

The Significance of Maternal Care for the Formation of Ethanol Preference in Rats Periodically Separated from Mothers during the First Half of the Nest Period

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The relationship between the process of ethanol preference formation in rat pups periodically separated from mothers during the first half of the nest period and the intensity of maternal behavior of females was studied. The progeny of females with poor maternal behavior and/or separated from mothers is characterized by slow somatic development and high level of ethanol consumption. The quality of maternal care was inessential for the formation of ethanol preference in rats separated from mothers.

Key Words: *maternal behavior; separation; ethanol; nest period*

The mechanisms of the relationship between poor maternal care and the formation of alcohol addiction in subsequent life are studied on the model of separation of pups from their mothers during the nursing period. Short-term separation reduces emotional reactivity, increases resistance to stress factors, and reduces the level of ethanol consumption and preference. On the other hand, long-term separation from the mother leads to opposite effects [5]. A negative correlation between the level of maternal care during nursing (care/grooming) and volume of ethanol consumed by adult animals was detected [2]. However, the level of maternal care (grooming) increases similarly after short and long separation of pups from mothers, though resistance to stress factors increases in the former case and decreases in the latter [3]. Hence, it is impossible to attribute the increase of ethanol addiction and

reduction of stress resistance emerging after a long separation from the mother to poor maternal care. However, the intensity of maternal behavior of females can determine the subsequent level of ethanol consumption and preference in progeny.

We studied the relationship between the process of ethanol preference formation in rat pups separated from mothers during the first half of the nest period and the intensity of maternal behavior of females.

MATERIALS AND METHODS

The study was carried out on the progeny of Wistar females (150-200 g) born after the first pregnancy. Six females with poor maternal behavior (PMB) and 9 with active maternal behavior (AMB) were selected. The intensity of maternal behavior was evaluated by the presence of a nest, its status (dry, clean), and presence of rat pups in the nest. The period needed for bringing back the pup into the nest was evaluated; the test was considered positive if the female spent no more than 5 min for this

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procedure. On day 21 after birth, the rat pups were separated from the mothers. The rats were kept under standard conditions ($21\pm 3^{\circ}\text{C}$, 12:12 light:darkness period, free access to water and food). A total of 99 pups were born from 15 females.

Somatic development was evaluated from day 1 to day 21 of life from 10.00 to 17.00 using a complex of tests developed at P. K. Anokhin Institute of Normal Physiology [1]. Before the test, the females were removed from home cages into individual ones. The pups were weighed and put into individual plastic boxes ($15\times 15\times 15$ cm). The boxes were illuminated similarly as the home cages at $37\pm 1^{\circ}\text{C}$. Somatic development was evaluated by daily changes in body weight, time of manifestation of the conchae and fur growth, separation of fingers on the fore and hind paws, eruption of maxillary and mandibular canines, complete coverage by fur, and eye opening. In order to evaluate the time course of somatic development, the period during which one of these signs appeared was divided into 3 parts: early, medium, and late time of the sign manifestation. Timely development of the sign was evaluated in points (2, 1, and 0 points, respectively). The timely somatic development score (TSDS) of a pup was calculated by summing up the points for all signs.

After birth, each litter was divided into 2 groups at random. Half of pups from litters of females with AMB and PMB was returned into home cages to mothers after evaluation of their development and two experimental groups (AN and PN, respectively) were formed from them. The other subgroups were daily separated from mothers from day 1 to day 10 after birth. Two other groups were formed from these pups (AS and PS, respectively). Daily separation included a period of tests for evaluation of their development and a period spent in an isolated box; its total duration was 180 min.

Spontaneous ethanol consumption was started in animals at the age of 3.5 months. The rats were put into individual cages with two drinking bowls for 10 days. One bowl was filled with water, the other with 15% ethanol solution in water. The volume of consumed water and ethanol was recorded daily. Three types of rats were distinguished: 1) preferring water (ethanol consumption $<10\%$); 2) preferring ethanol (ethanol consumption $>50\%$); and 3) intermediate type (ethanol consumption 11-49%). Statistical analysis of parametric data was carried out by evaluating the general effects of age, separation from mothers, and impact of maternal behavior (ANOVA). Binary data were compared using Fisher's test with χ^2 test.

