs have been shown to disrupt the preference for novelty normally displayed by undrugged rats [16,18], an effect which has been interpreted as a consequence of their aversive stimulus properties [17]. The aversive stimulus properties of scopolamine hydrobromide were directly investigated using a conditioned location avoidance test derived from the conditioned location preference procedure of Reicher and Holman [26].

EXPERIMENT 1

METHOD

Subjects

Eight naive New Zealand random-bred hooded male rats

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from the departmental breeding colony were used. Their age range was 166–174 days (mean=168 days) and their weight range was 310–404 g (mean=355 g). Ad lib food and water was available in the home cage.

Apparatus

Subjects were individually trained and tested in a Lafayette Shuttlebox (model A550), measuring 61×25×28 cm (L×W×H), which was divided across the middle by a partition which contained a guillotine door (9×12 cm, W×H). The floor consisted of 36 0.5 cm diameter stainless steel rods spaced 2 cm apart. A 15 W light bulb positioned immediately above the transparent perspex ceiling of both chambers gave 325 lux illumination in each chamber. One end wall was covered by 3 cm black and white vertical lines and the end wall on the other side by 3 cm black and white horizontal lines. A speaker positioned behind the shuttlebox provided background white noise of 80 dB. Micro-switches detected movements of the grid floor and Pye Hi-Log programming equipment automatically recorded time (in 0.5 sec units) spent on each side and the number of crossings from one side of the shuttlebox to the other.

Drugs

Scopolamine hydrobromide (Hyoscine hydrobromide 0.6 mg/ml McGaw Ethicals) was injected at a dose of 1.2 mg/kg body weight. Control injections were of sterile isotonic saline solution. Injection volume was 2 ml/kg body weight IP.

Procedure

Each subject received 24 consecutive daily sessions each of 20 min duration.

Adaptation. On days 1–4 each subject received one session per day in the experimental chamber without injection. Each subject was confined to the side with the vertical line stimulus (VS) or the side with the horizontal line stimulus (HS) on alternate days. Subjects were randomly assigned to two groups so that order of exposure to the two sides was counterbalanced.

Test for baseline stimulus preference. On days 5–6 the guillotine door was retracted and the subjects were individually placed in the apparatus with free access to both sides. On day 5 half of the subjects in each group were placed on the VS side and half on the HS side. On day 6 the sides of entry were reversed. Time spent in each side of the shuttlebox and the number of times the subject crossed from one side to the other was recorded.

Conditioning. On days 7–19 each subject was injected with either saline or scopolamine immediately before being confined to one half of the shuttlebox. Subjects were given scopolamine or saline on alternate days. After injection with scopolamine the rat was always placed on one side of the box, and placed on the other side when injected with saline. Subjects were thus confined to the VS or HS sides on alternate days. Subjects received six scopolamine sessions (120 min total) and six saline sessions (120 min total). Half of the subjects were placed in the VS side after scopolamine administration and half on the HS side. These two groups were divided such that order of injection was counterbalanced over days.

Post-test for conditioned effects. On days 20–24 the procedure followed during the test for initial stimulus preference was duplicated with one modification. The side of entry to the shuttlebox was not alternated. One subject from each of the four subgroups was entered on the VS side on every day of the post-test while the other was always entered on the HS side. No injections were given during this phase of the experiment.

RESULTS

The raw scores of location preference (i.e., time spent in each half of the apparatus) obtained in the post-test (days 19–21) after conditioning sessions were used to produce a single preference score for each animal for the side associated with saline injection. This score is the proportion:

$$\frac{T_{SAL}}{T_{SAL} + T_{SCOP}}$$

where T_{SAL} = time spent in side associated with saline injection
and T_{SCOP} = time spent in side associated with scopolamine injection.

Indifference, would produce a score of 0.50. (N.B., $T_{SAL} + T_{SCOP}$ does not equal total time in apparatus, because it excludes time spent crossing the midline). Scores obtained in the pre-test trials (days 5 and 6) prior to conditioning sessions were used in the same way to produce preference proportions for the side later to be associated with saline injection.

