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Discriminated Taste Aversion and Context: A Progress Report

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JÄRBE, T. U. C. AND R. J. LAMB. *Discriminated taste aversion and context: A progress report.* PHARMACOL BIOCHEM BEHAV **64**(2) 403–407, 1999.—The research described here concerns the interaction between the environment (context), the organism, and the effects of opiates, focusing on how conditioning and contextual cues affect drug controlled behaviors. This analysis applies the powerful tool of drug discrimination to a respondent conditioning procedure (discriminated taste aversion, DTA). Data show that the use of DTA is feasible in that it is sensitive to morphine dose and saccharin concentration. Swifter control over DTA was achieved by increasing the LiCl dose (UCS magnitude). It is also clear that morphine alone can serve as a discriminative stimulus not requiring saccharin as a contextual element (which has been the case for most DTA studies to date), or saccharin being part of a compounded stimulus. Pharmacological specificity was demonstrated in substitution tests with delta-9-tetrahydrocannabinol. This research continues a systematic experimental analysis of the interaction between drug-controlled behavior and context. © 1999 Elsevier Science Inc.

Taste aversion Saccharin Drug discrimination Morphine Δ^9-THC Rats

DRUG discrimination is the most commonly used paradigm for studying the stimulus functions of drugs in general, and drugs of abuse in particular (2). Although much is known about the general properties of drugs as discriminative stimuli [see (5,9)], and about specific drug classes, such as opioids, ethanol, and cannabinoids [see (2)], less is known about the interactions between drug stimuli and other classes of stimuli [see (6,14–16)]. Like other stimuli, drugs can be part of a compound stimulus, for example, rats can be taught to turn left to escape shock following the administration of pentobarbital when the maze is dark and right following administration of saline when the maze is lit [e.g., (5,6)]. Probably, in the less restricted environments in which we live our daily lives such compound stimuli are the rule rather than the exception.

Determining if the control over behavior by exteroceptive and drug stimuli is similar has scientific importance not only for our understanding of the addictive process and the interpretation of drug discrimination data (15), but also for our understanding of the role of private events, such as anxiety or anger, in the control of behavior in general. The methods employed and the interpretation of results in drug discrimination research are derived from methods and interpretations developed for exteroceptive stimuli based upon the correspondence in the control of behavior by public- and private-events suggested over 40 years ago (19,20). However, crucial elements of these two types of stimuli in the control of behavior remain to be ascertained (5,11,21). Still, although few comparative experiments have been carried out to date [e.g., (6,14, 16)], available data indicate that drugs and exteroceptive stimuli share many common attributes as discriminative stimuli.

Following the initial demonstrations by Lucki (13) and Riley and associates (10,18) that conditioned taste aversion (CTA) can serve as a baseline for drug discriminative responding (DTA), many subsequent reports have extended the analysis of drugs serving a stimulus function in the DTA paradigm [e.g., (3,4)]. Unfortunately, virtually nothing is known about how contextual stimuli and conditioning factors interact with drug discriminative stimuli in such a respondent or classical conditioning paradigm.

In the preface of the program for the 20th celebration meeting of the Society of the Stimulus Properties of Drugs (SSPD) held in New Orleans, LA, October 1997, the first SSPD President Professor D. Overton wrote, "In the meantime, how can we summarize our state of affairs? We have learned a lot about the descriptive phenomenology of SDL (state dependent learning) and DDs (drug discrimination) which is good. Our research has helped us to learn more about neurochemistry than we would otherwise have learned which is also good. And we have cooperated and kept each other company along the way, which is as it should be. But we

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have learned little about the psychological underpinnings of the SDL and DD phenomena. That remains to be accomplished. Good luck to you all as you continue to work towards that goal." The present research is aimed towards that goal.

METHODS AND EXPERIMENTS

The general methodology for the present DTA experiments is described below, followed by an outline of specific experimental procedures.

