Pharmacology Biochemistry & Behavior, Vol. 21, pp. 387–391, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Changes in Alcohol Intake Resulting From Prior Experiences With Alcohol Odor in Young Rats

JUAN CARLOS MOLINA, JOSEPH SERWATKA AND NORMAN E. SPEAR[1]

Department of Psychology, State University of New York at Binghamton Binghamton, NY 13901

Received 13 January 1984

MOLINA, J. C., J. SERWATKA AND N. E. SPEAR. *Changes in alcohol intake resulting from prior experiences with alcohol odor in young rats.* PHARMACOL BIOCHEM BEHAV 21(3) 387–391, 1984.—Twenty-one day old rats were exposed to either alcohol or lemon odor, paired or unpaired with lithium chloride (LiCl) induced toxicosis, and were tested 7 days later for odor preferences and ethanol intake. Additional control groups received neither the conditioned nor the unconditioned stimuli and were merely tested on either odor preference or alcohol consumption. Ethanol odor exposure per se resulted in an enhanced ingestion of a 5.6% ethanol solution. This effects was attenuated by pairing such exposure with internal malaise. Furthermore, ethanol odor-LiCl pairings decreased olfactory preferences for ethanol relative to lemon odor, whereas lemon-LiCl pairings increased ethanol odor preference relative to lemon odor. Order of testing also affected odor preferences. Rats previously tested on ethanol consumption demonstrated a strong rejection of the alcohol odor when compared to rats initially tested in the olfactory task. These results suggest that early learned and unlearned experiences with alcohol odor can not only affect subsequent ethanol odor preferences but can also lead to significant changes in alcohol consumption.

Alcohol Odor aversion Preweanling rats Lithium chloride toxicosis

DIFFERENT species of rodents at an early age utilize olfactory cues in processes related to orientation and physical contact with other members of the species as well as in the detection, selection, and consumption of food [1, 3, 19]. In turn, the functional importance of such cues can be modified by early experiences related with olfactory stimuli. Unreinforced exposures to odors has resulted in increased preferences towards the odors employed in hamsters [12], guinea pigs [7] and rats [3, 16, 19]. Generally, these changes in odor preference have only been observed after long-term exposures to such cues. Nevertheless, in a recent study, Caza and Spear [8] demonstrated that in young rats, a 3-minute exposure to a lemon odor was sufficient to increase preference for that odor relative to a novel odor.

During the last decade, it has also been demonstrated that rats at a very early age are capable of associating olfactory stimuli with various reinforcers and they are capable of retaining this information over long periods of time. Rudy and Cheatle [24,25] provided evidence which suggests that 2-day old rat pups are able to associate a novel odor with LiCl-induced illness. These pups also demonstrate retention of this associative learning over a 6-day interval. Furthermore, Johanson and Hall [18] observed that 1-day old rat pups are able to use olfactory cues as discriminative stimuli in an appetitive learning paradigm.

Data from a recent set of experiments in our lab (Molina *et al.*, in preparation) suggest that olfactory cues might also play a role in ethanol ingestion, and this role seems to vary as a function of the age of the organism. Twenty-one and 40-day old rats which were exposed during a 20-minute period to ethanol odor showed an increased resistance to associate an alcohol solution with internal malaise, as measured by alcohol intake. This pre-exposure effect was not observed in older animals (60 and 80 days of age). Additionally, young rats retain this olfactory information over a 40-day retention interval. These data suggest that early olfactory experience has a significant impact in the acquisition and retention of subsequent ethanol aversions in the developing rat.

Nachman *et al.* [21] also noted that the odor of ethanol is an important component in the development of alcohol preferences for certain strains of mice. In rats, forced drinking of alcohol has resulted in conditioned aversions, not only to the taste of alcohol, but also to the smell of this drug [6,13]. Our results coupled with this previous research demonstrate that both olfactory and taste components can singularly produce changes in ethanol intake, suggesting that olfaction plays a significant role in ethanol consumption. Considering our own data, it appears that there exists some type of ontogenetic factor that also contributes to the degree in which olfactory cues affect subsequent ethanol intake. This is congruent with

[1]Requests for reprints should be addressed to N. E. Spear.

previous investigations demonstrating overall food preferences and aversions resulting from early experiences with olfactory components present in the diets [2, 11, 20].

