Pharmacology Biochemistry & Behavior, Vol. 16, pp. 707–711, 1982. Printed in the U.S.A.

Alcohol-Morphine Interaction: Oral Intake in Genetically Selected Maudsley Rats[1]

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Received 29 May 1981

SATINDER, K. P. *Alcohol-morphine interactions: Oral intake in genetically selected Maudsley rats.* PHARMAC. BIOCHEM. BEHAV. **16**(5)707–711, 1982.—Alcohol and morphine intake in the same animals was investigated in genetically selected Maudsley lines of rats, to test the possibility of a reciprocity between these two drugs. The MR animals (selectively bred for high open-field defecation) showed higher preference for both the drugs as compared to their counterpart MNR animals (low open-field defecation). The results support the findings of many other investigators of a possible genetic link in alcohol-morphine preference. In light of this, genotypic specificity of this relationship is suggested as a tentative explanation.

Alcohol-opiate interaction Maudsley lines of rats Oral intake of alcohol and morphine

THE possibility of a link between alcohol and morphine dependence [6] has been suggested and/or investigated in humans [5], monkeys [26], rats [9, 12, 18, 23, 24], mice [1, 8, 10, 27], and hamsters [19]. Unfortunately, after a decade of research results remain inconclusive.

In support of the hypothesis strains of rats bred for a differential relapse to morphine drinking were found to show a similar differential relapse to alcohol drinking [18]. Similarly alcohol-susceptible and alcohol-resistant strains of mice have been shown to have a parallel susceptibility and resistance to morphine drinking [8]. Mice strains (C57 and DBA) known to differ in alcohol preference have shown corresponding preferences for morphine [15]. Suppressive actions of morphine on the drinking of alcohol [23], increases in alcohol consumption during narcotic withdrawal in rats [13], increases in morphine self-administration after alcohol withdrawal in monkeys [26], and morphine-induced reduction in alcohol intake in hamsters [19], have also been reported. Rats readily switched to alcohol after acquiring morphine self-administration behavior [24]. Morphine suppressed alcohol-induced convulsions in mice [1]. In rats, chronic administration of alcohol during the development of dependence on morphine suppressed naloxone-precipitated withdrawal [16]. Alcohol also suppressed the morphine withdrawal syndrome [14]. Cross-tolerance between alcohol and morphine has been demonstrated in mice [11] and rats [17].

In contradiction to the hypothesis, naloxone did not induce opiate withdrawal (jumping) syndrome in alcohol-dependent mice [10]. Administration of alcohol did not suppress the morphine consumption in the morphine preferring rats [9]. No correspondence between morphine and alcohol consumption was found in Tryon's S_1 and S_3 lines of rats [12].

The Maudsley Reactive (MR) genetic line of rats selectively bred for high open-field defecation [4] have been shown to have higher preference for both alcohol [3, 7, 20, 21] and morphine [22] as compared to the Maudsley Nonreactive (MNR, low open-field defecation) animals. These findings can be taken to support the link in alcohol-morphine preference. However, the acceptance of this evidence is constrained by the fact that the data pertaining to alcohol and morphine were collected under different situations. Hence, to seek more direct evidence for the possible relationship between these two drugs in these genetic lines, the present research investigated the oral intake of both drugs in the same animals.

METHOD

Animals

The animals were 96 experimentally naive rats, 48 each from two genetic lines (MNR/Har/Lu and MR/Har/Lu) and equally represented by both the sexes. The MNR and MR have been subject to genetic selection for low and high

[1]This research was supported in part by an operating Research Grant #A0321 from the Natural Sciences and Engineering Research Council of Canada to the author. This paper was presented at the American Psychological Association's Annual Meeting in Los Angeles, CA, August, 1981.

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open-field defecation, respectively. The animals were bred and reared in the laboratory, weaned at 28 days, and were 100 days of age at the start of the experiment. Before experimentation the animals were housed in same-sex pairs, with the genetic lines on separate cage racks. During experimentation the animals were coded and housed individually to ensure that the experimenter did not know the genetic origin of the animals. The laboratory temperature was thermostatically controlled at 22±1°C, and the humidity level was maintained at 40%. Fluorescent lights were on a 12 hour light-darkness cycle.

Experimental Design

To study the intake of both drugs in the same animals, successive presentation of drugs was considered best. To accomplish this littermates, in pairs, from each of the strain-sex groups, were presented with distilled water ad lib for six successive days. On the basis of average daily water consumption littermates were assigned to morphine-alcohol or alcohol-morphine order of drug intake to produce matched groups. Each animal was given forced presentation of the respective drug for 6 days followed by 2 days of choice presentation between the drug and distilled water. This forced-choice schedule of 8 days (6–2) was repeated four times for each animal and then the animal was switched to the other drug with the exact replication of drug schedule. To control the order effect of drug presentation one-half of the animals from each strain-sex group were presented with morphine (M) first followed by alcohol (A), i.e., M-A order, and the other half of the animals were presented with alcohol followed by morphine, i.e., A-M order.

