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Modulation of Conditioned Taste Aversion by Sodium Pentobarbital[1]

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CONCANNON, J. T. AND J. FREDA. *Modulation of conditioned taste aversion by sodium pentobarbital.* PHARMAC. BIOCHEM. BEHAV. 13(6) 761–764, 1980.—The effects of pentobarbital on the formation and expression of LiCl induced taste aversion were examined using a two-bottle preference test. Rats adapted to restricted fluid intake were offered a 15% sucrose solution 15 min after a pentobarbital or saline injection but prior to post-CS LiCl or control injections. All animals were tested 3 days later in either the same or opposite drug state, and were returned to the conditioning day drug state for a second test. The results showed that pentobarbital in testing disrupted evidence for taste aversion in a manner not simply accounted for by its dipsogenic effects. It was suggested that the present paradigm may prove to be a simple behavioral assay for screening putative anxiolytic drugs.

Pentobarbital Conditioned taste aversion Lithium chloride Anxiolytic drugs Conflict tests

SODIUM pentobarbital, a potent central nervous system (CNS) depressant, is an agent possessing potent dipsogenic and conflict-reducing properties. For example, pentobarbital can increase fluid consumption in deprived and non-deprived rats [8,10], and can increase punished responding in conflict-like situations [2]. This latter finding suggests that pentobarbital has anxiolytic properties, as seen, for example, in decreased response suppression in the conditioned emotional response (CER) paradigm [5], a property shared with amobarbital [9] and other CNS depressants (e.g., the benzodiazepine chlordiazepoxide [9]).

It may be of considerable theoretical interest to know whether pentobarbital's consummatory-potentiating (i.e., dipsogenic) and/or putative anxiolytic effects might disrupt evidence for conditioning in the conditioned taste aversion (CTA) paradigm, which is a consummatory-based paradigm that contains elements of both conflict and response suppression. That is, pentobarbital's direct, non-associative increase in fluid consumption may bias a previously poisoned animal to drink relatively more of the poisoned substance than would a previously poisoned animal injected with saline prior to CTA testing [4]. If CTA testing were performed using only a single-bottle test, in which absolute fluid intake is indexed, then pentobarbital's non-associative dipsogenic effect may be impossible to separate from pentobarbital's specific disruption of the CS-UCS association [3]. If, on the other hand, this non-associative influence could be minimized (e.g., by use of a 2-bottle test), then it is easier to see that administration of pentobarbital prior to CTA testing might attenuate evidence for CTA due to its direct anxiolytic properties. Of paramount importance for the anxiolytic interpretation, furthermore, is that pentobarbital be most effective in animals receiving a CS-UCS pairing, whereas it should be either completely ineffective or relatively less effective in animals injected with a control substance post-CS exposure.

Accordingly, our study was designed to determine whether pentobarbital would prevent against detection of CTA when administered during testing [4], and/or interfere with taste aversion conditioning per se. To address these issues we employed a factorial combination of pentobarbital or saline prior to training or testing in poisoned and non-poisoned subjects. Furthermore, we included a second retention test to assess the relative permanence of the influence of pentobarbital on manifestation of CTA conditioning. Hence, this design will allow for a determination of the effects of pentobarbital on neophobia and on original conditioning, in addition to allowing for a determination of pentobarbital's effects on manifestation of CTA. Lastly, pentobarbital's effects will be assessed using sensitive two-bottle retention tests in the attempt to minimize motivational (i.e. dipsogenic) confounds (based on absolute CS intake) in this associative learning paradigm.

METHOD

Animals

The subjects were 48 experimentally-naive male Sprague-Dawley rats bred and raised in the Department colony. The animals were approximately 125 days old and weighed between 312 and 429 g at the beginning of the experiment. All rats were housed in individual wire mesh cages

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(25×18×18 cm) in the vivarium which was maintained at approximately 70°F at 55% relative humidity. Food was available ad lib in the home cages throughout the duration of the experiment. The animals received all treatments during the lights "on" portion (i.e., between 0630 and 2000 hr) of their light-dark regimen and were run in two replications with 24 animals per replication.

Apparatus

The experiment was conducted in one of six identical 24×14×20 cm chambers of a 6-compartment wooden drinking box. Each of the compartments had a wire mesh floor and a clear Plexiglas cover. On the front of each compartment were 3 13-mm diameter holes, one located in the center of the wall and the other two equidistant from the center hole and the side walls of the chambers. The center hole was used for insertion of the drinking tubes for the pre-conditioning days, the single conditioning day, and the water recovery days; the side holes were used for the 2-bottle preference tests.

