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Effects of Ethyl Alcohol on Behaviour in Nursing Female Mice

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Abstract. Effects of administering 10% ethyl alcohol as drinking fluid to mice during pregnancy and lactation have been examined by ethological analysis of behaviour of nursing females in their home cages at 1 day, 5 – 7 days and 12 – 14 days postpartum. The treatment with alcohol did not affect gestation period or litter size, and fluid intake of treated mice remained similar to that of controls, the average intake of alcohol amounting to 29 mg/g body weight during lactation.

Increase in frequency of exploration at 1 day postpartum was the only significant behavioural effect of alcohol on the nursing female mice. Duration of Non-Social Behaviour was unaltered, and no effects of the treatment on Maternal Behaviour or on Social and Sexual Investigation of male partners could be demonstrated.

Behaviour of nursing females changed with increase in age of their pups. This occurred to a similar extent in treated and control animals. Maternal and Non-Social Behaviours declined in frequency as the pups became older although the time spent in these behaviours remained fairly constant. Social Investigation of the male partner declined both in frequency and duration while females were nursing their pups.

Key words: Ethyl alcohol – Maternal behaviour – Mouse

Exposure of developing animals to ethanol has been shown to influence their subsequent behaviour (Branchey and Friedhoff, 1976; Elis and Krsiak, 1975; Yanai and Ginsburg, 1977; Ewart and Cutler, 1979), but it is not as yet clear to what extent this effect is due to direct effects of ethanol on the nervous system of the young or to ethanol-induced changes in behaviour of the nursing mothers. Changes reported in behaviour of the offspring of rodents treated with alcohol range from a reduction of social investigation to an increase in exploratory activity or in aggressiveness.

The present experiments are concerned with an examination of behaviour in nursing female mice during the first 14 days of lactation when ethyl alcohol had been administered as a 10% solution in their drinking water. Behavioural effects have been assessed by ethological techniques as described by Mackintosh et al. (1977).

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Materials and Methods

Adult CFW mice, 20 females and 20 males supplied by Tucks Ltd. were distributed at random into 2 groups being housed for 2 weeks in groups of 10 animals of the same sex in stock boxes. Mice of one group received 10% ethyl alcohol as the sole drinking fluid and in the other group were given tap water. Diet 41B was freely available. Mice were maintained under a reversed lighting regime with a strong white light from 15.00 to 3.00 h and darkness for the remainder of the period.

Breeding was commenced with mice being housed in pairs of one male with one female in transparent Macrolon observation cages measuring $33 \times 15 \times 13\,cm^3$. The 20 mice in the treated group continued to receive a 10% solution of ethyl alcohol and the 20 controls were given tap water. Nest material was added to the cages when the females had reached an advanced stage of pregnancy, and on the day of birth all offspring were examined and counted. The number of mouse pups in each litter was reduced to six when the offspring had reached 6 days of age. Fluid intake of the pregnant and lactating female mice was measured up to the 14th day postpartum.

Behaviour of the nursing females within their transparent home-cages was examined at 3 time periods, when pups were 1 day old, 5 – 7 days old and 12 – 14 days old. It was not possible for observations to be blind since cages had been marked. Behavioural observations were made for 5 min periods twice daily, once in the morning and once in the afternoon. The behaviour shown by each mouse was recorded by means of a tape recorder using the check list of elements shown in Table 1. These behavioural elements were grouped into three major categories: Non-Social Behaviour, Social Investigation and Sexual Behaviour and Maternal Behaviour. The elements on Non-Social Behaviour and of Social Investigation are those described by Mackintosh et al. (1977). Maternal Behaviour was based on the

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Table 1. Categories adopted for the description of behaviour and their respective postural elements

Category	Elements
Non-social behaviour	Explore, scan, wash, self-groom, scratch, eat, dig, push-dig, on bars, off bars, sit, flop
Social investigation and sexual behaviour	Attend, nose, investigate sniff, push-under, groom, stretch-attend, follow, crawl over, push past, attempt mount, mount, genital groom
Maternal behaviour	Attend young, investigate young, sniff young, groom young, crouch over young, retrieve, transport material into nest, rearrange nest material, transport material away from nest, undirected carrying of young

broad categories of cleaning, retrieving and nursing pups and of nest-building as described by Noirot (1972), and this was subdivided into ten descriptive elements. The acts of attending, investigating, sniffing and grooming of the young were similar to those described by Mackintosh et al. (1977) for Social Investigation in adult mice. Nest-building was subdivided into three acts of transporting material into the nest, away from the nest and of re-arranging nest material. Crouching over the young, retrieving of pups and undirected carrying of young were the other three elements of Maternal behaviour. A record was made of the mean frequency of behavioural elements observed from mice of each treatment group and the significance of differences between means estimated by the non-parametric Mann-Whitney U test. The duration and frequency of each behavioural category were also recorded using a machine especially constructed for that purpose, and the significance of differences between groups estimated by the same procedure.

Results

Administration of 10% ethyl alcohol as the sole drinking fluid to breeding mice was without significant effect on the period of gestation or on the size of the litters, there being on average 9.8 pups/litter for treated and 9.4 pups/litter for control mice. Fluid intake by breeding pairs of alcohol-treated mice did not differ significantly from that of controls, the volume of fluid consumed amounting to 7 ml/24 h on average before birth of the litters and 9 ml/24 h during the first 10 days of lactation. The mean daily intake of alcohol by nursing females during lactation was 29 mg/g body weight.