Alcohol solution was 10% (v/v) and morphine solution was 0.5 mg/ml morphine in sucrose. The amount of sucrose in morphine solution was matched for caloric value of 10% alcohol solution. Morphine-sucrose solution also masked the bitter taste of morphine.

To investigate if the presence of sucrose in morphine solution will influence the morphine intake, choice between two equiaversive substances (0.5 mg/ml morphine sulfate and 0.25 mg/ml quinine sulfate) was also studied in the same level of sucrose solution. Equiaversiveness for these levels of morphine and quinine has already been demonstrated for these genetic lines [22]. Animals were given distilled water for six days to allow adaptation to the new cage setting and taste of distilled water. For the next 24 days (same number of days as the forced intake of each drug) all the animals were given choice between morphine and quinine in sucrose solution of the same concentration.

Half the number of animals were used to study the alcohol-morphine interaction and the remaining half were used to study the morphine-quinine intake.

Procedure

The cage setting described in an earlier study [20] was used. Drug solutions were prepared every day just before administration. The animals were disturbed only at 24 hour intervals to record body weight and intake of drug solution, to replenish food and to empty, clean and refill the drinking bottles. During the entire experiment 3 bottles per cage were used. On forced intake days two bottles contained the appropriate drug solution and on choice days one bottle contained the drug, the second distilled water or quinine and third always remained empty. The order of bottles was changed every day in a systematic rotation.

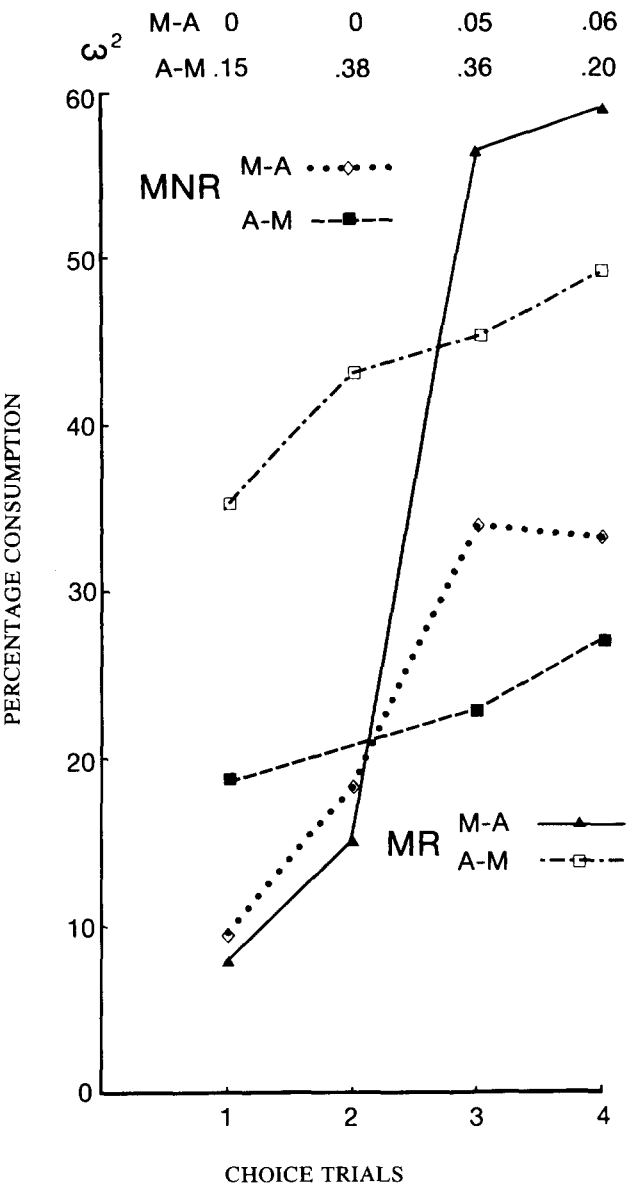

FIG. 1. Mean percentage consumption of alcohol on choice trials in the MNR and the MR genetic lines for M-A and A-M drug presentation orders.

RESULTS

Intake of alcohol and morphine solutions expressed as a percentage of the total fluid intake was calculated for each animal for the 2-day choice trials and means according to genetic lines and drug presentation orders are presented in Fig. 1 (alcohol) and 2 (morphine).