The mean daily preference for the 0 delay saline-associated side is shown in Fig. 1. Prior to conditioning the subjects showed indifference, spending equal time on both sides of the apparatus. Following conditioning, however, there was a significant increased preference for the saline side, shown by a one way ANOVA, $F(1,35)=9.47$, $p<0.05$ [20]. This preference declined to indifference over the 4 extinction days.

Midline crossings declined from a mean of 27.6 in Session 1, to a mean of 20.75 in Session 6, but this change was not significant.

EXPERIMENT 2

At zero delay between drug injection and pairing with one side of the shuttle box, significant location avoidance was shown (Experiment 1). Following Reicher and Holman [26] a second experiment was run, in which a 20 min delay occurred between injection and confinement to examine the time course of the aversive effect.

METHOD

Subjects

Eight subjects of the same strain and sex used in Experiment 1 were used. Their age at time of testing was 140 days. Their weight range was 325–440 g (mean=371 g).

Apparatus and Drugs

These were the same as in Experiment 1.

Procedure

The procedure followed in Experiment 1 was duplicated with one modification. During the conditioning phase of the experiment the subjects were injected and then returned to the home cage for 20 min before being confined to one side or the other of the shuttlebox.

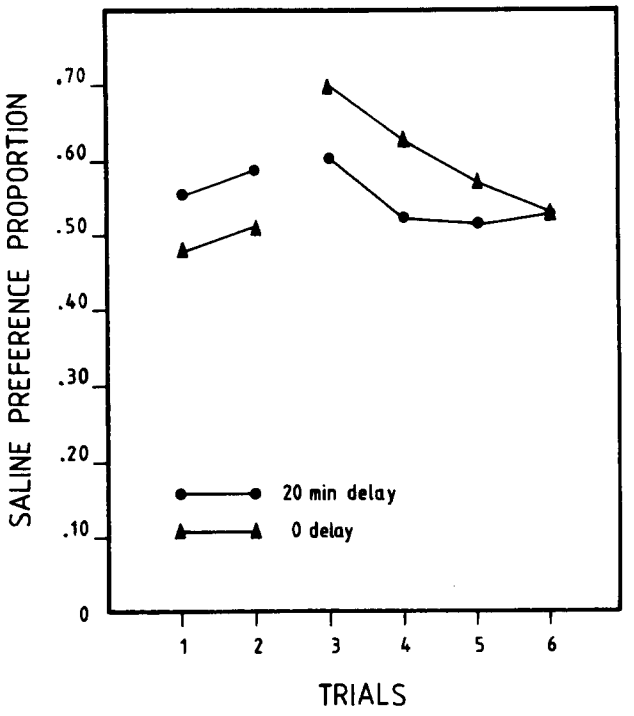

FIG. 1. Proportion of time spent on the saline associated side before and after conditioning trials as a function of delay between injection and exposure.

RESULTS

Time spent on left and right sides was used to calculate a preference proportion for the saline-associated side, as in Experiment 1, and the mean values are shown in Fig. 1. The mean of the two pre-conditioning sessions was 0.57, indicating an initial bias towards the side to be associated with saline. On Session 3, immediately post-conditioning, there was a slight apparent elevation of preference for the saline associated side (mean=0.60), but overall there were no significant differences, $F(6,35)=1.25$.

Mean daily midline crossings showed a non-significant decline from Session 1 to Session 6, as in Experiment 1.

GENERAL DISCUSSION

When exposed to a particular location immediately following an injection of scopolamine, rats subsequently avoided that location in undrugged, free-choice tests. This conditioned avoidance extinguished over four non-reinforced sessions. When the injection preceded conditioning by 20 min no conditioned avoidance was observed.

From this evidence it can be inferred that it is the immediate, post-injection drug state produced by scopolamine which is aversive. Whatever the stimulus properties of the drug-state 20 min after injection, they were insufficiently aversive to generate conditioned location avoidance, at least using the dose of the present experiment. Berger [3] also found scopolamine ineffective in producing a conditioned taste aversion when injected 30 min prior to consumption of the test solution. These data suggest a dissociation between the aversive stimulus properties of scopolamine and its other behavioral and pharmacological effects which are often reported to increase to maximum magnitude 20 to 30 min post-injection. The extent to which these effects on location preference are due to the peripheral anticholinergic effects of scopolamine remains to be determined by subsequent research using quaternary analogues which do not readily pass the blood-brain barrier. The aversive stimulus properties of scopolamine appear to be weaker than those of amphetamine, which persist over a 20 min post-injection delay [26].