Procedure

The complete experimental procedure is outlined in Table 1. For 6 consecutive days prior to conditioning all animals were placed in the wooden drinking boxes on a restricted fluid access schedule, which remained in effect throughout the duration of the experiment. The first day of the pre-conditioning phase consisted of a 30-min water access period, while days 2–6 allowed for 10 min water access. All water access was given while the drinking tube remained in the center position and while the lights were on in the experimental room. Intake was measured using 100-ml calibrated drinking devices (1.0 ml accuracy) with non-drip ball bearing spouts. After the last pre-conditioning session all animals were matched for fluid intake and body weight by the randomized blocks method and were assigned to one of 8 treatment groups with 6 subjects per group.

Fifteen minutes prior to Conditioning Day sucrose access one-half of the animals were administered an intraperitoneal (IP) injection of Sodium Pentobarbital (15.0 mg/kg) while the other half received a matched volume of physiological saline (1.0 ml/kg). All animals were then placed in their holding cage to await the onset of drug action. Animals were then given a 10-min access period to a 15% (w/v) sucrose solution, which was followed immediately by an IP injection (1.0 ml/kg) of 3.0 MEq Lithium Chloride (LiCL) for one-half of the animals or by an equal volume of physiological saline (Control) for the other half of the animals. On post-conditioning days 1 and 2 all animals were allowed 10 min water access recovery sessions in which no drugs were present.

Testing was conducted on the two days following the two water recovery days. On Test Day One all animals were given a 20-min access to sucrose *and* water with the position of the bottles (i.e. left or right of center) being counterbalanced across groups. Fifteen min prior to the 2-bottle test one-half of the pentobarbital (D) and saline (N) animals who received the LiCl or Control (saline) injection received a pentobarbital (D) injection while the other half received a saline (N) injection. Thus, Test Day One was represented by a 2×2×2 factorial design with the main factors being post-sucrose UCS (LiCl vs Control), Drug in Conditioning (pentobarbital vs saline), and Drug in Testing (pentobarbital vs saline).

The procedure for Test Day Two was identical to that for Test Day One except that all animals were given the drug that they had received on Conditioning Day (i.e., pentobarbital or saline). Thus Test Day Two intake could be examined in a 2×2×2 factorial design with the main factors being post-sucrose UCS (LiCl vs Control), Drug on Test Day One (pentobarbital vs saline), and Drug on Test Day Two (pentobarbital vs saline).

RESULTS

The mean absolute sucrose intake during Conditioning Day for all eight treatment groups (Table 2) was examined using a one-way analysis of variance (ANOVA) which failed to generate any significant between-group differences, $F(7,40)<1.00$, on original sucrose consumption. Hence, pentobarbital did not increase intake on the "neophobia" test of 10 min duration, although such an effect might have occurred if the conditioning session were longer in duration (e.g., 30 *min*).

Mean sucrose indices (SIs) were calculated for Test Days One and Two by dividing the amount of sucrose intake by the total amount of fluid (i.e., sucrose plus water) consumed on the test day. This index shows a preference for sucrose if the ratio is greater than .50 and an aversion to sucrose if the ratio is significantly less than .50. The results using this index (Table 2) are strikingly similar for Test Days One and Two. That is, groups receiving post-sucrose LiCl show a CTA, as indicated by their low SIs relative to the Control (saline) groups. Secondly, pentobarbital leads to a higher SI in those animals that received pentobarbital in testing, regardless of the drug treatment received in Conditioning or on the previous test day. Finally, the presence of pentobarbital in testing tends to disrupt evidence for CTA, since the LiCl animals receiving pentobarbital in testing exceed their comparison groups receiving saline in testing to a degree greater than do the Control animals receiving pentobarbital in testing (i.e., the UCS interacts with Drug in Testing).

These results were verified statistically with the aid of 2×2×2 factorial ANOVAs (UCS × Drug in Conditioning × Drug in Testing, for Test Day One; UCS × Drug on Test Day One × Drug on Test Day Two, for Test Day Two) of the SIs. For Test Day One, the analyses generated reliable effects of UCS, $F(1,40)=56.36$, $p<0.001$, Drug in Testing, $F(1,40)=54.95$, $p<0.001$, and the interaction between UCS and Drug in Testing, $F(1,40)=4.27$, $p<0.05$. The nature of the UCS × Drug in Testing interaction was further examined using a Duncan's Multiple Range Test ($p\leq0.05$). The results of this test showed that the saline animals had statistically lower SIs than did pentobarbital animals for the Control groups and for the LiCl groups, although the differences were more highly reliable for the LiCl groups (means: D: Control = .85; N: Control = .56; D: LiCl = .55; and N: LiCl = .03, respectively). For Test Day Two the analyses generated reliable effects of UCS, $F(1,40)=88.43$, $p<0.001$, Drug on Test Day Two, $F(1,40)=74.57$, $p<0.001$, and the interaction between UCS and Drug on Test Day Two, $F(1,40)=42.25$, $p<0.001$. Furthermore, there was a marginally significant effect of the interaction of UCS × Drug on Test Day One × Drug on Test Day Two, $F(1,40)=3.73$, $p=0.06$. The nature of the UCS × Drug on Test Day Two interaction, examined with the aid of a Duncan's Multiple Range Test, showed that the saline and pentobarbital Control animals did not differ in their SIs, whereas pentobarbital on Test Day Two attenuated the taste aversion seen in the LiCl groups. The nature of the marginally significant third-order interac-