Subjects

Male Sprague–Dawley rats were used as subjects for these DTA experiments. Upon arrival to our vivarium, the weights of the rats ranged between 275 to 325 g. Rats were individually housed in a colony room having a 12 L:12 D cycle. Daily access to water was limited (see below); because of this restriction in water supply, the weights were maintained at about 80% of rats' expected free-feeding body weight. Rats had unlimited access to pellet food.

Apparatus

Depending on the experiment, there was one novel taste stimulus [saccharin (*o*-sulfobenzimide sodium salt) dissolved in tap water, 0.2–0.0001% w/v, presented in Nalgene 250 ml bottles with #7 rubber stoppers], and one novel training drug stimulus (morphine sulfate 0–18 mg/kg dissolved in normal saline and injected IP in a volume of 1 ml/kg). In the first two studies described below, the protocol also included a novel tongue/tactile stimulus consisting of a ball bearing nozzle [see (7,12)]. The diameter of the drinking opening of the stainless steel nonball bearing nozzle was 3 mm. The outer diameter for the nozzle tube was 7 mm. The length of the tube protruding into the rat cage was around 50 mm for both types of nozzles.

Procedure

During the 1-week acclimatization period, water was freely available. After acclimatization access to water was restricted to two sessions per day, for a total of 1-h access per day during weekdays. The morning session lasted 30 min, and was the experimental session. The afternoon session was 30 min without experimental manipulations. With the exception of the first study (see below), or when conditioning only the saccharin element as in Experiment 5, IP injections of morphine sulfate (dissolved in saline and injected in a volume of 1 ml/kg), or saline occurred 20 min prior to the 30-min morning fluid presentation (water or saccharin). Immediately after this drinking period, the rats were injected IP with either 10 ml/kg of lithium chloride (LiCl, dissolved in normal saline) or the control solution (10 ml/kg 0.9% saline). In the first study, the injection-to-session interval was the experimental question and three injection-to-session intervals after morphine were examined, viz., 5, 10, and 20 min postinjection. When conditioning involved saccharin alone, morning drinking sessions were preceded by an IP injection of 1 ml/kg saline.

Rats were trained Monday through Friday, and drug/saline presentations alternated daily. On weekends, the animals were given free access to water from Friday afternoon to Sunday afternoon. Training and testing took place in the rats' home cages in the vivarium. Rats usually consume between 10 to 20 ml of fluid during a session when saccharin or water are not paired with LiCl. Discrimination is considered evident whenever there is a statistically significant difference in fluid consumption between LiCl and saline sessions. A discrimination was considered robust when the difference in fluid consumption between LiCl and saline sessions differ by a factor of at least three, i.e., consumption is three times as much in the non-LiCl condition compared to the paired LiCl condition. To control for potential unconditioned effects of morphine and saccharin, control rats were included. The only difference between the two types of groups (experimental and control animals), is that the control group receives postsession saline in lieu of postsession LiCl in the experimental group. In all other respects they were treated similarly. In Experiment 4, (−)-delta-9-tetrahydrocannabinol (Δ^9-THC) was examined. Δ^9-THC was injected IP 2 ml/kg 30 min prior to drinking as a suspension consisting of 5% propylene glycol, 3% Tween-80, and saline. The amount of Tween-80 was increased to 4% at the expense of saline and the volume administered increased to 3 ml/kg for the highest dose (10 mg/kg) of Δ^9-THC tested. Below we describe the experiments in the chronological order by which they were carried out (Fig. 1).

RESULTS AND DISCUSSION

Experiment 1

In our first DTA experiment, the effects of the morphine pretreatment interval used on the stimulus control exerted by a multielemental stimulus consisting of morphine (5.6 mg/kg), saccharin (0.2%, w/v), and a ball-bearing drinking nozzle in a discriminated taste aversion procedure was examined. In this discriminated multielemental aversion procedure, rats received injections of LiCl (0.15 M) following presentation of this multielemental stimulus, and injections of saline following the saline, tap water, and nonball bearing nozzle composite stimulus. These experimental rats were compared to control rats that received saline injections rather than LiCl injections following presentation of this multielemental stimulus. Morphine pretreatment times of 5, 10, and 20 min were examined in groups of 12 experimental and 6 control rats. The discrimination was rapidly learned under all three pretreatment intervals. By the third saccharin session, the average intake of the saccharin solution was around 1 g in all of the three experimental groups.

