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Biochemically Instigated Avoidance of Alcohol in Hats

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BIOCHEMICALLY INSTIGATED AVOIDANCE OF ALCOHOL IN RATS

by

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INTRODUCTION

X-irradiation has been demonstrated to be an aversive stimulus. When paired with a discriminable solution it results in the subsequent avoidance of that solution. This has been demonstrated in the literature for a variety of solutions including saccharin (McLaurin and Scarborough, 1963; McLaurin, Scarborough and Farley, 1963; McLaurin, 1964; Scarborough and McLaurin, 1964; Garcia, Kimeldorf and Koelling, 1955), chocolate in milk (Kimeldorf, Garcia and Rubadeau, 1960), Na Cl (Perry, 1963), ethanol (Peacock and Watson, 1964), kool-aid (Harlow, 1962), chocolate on food (Hulse and Dempsey, 1964), morphine (Mountjoy and Roberts, 1967). Not only has the avoidance been obtained with a variety of solutions but also the phenomenon has been seen in a variety of organisms such as the rat (Garcia et al, 1955; McLaurin et al, 1963; McLaurin et al, 1963; McLaurin, 1964; Scarborough et al, 1964), cat (Kimeldorf, 1963), mouse (Peacock and Watson, 1964), and monkey (Harlow, 1962). Thus, the resulting avoidance phenomenon from the pairing of X-irradiation with a discriminable solution appears to be neither solution nor organism specific.

One of the more traditional explanations of this phenomenon has been based on the classical conditioning paradigm in which the conditioned stimulus (CS) is presented either prior to or simultaneously

with the unconditioned stimulus (UCS), resulting in the CS taking on some of the characteristics of the UCS. More specifically, as a result of pairing the CS, saccharin takes on some of the aversive qualities of the X-irradiation, the UCS, and results in the subsequent avoidance of saccharin.

Results of research reported by Scarborough, Whaley and Rogers, (1964) indicated that it was possible to get avoidance of discriminable solutions if the CS was presented after the UCS. More specifically, the CS could be presented up to twelve hours following the termination of the actual irradiation with appreciable avoidance resulting. This paradigm in which the CS follows the UCS is traditionally known as "backward conditioning." Most attempts to obtain backward conditioning using variables other than saccharin avoidance have been either unsuccessful or very temporary and likewise seemed untenable to the authors who suggested an alternate explanation of their results. They hypothesized, since their evidence showed that the avoidance effect could be initiated after the termination of the X-irradiation per se, that some other physiological mechanism was involved which remained active after the termination of the irradiation. They went on to suggest that a physiological disturbance of a humoral nature was the possible locus of this phenomena.

Research by Garcia and Kimeldorf, (1960) lends some support to the humoral hypothesis. They isolated and radiated various regions of the body such as the head, thorax, pelvic and abdominal regions. They found that avoidance was obtained at much lower dosages of

radiation in the abdominal region than in the other isolated areas. In addition to this, they found that total body radiation resulted in a cumulative effect with nearly 100% avoidance. This evidence appears to indicate that the mechanism for avoidance is located in the abdominal region which is the locus of the reticulo-endothelial system (RES), one of the more extensive humoral systems in the body.

The RES consists of phagocytic cells which are located in the liver, bone marrow, lymph nodes and spleen tissue of the body. Functionally, they act as protective agents against foreign substances and destroy any degenerating red blood cells. Although the cells of this system are dispersed through several tissues of the body, the action of the system is unified in that if some of the cells are destroyed, other cells in the system will attempt to make up for the loss.

Although the RES has been investigated quite extensively in terms of its physiological function, relatively little research has been directed towards its possible role as a locus for behavior change. McKenna and Zweifach (1956), found that through the use of biological colloidal agents the RES could be "overloaded" in the sense that the phagocytic cells are forced to take on colloidal particles to the point where no further uptake of particles is possible. By doing this, the RES becomes non-functional until these colloidal particles are destroyed. Behaviorally, the consequences were a lowering of the ability of animals to survive resistance to drum trauma created by exposing the animals to sub-lethal dosages of drum trauma.

Reichard, Gordon and Tessmer, (1960) concurred with McKenna and Zweifach's conclusion and produced more direct evidence of the possible role of the RES in behavior change. They subjected rats to sub-lethal doses of stress through the employment of a Noble-Collip tumbling apparatus. Following this, tissue from the spleen, liver and plasma were removed, acidified and injected into normal rats. The rats were then placed in the Noble-Collip tumbling apparatus and data were collected on lethal dosages. These data were then compared with data of naive rats who were placed in the tumbling apparatus. The results showed that the rats injected with the boiled filtrate containing portions of the RES system could survive more stress than the naive rats. The authors concluded that the RES had played a major role in developing the resistance to stress.

