Social Interactions Affect the Ethanol Preference Forming in Rats

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Abstract
Model experiments with animals have showed that social relationships in communities of alcoholized animals are disturbed. The goal of this work was to study patterns of alcohol dependence development during individual alcohol consumption in animals housed in the groups where other members were not alcoholized in comparison to the groups of animals where all members were alcoholized. After 14 days of isolation 48 male Wistar rats were placed by 3 into standard cages. Hierarchical relationships between the rats were observed for the first 15 min after placing them into one cage. After three days of housing in groups the interactions between animals were studied again using the resident–intruder test. On the basis of the interactions recorded in all tests, the animal rank was determined. Studies were carried out in experimental groups of three types: 1) only one rat of each group was given 10% ethanol, whereas the other members received water. Such individual alcoholization was studied in animals of different hierarchical levels. 2) 10% ethanol was given to all animals living together. 3) all rats received water – control group. The level of alcohol preference was assessed in the «two bottle» test before and after 27 and 54 days of experiment. It was found, that social interactions significantly affect the development of ethanol preference in rats. Initial high anxiety level as well as subordinate and subordinate ranks predispose to ethanol intake. In groups of drinking together rats alcohol preference develops significantly higher than in case of drinking alone rat housed with alcohol free cage mates. Housing of drinking alone rat with alcohol free rats induces the aversive reaction to ethanol instead of their rank status.

Keywords: Animal models; Alcoholism; Ethanol preference; Zoosocial interaction; Hierarchical relations in rats

Introduction
The study of alcoholism as an addictive behavior requires the study of zoosocial relationships. Model experiments with animals showed that hierarchical relationships in communities of alcoholized animals are disturbed [1,2]. It was found that, when housed in groups, the subdominant animals more readily become ethanol dependent than the dominant animals: the former consume more ethanol and earlier become alcohol addicts [1]. In addition, an increase in physical aggression as a result of alcoholization in monkeys [3] and under some conditions (increase of maternal aggression in ethanol drinking lactating female rats) in rodents was demonstrated [4].

It was assumed that ethanol, in general, stimulates social activity [5]. It is known that, in animal colonies alcohol dependence develops at different rates in different animals depending on their social status [6]. In isolation, conversely, alcohol preference is observed in the dominant animals [7,8].

The question as to how group or individual ethanol consumption under conditions of group housing affects the development of alcohol addiction in animals remains to be answered. The goal of this work was to study patterns of alcohol dependence development during individual alcohol consumption in animals housed in the groups where other members were not alcoholized in comparison to the alcohol dependence development in the groups of animals where all members were alcoholized.

Materials and Methods
Experiments were carried out on 48 adult male Wistar rats weighing 250–300 g. To increase the intensity of aggressive interactions rats were isolated from the other animals for two weeks. The separated animals were kept in individual metal cages (200 × 150 mm) and received water and food ad libitum. After 14 days of isolation rats were placed in cages 570 × 350 mm; (three animals in each cage) with fresh litter.

To analyze the social interactions, four parameters were determined: the number of attacks, the number of defensive and submissive postures, and interactions expressed in grooming and sniffing of partner’s body. We have estimated the forming of social hierarchical relations among three rats during the 3 day period. On the first day after 14 day isolation we observed hierarchical relations in three rats during first 15 minutes after housing into one cage. Then after 3 days of group setting we measured hierarchical relations in the same rats using the resident–intruder test with male and female animal each for 15 minutes. When the male intruder was out of the cage, a female was placed into the cage and the behavior of rats, including the copulative acts, was recorded. On the basis of the interactions between animals recorded in all tests, the rank of animals (dominant, subdominant, and subordinate) was determined.

The 54 day alcoholization period started after final hierarchical estimation and other baseline tests. Studies were performed in experimental groups of three types. In one type of groups, only one rat of each group was given alcohol, whereas the other members received water. Such individual alcoholization was studied in animals of different hierarchical levels. In the second type of groups, alcohol solution was given to all animals living together. In the third type of groups (control) all rats received water. So all rats had a daily 1 hour access to any liquid (ethanol or water).

In the constant housing cages water was absent, while food ration

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was standard. For drinking each rat was placed daily for 1 h in an individual cage, where it had access to liquid. In our experiments, we used the forced alcoholization model. For two months all the rats subjected to alcoholization had no access to any liquid except for 10% ethanol. All other rats received water. The level of alcohol preference was assessed in the "two bottle" test. A rat was placed in an individual cage with two waterers, one of which was filled with water and the other one contained 10% alcohol. The volume of liquid consumed from each waterer determined and the number of approaches to each waterer were counted for 15 min. During the experiment, this test was performed three times—before as well as 27 and 54 days after the beginning of forced alcoholization.

The dynamics of different parameters of behavioral activity was monitored in the "Suok test" [9]. In this test, a rat was placed on a 5 cm wide rod, which is divided into equal segments and elevated to a height of 50 cm above the floor, and a number of parameters reflecting the locomotor activity, exploratory activity, which reflect anxiety level were recorded for 5 min. The latter included the number of visited segments, passes through the central area, stops, looks down, events of orientation in space, as well as the level of vertical activity, the time of quitting the central area, defecation, and urination. The test was performed twice for all rats, before and 54 days after the beginning of forced alcoholization.

Statistical analysis was carried out by "Statistica for Windows 6.0"; the Mann-Whitney nonparametric criteria was used for independent sample comparison. Wilcoxon test was used to analyze differences in different tests within one group.