In subsequent testing with each individual stimulus element and combinations of two stimulus elements, stimulus control was clearly exerted by both morphine and saccharin. Experimental rats drank less saccharin than the controls, and less saccharin than water. Similarly, experimental rats drank less fluid following morphine administration than following saline administration, and less fluid than control rats did following morphine administration. Control by the nozzle type was also apparent in significant interactions between the nozzle, morphine, or saccharin and pairing with LiCl.

In general, pretreatment time did not influence the stimulus control that developed. However, at the shorter pretreatment times there was some indication that a conditioned taste aversion to morphine was developing in the control rats with the two shorter pretreatment times. In conclusion, our first DTA study indicated (a) that our discriminated taste aversion procedure may be a viable method for studying the contextual control of how drugs function as discriminative stimuli; and (b) that longer drug pretreatment times may be desirable in such procedures. Järbe and Lamb (7) provide a full account of the data.

Experiment 2

In our second study employing the DTA technique, the effects of saccharin concentration on the stimulus control by a compound stimulus consisting of morphine (5.6 mg/kg), saccharin (0.01, 0.03, or 0.10 %, w/v), and a ball bearing drinking nozzle in the DTA procedure were examined. In experimental rats (n = 12), injections of LiCl (0.15 M) followed this compound stimulus, while in control rats (n = 6) saline injections followed this compound stimulus. For reasons discussed above, we choose the 20-min presession interval for morphine administration. DTA acquisition was more rapid with higher saccharin concentrations. In testing with each individual stimulus element, stimulus control was clearly exerted by all three stimulus elements. When another stimulus element was presented jointly with saccharin, behavioral control was similar to that of saccharin alone. Behavioral control by saccharin increased with saccharin concentration. However, behavioral control by the two other stimulus elements was relatively unaffected by increasing the saliency of the saccharin element. Yet, stimulus control was evident with all three stimuli. For a detailed description of these data, see Lamb and Järbe (12).

Experiment 3

In the two DTA experiments described above, the saccharin component of the composite was the single element most strongly controlling drinking. Therefore, one might raise the question if taste always will be the main controlling factor whenever present because rodents would be biologically prepared to more easily making an association between taste/ingestion and malaise and that other correlating events would play the role of supporting, added features. Rephrased in other words, the question is "Can drug, i.e., morphine 5.6 mg/kg, become the major controlling factor when ingestion of fluid is followed by malaise?" in our DTA procedure.

To examine this question, we used two groups of rats (n = 8) of the same gender and strain as those used in the previous DTA studies. As before, the experimental rats were given IP morphine (5.6 mg/kg) 20 min prior to being exposed to a saccharin solution (0.003%) followed 30 min later by 10 ml/kg 0.15 M LiCl. On alternate days, 1 ml/kg saline was followed 20 min later by a presentation of regular tap water and 30 min thereafter the animals were given an IP injection of 10 ml/kg saline. The control animals were treated in an identical manner, except 10 ml/kg saline replaced LiCl on morphine/saccharin sessions.

As can be seen from the figure, the variances in consumption between saccharin and tap water were not overlapping in the experimental group by conditioning trial 3. By conditioning trial 6 and onwards, consumption of saccharin was very low in the experimental (morphine plus saccharin followed by LiCl) animals, whereas no consistent difference(s) in the amount fluid ingested emerged among the controls.

