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Toward Understanding Ethanol's Capacity to be Reinforcing: A Conditioned Place Preference Following Injections of Ethanol

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REID, L. D., G. A. HUNTER, C. M. BEAMAN AND C. L. HUBBELL. *Toward understanding ethanol's capacity to be reinforcing: A conditioned place preference following injections of ethanol.* PHARMACOL BIOCHEM BEHAV **22**(3) 483–487, 1985.—Rats that had previously consumed a 6% ethanol (ETOH) solution daily for 26 days and rats without such a history served as subjects in a test for the ability of ETOH to establish a conditioned place preference. The time of putative conditioning was from 4 to 8 min after injections of ETOH, 1 g/kg. The combination of programming the period of putative conditioning to be shortly after injections and using rats habituated to drinking ETOH allowed a conditioned place preference to emerge after only a few conditioning trials. Such a result potentially reveals features of the way ETOH achieves its reinforcing capability and sets the stage for understanding the mechanism of that reinforcement.

Ethanol Conditioned place preference Alcoholism CPP-test

FROM the perspective of modern theory of addiction, established following studies of the prototypic addictive agent morphine (e.g., [15], and from analyses of the effects of ethanol (ETOH) (e.g., [1], it is presumed that ETOH reinforces ETOH-intake by way of its capacity to produce an increment in positive affect. (The term addiction is used rather than dependency, the popular alternative to addiction. The term dependency has implications evoking need-reduction theory of reinforcement, a theory that has been found wanting since the 1950s, also, see Goldstein [7]. Additionally, the use of the term dependency seems to force the distinction between physical and psychological dependence, a dualistic distinction that is at variance with a monistic resolution of the mind-brain problem.) Stated another way, one or more of ETOH's actions set the circumstances for positive reinforcement of ETOH-intake, thereby leading to an escalation of ETOH-intake which eventually leads to the full addiction syndrome known as alcoholism. If these critical affective, reinforcing events which maintain intake of ETOH are to be studied in the laboratory, a technique must be developed for indexing ETOH's capacity to elicit positive affect (positive reinforcement when there is a contingency).

In addition to studies of ETOH-intake in laboratory settings, there have been a number of attempts to more precisely index ETOH's capacity to elicit positive affect. One of those attempts involves observing subjects pressing for intracranial stimulation (ICS). It has been concluded that any drug increasing responding for positively reinforcing ICS is also a drug that has a capacity to elicit increments in activity in the brain areas activated by the ICS [5,20]. Such a drug, therefore, would have the capacity, in its own right, to be positively reinforcing. Consequently, the effects of ETOH on responding for ICS have been studied [6, 8, 11, 18, 19], although not extensively. The initial results of those studies indicate that doses of ETOH do, indeed, increase responding for ICS; however, the effects are often small and inconsistent.

Flanagan [6], for example, tested for the effects of doses of ETOH on pressing for hypothalamic ICS. Doses of ETOH ranging from 0.25 to 2.5 g/kg, in large volumes of saline, were injected interperitoneally (IP), 15 min before a session with ICS. Doses of 0.25 and 0.5 g/kg produced slight increments in pressing and doses of 1.0 g/kg and greater, reduced rate of pressing for ICS. Subsequently, we ([10]; Kelly, Flanagan, and Reid, unpublished manuscript) tested rats pressing for ICS after doses of ETOH, 0.5 and 1.0 g/kg (IP, 15 min before testing), both before and after forcing the subjects (by way of fluid deprivation) to consume ETOH-solutions daily for 30 days. Their responsiveness under ETOH was compared to rats that were tested concurrently, but did not take ETOH during the period intervening between tests. Prior to the 30-day period, the 0.5-g/kg dose produced slight increments in pressing for ICS compared to the effects of injections of placebo of the day before. Prior to the period, the dose of 1.0 g/kg reduced pressing. After the 30-day period, the doses produced similar effects in the rats not taking ETOH. In those rats that took ETOH for 30 days, however, both the doses of 0.5 and 1.0 g/kg produced a mean increment in

pressing. These doses were tested with only four rats per group and the results indicated a change in responsiveness to ETOH following ETOH-consumption which just failed to reach standards for statistical significance ($p=0.07$). The results seem to indicate that ETOH is more apt to facilitate pressing for ICS among rats that have consumed ETOH.