These results provide further evidence showing the conditioning of an exteroceptive, spatial cue to an interoceptive, drug-induced US. Despite initial claims that such associations could not be formed by rats [13,14] it is now clear that exteroceptive CS-interoceptive US associations can be formed, using several different conditioning procedures [12, 26, 27].

The conclusion that scopolamine is aversive rests on the assumption that the subjects were avoiding the scopolamine-associated side, rather than approaching the saline associated side. Given the extensive prior habituation, and the pre-conditioning demonstration of equal preference for the two sides it is hard to argue that the drug treatment should have left the scopolamine side neutral while making the saline side positive in some way. For example, impaired habituation to or memory of [9,10] the drug-associated side by scopolamine would have rendered that side more novel and thus preferred at least on the first post-test day [17].

This demonstration of the aversive stimulus properties of scopolamine provides direct support for Hughes' [17] re-evaluation of the effects of anticholinergic drugs on some measures of habituation to novelty. Hughes has argued that the effects of many psychotropic drugs (particularly anticholinergics) on certain indices of habituation to, and preference for novelty can be more parsimoniously interpreted as due to the aversive effects of the drugs acting to suppress preferences by enhancing novelty-avoidance in a manner analogous to the effects of electric shock [1,25], rather than by appealing to complex central neuropharmacological mechanisms (e.g., [8, 9, 10]).

Unconditioned aversive effects are one of a number of stimulus properties of drugs which may be important in producing their behavioral effects [11, 15, 29, 30, 33]. The role which these stimulus properties may play in determining the outcome of an experiment is frequently overlooked. This especially applies to experiments concerned with drug effects on associative processes such as habituation, conditioning and memory, where explanations based on neuropharmacological changes in central synaptic and neuronal physiology are often preferred.

REFERENCES

1. Aitken, P. P. Aversive stimulation and rats' preference for familiarity. *Psychon. Sci.* **28:** 281–282, 1972.

2. Azrin, N. H., and D. F. Hake. Positive conditioned suppression: conditioned suppression using positive reinforcers as the unconditioned stimuli. *J. exp. Analysis Behav.* **12:** 167–173, 1969.