Subsequently, we tested the two elements of the composite separately. We observed that morphine exerted stronger control over drinking than did saccharin by comparing the consumption of fluid under the conditions of "morphine–water" and "saline–saccharin" in the experimental animals. However, some control by saccharin was also observed among the experimental animals, i.e., the experimental rats drank less of the saccharin solution compared to plain tap water. These comparisons were not significant for the control rats. To reiterate, these results are important because they show that conditions can be created where morphine exerts stronger control than saccharin, even though saccharin played a significant

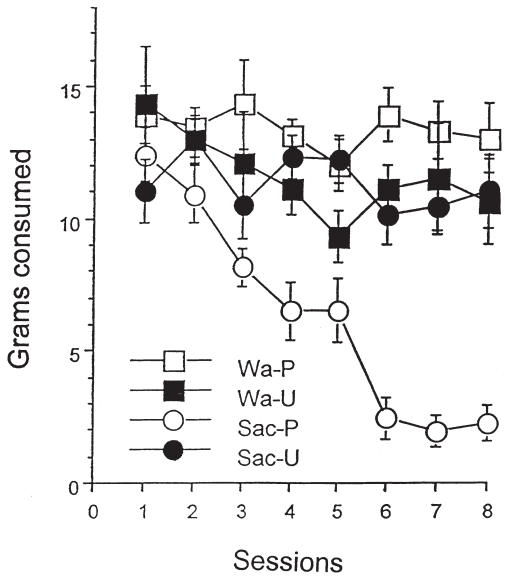

FIG. 1. Acquisition of a DTA between morphine/saccharin/LiCl vs. saline/water/NaCl in paired, experimental rats (n = 8). Controls (unpaired rats; n = 8) were treated similarly except NaCl replaced LiCl. Wa-P = water condition for the paired rats; WA-UP = water condition for the unpaired rats; Sac-P = saccharin condition for the paired rats; and Sac-UP = saccharin condition for the unpaired rats. The condition "Morphine-Saccharin-LiCl" alternated on a daily basis with the condition "Saline-Water-NaCl." Error bars indicate ±SEM. Animals were adult male Sprague–Dawley rats from Taconic Farms (Germantown, NY).

part of the stimulus compound. As part of this third DTA study, we also examined if acquisition of a DTA could be accelerated by using only paired LiCl sessions and thus forsake alternating "safe" morning sessions where drinking was not followed by LiCl (which would be the equivalent to a state dependency design in this situation). Indeed, acquisition of DTA was faster for such groups, but problems were encountered in tests for stimulus generalization in that rats trained with these procedures did not sample the liquids. Hence, the response did not show state dependency because in initial tests without the morphine cue there was clearly a transfer to the nondrugged state (i.e., suppression of drinking). Therefore, explicit discrimination training seems necessary in this procedure for assessing generalization gradients. Additionally, two higher doses of morphine were examined for the establishment of DTA behavioral control, viz., 10 and 18 mg/kg morphine (n = 8). Although morphine control of fluid ingestion seemed evident with these higher doses, the unconditioned drug effects make interpretation(s) difficult. Thus, using doses of morphine 10 mg/kg and above in drug naive animals does not seem viable under the present experimental conditions.

Experiment 4

In a fourth study we used different groups of rats (n = 8) that were conditioned with different doses of LiCl (range 30 to 180 mg/kg). In the previous experiments the dose of LiCl had been 0.15 M (~63 mg/kg). We had become concerned that this dose might be less than optimal for this kind of work. Results indicated that indeed a higher dose of LiCl resulted in a faster acquisition of the discrimination between 5.6 mg/kg

morphine and saline. Additionally, we found that the control of drinking is more robust with the higher doses of LiCl. Across the tested groups (60 to 180 mg/kg conditions), we found that the control by morphine is dose related. In tests with Δ^9-THC, the main psychoactive constituent in marijuana, we found that stimulus control is specific to morphine (and presumably pharmacologically related agents). The dose effect curve for Δ^9-THC (dose range 0.3 to 10 mg/kg) was very similar in both the control and the experimental animals. In contrast, morphine in doses of 1.75 mg/kg and above clearly suppressed drinking among the LiCl-treated animals but did not suppress drinking among the controls. The test doses of morphine examined ranged between 0.3 to 10 mg/kg.