The preliminary study indicating that ETOH more readily facilitated pressing for ICS in rats having a history of ETOH-consumption was not extended, except for a test of the idea that the resulting increase following ETOH-injections was due to tolerance to the debilitating effects on motor capabilities ([10]; Kelly and Reid, unpublished manuscript). Contrary to what we presumed at the outset of the tests, 30 days of opportunity to drink ETOH did not produce tolerance to the debilitating effects of 1.0-g/kg doses of ETOH on our tests of motor skills. This dose of ETOH, for example, reduced pressing rates for water both before and after a 30-day ETOH consumption period. Yet, pressing for ICS was facilitated following the 30-day period of consumption by the 1.0-g/kg dose. From these preliminary results, it appears that experience with drinking ETOH somehow modifies the ability of ETOH to be a reinforcer (as indexed by modification of responsiveness for ICS) and this ability is not related to tolerance to the debilitating effects of ETOH on motor skills.

There has been another approach used in an attempt to measure ETOH's capacity to elicit positive affect, the conditioned place preference-test (CPP-test) [12]. The CPP-test is a procedure in which a subject is put into a particular place at a specific time after administration of a drug. Subsequently, subjects are tested to see if they prefer the place of the drug-experience. If the drug-experience is one of positive affect, it is presumed that subjects will spend more time in the place of the drug-experience. The initial attempts [2,17] to establish a CPP using the effects of ETOH as the unconditioned stimulus have not been completely successful. Stewart and Grupp [17], for example, were able to establish a CPP with ETOH-injections, but only when rats had food available in the place of putative conditioning.

Shippenberg *et al.* [13,14] confirmed that injections of ETOH produce a transient excitation followed by sedation. Rats were also trained to respond in a discrimination procedure so that they made one response when under the influence of ETOH and another under saline. Some rats were trained when the ETOH was producing the initial excitatory effects (about 6 min after injection) while others were trained when ETOH was producing sedation (about 30 min after injection). It was found that the introceptive cue of excitation did not generalize to that of sedation. There was some indication that naloxone, the prototypic antagonist of morphine and related opioids, blocked features of the excitatory phase of ETOH's action, but did not modify those of the sedative phase. These data lead to the suggestion that the initial effects of ETOH are distinguishable from subsequent effects, and further suggest that these features may be differentially related to ETOH's ability to be positively reinforcing.

We reasoned, from the early tests with ETOH and ICS, that a CPP is more apt to be established in rats having a history of ETOH-intake. Consequently, we [9] tested that, using rats which either did or did not have experience drinking ETOH containing beverages. A dose of 0.5 g/kg, IP, was used with the conditioning period being 30 min, starting 15 min after injections. This is a time at or following peak levels of ETOH in brain. No reliable CPP was established with that test. We reasoned from (a) this failure to establish a CPP, (b) some recent tests with ETOH and ICS showing facilitation of pressing shortly after injections, and (c) the data of Shippenberg *et al.* [13,14] potentially showing that the time just following injections to be the period of positive affect, that a CPP could be established, provided the period of putative conditioning was shortly after injections. Also, from the early studies of ETOH and pressing for ICS, it was hypothesized that injections of ETOH would be considerably more effective in rats having had experience drinking ETOH. Therefore, we tested this reasoning and report the results here, the first clear demonstration that a CPP can be established in a laboratory setting with ETOH (see, however, [3]).

METHOD

Subjects

Forty-eight, male, Sprague-Dawley derived rats participated in a 2-day, preliminary procedure that yielded baseline-scores. On the basis of those scores, 40 rats were selected for further study (procedure and criteria for selection given subsequently).