The present study was designed to further examine preweanlings' learned and unlearned experiences with alcohol odor and their subsequent effects upon alcohol ingestion and odor preferences.

METHOD

Subjects

The subjects were 61 male and 70 female Sprague-Dawley derived rats from 14 litters (litter size=8–10 pups). All pups were born and reared at the breeding colony of the State University of New York at Binghamton. They were housed with their parents and conspecifics in standard opaque maternity cages partially filled with pine shavings until postnatal day 23, at which time they were weaned. After weaning and throughout the entire experiment, pups were kept in the same maternity cages in which they were previously housed with their parents. All subjects were maintained on a 16 hour light/8 hour dark illumination cycle, where light onset occurred at 0600 hours.

Apparatus

Odor exposure took place in standard wire mesh cages (24.5×17.5×17.5 cm) lined with brown plastic bags. Odorants (1.5 cc of absolute ethanol or 2.0 cc of Regal Lemon Extract) were presented in a cotton ball located in the interior of an acrylic tray which was attached to a clear Plexiglas top covering the entire apparatus. Odorants were presented in this fashion in order to prevent rat pups from licking the stimulus or carrying individual traces of the odors on its pelage due to any direct physical contact with the cotton.

Odor preference tests were conducted in a 33.0×14.0×15.0 cm clear Plexiglas box fitted with a screen floor. This apparatus was divided into 3 equal sections. In each of the 2 end sections, cotton scented with ethanol (1.5 cc) or lemon (2.0 cc) was placed in trays separated by 3 mm from the screen floor. Located beneath the middle section was a tray of unscented cotton. Water and ethanol drinking sessions took place in standard individual wire mesh cages which had spring grip clamps that supported 10 ml (±0.1 ml) graduated tubes equipped with plastic stoppers and stainless steel spouts.

Procedure

Subjects were randomly assigned to 8 treatment and 2 control conditions designated by the conditioned olfactory stimulus [Ethanol (E) or Lemon (L)]; the unconditioned stimulus [Paired Injection of LiCl (P) or Unpaired (U)] and the order of testing [Odor Preference—Ethanol Ingestion (O-I) or vice versa (I-O)]. Control animals received neither the conditioned nor unconditioned stimuli. One control group was tested for odor preference (C-O, n=11) while the other control group was tested only for ethanol ingestion (C-I, n=12). On conditioning day (postnatal day 21) the pups were individually placed in wire mesh cages lined with plastic bags. These cages were then covered with plastic tops containing either unscented cotton, cotton scented with lemon, or cotton scented with ethanol. Five minutes later, pups assigned to the lemon-lithium or ethanol-lithium paired conditions (EP-OI, n=14; EP-IO, n=14; LP-OI, n=13; LP-IO, n=13) were removed from the apparatus and administered a 10 mg/kg intraperitoneal injection of 0.3 m LiCl. Animals were then returned to the apparatus for 30 additional minutes. The rest of the experimental animals (EU-OI, n=14; EU-IO, n=14; LU-OI, n=13; LU-IO, n=13) were handled exactly as those in the paired groups with the sole exception being that they were not injected after the first 5 minutes of exposure. After completion of the conditioning trial and 60 minutes before being returned to their respective maternity cages, all groups were separately placed in clear plastic cages containing pine shavings. This was done in order to avoid immediate contact between subjects exposed to different odors as well as to permit LiCl injected rats to recover from the toxic effects before being reunited with the remaining subjects. As previously demonstrated by Coombes *et al.* [10], unpoisoned pups can develop aversions by being exposed to animals which had prior administration of a poisoning agent.

