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Open-Field Emotional Reactivity and Alcohol Intake in Rats[1]

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SATINDER, K. P. *Open-field emotional reactivity and alcohol intake in rats.* PHARMAC. BIOCHEM. BEHAV. **17**(5) 961–965, 1982.—The relationship between open-field emotional reactivity and alcohol intake was investigated. Randomly selected, high and low open-field defecation (OFD) groups from each of the MNR, MR and RCA genetic lines of rats were used. No functional relation between OFD as a measure of emotional reactivity and alcohol intake was found. Differences in alcohol intake among genetic lines were confirmed. It is concluded that emotional reactivity is a mediating process related independently to both OFD and alcohol intake.

Open-field reactivity Alcohol intake Genetic differences Sex differences Defecation

THE relationship between emotional reactivity and alcohol intake has been investigated in man [12], monkey [10], rat [3, 6, 7, 11, 17, 18, 20, 23], and mouse [13, 14, 15, 24]. Rat emotional reactivity which may be considered as an index of sensitivity to stress situations, has been operationally defined as the number of fecal boli excreted by an animal when placed in a strange situation, e.g., open-field [4,9]. Organisms highly susceptible to stress indicate higher levels of alcohol intake [17].

Previous research [3, 7, 17, 18, 20, 23] shows that Maudsley Reactive (MR) rats have consistently higher alcohol intake than their Maudsley Nonreactive (MNR) counterparts. The MR and MNR lines have been genetically selected for high and low open-field defecation (OFD), respectively. However, these investigations have not used OFD as a criterion to study alcohol intake. Therefore the purpose of the present study was to investigate the relationship between OFD and alcohol intake.

METHOD

Subjects

Open-field testing. Eight hundred fifty three experimentally naive rats from three genetic lines (MNR, MR, RCA) and both the sexes were used as subjects. The number of animals from each of the strain-sex groups are listed in Table 1. The reactive (MR) and nonreactive (MNR) genetic lines were established by selective breeding for extreme defecation scores in an open-field test [4]. These genetic lines were maintained by inbreeding until their arrival in the author's laboratory in 1968. In my laboratory, they are outbred within each of the genetic lines. Extensive summaries of the findings using these genetic lines have previously been provided [5,8] and further details regarding their genetic history are given elsewhere [21]. The RCA line represents a non-selected randomly bred control group to account for the differential effects of genetic selection. All animals were bred and reared in the laboratory, weaned at 28 days of age, and were 100 days of age at the start of the experiment. Before experimentation the animals were housed as same-sex pairs. Further details regarding the animals husbandry, care, and maintenance have been reported ([22], Experiment 2).

Alcohol intake. One hundred and eighty animals equally representing three genetic lines, both the sexes and three experimental groups representing three levels of OFD were used in this study. These animals were selected out of the 853 animals tested for open-field defecation. The selection method is described under Experimental Design below.

Animals were coded and housed individually, in both the phases (open-field testing and alcohol intake) of the experiment, to ensure that the experimenter did not know the genetic origin of the animals. The laboratory temperature was controlled at 22±1°C. The humidity level was maintained at 40% and the fluorescent lighting was on a 12:12 hr light/dark cycle. Animals were maintained on ad lib food and water.

Experimental Design

To investigate the relationship between open-field defecation and alcohol intake, animals from the three genetic lines were tested for OFD and assigned to groups representing different levels of OFD score. At least two groups were needed. One group represented the genetic lines as they are, i.e., randomly selected animals irrespective of OFD score. A second group was required which minimized the differences in OFD among the genetic lines, i.e., animals with the same OFD score. Although only one group of same-score animals would be sufficient, in order to seek more definitive answer two same-score groups representing low and high levels of

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OFD were included. The reason for the inclusion of same-score groups in the experimental design was that if OFD and alcohol intake are related, then elimination of variation in OFD (same-score groups) among genetic lines should lead to reduction or elimination of variation in alcohol intake among genetic lines. Whereas randomly selected animals representing the genetic lines without OFD selection, should retain variation in alcohol intake as previously reported [17,18]. The low and high OFD same-score groups should show subsequent low and high levels of alcohol intake, respectively.

Therefore the design included three experimental groups. One group involving the animals with zero defecation scores representing low emotional reactivity, another involving the animals with defecation scores of 4 representing high emotional reactivity, and a third involving animals randomly selected to represent the genetic lines without OFD selection.

As the 100 day old animals became available, they were tested for open-field behavior and animals meeting the criterion of the respective experimental groups were selected.