All rats were purchased, as young adults (about 200 g in body weight), from Taconic Farms (Germantown, NY). All were housed individually in standard, hanging cages with food always available. The cages were kept in a windowless colony room maintained at 24°C and having 12 hr of artificial light/day beginning at 1000 hr.

Prior to the conditioning procedures, one-half of the rats had the experience of drinking an ETOH solution while the other half did not. The experienced rats (drinkers) had a 1-hr opportunity to drink a 6% solution of ETOH followed by 3 hr of access to tap water daily for 26 days, 35 days prior to participating in the conditioning procedures. Rats typically consumed an average of 8 to 11 ml/day of this ETOH-solution when forced to drink by the moderate deprivation schedule. Except for the aforementioned 26 days, subjects had tap water always available. The rats with no prior experience with ETOH (non-drinkers) were housed in the same colony room for a comparable period. The rats' mean body weight with the beginning of the conditioning procedure was 406 g.

Apparatus

The apparatus is the same one used by Stapleton *et al.* [16] to demonstrate that an enkephalin analogue was capable of eliciting positive affect. There were four nearly identical boxes. Each box was an alley 94×19×28.5 cm. There were three sections to an alley. The middle section (9×19×28.5 cm) had a wooden floor and was painted grey. This middle section could be separated from the two sides by guillotine doors. One side had a hardware cloth floor with 3.6 by 1.0 cm grids, was painted with horizontal black and white stripes (1.8 cm wide), and called the horizontal side. The other side had hardware cloth floor having a different grid-size (1.0 by 1.0 cm) and was painted with vertical black and white stripes (the vertical side). Both sides were 42.5×19×28.5 cm.

Procedure

The conditioning and testing procedure occurred between 1500 and 1800 hr daily, across a 3-week period. On the 1st day of this procedure, a rat was placed in the center compartment of the box and the guillotine doors were then removed allowing the rat access to the entire alley for 10 min.

On the next day, this was repeated, and the time a rat spent on the horizontal side of the apparatus was recorded. The middle of the grey area was the demarcation between the two sides of the alley. A rat was considered to be on a given side until it moved its head and front paws from that side. The baseline scores of the 2nd day in the alley were used for comparison of effects of putative conditioning and were used to select subjects for groups.

There were four groups, two of which were controls. One-half of the subjects of each control group were drinkers and the other half were nondrinkers. One experimental group consisted of only drinkers and the other group was only nondrinkers. Given these limitations, rats were assigned randomly to their respective groups. The two rats of each group showing the most extreme baseline scores were excluded, thereby yielding groups of 10 rats with mean baseline scores close to 300 sec and no extreme scores (limits of range of baseline scores across all groups=234 to 358 sec). There were no reliable differences in baseline scores among the resultant groups, $F(3,36)<1$.

During each of the 3 weeks immediately following determination of baseline, rats received 4 days of their respective treatments and a test-day, followed by 2 days of no experimental procedure. On the 1st and 3rd days of the treatment sequence, a rat was confined to the horizontal side of the alley for 4 min, beginning 4 min after an injection (a time used by Shippenberg *et al.* [13]. On the 2nd and 4th days, a rat was confined to the vertical side again for 4 min, 4 min after an injection. What distinguished the groups was the kind of injection they received before being placed in a side of the alley.

Rats received injections of either physiological saline or an ETOH-solution. The ETOH-injections were 10% U.S.P. ethyl alcohol, dehydrated (U.S. Industrial Chemical Co.) in physiological saline at a dose of 1.0 g/kg. The saline-injections were the same volume as ETOH-injections, 10 ml/kg.