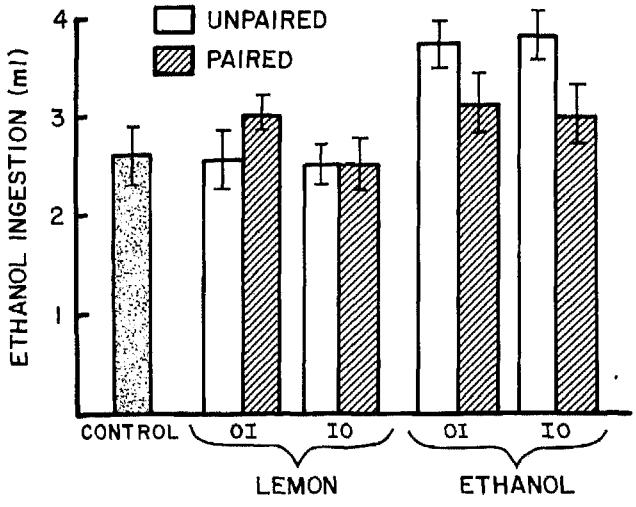

FIG. 1. Mean ethanol ingestion as a function of CS exposure (ethanol or lemon odor, US pairing (paired or unpaired LiCl administration) and Order of Testing (OI: Olfactory Preference—Ethanol Ingestion or OI: Ethanol Ingestion—Olfactory Preference).

Animals assigned to the unpaired LiCl groups (EU-OI, EU-IO, LU-OI, LU-IO) received the LiCl administration 24 hours after the CS exposure. Once again all subjects were separated from their conspecifics for a 60-minute interval before being returned to their maternity cages.

On Day 23, all subjects were weaned by taking the parents out of the home cages. All other aspects related with the maternity cages stayed the same. Observations within our laboratory suggest that weaning after such experimental conditions does not appear to affect the behavioral consequences of conditioning.

On postnatal days 26 and 27 all rats were given one daily adaptation session during which they had 20 minutes' access to room temperature tap water. On postnatal day 28, animals in the ethanol and lemon exposed groups were tested for olfactory preferences as well as for alcohol ingestion. Tests were separated by an interval of 60 minutes. Rats assigned to the control groups were only tested for ethanol intake or odor preference. All animals were placed on a 22-hour water

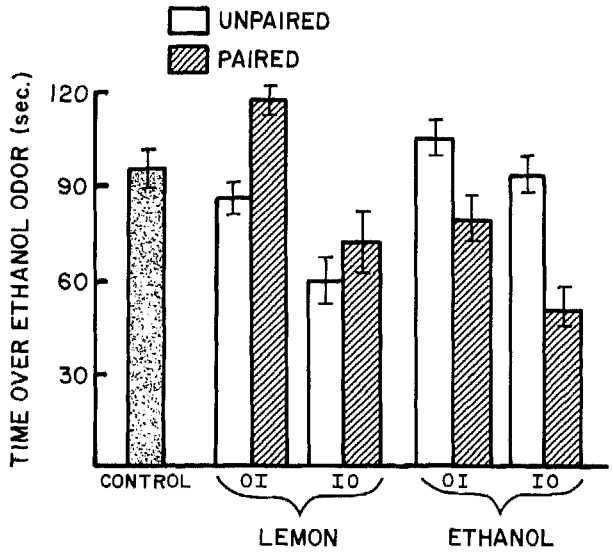

FIG. 2. Mean time spent over ethanol odor as a function of CS exposure (ethanol or lemon odor), US pairing (paired or unpaired LiCl administration) and Order of Testing (OI: Olfactory Preference—Ethanol Ingestion or IO: Ethanol Ingestion—Olfactory Preference).

deprivation schedule prior to these adaptation sessions as well as the ethanol intake session.

During olfactory testing, rats were individually placed in the center of the test chamber. The amount of time spent localized over the three sections was recorded during a 5-minute period. An animal was considered to be over a particular odor when its nose and front paws were above that section.

Alcohol consumption was tested by placing the 22-hour water deprived animals in the same cages in which water adaptation sessions were performed. After a 5-minute adaptation period, all rats were presented with drinking tubes containing a 5.6% v/v absolute ethanol solution for a period of 20 minutes. The amount of alcohol ingested during this time period was recorded. A one-bottle test was employed instead of a preference test between water and ethanol since pilot experiments in our laboratory showed that rats of this age will strongly reject even a 3% v/v ethanol solution when having simultaneous access to water. Furthermore, the 5.6% v/v concentration was employed due to the fact that in a previous one-bottle dose-response study (Molina *et al.*, in preparation) we observed that this alcohol solution presented alone was ingested at about 50% the rate of water ingestion during an equivalent amount of time.