Subsequently the animals were choice tested separately and simultaneously for solutions of 5 and 10% alcohol. The levels of alcohol solution were included in this study because these genetic lines have been found to differ in their choice for these two concentrations [17]. Alcohol intake was counterbalanced with one-half of the animals from each of the strain-sex OFD groups tested for 5% solution first and the remaining half of the animals for 10% solution first. Hence, the experimental design was a 3 (genetic line) × 2 (sex) × (OFD score) × 2 (order of 5 and 10%) factorial, with 5 animals in each factorial cell.

Apparatus

Open-field. The open-field was 90 cm on each side and divided into 16 equal squares marked on the floor. The open-field was made of plywood and melamine plastic, and the walls were 45 cm high. The front wall was sliding door of transparent Plexiglas, which served as an observation screen and as a door for cleaning the open-field. The open-field was lighted by four 90-cm-long fluorescent lights placed 90-cm above the floor level and provided an illumination of 230 ft-c. at the floor center of the arena. A white-noise generator produced 65 dB (re 0.002 μbar) of masking noise. (Sound intensity was measured at the center of the open-field floor with a General Radio sound-level meter, Type 1551-C).

Alcohol intake. A stainless-steel cage, 25×18×18 cm, was fixed with three metal holders and three calibrated fluid bottles in front and outside of the cage. A food hopper was fixed on the inner back wall of the cage. The food hopper protected the food from any contamination from urine or feces.

Procedure

Open-field. Each animal was placed under a Plexiglas container in the center of the open-field. Both illumination and sound stimuli were turned on and at the same time the container covering the animal was lifted. During a 2-min trial the number of defecations (fecal boli) and sections crossed (all four feet in one section) were recorded, by an observer. The open-field was cleaned after every trial. A single open-field trial was considered appropriate to test the initial emotional reactivity [1].

Alcohol intake. For each of the animals the alcohol intake schedule was as follows:

Day	1	Distilled water
	2	Alcohol intake of 5 or 10%
	3	Distilled water
	4–8	Choice intake between alcohol solution presented on Day 2 and distilled water
	9	Distilled water
	10	Alcohol intake of the other concentration
	11	Distilled water
	12–16	Choice intake between alcohol solution presented on Day 10 and distilled water
	17	Distilled water
	18–22	Simultaneous choice between 5 and 10% alcohol solutions

The rationale behind this mixed schedule was to provide acclimatization to new settings and fluids. Exposure to distilled water on the first day provided acclimatization to a new cage setting and fluid. The exclusive intake of each alcohol concentration before the choice intake was to ensure that the animals experienced the taste of that solution. Return to distilled water before choice trials allowed the animals to make up any fluid deficiency they might have developed during exclusive alcohol intake. Alcohol solutions were prepared every day just before administration. The animals were distrubed only at 24 hour intervals to record body weight and intake of fluids, to replenish food and to empty, clean, and refill the drinking bottles.

During choice intake three bottles were used. Two bottles contained the two fluids for choice and the third bottle remain empty. The order of bottles was changed every day in a systematic rotation.

RESULTS AND DISCUSSION

Open-Field Behavior

Mean of defecation scores, frequency of sections crossed (activity), and body weight at the time of open-field testing, are presented in Table 1. The MR and the MNR lines had the highest and the lowest defecation score, respectively, $F(2,850)=345.5$, $p<0.00001$, whereas the reverse was true for the open-field activity ($p<0.0001$). These findings confirm the open-field differences observed between these genetic lines in their genetic selection [4]. The RCA and the MNR lines were the heaviest and the lightest in body weight, respectively ($p<0.00001$). All the three genetic lines differed significantly from each other on all the three measures of defecation, activity and body weight. Relative positions of the genetic lines were the same on all the three measures in both the sexes but overall sex differences were significant in activity and body weight but not in defecation. Sex differences within each of the genetic lines were of varying magnitude as shown in Table 1.

Alcohol Intake

Intake of 5% and 10% alcohol-water choices, and 10% alcohol in 5–10% alcohol choice, expressed as percentage of the total fluid intake were calculated for each animal for each of the 5 day choice periods. Means and standard errors of 5% (A), 10% (B), and 10% of 5–10% (C) alcohol intake, and means of OFD, sections crossed, for each of the three experimental (defecation) groups are presented separately and combined according to the genetic lines in Fig. 1.