3. Berger, B. D. Conditioning of food aversions by injections of psychoactive drugs. *J. comp. physiol. Psychol.* **81:** 21–26, 1972.
4. Berger, B. D., C. D. Wise and L. Stein. Area postrema damage and bait shyness. *J. comp. physiol. Psychol.* **82:** 475–479, 1973.
5. Blackman, D. E. Conditioned suppression and the effects of classical conditioning on operant behaviour. In: *Handbook of Operant Behavior*, edited by W. K. Hönig and J. E. R. Staddon. Englewood Cliffs, NJ: Prentice-Hall, 1977, pp. 340–363.
6. Cameron, O. G. and J. E. Appel. Conditioned suppression of bar-pressing by stimuli associated with drugs. *J. exp. Analysis Behav.* **17:** 127–137, 1972.
7. Cappell, H. and A. F. Le Blanc. Punishment of saccharin drinking by amphetamine in rats, and its reversal by chlordiazepoxide. *J. comp. physiol. Psychol.* **85:** 97–104, 1973.
8. Carlton, P. L. Cholinergic mechanisms in the control of behavior by the brain. *Psychol. Rev.* **70:** 19–39, 1963.
9. Carlton, P. L. Brain acetylcholine and habituation. In: *Progress in Brain Research*, Vol. 28, edited by P. B. Bradley and M. Fink. Amsterdam: Elsevier, 1968.
10. Carlton, P. L. Brain acetylcholine and inhibition. In: *Reinforcement and Behavior*, edited by J. Tapp. New York: Academic Press, 1969.
11. Colpaert, F. A. and J. A. Rosecrans. *Stimulus Properties of Drugs: Ten Years of Progress*. Amsterdam: Elsevier/North Holland, 1978.
12. D'Mello, G. D. and J. P. Stolerman. Methodological issues in drug discrimination research. In: *Stimulus Properties of Drugs: Ten Years of Progress*, edited by F. C. Colpaert and J. A. Rosecrans. Amsterdam: Elsevier/North Holland, 1978, pp. 243–252.
13. Garcia, J. and F. R. Ervin. Gustatory-visceral and telereceptive-cutaneous conditioning: adaptation in internal milieus. *Communs behav. Biol.* **1:** 389–415, 1968.
14. Garcia, J. and R. A. Koelling. Relation of cue to consequence in avoidance learning. *Psychon. Sci.* **4:** 123–124, 1966.
15. Ho, B. Y., D. W. Richards and D. L. Chute, editors. *Drug Discrimination and State-dependent Learning*. New York: Academic Press, 1978.
16. Horsburgh, R. J. and R. N. Hughes. Modification of novelty preferences in rats by current and prior treatment with scopolamine and methylscopolamine. *Psychopharmacology*, in press.
17. Hughes, R. N. Scopolamine effects on preferences for novelty: A possible alternative to central cholinergic involvement in habituation. *IRCS J. med. Sci.* **6:** 165–168, 1978.
18. Hughes, R. N., N. M. Blampied and W. J. Stewart. Scopolamine induced changes in activity and reactions to novelty. *Pharmac. Biochem. Behav.* **3:** 731–734, 1975.
19. Katz, R. L. and G. Gormezano. A rapid and inexpensive technique for assessing the reinforcing effects of opiate drugs. *Pharmac. Biochem. Behav.* **11:** 231–233, 1979.
20. Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences*. Belmont, CA: Brooks/Cole, 1968.
21. Klein, S. B., G. C. Damato, C. Halstead, I. Stephens and P. J. Mikulka. Acquisition of conditioned aversion as a function of age and measurement technique. *Physiol. Psychol.* **3:** 379–384, 1975.
22. Kral, P. A. Effects of scopolamine injection during CS-US interval on conditioning. *Psychol. Rep.* **28:** 690, 1971.
23. McGlone, J. J., S. Ritter and K. W. Kelley. Area postrema lesion eliminates the antiaggressive and aversive properties of lithium. *Soc. Neurosci. Abstr.* **5:** 655, 1979.
24. Mucha, R. F. and D. van der Kooy. Reinforcing effects of intravenous and intracranial opiates revealed by a place preference paradigm. *Soc. Neurosci. Abstr.* **5:** 657, 1979.
25. Pinel, J. P. J. and R. F. Mucha. Activity and reactivity in rats at various intervals after footshock. *Can. J. Psychol.* **27:** 112–118, 1973.
26. Reicher, M. A. and F. W. Holman. Location preference and flavor aversion reinforced by amphetamine in rats. *Anim. Learn. Behav.* **5:** 343–346, 1977.
27. Revusky, S. and L. Parker. Aversions to unflavored water and cup drinking produced by delayed sickness. *J. exp. Psychol.: Anim. Behav. Processes* **2:** 342–353, 1976.
28. Rossi, N. A. and L. D. Reid. Affective states associated with morphine injections. *Physiol. Psychol.* **4:** 269–274, 1976.
29. Schuster, C. R. and R. L. Balster. The discriminative properties of drugs. In: *Advances in Behavioral Pharmacology*, Vol. 1, edited by T. Thompson and P. B. Dews. New York: Academic Press, 1977, pp. 85–138.
30. Thompson, T. and R. Pickens, editors. *Stimulus Properties of Drugs*. New York: Appleton-Century-Crofts, 1971.
31. Thompson, T. and C. R. Schuster. Morphine self-administration, food reinforced and avoidance behaviors in rhesus monkeys. *Psychopharmacologia* **5:** 87–94, 1964.
32. Vogel, J. R., R. A. Hughes and P. L. Carlton. Scopolamine, atropine and conditioned fear. *Psychopharmacologia* **10:** 409–416, 1967.
33. Winter, J. C. Drug-induced stimulus control. In: *Contemporary Research in Behavioral Pharmacology*, edited by D. E. Blackman and D. J. Sanger. New York: Plenum, 1978, pp. 201–237.