RESULTS

Ethanol Ingestion

A One Way Analysis of Variance (ANOVA) was performed in order to calculate the within groups mean error term for the 9 treatment conditions. Using this mean error term a 3 way ANOVA (CS×US×Test Order) was performed. No significant differences were found for water consumption across both adaptation sessions.

Ethanol intake was significantly affected by ethanol odor exposure. Rats exposed to the ethanol odor consumed more ethanol than subjects exposed to either lemon odor or no odor, $F(1,110)=15.4$, $p<0.001$. Furthermore, a significant CS×US interaction was revealed, $F(1,110)=21.6$, $p<0.05$. Post-hoc comparisons employing a Fisher test with an α set at 0.05 demonstrated an overall conditioning effect, i.e., group EP-IO differed significantly from goup EU-IO while differences between groups EP-OI and EU-OI approached significance. Taken together, these results indicate that rats which received ethanol odor-LiCl pairings ingested less ethanol than rats which received these stimuli unpaired.

No significant effects were found when analyzing US pairing, Order of testing or the interactions, CS×Order of testing, US×Order of testing, and CS×US×Order of testing.

These results suggest that passive exposure to ethanol odor can result in an enhanced preference towards ethanol solutions while this effect can be attenuated by pairing such exposure with internal malaise.

Olfactory Preference

Total amount of time spent over the ethanol-scented cotton was computed for each animal. The same 3 way ANOVA previously used to analyze ethanol ingestion revealed a significant interaction between CS exposure and LiCl pairing, $F(1,111)=38.9$, $p<0.001$. A Fisher test ($\alpha=0.05$) confirmed the locus of this interaction by specifying an overall odor conditioning effect. Groups aversively conditioned to the ethanol odor (EP-OI and EP-IO) spent less time over that odor than did their respective controls (EU-OI and EU-IO and C-O). Opposite differences were seen between animals conditioned to the lemon odor (LP-OI and LP-IO) and their respective controls (EU-OI, EU-IO and C-O) with the exception being that the difference between groups LP-IO and LU-IO, although in the same direction as the above results, did not reach significance. In other words, ethanol-LiCl pairing decreased preference for ethanol relative to lemon odor, whereas lemon-LiCl pairings increased ethanol odor preference relative to lemon odor.

Order of testing was also found to exert a significant effect upon odor preference, $F(1,111)=38.5$, $p<0.001$. Rats previously tested on ethanol intake spent less time over the ethanol odor than rats that were initially tested in the olfactory task. During olfactory testing we observed that rats who had already been exposed to the drinking session, showed clear signs of ethanol intoxication (motor incoordination, hyperactivity, tail stiffening). Probably these animals avoided ethanol odor as a consequence of a previous association of ethanol flavor with subsequent gastrointestinal distress. This is congruent with previous studies which demonstrated that forced alcohol drinking results in aversions toward the taste and smell of alcohol [6,13].

CS exposure, US pairing and the interactions, CS×Order of Testing, US×Order of testing, and CS×US×Order of testing failed to exert a significant effect upon the time spent over ethanol odor.

DISCUSSION

The results of this study demonstrate that early experiences with alcohol odor can substantially affect ethanol odor preferences as well as ethanol ingestion. Under the present experimental conditions, unpaired experiences between alcohol odor and onset of internal distress failed to affect subsequent alcohol odor preference while significantly enhancing consumption of an ethanol solution. Furthermore, pairing alcohol with a LiCl injection not only resulted in a

learned aversion to this olfactory stimulus, but also inhibited the enhanced ethanol consumption observed in groups which received unpaired ethanol-LiCl experiences. It should be noted that animals which received this pairing but were tested after the olfactory test did not differ significantly from the corresponding unpaired control. This could be a result of the order of testing since subjects in these groups experienced a 5-min presentation of the ethanol odor, unreinforced during testing. This "extinction trial" may have had an effect on the conditioned aversion to ethanol consumption. Regardless of this, these results appear to be specific to the ethanol odor experiences since both paired and unpaired lemon odor-toxicosis presentations failed to affect ethanol intake, while evidence of a lemon odor aversion was obtained through the olfactory preference test.