One control group received saline-injections prior to each treatment (S-S). The other control group received ETOH-injections prior to each treatment (E-E). One-half of each experimental group received saline on Days 1 and 3, the other half on Days 2 and 4. On alternate days of the regimen, these rats received ETOH. The consequences of the differential injections were that rats of each experimental group had the opportunity to associate the effects of ETOH-injections to only one side of the alley (half to horizontal, half to vertical), whereas the control groups had either (a) no such opportunity (S-S controls) or (b) an equal opportunity to associate ETOH-effects to both sides of the alley (E-E controls).

On test days, rats were allowed access to the entire alley for 10 min (600 sec) as they were at baseline. The time they spent on the horizontal side was recorded. For experimental rats that received ETOH-injections prior to being placed on the vertical side, their scores (time on horizontal side) were subtracted from 600 sec so that the resultant scores reflect the time spent on the side of putative conditioning. With this simple transformation of these rats' scores, any score greater than 300 indicates that an experimental rat spent the most time on the side of putative conditioning (potentially indicating a preference) whereas any score less than 300 sec indicates a rat spent less time on the side of putative conditioning (potentially indicating an aversion).

An initial analysis of the data indicated that the mean scores of the two control groups did not differ reliably from one another, $F(1,18)<1$. Consequently, they can be considered a single group (CON). The scores, therefore, conform to an experimental design for a 3 by 4 factorial analysis of variance (ANOVA) for repeated measures having a factor for the three groups (CON, drinkers, nondrinkers) and a factor for the repeated tests (baseline, Tests 1, 2, and 3).

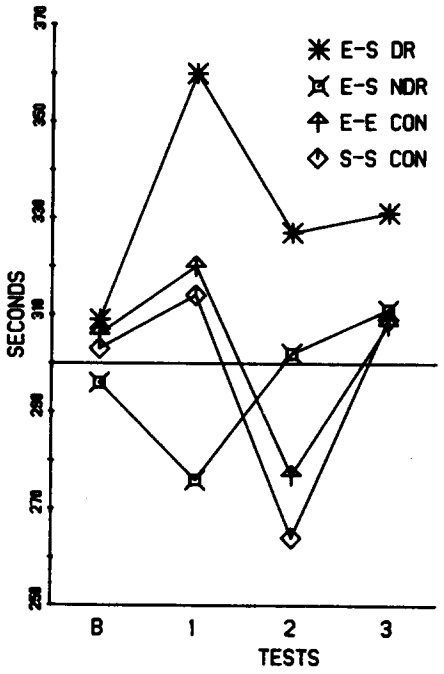

FIG. 1. Seconds on the side of putative conditioning for the four groups of this experiment. Measures were taken before conditioning (baseline, designated B) and periodically after conditioning trials (Tests, labeled 1 through 3). There were two control groups, one received saline (S-S CON and one received ethanol (E-E CON). There were two experimental groups both getting ethanol with some trials and saline with others (E-S). One experimental group had a history of drinking (DR) ethanol containing solutions and one did not (NDR). A score greater than 300 sec indicates a preference for the place where ethanol's effects were experienced by the experimental groups.

RESULTS

From Fig. 1, it can be seen that the mean scores of the two control groups (S-S and E-E) are very similar to each other at each test. The mean scores for the experimental group that were drinkers were greater than 300 sec and greater than control group scores on Tests 1 through 3 indicating that these rats developed a preference for the place of the ETOH-experience. The mean scores of the other experimental group, the nondrinkers, indicate that injections of ETOH produced no sign of having established a CPP.

The ANOVA yielded an $F(2,37)$ for the factor associated with groups=3.38, $p=0.045$; $F(3,111)$ for the factor associated with the repeated tests=1.48, $p=0.22$; and $F(6,111)$ for the interaction=2.32, $p=0.038$. As a further analysis, an ANOVA was computed using only the scores of the control group and the experimental group that had previously consumed ETOH. The resultant $F(1,28)$ for the factor of groups (CON versus drinkers)=4.92, $p=0.035$. Given that groups did not differ at baseline, it is clear that the experience of

ETOH on only one side of the alley changed rats' preference for the side of the ETOH-experience. To determine if the effect seen at Test 1 (after only 4 days, two with ETOH-injections) was significant, a t-test for dependent scores was used to compare baseline scores of the experimental group, drinkers, to their scores at Test 1. The resultant t-value is $t(9)=2.98$, $p=0.02$. A t-test for comparing independent scores was calculated for the values of the control-group and the experimental-group, drinkers, at Test 1. The resultant value is $t(28)=2.13$, $p=0.04$.