The MR line showed a higher preference for both the drugs than the MNR line, $F(1,40)=17.1$, $p<0.0002$, in an overall 2 (genetic line) × 2 (sex) × 2 (morphine or alcohol start) × 2 (alcohol vs morphine intake) × 4 (choice trials for each drug) complete factorial analysis of variance. However, differences between the genetic lines were larger for alcohol preference ($p<0.007$) than morphine preference ($p<0.016$).

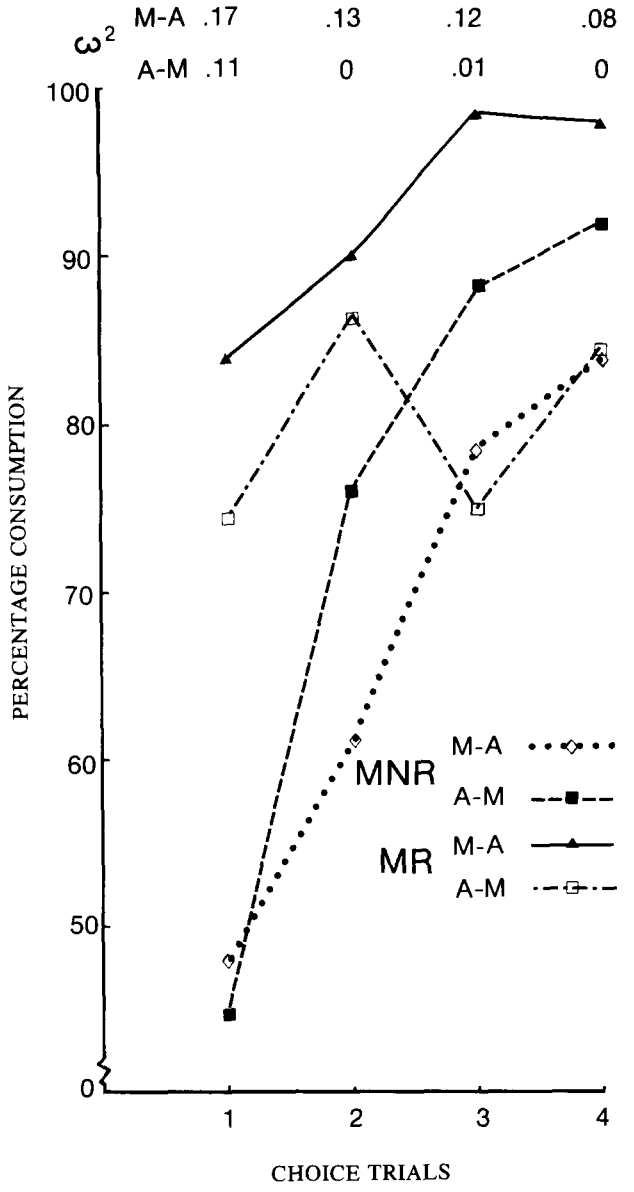

FIG. 2. Mean percentage consumption of morphine on choice trials in the MNR and the MR genetic lines for M-A and A-M drug presentation orders.

Animals of both the genetic lines showed higher preference for morphine than alcohol. Over 4 choice trials the percentage intake of both the drugs in both the genetic lines increased (alcohol: MNR, $F(3,60)=6.4$, $p<0.0008$; MR ($p<0.00001$); morphine: MNR ($p<0.00001$), MR ($p>0.4$). Because the increases among the choice trials were different, significant interactions between genetic lines and percentage intake over choice trials were found for alcohol, $F(3,120)=2.8$, $p<0.04$, and morphine ($p<0.012$).

Higher preference for both alcohol (Fig. 1) and morphine (Fig. 2) intake by the MR animals as compared to the MNR animals supports the hypothesis of common basis for the two

TABLE 1

MEANS OF PERCENTAGE INTAKE OF MORPHINE IN BOTH MNR AND MR LINES IN BLOCKS OF 6 DAYS EACH

	Days			
Genetic Lines	1–6	7–12	13–18	19–24
MNR	50.3	68.8*	69.5*	71.3†
MR	65.5*	70.1†	73.0†	77.5†

p Values indicate significance of differences in preference between the two drug solutions. *$p<0.05$; †$p<0.01$.

drugs. These results also confirm the earlier findings in independent studies of higher alcohol [3, 7, 20, 21] and morphine [22] intake in the MR as compared to the MNR animals.

Differences due to sex or the drug presentation order were not significant. But the males in MNR line showed steeper increase in percentage intake of morphine over choice trials than the females, thus producing an interaction between choice trials and sex ($p<0.003$). In the MR line the animals starting with morphine intake showed a steeper increase in subsequent alcohol intake than the animals starting with alcohol intake, thus showing an interaction between choice trials and drug presentation order ($p<0.0003$).