Order of testing also was a main factor contributing to the establishment of olfactory preferences. When ethanol intake preceded the olfactory test rats demonstrated a strong rejection to the ethanol odor (and hence, acceptance of lemon) even after having received prior reinforced lemon experiences. As previously stated in the results, these animals exhibited clear behavioral symptoms of ethanol intoxication. Previous investigators have demonstrated that forced alcohol intake results in learned aversions to the taste and smell of this drug [6,13]. Of considerable importance is the fact that all ethanol solutions employed in this study were prepared with absolute alcohol which contains small amounts of benzene. Additional research [9] suggests that rats are able to discriminate between 95% and absolute solutions laced with benzene. Therefore, absolute ethanol could have provided two different orosensory cues, one related to ethanol and a second one related to benzene. Furthermore, it should also be noted that ethanol has been employed as an effective aversive US in taste aversion experiments [4, 5, 17, 26]. Therefore, groups which received the ethanol intake test first could have associated the orosensory characteristics of alcohol and/or benzene with aversive consequences resulting from the ingestion and hence demonstrated aversions toward the smell of this stimulus.

Taking into account that (a) olfactory experiences modified subsequent ethanol intake and (b) strong olfactory aversions resulted from previous ethanol intake (an experience in which probably the most salient cue was the ethanol taste), these results suggest an interaction between olfactory and gustatory cues in the control of alcohol preference. Recent electrophysiological and anatomical studies support the hypothesis of a central nervous process in which olfactory and gustatory information could be integrated. It has been found that the substantia innominata in the rat brain receives afferents from the pontine taste nuclei as well as from the main olfactory bulbs and the prepiriform cortex, these later structures being related to the processing and transmission of olfactory inputs [14, 15, 22]. In turn, neurons in the substantia innominata region project to the gustatory cortex and are activated by the site of edible liquids, and yet remain unresponsive when nonfood objects are presented to the animal [23]. Thus, it has been proposed that units in this particular brain region could function as multimodal units integrating sensorial information relevant to feeding and drinking behavior [15].

Independent of the mechanisms by which olfactory experiences might affect ethanol intake and how ethanol ingestion might result in an ethanol odor aversion, however, the present results confirm and extend previous evidence that early experiences with odors can produce changes in the consumption and preference of substances containing such sensorial cues. To our knowledge this is the first set of results indicating that early learned and unlearned experiences with ethanol odor can significantly increase, or decrease, consumption of alcohol solutions.

ACKNOWLEDGEMENTS

This research was supported by a grant from the National Institute of Mental Health (1 RO1 MH35219) to Norman E. Spear, a BRSG Grant (SO7RR07149-10) awarded by the Biomedical Research Support Grant Program Division of Research Resources, National Institutes of Health to Norman E. Spear and Juan C. Molina, and by a foreign fellowship awarded by the National Council of Research of Argentina (CONICET) to Juan C. Molina. The authors are grateful for the assistance of Norman Richter in conducting the research and to Teri Tanenhaus in preparing this report.