One of the more sweeping demonstrations of the possible role of a humoral system in behavior change was done through the use of a parabion technique by Hunt and Kimeldorf, (1967). They linked two rats together through a cutaneous vascular anastomosis which morphologically ran from the upper neck to the back of the tail. This meant that the circulatory system was the only common connection between the two rats. Following this, the animals were shielded from each other and one rat was irradiated with saccharin available to both animals. The other rat was then tested and the results showed he avoided saccharin which had been paired with the irradiation of his partner. In addition, two control groups were used in which one had the cutaneous vascular anastomosis but H_2O was present during irradiation rather than saccharin. In the second group the animals were just clamped

together and saccharin was present during irradiation of the partner. In neither case was there any avoidance. Thus, the results of the total study showed only the method which could have involved the humoral factor resulted in subsequent avoidance of saccharin.

As has been described, avoidance of preferable saccharin solutions have been obtained through the use of X-irradiation. Ancillary evidence has also been cited which tends to point up the possibility that colloidal agents act in a similar manner. Feinberg, (1966) in his investigation found that avoidance with the use of X-irradiation was equivalent to that of the use of proferrin, a colloidal agent, but showed an additive effect when used together. His results were interpreted to support the hypothesis that the drug and X-irradiation bring about saccharin avoidance because they both operate through a common biological system.

If this interpretation is correct then the use of proferrin to obtain avoidance with a variety of solutions should be possible.

It is the purpose of this work to add to the data by using alcohol as the discriminable solution and proferrin as the biological agent.

METHOD

Subjects

The subjects were male hooded rats weighing approximately 250 grams each and randomly selected from a group of rats which were being bred for their alcohol preference. Although the rats did not drink alcohol at the 100% level in preference to tap water, they drank significantly more alcohol relative to non-selectively bred animals from the colony at Fort Custer. All animals were kept in individual home cages and had free access to tap water as well as lab blox, except as dictated by the experimental procedures. All animals were approximately 115 days old.

Apparatus

All testing took place in individual home cages which were placed in a rack isolated from other animals. Tap water and a 95% solution ethyl alcohol were used to prepare the 10% alcohol solution according to the procedure described by Thor, Weisman and Boshka (1966). Tap water was used as the alternate solution. The fluids were presented to the animals in uniform glass bottles with uniform metal tips as dictated by the experimental procedure. Proferrin, a saccharated iron compound which takes the form of a colloid, was used to induce blockage of the RES in the experimental group while physiological saline was used in the control group. Both the proferrin

and saline were injected with a microsyringe.

Procedure

Ten animals were deprived of liquids for a twenty-two hour period at which time they were presented with a two-choice situation of tap water and a 10% alcohol solution. This baseline was taken for five days with the bottle positions being altered each day. The data collected were grams of solution drunk which was then transformed into an alcohol index (AI).

$$\text{ALCOHOL INDEX} = \frac{\text{total alcohol consumed}}{\text{total fluid consumed}} \times 100$$

Twenty one and one-half hours following the last day of baseline an incision was made to expose the jugular vein at the sternal level. In five of the animals, .2 cc of proferrin solution was injected while .2 cc of saline was injected in the remaining five. The incision was then closed with wound clips and the animal placed back in his home cage. Two hours following the injection a bottle containing a 10% solution of alcohol was placed on the home cage. At the end of twelve hours the food was returned and twenty-two hours later the animals were again subjected to a two-choice drinking situation of tap water and 10% alcohol solution. This same procedure was followed over the next five days and as in baseline all data were transformed into an AI.

Results

Figure 1 is a graph which represents all the alcohol indices collected during the present research. Included are the results of the control and experimental groups under both baseline and treatment conditions. Under baseline conditions the proferrin group appeared to have higher mean alcohol indices. In the treatment condition the proferrin injected group seem to diverge from the saline injected group.

Since the alcohol indices were percents, they were not in congruence with the assumptions underlying analysis of variance test. Thus, it was necessary to transform these indices to a more appropriate scale. The transformation selected was the arcsin transformation deemed suitable by Winer (1962, p. 221) for use with data which are expressed in terms of proportions.

The first analysis done was to test for significant differences between the baseline and treatment conditions for those animals injected with saline. A single factor analysis with repeated measures was used as outlined by Winer (1962, p. 106). As can be seen in Table I the results of this analysis proved to be insignificant and thus there was no significant change in the saline injected animals' drinking preferences from the baseline to the treatment condition.