RESULTS

Daily body weight gain was observed during the nest period in all examined animals. Analysis of dispersions showed the main impact of age in the groups: $F_{(20,924)}=1915.3$, $p=0.00001$ for AN group; $F_{(20,819)}=1446.1$, $p=0.00001$ for AS group; $F_{(20,420)}=1622.4$, $p=0.00001$ for PN group; and $F_{(20,420)}=1150$, $p=0.00001$ for PS group. Body weight gain was inhibited by poor maternal behavior and by separation from the mother. The significance of maternal behavior and separation factors was $F_{(1,2583)}=8.2651$, $p=0.00407$ and $F_{(1,2583)}=72.304$, $p=0.00001$, respectively. The level of interactions between the separation and maternal behavior factors was negligible ($p>0.05$). Unidimensional analysis of dispersions showed the impact of separation from the mothers for the TSDS value. This is seen from the significance of the separation factor ($F_{(1,95)}=4.05$, $p=0.047$). The results of post-hoc analysis (Fisher's LSD test; Fig. 1) are presented. Separation caused significant inhibition of somatic development only in the progeny of mothers with AMB. The inhibition of development was less pronounced in the progeny of mothers with PMB. However, TSDS in PS group was significantly lower than in AN group. Activity of maternal behavior was inessential for somatic development. The maternal behavior factor was $F_{(1,95)}=2.84$, $p=0.095$. The absence of appreciable differences in TSDS between AN and PN groups and between AS and PS groups confirms this (Fig. 1). Interactions between the separation and maternal behavior factors were negligible ($p>0.05$).

Comparison of the mean volumes of ethanol, consumed by rats from groups AN and PN, showed

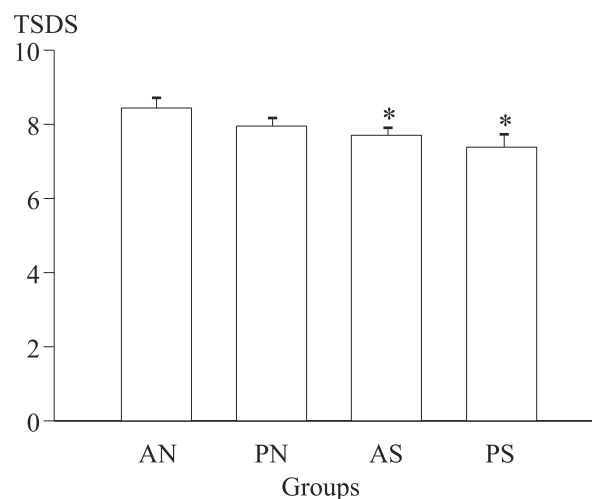


Fig. 1. Mean TSDS values in AN, AS, PN, and PS rat pups. Here and in Fig. 2: * $p<0.05$ compared to AN.

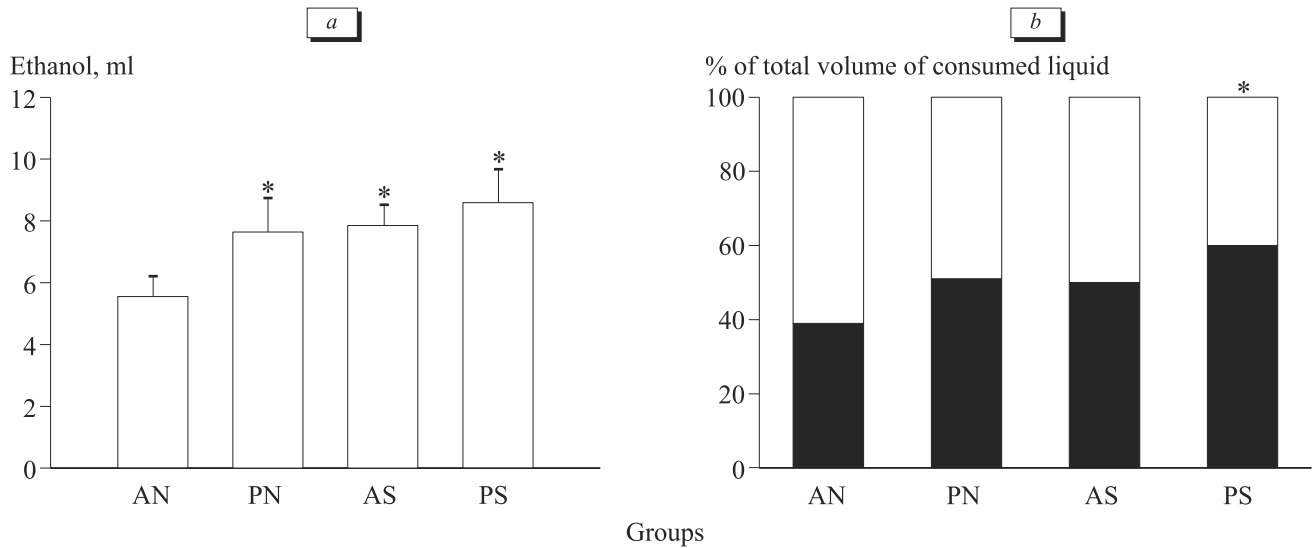


Fig. 2. Effects of repeated separation from the mother on ethanol consumption (a) and preference (b) by the progeny of female rats with AMB and PMB. b) light section: water; dark section: ethanol.