REFERENCES

1. Alberts, J. Ontogeny of olfaction: Reciprocal roles of sensation and behavior in the development of perception. In: *The Development of Perception: Psychobiological Perspectives*, vol I, edited by R. Aslin, J. Alberts and M. Peterson. New York: Academic Press, 1981, pp. 321–357.
2. Bronstein, P. and D. Crokett. Exposure to the odor of food determines the eating preferences of rat pups. *Behav Biol* **18:** 387–392, 1976.
3. Brunjes, P. and J. Alberts. Olfactory stimulation induces filial preferences for huddling in rat pups. *J Comp Physiol Psychol* **93:** 548–555, 1979.
4. Cappell, H., A. LeBlanc and L. Endrenyi. Aversive conditioning by psychoactive drugs: Effects of morphine, alcohol and chlordiazepoxide. *Psychopharmacologia* **29:** 239–246, 1973.
5. Cappell, H. and A. LeBlanc. Gustatory avoidance conditioning by drugs of abuse: Relationships to general issues in research on drug dependence. In: *Food Aversion Learning*, edited by N. Milgram, L. Krames and T. Alloway. New York: Plenum Press, 1977, pp. 133–167.
6. Carey, R. A decrease in ethanol preference in rats resulting from forced ethanol drinking under fluid deprivation. *Physiol Behav* **8:** 373–375, 1972.
7. Carter, C. and J. Marr. Olfactory imprinting and age variables in the guinea pig. *Anim Behav* **18:** 238–244, 1970.
8. Caza, P. and N. Spear. Short-term exposure to an odor increases its subsequent preference in preweanling rats: A descriptive profile of the phenomenon. *Dev Psychobiol*, in revision.
9. Cicero, T. H. and S. Hill. Ethanol self-selection in rats: A distinction between absolute and 95 percent ethanol. *Physiol Behav* **5:** 787–791, 1970.
10. Coombes, S., S. Revusky and B. Lett. Long delay taste aversions in an unpoisoned rat: Exposure to a poisoned rat as the US. *Learn Motiv* **11:** 256–266, 1980.
11. Cooper, A. and S. Hathorn. Modification of flavor preference by olfactory pre-exposure in normal and zinc sulfate treated mice. *Bull Psychon Soc* **10:** 369–370, 1977.
12. Cornwell, C. Golden hamster pups adapt to complex rearing odors. *Behav Biol* **14:** 175–188, 1975.
13. Deutsch, J. and A. Eisner. Ethanol self administration in the rat induced by forced drinking of ethanol. *Behav Biol* **20:** 81–90, 1977.

14. Ferreyra Moyano, H. and J. Molina. Axonal properties and conduction properties of olfactory peduncle neurons in the rat. *Exp Brain Res* **39:** 241–248, 1980.
15. Ferreyra Moyano, H. and J. Molina. Olfactory connections of substantia innominata and nucleus of the horizontal limb of the diagonal band in the rat: An electrophysiological study. *Neurosci Lett* **34:** 241–246, 1982.
16. Galef, B. and H. Kaner. Establishment and maintenance of preference for natural and artificial olfactory stimuli in juvenile rats. *J Comp Physiol Psychol* **94:** 588–595, 1980.
17. Gamzu, E. The multifacted nature of taste-aversion inducing agents: Is there a single common factor? In: *Learning Mechanisms in Food Selection*, edited by L. Barker, M. Best and M. Domjan. Waco: Baylor University Press, 1977, pp. 477–509.
18. Johanson, I. and W. Hall. Appetitive learning in 1-day old rat pups. *Science* **205:** 419–421, 1979.
19. Leon, M., B. Galef and J. Behse. Establishment of pheromonal bonds and diet choice by odor-exposure. *Physiol Behav* **18:** 387–391, 1977.
20. Martin, L. The relationship between neurobehavioral ontogeny and the expression of learning in rats. Unpublished Ph.D. dissertation, Indiana University, Bloomington, 1980.
21. Nachman, M., C. Larue and J. Le-Magnen. The role of olfactory and orosensory factors in the alcohol preference of inbred strains of mice. *Physiol Behav* **6:** 53–59, 1971.
22. Norgren, R. Gustatory afferents to ventral forebrain. *Brain Res* **81:** 285–295, 1974.
23. Rolls, E., M. Sanghera and A. Rope-Hall. The latency of activation of neurons in the lateral hypothalamus and substantia innominata during feeding in the monkey. *Brain Res* **164:** 121–135, 1979.
24. Rudy, J. and M. Cheatle. Odor aversion learning in neonatal rats. *Science* **198:** 845–846, 1977.
25. Rudy, J. and M. Cheatle. Ontogeny of associative learning: Acquisition of odor aversions by neonatal rats. In: *Ontogeny of Learning and Memory*, edited by N. Spear and B. Campbell. New Jersey: Lawrence Erlbaum Associates, 1978, pp. 157–188.
26. Sherman, J., C. Hickis, A. Rice, K. Rusiniak and J. Garcia. Preferences and aversions for stimuli paired with ethanol in hungry rats. *Anim Learn Behav* **11:** 101–106, 1983.