TABLE 1

SCHEMATIC REPRESENTATION OF THE PROCEDURE FOR THE CTA PARADIGM

Pre-conditioning days (Days 1–6)	Conditioning day (Day 7)	Recovery days (Days 8&9)	Test days: Day 1 (Day 10)	Test days: Day 2 (Day 11)
All subjects	N-Control	All subjects:	Control groups:	Control groups:
	D-Control		1. NN	1. NNN
Day 1: 30 min		10 min	2. ND	2. NDN
water access	N-LiCl	water	3. DN	3. DND
		access	4. DD	4. DDD
Days 2–6: 10 min water access	D-LiCl			
			LiCl groups	LiCl groups
			5. NN	5. NNN
			6. ND	6. NDN
			7. DN	7. DND
			8. DD	8. DDD

D=pentobarbital, N=saline for pre-conditioning/pre-testing injections.
LiCl=Lithium Chloride, Control=saline for post-sucrose injections.

TABLE 2

GROUP MEAN AND STANDARD ERROR OF THE MEAN FOR DIFFERENT PERFORMANCE INDICES

		Test day one			Test day two		
	CON SUC	SI	SUC	TF	SI	SUC	TF
Group							
1	12.50 ± 1.09	.59 ± .06	10.00 ± 1.24	19.00 ± 1.84	.81 ± .13	14.67 ± 0.82	18.17 ± 0.60
2	10.00 ± 2.04	.90 ± .03	17.17 ± 1.25	19.17 ± 1.40	.69 ± .20	11.17 ± 1.49	16.00 ± 0.86
3	11.67 ± 1.93	.52 ± .11	8.33 ± 2.12	15.17 ± 1.04	.81 ± .16	16.33 ± 1.65	20.50 ± 2.33
4	11.67 ± 0.80	.80 ± .08	13.17 ± 1.25	16.83 ± 1.01	.89 ± .06	18.33 ± 0.71	20.67 ± 1.14
5	12.67 ± 1.80	.04 ± .01	.67 ± 0.33	16.00 ± 1.41	.05 ± .07	1.00 ± 0.63	18.83 ± 0.91
6	13.67 ± 1.87	.49 ± .11	9.17 ± 2.02	20.00 ± 1.69	.03 ± .03	0.50 ± 0.22	15.50 ± 0.99
7	15.00 ± 1.06	.03 ± .02	.50 ± 0.34	17.67 ± 0.99	.81 ± .17	13.50 ± 1.28	17.00 ± 1.29
8	14.67 ± 2.81	.61 ± .10	9.83 ± 1.85	16.50 ± 1.67	.63 ± .26	14.50 ± 2.59	23.00 ± 3.53

CON SUC=Conditioning Day Sucrose Intake (ml).
SI=Sucrose Index.
SUC=Absolute Sucrose Intake (ml).
TF=Total Fluid Intake (ml).

tion of UCS × Drug on Test Day One × Drug on Test Day Two was examined by breaking down the UCS × Drug on Test Day Two interaction at the different levels of Drug on Test Day One and applying Duncan's Multiple Range Test. These results substantiated those for the UCS × Drug on Test Day Two interaction, since the NNN and NDN LiCl groups showed low SIs, and the DND and DDD LiCl animals showed higher SIs, although the Control groups did not differ in their SIs. In agreement with the results for Test Day One, evidence for CTA is most apparent in LiCl animals receiving saline or Test Day Two, while the presence of pentobarbital on Test Day Two disrupts evidence for CTA in the LiCl groups.