Table II portrays a second analysis using the same single factor design with repeated measures on the baseline and treatment conditions for those animals which received proferrin injections.

This analysis proved to be significant at the .05 level. Since this analysis proved to be significant it could be seen that a significant change in drinking preferences took place after the proferrin injection.

Two other analyses were enacted using a two-factor design having repeated measures as described by Winer (1962, p. 302). The first analysis was to test for significant differences between the control and experimental groups under the baseline conditions. Table III shows the result of this test to be significant. Thus, the control and experimental groups appeared to have obtained higher mean alcohol indices during the baseline portion of the research.

The second analysis using the same two-factor repeated measure design was done to test for significant differences between the saline injected and proferrin injected groups after their respective injections. This analysis is summarized in Table IV. When this analysis was done an F of 74.6759 was obtained which was significant at the .01 level. A second significant factor was found for the repeated measures and indicates that during the treatment conditions the proferrin injected animals drank significantly less alcohol than the saline injected animals.

Conclusions

The results of the present research indicate that proferrin has a differential effect on the drinking preferences of rats using alcohol and water as the discriminative solutions. In a comparison of the treatment versus baseline condition the group given the saline injection

showed no significant change in preference. On the other hand, the group given the proferrin injection showed a significant (.05 level) decrease in alcohol preferance following the injection.

When the two baseline groups were compared with each other there was a significant difference found, indicating that by chance higher mean alcohol indices were obtained by the experimental group. A significant difference (.01 level) was found when the saline and proferrin groups were compared in the treatment conditions. Here again the statistic shows that the rats which were given the proferrin treatment had lower alcohol indices than the saline treated animals.

It was also found that the repeated measure factor was significant (.01). This would be expected from a visual analysis of Figure 1 since the proferrin injected animals showed a systematic change over the five treatment days.

This curve looks like a typical extinction curve and appears to be very similar to those obtained by investigators using X-irradiation as the unconditioned stimulus and a variety of discriminable solutions such as saccharin (McLaurin and Scarborough, 1963; McLaurin, Scarborough and Farley, 1963; McLaurin, 1964; Scarborough and McLaurin, 1964; Garcia, Kimeldorf and Koelling, 1955), chocolate in milk (Kimeldorf, Garcia and Rubadeau, 1960), Na Cl (Perry, 1963), ethanol (Peacock and Watson, 1964), kool-aid (Harlow, 1962), chocolate on food (Hulse and Dempsey, 1964), morphine (Mountjoy and Roberts, 1967). The curves also showed a likeness to the ones obtained by Feinberg, (1966) using proferrin and a combination of proferrin and X-irradiation. Because of the consistency of these curves it appears that proferrin