Once the drug/dose testing was accomplished the animals were put under extinction conditions, i.e., morphine-drinking sessions were no longer followed by an administration of LiCl, but rather saline only was given. The results indicated no major effect in terms of number of sessions to extinction between the various LiCl conditions. It was expected that sessions to extinction would be related to LiCl dose (UCS magnitude). It is possible that the fairly extensive drug and dose testing occurring between acquisition and extinction may have obscured the predicted outcome. Once suppression of drinking during morphine sessions was no longer evident (extinction), the animals were again tested with 3 and 10 mg/kg of morphine. After extinction, the effects of morphine (3 and 10 mg/kg) were similar across groups, i.e., one-way ANOVAs were nonsignificant ($p > 0.05$). In the only other study where an extinction procedure was used to manipulate drug discriminative responding, rats discriminating a higher dose of chlordiazepoxide extinguished lever pressing for food faster than the group trained with a lower dose of the drug using an operant approach (17).

This fourth study is important not only because of the useful information about CS–UCS intensity relationships gathered, but also because of the demonstration that drug (morphine) alone served the discriminative function in our DTA. Previous studies have either relied on saccharin as a contextual element, or saccharin being part of a compounded stimulus as in our previous studies (experiments 1–3). Furthermore, the pharmacological specificity seen in tests with Δ^9-THC is reassuring in that it agrees with previous data obtained with more conventional drug discrimination studies employing operant methodology (1,8).

Experiment 5

DTA experiments to be carried out in the future will use combinations of morphine and saccharin designed to investigate the procedure of interest under various stimuli intensity combinations. That is, (a) conditions favoring dominance by each stimulus will be examined; (b) as well as conditions where the two stimuli are of relatively equal saliency, while absolute stimulus intensity is varied systematically.

Therefore, in a fifth study we examined the acquisition of a discriminated taste aversion as a function of morphine dose. Thus, different groups of rats ($n = 8$) received 0, 1, 1.8, 3 or 5.6 mg/kg morphine followed by LiCl (120 mg/kg) on alternating days of water presentation. On alternate days the drinking session was preceded by an injection of 1 ml/kg saline, and when the drinking session was over, the animals were given 10 ml/kg saline. Acquisition of the discriminated taste aversion was dose related such that the higher the morphine dose, the faster the formation of the aversion. The zero morphine dose was included to control for the possible temporal control of responding by the alternating sessions. That is, these animals were always pretreated with saline followed by alternating days of postsession treatment with LiCl and saline. We found that the two conditions (water followed by saline and water followed by LiCl) were indistinguishable, as revealed by the two plots virtually overlapping each other throughout the 100 alternating sessions of LiCl (50 sessions) and saline (50 sessions) examined (data not shown). Such results argue against drinking in our DTA procedure being controlled by daily alternating treatment per se.

A similar study is in progress where different concentrations of saccharin (range: 0.01 to 0.0001%) are being used as discriminative stimuli. Preliminary results also suggest a tendency for a concentration-dependent acquisition for the discrimination between saccharin and water. This would be analogous to the dose (concentration)-dependent acquisition of the above-described morphine DTA.

In summary, the data presented here have shown that the use of DTA is feasible in that it is sensitive to morphine dose and saccharin concentration. We have also established better control over the DTA by increasing the LiCl dose (UCS magnitude). It is also clear that morphine alone can serve as a discriminative stimulus not requiring saccharin as a contextual element (which has been the case for most DTA studies to date), or saccharin as part of a compounded stimulus (as in Experiments 1–3 described above). Pharmacological specificity was demonstrated in substitution tests with Δ^9-THC. This research continues a systematic experimental analysis of the interaction between drug-controlled behavior and context. Specifically, the control of behavior by drugs functioning as discriminative stimuli is examined. In particular, control of behavior will be examined in overshadowing, blocking, reversal learning, and context-dependent extinction paradigms. In the future, it is our intention to also include analyses of other stimulus–stimulus associations, such as those captured in designs commonly referred to as sensory preconditioning and second-, or higher order conditioning experiments.

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