In addition to the SI, two other measures of performance during CTA testing are also presented in Table 2, i.e., (a) absolute sucrose intake (SUC), and (b) total fluid intake (TF: sucrose plus water). The results for absolute sucrose intake on Test Days One and Two are similar to those using the SIs, except for the fact that UCS did not interact with Drug in Testing. That is, the presence of pentobarbital in testing elevated sucrose consumption (relative to saline) to the same degree in animals receiving either LiCl or the Control injection post-sucrose consumption. On the other hand, the results of the manipulations on total fluid intake were inconsistent across the two test days. For Test Day One, animals receiving pentobarbital in Conditioning drank less total fluid

than those animals receiving saline in Conditioning (means = 16.54 and 18.54 ml, respectively). Pentobarbital on Test Day Two increased total fluid intake (means = 20.29 and 17.12 ml for pentobarbital and saline, respectively). Lastly, animals experiencing the same drug on Test Day One and Test Day Two drank more total fluid (mean = 20.16 ml) than animals experiencing a drug state change between Test Day One and Test Day Two (mean = 17.25 ml).

DISCUSSION

The major result of this experiment is that, within the present paradigm, the presence of pentobarbital in testing is able to block, at least temporarily, the manifestation of CTA in the LiCl groups. Conversely, of course, CTA is most evident whenever saline was present in testing (on either Test Day) in the poisoned groups. These results occurred even though pentobarbital had no reliable effect on sucrose consumption upon Conditioning Day or on original conditioning of the CTA—effects not assessed in a previous CTA study [4]. Finally, these results were not due solely to unconditional effect of pentobarbital on sucrose indices, since the critical differences in the LiCl groups existed over and above the effects of pentobarbital on the Control groups, as seen in the UCS × Drug on Test Day interactions.

As already stated in the Introduction, pentobarbital can increase absolute fluid consumption in deprived and non-deprived rats when using a single-bottle testing procedure. Indeed, there was some indication that pentobarbital in testing increased total fluid intake during Test Day Two in the present experiment, although this was not found during Test Day One. Furthermore, the presence of pentobarbital in testing increased absolute sucrose intake on both Test Days in a non-differential fashion for the LiCl and Control groups which is indicative of pentobarbital's non-associative, dipsogenic effects. Only when the SI was examined did the critical UCS × Drug on Test Day interactions emerge, suggesting that this is the most sensitive index for determining the anxiolytic properties of pentobarbital unconfounded by non-associative, dipsogenic effects of pentobarbital. Hence, we stress the point that anxiolytic effects of pentobarbital be examined using the sensitive 2-bottle CTA test [3] in a paradigm including animals who never receive poisoning.

With procedural problems aside, the remaining task is to specify the mechanism of action of pentobarbital in testing that allowed for the altered manifestation of CTA. The most apparent explanation is that pentobarbital in testing is acting as an anxiolytic agent by decreasing the conditioned anxiety (fear) and/or conflict associated with the choice between the two fluids. The former type of anxiolytic effect has already been well documented for CNS depressants by showing that they decrease response suppression for animals drugged in testing after receiving response non-contingent classical (i.e., CER) conditioning [1, 5, 9]. In this light CTA conditioning may be viewed as simply another type of classical conditioning in which aversive events (e.g., LiCl) are administered in the presence of the to-be-conditioned stimulus independent of an animal's behavior. This classically-conditioned fear in turn probably serves as the source of suppression of CS intake during non-reinforced extinction trials—behavior potentially laden with anxiety. It is important to note that, as in our present study, anxiolytic agents can prevent against manifestation of conditioned suppression even though they do not affect original conditioning per se ([9], but see [1,7]). The latter type of "anxiolytic" action mentioned above is more appropriately described as "anti-punishment" or "anti-conflict" behavior, since, unlike conditioned suppression, it depends on the reduction of contemporaneous responding associated with response-contingent application of aversive stimulation (i.e., punishment). Animals in the traditional Geller-Seifter [2] conflict procedure, for example, are faced with the choice of not responding or responding for combined appetitive reinforcement *and* electric shock, a choice associated with considerable anxiety. Similarly, animals in CTA testing are faced with the choice between not drinking or drinking a previously preferred, yet poisoned substance, and may therefore be considered to be experiencing experimentally-induced conflict. Regardless of its source, conflict-like behavior has been consistently shown to be reduced by anxiolytic agents such as pentobarbital (present experiment), ethanol, and chlordiazepoxide as is the case for these drugs when administered before conditioned suppression tests. Since conventional conflict paradigms are laden with interpretative difficulties [6] it would seem more reasonable to use conditioned suppression paradigms including CTA to measure anxiolytic effects of drugs since these paradigms are so simple to implement. Accordingly, we tentatively conclude that the CTA paradigm utilized herein is a simple, sensitive behavioral assay for studying the anxiolytic effects of pentobarbital uncomplicated by the interpretative problems of conventional conflict tests or the motivational problems associated with reliance on absolute fluid intake in some versions of CTA testing.

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