The results for alcohol intake, open-field defecation and

TABLE 1

MEANS AND STANDARD ERROR

Measures	MNR (N=395) Mean	Females (n=183)	Males (n=212)	p for Sex Differences	RCA (N=194) Mean	Females (n=91)	Males (n=103)	p for Sex Differences	MR (N=264) Mean	Females (n=134)	Males (n=130)	p for Sex Differences
Defecation	0.5 (0.1)	0.3 (0.1)	0.6 (0.1)	<0.005	2.9 (0.1)	3.2 (0.2)	2.7 (0.2)	>0.1	4.0 (0.1)	4.1 (0.2)	3.9 (0.2)	>0.5
Number of Sections Crossed	26 (1)	28 (1)	24 (1)	<0.0001	22 (1)	25 (1)	20 (1)	<0.01	17 (1)	18 (1)	16 (1)	>0.2
Body Weight (g)	193 (2)	151 (1)	230 (1)	<0.0001	307 (5)	238 (2)	368 (2)	<0.0001	226 (4)	169 (1)	285 (2)	<0.0001

activity were evaluated by a 3 (genetic line) × 2 (sex) × 3 (experimental group) × 2 (order of alcohol presentation) complete factorial analysis of variance. Differences among genetic lines were significant on all the three measures of alcohol. $F_{min}(2,144)=13.5, p<0.001$, defecation ($p<0.00001$) and activity ($p<0.005$). Differences among the experimental groups (low, randomly selected and high defecation) were significant for defecation ($p<0.0001$), but not for any of the measures of alcohol intake or activity. No significant or systematic differences were observed between sexes and order of alcohol presentation.

Pearson and Spearman correlation coefficients between defecation, activity, and alcohol intake measures were calculated for all the groups and all the variables. No significant systematic correlations emerged.

The ω^2 (variance attributable to differences among genetic lines) values presented in Fig. 1 show that the differences among randomly selected groups (Fig. 1, column 2) were the lowest in 5% alcohol choice (A) and second to the lowest in the other two measures of (B,C) alcohol intake. Had the relationship between emotional reactivity (defecation) and alcohol intake been found, these differences should have been the largest, because the differences among genetic lines in emotional reactivity (defecation) were the largest in these randomly selected groups. In comparison to the randomly selected groups, differences among genetic lines should have been the smallest in both the low (zero defecation) and high (four defecations) defecation groups. In fact all the ω^2 values for low defecation groups were larger than the corresponding randomly selected groups. Furthermore, if there was a positive relationship between emotional reactivity and alcohol intake then the alcohol intake in all the genetic lines should have been higher in the high defecation groups as compared to the respective low defecation groups. The RCA (random bred control line) animals of the high defecation group did show higher intake on all the three measures as compared to the respective low defecation group, but none of these differences were statistically significant. The MR (selectively bred for high defecation) animals of the high defecation group showed lower alcohol intake as compared to the MR animals in the low defecation group. The MNR animals did not show any systematic trend.

Differences between the MNR and RCA lines were statis-

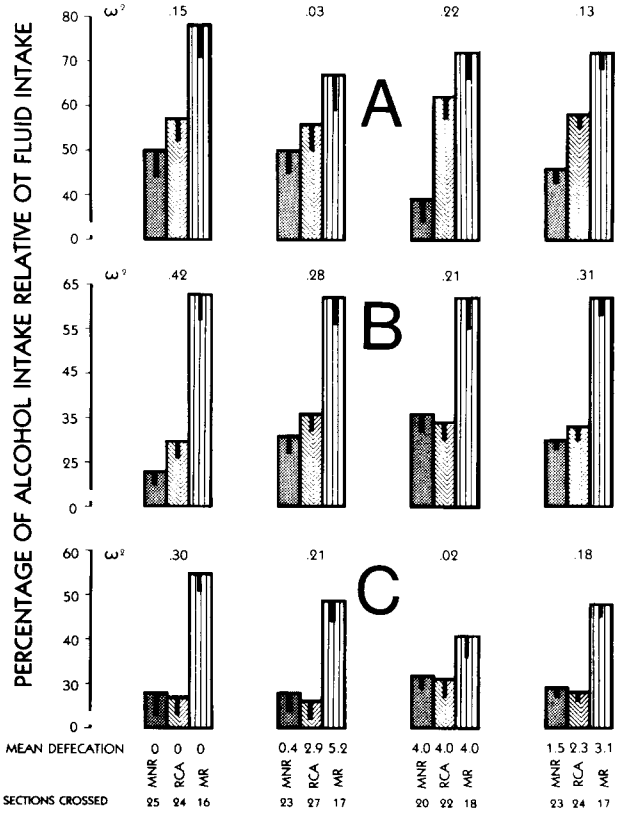

FIG. 1. Mean percentage of alcohol consumption relative to fluid intake of 5% (A), 10% (B), and 10% in 5–10% (C) alcohol choice for low same OFD (first column), randomly selected (second column), high same OFD (third column) experimental groups separately, and all experimental groups combined (fourth column). Defecation score and activity (sections crosses) of the respective genetic line-experimental groups at bottom of the figure. The ω^2 values reflect variance attributable to genetic lines for the respective group. Solid bar within bar graph indicates standard error for the respective group mean.