that the AN rats consumed less ethanol than PN ones (Fig. 2, a; $p < 0.05$). It is remarkable that these groups did not differ by the mean volume of consumed water and total volume of consumed liquid. No appreciable difference in ethanol preference (percentage of consumed ethanol vs. total volume of consumed fluid) was detected between the AN and PN groups (Fig. 2, b). The percentage of rats preferring ethanol was 2.3 times higher ($p < 0.05$) in the progeny of females with PMB in comparison with the progeny of females with AMB (Fig. 3). On the other hand, the number of intermediate type rats and water-preferring rats was 1.7 and 2.9 times lower in the PN vs. AN group ($p < 0.05$). Hence,

higher mean level of consumed ethanol in the progeny of females with PMB is explained by a higher percentage of ethanol preferring rats in comparison with water-preferring and intermediate type rats in their litter.

Separation from the mother was essential for ethanol consumption: the AS rats consumed more ethanol than AN rats (Fig. 2, a), but the separation was inessential for the volume of water and total volume of fluid consumed. The levels of ethanol preference in the AN and AS groups virtually did not differ (Fig. 2, b), but the separation caused a significant redistribution of animals by the types (Fig. 3). The percentage of ethanol-preferring rats in the AS group was 2.1 times higher than in AN group ($p < 0.05$). The percentage of intermediate rats and water-preferring rats in the AS group decreased 1.5 and 2.3 times, respectively ($p < 0.05$) in comparison with AN group. These data are in line with the results of a previous study [4]. Separation from the mother during the nursing period promoted an increase in the percentage of ethanol-preferring rats under conditions of free choice, while the percentage of rats consuming ethanol in moderate amounts and preferring water decreased. As a result, the rats separated from mothers generally consumed more ethanol than animals not separated from mothers.

The mean level of ethanol consumption in PS rats is comparable to that in PN and AS animals and is significantly higher than in AN group (Fig. 2, a). Ethanol preference was the highest in PS group (Fig. 2, b), though it also did not differ much from the levels in PN and AS groups. However, ethanol preference in PS group was higher than in AN group. Distribution of types in the PS group

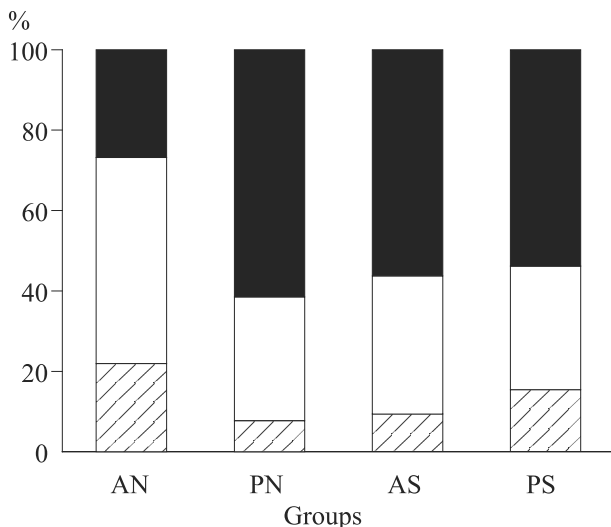


Fig. 3. Effect of repeated separation from mothers on distribution of rats by ethanol consumption types in litters of females with AMB and PMB. Dark sections: ethanol preference; light sections: intermediate type; cross-hatched sections: water preference.

was identical to that in AS and PN groups (Fig. 3). Hence, high level of ethanol consumption by the progeny of females with PMB after separation from mothers was explained not only by predominance of ethanol-preferring animals, but also by a higher mean level of ethanol preference.

A negative linear correlation ($r=-0.24$, $p<0.01$) was detected between the volume of ethanol consumed by the rats and the TSDS values. No correlation with the mean body weight increment during the nest period was detected ($r=-0.12$, $p=0.23$).

These data indicate that the progeny of females with a poor maternal instinct and/or separated from mothers during the nest period is characterized by delayed somatic development during the nest period and by a higher level of ethanol consumption. Our findings are in line with the data of other scientists [2] indicating an inverse relationship between the level of maternal care during nursing and volume of ethanol consumed by adult animals. How-

ever, no relationship between ethanol consumption or preference and the conditions of development during the nest period (mothers with active or poor maternal behavior) was detected in rats with a history of repeated separation from mothers during the nest period. Hence, the formation of ethanol preference in rat pups separated from the mother during the first half of the nest period does not depend on activity of maternal behavior of females.

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