**Results**

The results of the "two bottle" test showed that the level of alcohol preference after the two month forced alcoholization of the rats that drank alcohol but lived together with the rats that drank water significantly differed from the level of alcohol preference of all other rats. We have measured the percentage of drunken ethanol to total liquid consumption (Figure 1). It was found that rats that consumed alcohol in the group of abstinent rats significantly (P=0.04) reduced their ethanol intake in the "two bottle" test. In contrast, those rats that consumed alcohol together with their cage mates significantly (P=0.006) increased ethanol intake. In addition, the increase of alcoholization in the two-bottle test after two months has been observed in control rats as well.

Figure 2 shows the dependence of alcohol preference on the hierarchical levels in animals of different experimental groups. After 27 days, no significant difference in the average level of ethanol preference among rats of different ranks has been observed in the ethanol group. Meanwhile a tendency to increase alcohol intake was found in all rank rats (Figure 2a). Alcohol intake in control group after 27 days did not differ from the baseline (Figure 2b). In the group where one rat consumed ethanol alone with water cage mates (ethanol+water), only subordinates significantly (P=0.04) decreased their ethanol preference while dominants and subordinates did not change. So after 27 days in rats of all ranks ethanol preference in ethanol+water group was that same compared in controls.

In 54 days after the beginning of forced alcoholization, a considerable increase in the alcohol preference among the subdominant and subordinate animals, but not among the dominants, was observed in the group of rats where all members consumed alcohol (Figure 2a). A similar tendency, though at a lower level, was observed among the control animals that received only water (Figure 2b). Subordinates alcohol intake in the ethanol group significantly differed (P=0.04) from subordinates from the control group. Meanwhile, the amount of drunk ethanol during the "two bottle" test became statistically significant in dominant spices compared to subdominant (P=0.02) and subordinate (P=0.06) rats (Figure 2b). The prominent decrease of ethanol intake after two months was found in rats of all ranks drinking alone (Figure 2c).

A significant decrease in the level of alcohol preference was observed in the rats of all ranks that consumed alcohol and were kept together with the rats that received water (Figure 2c). It is important that in the "two bottle" test alcohol consumption remained at a level up to 10% even among non preferred animals from control group, in contrast to the rats with aversive reaction that received alcohol from ethanol+water group. After two month of forced alcoholization the latter drank only water in the "two bottle" test.

The analysis of behavioral parameters estimated in the Suok test revealed no significant differences in the level of locomotor activity and anxiety in rats of different groups. Up to the results of the "two bottle test" in the end of the experiment all rats from the ethanol group (all rats consumed ethanol) were divided into two subgroups: alcohol preferred rats (consumed more than 45% of ethanol of total liquid) and alcohol non preferred rats ((consumed less than 45% of ethanol of total liquid). The same division was made to water controls. The group of drinking alone rats with their consuming water cage mates was not divided because none of them has showed alcohol preference in the "two bottle" test in the end of the experiment. As it is shown in Figure 3, in the ethanol group alcohol preferred rats before the experiment have showed strong tendency to lower locomotor activity compared to alcohol non proffered rats (p=0.08). The same tendency
was observed to ethanol preferred water controls. Thus, the rats with a predisposition to alcohol preference had the initially lower locomotor activity. In the end of the experiment the decrease of locomotor activity was observed in rats of all subgroups, compared to the first measurements. No significant differences in the locomotor activity between the subgroups were found. The decreased locomotor and exploratory activity in rats under stressful conditions of the “Suok test” may reflect their high anxiety level [9].

In our study we have found two groups of factors influencing ethanol consumption in rats. The first one is initial anxiety level. Up to the “Suok test” data rats with higher anxiety level consumed more alcohol in the end of the experiment. Anxiolytic ethanol action may be one of patterns which promoted alcohol preference forming. It was shown earlier that ethanol consumption is positively correlated with high anxiety [10,11].

Another group of factors is connected with social interrelations. Principal similarity between ethanol and control groups where subdominants and subordinates have demonstrated higher alcohol preference compared to dominants may be explained by social stress conditions during hierarchical relations [12]. Due to the previous publications it was shown that subdominant and subordinate animals are more sensitive to ethanol consumption [1]. The most interesting and new fact obtained in this study is the aversive reaction occurred in the group of ethanol drinking rats housed with water drinking cage mates. We may suppose that drinking alone rat has a specific alcohol odor which differs this rat from the cage mates. Odor is one of the main patterns influencing rodent behavior [13]. One can speculate that this alcohol odor may be a reason of social interrelation destruction and becomes a factor inducing alcohol aversion in the whole group. It is interesting that subdominant rats were more sensitive to odor factor because they reduce their ethanol intake earlier (just after 27th day) in the experiment, in contrast to the situation where all rats consumed ethanol. We can consider that odor factor did not act in the ethanol group, where alcohol preference depended only on social relations and anxiety level. The total alcohol intake increase in the ethanol group may be explained by the some kind of alcohol socialization described in humans. It was shown that interrelations between alcoholic spouses are better that in case when only one spouse is an alcoholic [14].

Thus, zoosocial interactions significantly affect the development of ethanol preference in rats. In the case of group alcoholization, ethanol preference is observed more frequently than in case of drinking alone rat housed with alcohol free cage mates. The development of alcohol preference was found to be related to the initial high anxiety level and
hierarchical animal rank: after two months of forced alcoholization it significantly increased in the subdominant and subordinate animals but not in the dominants. In the case of individual alcohol intake among two abstinent rats an aversive response to ethanol has developed irrespective to the hierarchical level.

Conclusions

1. Zoosocial interactions significantly influence ethanol preference in rats.
2. Initial high anxiety level as well as subordinate and subordinate ranks predispose to ethanol intake.
3. In groups of drinking together rats ethanol preference develops significantly higher than in case of drinking alone rat housed with alcohol free cage mates.
4. Housing of drinking alone rat with alcohol free rats induces the aversive reaction to ethanol instead of their rank status.

References