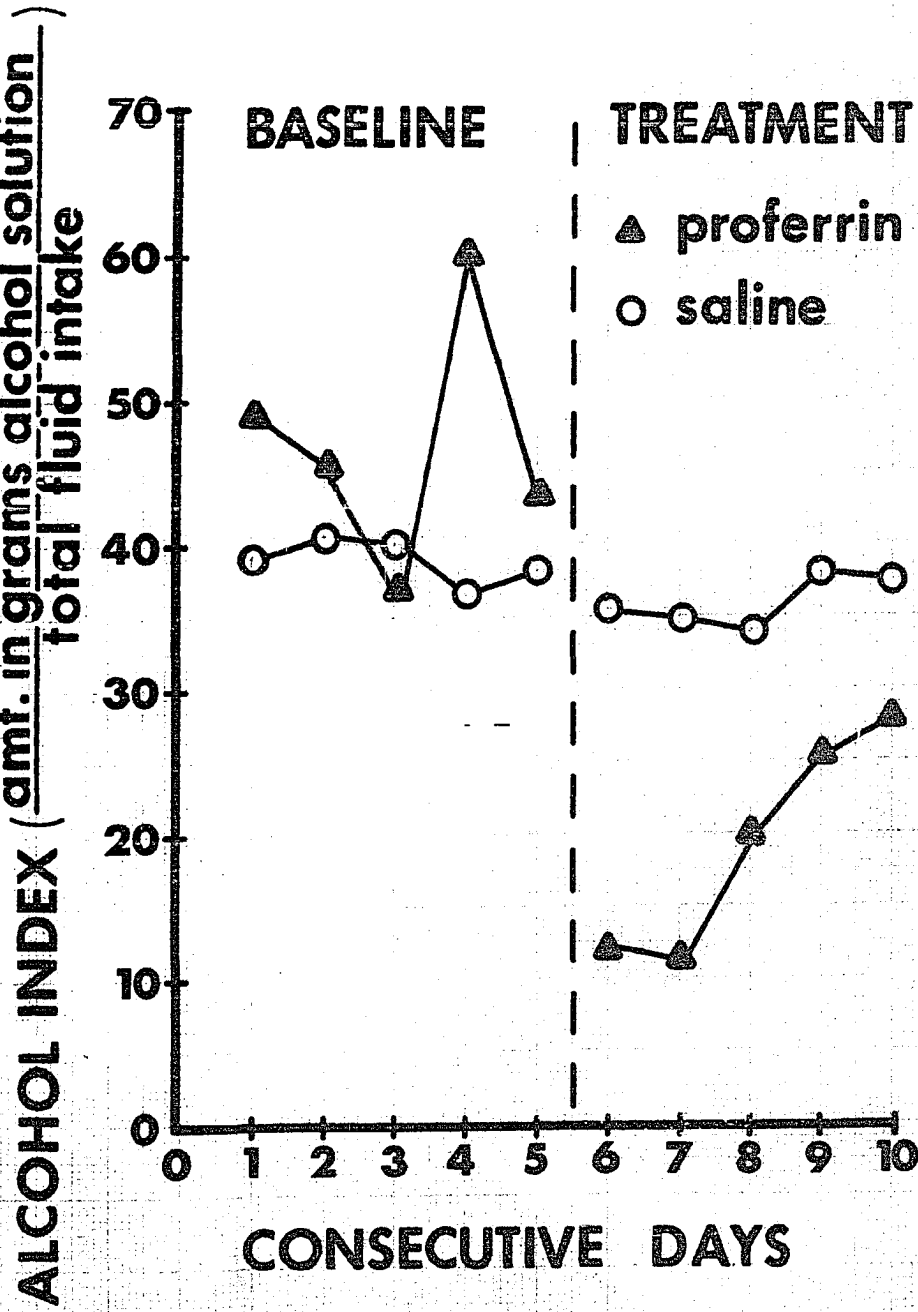
works in a similar manner as an aversive stimulus whether alcohol or saccharin is the discriminable solution.

If this hypothesis is true, then future research should first be directed towards finding out whether proferrin is like other aversive stimuli such as shock or loud noise. Questions must be answered in terms of dose response curves, and the effects of repeated injections. Technical problems such as these must be answered to further enhance the usability of proferrin as an aversive stimulus which can modify behavior.

A second avenue of research open to investigation is the type of responses which can be modified through the use of proferrin. Are the responses limited to consummatory responses or will other responses be amenable to change through proferrin injections? The present research indicates that proferrin is not solution specific and this lends itself to research in alcoholism as well as numerous types of drug addictions.

These investigations of alcoholism and drug addictions will necessitate the eventual transfer of research to a higher level animal. Drug research with monkeys would give more concise information on the effects of the proferrin for clinical application. The information could then be used in applying the proferrin to alcohol and drug addiction research at the human level.

APPENDIX A



APPENDIX B

Table I

Baseline vs Treatment Single Factor Analysis for Control Group

Source of variation	SS	df	MS	F
Between Groups	.0355	4		
Within Groups	.1115	5		
Drug	.0077	1	.0077	.2972
Residual	.1038	4	.0259	

Not Significant
$F_{.95} = 7.71$

Table II

Baseline vs Treatment Single Factor Analysis for Experimental Group

Source of variation	SS	df	MS	F
Between Groups	.6108	4		
Within Groups	1.4619	5		
Drug	.9714	1	.9714	7.9233*
Residual	.4905	4	.1226	

*$F_{.95} = 7.71$

Table III

Two Factor Analysis of Control vs Experimental Groups
Under Baseline Conditions

Source of variation	SS	df	MS	F
Between Subjects	169.1303	9		
A	.3799	1	.3799	5.0574*
subjects with groups	5.9438	8	.0742	
Within Subjects	2.2272	40		
B	.2835	4	.0070	.1521
AB	.4693	4	.0117	.2543
B x subjects within	1.4744	32	.0460	

*$F_{.95} = 5.32$

Table IV

Two Factor Analysis of Control vs Experimental Groups
Under Treatment Conditions

Source of variation	SS	df	MS	F
Between Subjects	2.3853	9		
A	4.3088	1	4.3088	74.6759*
subjects with groups	.4618	8	.0577	
Within Subjects	1.5609	40		
B	.5187	4	.1296	5.4453
AB	.2794	4	.0698	2.9327**
B x subjects within	.7628	32	.0238	

*A $F_{.99} = 11.3$
**AB $F_{.95} = 2.61$
B $F_{.95}(4.32) = 2.69$

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