tically significant only in the 5% alcohol intake condition in the high defecation group. Differences between the MR and RCA lines were statistically significant for 10% alcohol-water choice but not for 5% alcohol-water choice in all these experimental groups. Whereas for 10% intake of 5–10% alcohol choice, the MR and RCA lines differed significantly in low defecation and randomly selected groups. Differences between the MNR and MR lines were significant in all cases except for 5% alcohol intake in randomly selected group and 10% of the 5–10% alcohol choice in high defecation group. Differences among all three genetic lines were significant in the same alcohol intake conditions as between the MNR and MR lines, indicating that the differences among genetic lines were mainly attributable to the differences between the two Maudsley lines.

The present findings confirm previous results showing higher alcohol intake in the MR (selectively bred for high defecation) line as compared to the MNR (selectively bred for low defecation) line [3, 7, 17, 18, 20, 23]. However, these findings fail to show any reasonable degree of relationship between alcohol intake and OFD as a measure of emotional reactivity. The lack of this relationship between alcohol intake and emotional reactivity suggests six alternative explanations.

The MR and MNR Lines Are not Reactive and Nonreactive Animals

Evidence presented by Eysenck and Broadhurst [8], Broadhurst [5], and more recently, findings from our laboratory (research in progress) clearly support the prediction that the reactive animals will behave in a more fearful and emotional manner, relative always to the nonreactive animals, in situations quite different from the open-field test in which they have been selected [8]. Hence, it is reasonable to accept that the MR and MNR lines are reactive and nonreactive groups of animals, respectively.

There is No Relationship Between Alcohol Intake and Emotional Reactivity

Consistent and reliable evidence, from various laboratories [3, 7, 17, 18, 20, 23] over an extended period of time, clearly indicates that the MR line shows preference for alcohol over the MNR line. This fact in collaboration with the evidence presented in (a) above, can be taken to indicate that there is a relationship between emotional reactivity (as manifested by the Maudsley lines) and alcohol intake.

The Relation Between Emotional Reactivity and Alcohol Intake in the Maudsley Lines Is a Fortuitous Association Arising in the Process of Genetic Selection.

The relation between emotional reactivity and alcohol intake reported in other genetic lines of rats [6,11] would indicate that this relation in the Maudsley lines is not merely a fortuitous association arising in the process of genetic selection.

Open-Field Defecation Is Not the Appropriate Criterion of Emotional Reactivity

Ample evidence has been reported to show OFD as a valid measure of emotionality in rats [2, 4, 5, 8, 9] but the issue is not resolved yet [1,19]. However this is an empirical question. Emotionality could be systematically varied and the influence on OFD observed. Research addressing to this question is in progress in our laboratory.

Single Open-Field Trial Was Not Sufficient

A single open-field trial was considered appropriate to test initial emotional reactivity [1]. But it is possible that it did not provide adequate differentiation of OFD groups. An investigation involving 4 daily open-field trials followed by a test for alcohol intake is already in progress in our laboratory.

Emotional Reactivity Is a Mediating Process, Relating Independently to Both OFD and Alcohol Intake

As indicated above, alcohol intake is related to emotional reactivity as manifested by the Maudsley lines with OFD as a genetic selection criterion for emotional reactivity. From this it may be deduced that alcohol intake and OFD are related to emotional reactivity but not to each other since no relationship between alcohol intake and OFD was found. However, it is plausible that emotional reactivity is a mediating process between alcohol intake and OFD. This suggested explanation would also provide better appreciation of the lack of relationship between OFD and avoidance learning [19].

The fact that experimental groups differed in defecation, but not in alcohol intake and activity, may indicate that emotional reactivity is not a unitary factor. In summary, both OFD and alcohol intake may be related to emotional reactivity, but they are not related to each other, it is plausible that emotional reactivity is a nonunitary mediating process.

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