An ANOVA of the scores of the control-groups arranged according to whether or not they had a history of drinking ETOH solutions, yields no values indicating that the groups differed in their performance across the tests, e.g., the F-value for the factor of drinking history is $F(1,16)=2.2$, $p=0.16$. Although there is no reason to consider drinking history as a relevant source of variance among control groups, it may be of interest to compare the scores of the control-group with a history of drinking to those of the experimental group with the same ETOH experience. A t-test for comparing mean scores across the three tests between those control drinkers and those of experimental drinkers yields a $t(19)=2.76$, $p=0.013$. This comparison of rats' scores with the same drinking history, but different conditioning procedures, provides further confirmation that a reliable CPP can be established using injections of ETOH.

In contrast, an ANOVA of only the scores of the nondrinkers and the controls yields no F-value indicating statistical significance. The only t-value indicating a reliable effect (from the comparisons of baseline score of NDR-group to their test-scores or comparisons of NDR-group to controls at Tests 1–3) was the t-value for comparison of scores at Test 1 between control and experimental, nondrinkers, is $t(28)=2.28$, $p=0.03$. From this analysis, there is no reason to conclude that ETOH produced a CPP in the NDR group and may have even produced a small aversion with first injections.

DISCUSSION

The variation in the control groups' preferences across testing (Fig. 1) probably reflects some uncontrolled sources of variance producing some slight preference toward a side with the 1st and 2nd test. It is doubtful that the variance across tests among the control-groups' scores are random sources of variance, since the factor of tests of an ANOVA of control groups' scores has associated with it an $F(3,54)=3.53$, $p=0.02$. There is no reason to suspect that whatever factors were operating on the control groups' performance were unique to them, since subjects of the experimental groups and control groups were tested at the same time and order of conditioning and testing (with respect to the four alleys) was by random assignment (but consistent after the procedure began). Perhaps, odors left by an occasionally frightened rat might have been a source of uncontrolled variance [4]. Regardless of the reason for variance in means across baseline and test-periods of the controls, it is clear that the effects of ETOH-injections (when these effects are arranged so that they may be unconditioned stimuli) emerge as significant sources of variance. Nevertheless, the variation in control groups' performance does reflect a potential problem with the CPP-test.

The results of these tests highlight both the strengths and the weaknesses of the CPP-test. The CPP-test has shown itself to be sensitive to ETOH's reinforcing effect when potentially relevant variables are properly arranged. The CPP-test was originally designed to assess the affective state elicited at various times after injections of morphine [12]. When the time after injections of ETOH that rats are putatively conditioned is such that ETOH-levels in brain have peaked, we and others [2,17] are unable to get a CPP. When the time after injection is such that ETOH could be having an initial impact on brain, we can observe a CPP provided that the rats have been inured to the effects of ETOH. So, a strength of the CPP-test, i.e., its ability to provide a test of discrete periods following injections, allows the tentative conclusion that ETOH achieves its positive reinforcing capability as a consequence of events occurring with ETOH's initial interaction with brain mechanisms.

It would take an enormous study using the CPP-test to detail precisely, in small units, the complex interaction between the variables of dose by time after dosing by level of ETOH-experience that will produce clear signs of ETOH's capability to produce positive affect. At this stage, we can guess that it may take only limited experience with ETOH before some asymptotic state is achieved that allows moderate doses to be effective in producing positive affect. In this test, the drinkers had consumed ETOH a number of days prior to the procedures of the CPP. The aversiveness associated with ETOH-injections among the nondrinkers, evidently, waned with the first two or three injections. We further guess that small doses of ETOH may not have an initial aversiveness associated with them in either inured or naive rats. We feel confident in concluding, however, that there will always be a range of large, nonlethal doses that will elicit signs of aversion. Additionally, we guess that positive affect will be a property of the initial effects of ETOH on brain.