Because of a significant interaction ($p<0.002$) between choice trials and drug presentation order in the overall analysis, results were further evaluated separately for alcohol and morphine intake according to drug presentation order. The differences between genetic lines in both alcohol (Fig. 1) and morphine (Fig. 2) intake were significant only in the groups starting with alcohol for alcohol intake and for morphine intake in groups starting with morphine intake first. For example, morphine intake in the MNR and MR animals was 68 vs 93% ($p<0.009$) in M-A groups and 75 vs 80% ($p>0.6$) in A-M groups. Correspondingly alcohol intake was 22 vs 43% ($p<0.0004$) in A-M groups and 24 vs 35% ($p>0.3$) in M-A groups. The ω^2 values (variance attributable to differences between genetic lines for drug presentation orders) presented at the top of Figs. 1 and 2 separately for M-A and A-M orders make this differentiation clear for each of the 4 choice trials of each drug (ω^2 value of 0.12 yields F ratio to be significant at $p<0.05$).

Steeper increase in alcohol intake (Fig. 1) of the M-A animals of both genetic lines as compared to the respective A-M groups, shows the effect of the change in taste of drug solution, i.e., change from morphine in sucrose solution to alcohol in plain distilled water. Similar increase in morphine intake of the MNR animals (both experimental groups) indicates slower habituation to the effects of morphine. Although, it may be argued that the taste was probably the reason, but, in an earlier study ([22], Fig. 2) delayed habituation was found for morphine and not for an equiaversive solution of quinine.

Intake of both morphine and quinine solutions expressed as a percentage of the total fluid intake was calculated for each animal for each of 24 days of choice between these two solutions. Means for morphine intake based on 6 day blocks are presented in Table 1. It is clear from the data that animals of both the genetic lines preferred morphine solution over quinine solution although both the solutions have been found

equiaversive [22]. These results show that preference was for morphine and not for sucrose.

DISCUSSION

These findings may be taken to indicate that the link in alcohol-morphine preference has a specific genetic component, because a large number of previous investigations supporting the hypothesis have used genetic animal models. Further research to verify the genotypic-specificity of this hypothesis is already planned in our laboratory. Although much more data are required to fully test the genetic hypothesis, but it may be speculated that it is a partial overlap, i.e., polygenic partial pleiotrophy. Out of the five studies, including the present, four support the common basis ([8, 15, 18], and the present findings). The only exception is a study by Hill [12] in which alcohol-morphine interaction was tested in Tryon's S_1 and S_3 genetic lines, originally selectively inbred for maze learning ability [25]. Unfortunately this study [12] is seriously flawed for various reasons. For example: (a) the assumption that adulteration of alcohol and morphine solutions by 1% saccharine minimized the differences in taste qualities of both the drugs is not valid unless data confirming this are presented. To verify this, a test of equiaversiveness, as previously reported [22] to test the equiaversiveness between quinine and morphine, is required. (b) The design of the study is unnecessarily complicated without appropriate controls. For example, forced consumption (Phase III) was introduced to compare prior (Phase II) and subsequent (Phase IV) choice consumption, but no control was introduced to balance the effect of forced consumption. (c) The length of each of the 4 phases is not given, which makes it impossible to examine if enough adaptation for drug intake was provided to get reliable data. Incidentally alcohol consumption in S_1 and S_3 as reported in this study [12] contrasts with that reported by Drewek and Broadhurst [7] for these lines.

Empirical evidence supporting the common basis hypothesis is of a larger magnitude [1, 8, 11, 13, 14, 15, 16, 17, 18, 19, 23, 24, 26] as compared to the evidence contradicting the hypothesis [9, 10, 12]. It is to be noted that working with Swiss Webster mice, Goldstein and Judson [10] contradicted the hypothesis, whereas Blum, *et al.* [1,2] supported the hypothesis with findings from the same line of mice. Furthermore, Gelfand and Amit [9] in an attempt to replicate a previous study [23] used a different strain of rats (hooded vs Wistar derived), whereas it is imperative that in such a situation, the same line of animals be used.

In conclusion, it may be stated that the present findings along with previous results support the possibility of common basis of alcohol and morphine intake. For an ultimate validation of the hypothesis the measures of preference, intoxication, tolerance, dependence and withdrawal must corroborate each other, and in addition thorough analysis of the genetic variables should be carried out. The main contribution of the present study is the fact that it is the first investigation in which alcohol and morphine preference has been investigated in the same experiment and in the same animals.

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