With respect to the weaknesses of the CPP-test, we have already alluded to the variability in the control groups' mean scores across testing. This variability points to the possibility for a number of factors, perhaps normally not controlled, which may become nuisance variables. This makes it necessary to have control groups run and tested concurrently with treatment groups. One can not merely rely on within group comparisons. The magnitude of the CPP with ETOH-injections is not large. This may be due to a limitation of the test or to the fact that ETOH does not produce a dramatic increment in affect. We do not have enough data with doses of potential euphorigens to make comparisons about the magnitude of CPPs. Also, we may not have used the optimal conditioning period and only further research can determine that period. Because with each test there were rats in the experimental groups whose performance did not conform to the others, we recommend larger numbers of subjects per group than used here to allow the main effect to clearly override the individual differences that seem to be characteristic after ETOH-injections.

The rats received large injections of a dilute, but still rather potent, ETOH-solution. One wonders about the effects of such large doses given daily. It is clear from the rat's behavior that the injections are uncomfortable, but the discomfort was apparently very temporary because rats could be handled easily just after injections. Across the days of injections and testing all rats gained weight indicating that the rats were healthy. In another study, however, one rat did become ill following this kind of ETOH-injection and was then sacrificed. The autopsy showed that the rat probably became ill due to damage to the gut. We conclude from these observations that the injections are reasonably safe. Never-

theless, there is some greater risk than is usually involved with smaller injection volumes, a conclusion which argues for relatively large numbers of subjects in a group. Also, this may account for the reduced effects on Tests 2 and 3.

Some comment is in order as to why the CPP did not grow in magnitude with continued putative conditioning and testing. There are some reasons to suppose that the test itself may not be particularly sensitive to variations in the level of positive affect, while being very sensitive to its presence or absence. The competing cues which elicit exploration may place a ceiling on the measured CPP. There is a possibility that the large injection volumes caused an occasional malaise that would detract from the putative positive affect elicitable by ETOH (the mean decrease seen at Tests 2 and 3 with the experimental group drinkers is due to one or two rats showing uncharacteristically low scores). Relatedly, a pattern of ETOH-assimilation that is different than the pattern of usual voluntary intake was imposed on the rats. When allowed access to ETOH, rats show a pattern of large drinking bouts interspersed by 3 to 5 days of lower consumption [8]. When we inject large doses every other day, there is a possibility of injecting a dose at a time when a large dose may not have otherwise been taken, thereby, setting the circumstances for something less than elicitation of positive affect. With the strong possibility of an ethanol-CPP being clearly established (as this study accomplishes), it seems reasonable to suppose that studies will be done to determine the optimal pattern of dosing for showing a robust CPP. The results of such studies will delineate ETOH's capabiltiy to elicit positive affect and thereby allow studies of the mechanisms involved.

We have stressed potential methodological problems, because we think that this study demonstrates the possibility of studying ETOH's potential for reinforcement in relative isolation from ETOH's other effects. Although the CPP-test appears simple, it is apparent that considerable care must be taken to control for nuisance variables. Also, it is apparent that the test is sensitive to relevant variables (such as time after injections), and that knowledge is derivable concerning the critical variables for ETOH's elicitation of a CPP. Such knowledge is the basic step in making the test useful for studying other details of ETOH's ability to evoke positive affect. In summary, this initial demonstration of a CPP following ETOH-injections (a) provides a basis for further study of ETOH's capability of eliciting positive affect, and (b) indicates that a rigorous analysis of ETOH's reinforcing capacity might show that it is "getting drunk that is fun, not being drunk."

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