Behaviour shown by female mice of the control and alcohol-treated groups at 1 day after birth of the litters is shown in Table 2. Alcohol-treated females showed a significantly higher frequency than controls in the individual elements of 'scan' and 'explore on bars' and in all forms of exploration. Other Non-Social elements were not significantly raised in frequency in treated females, and the mean duration of Non-Social elements was similar in the treated to that in the control group. Neither Maternal Behaviour nor Social Investigation of the male partner were significantly affected by the treatment with alcohol.

Table 2. Behaviour of female mice with 1 day-old offspring

	Mean frequency of behavioural elements	
	Control group	Alcohol-treated group
No. of observations	18	20
All Non-Social elements	29.2	44.6
Explore	9.7	14.3
Scan	5.3	13.3[a]
Explore on bars	0.9	5.7[b]
All forms of exploration	15.9	33.3[a]
Other Non-Social elements	13.3	11.3
All elements of Social Investigation of male partner	5.4	5.3
All elements of pup-directed behaviour	2.7	4.1
All elements of nest-building behaviour	4.8	8.6
Mean duration of Non-Social elements (s)	201.6	202.9
Mean duration of Social Investigation of partner (s)	10.3	5.9
Mean duration of Maternal behaviour (s)	80.2	91.2

[a] $P < 0.05$ between treated and control mice by the Mann-Whitney U-test

[b] $P < 0.01$ between treated and control mice by the chi-squared test

Mouse pups at 1 day of age were actively suckling and displayed little other co-ordinated movement. Adult male partners shared with the female such activities as nest-building, nursing and retrieving the young. The quality of nests and their positions in the cage were similar in treated and control groups.

At 5–7 days of age, mouse pups showed co-ordinated movements, climbing over one another, suckling actively but not leaving the nest. Table 3 shows the behaviour of the nursing females at this stage. There were no significant differences in behaviour between females of the treated and control groups, exploratory activity in treated females having returned to normal levels.

Table 3. Behaviour of female mice with 5–7 day-old offspring

	Mean frequency of behavioural elements	
	Control group	Alcohol-treated group
No. of observations	16	18
All Non-Social elements	36.6	30.2
All forms of exploration	28.2	16.6
Other Non-Social elements	8.4	13.6
All elements of Social Investigation of male partner	1.9	2.2
All elements of pup-directed behaviour	2.5	1.2
All elements of nest-building behaviour	0.7	3.6
Mean duration of Non-Social elements (s)	239.2	209.9
Mean duration of Social Investigation of partner (s)	1.8	3.4
Mean duration of Maternal behaviour (s)	59.0	86.7

Table 4. Behaviour of female mice with 12–14 day-old offspring

	Mean frequency of behavioural elements	
	Control group	Alcohol-treated group
No. of observations	16	20
All Non-Social elements	15.7	15.4
All forms of exploration	8.1	6.8
Other Non-Social elements	7.6	8.6
All elements of Social Investigation of male partner	0.7	0.5
All elements of pup-directed behaviour	1.4	1.3
All elements of nest-building behaviour	1.9	1.1
Mean duration of Non-Social elements (s)	195.3	203.4
Mean duration of Social Investigation of partner (s)	3.6	1.0
Mean duration of Maternal behaviour (s)	101.1	95.7

Table 4 summarizes the behaviour of the nursing females when pups had reached 12–14 days of age. No significant differences between control and treated groups could be seen. Mouse pups were now showing activities such a self-grooming, washing and scratching and some were beginning to leave the nest although their eyes had not yet opened. Eye-opening occurred when they had reached 15–16 days of age.

In both alcohol-treated and control groups behaviour of the nursing females changed with increase in age of the pups (Tables 2–4). Non-Social behaviour of the nursing females remained fairly constant in duration up to 14 days after birth of the young, but the frequency of Non-Social elements declined when pups had reached 12–14 days of age. In the alcohol-treated group, frequency of Non-Social elements was significantly less at 12–14 days than at 1 day postpartum ($P < 0.01$). The same trend could be seen in the control group although changes in the frequency of elements were less marked.

Social Investigation and Sexual behaviour by nursing females declined both in frequency and duration during the period of 1–14 days after birth of the litters. The frequency of these elements in control and treated groups was significantly less at 12–14 days postpartum than at 1 day, and duration of Social Investigation was significantly reduced in controls at 5–7 days.

Maternal behaviour remained fairly constant in duration up to 14 days after birth of the young, but the frequency of Maternal elements declined as pups became older. Maternal elements were significantly reduced in frequency at 5–7 days in controls and at 12–14 days in the treated group ($P < 0.05$) when compared with the frequency at 1 day postpartum.

Discussion

Chronic treatment of mice with a 10% solution of ethyl alcohol in their drinking fluid throughout pregnancy and lactation was without detectable effect on maternal behaviour in the present investigations. However, effects of the treatment on pup retrieval following removal from the nest were not examined, since removal of pups can influcne behaviour of the mother (Lee and Williams, 1977). Abel (1978) found that daily intubation of pregnant and lactating rats with alcohol at a dose level of 1 g/kg had no effect on pup retrieval, but that a dose level of 2 g/kg did delay retrieval of the young. In the present experiments in which environment of mother and pups was not disturbed, administration of alcohol brought about only one significant change in behaviour of the nursing females. Exploratory activity was increased in frequency in alcohol-treated mothers at 1 day after birth of their pups. Other elements of Non-Social behaviour were not affected, and the duration of exploration was unchanged. These behavioural changes brought about by the treatment with alcohol were so slight that they are unlikely to have influenced the offspring, and alterations in behaviour which did occur in the pups (Cutler

et al., 1979) are most probably due to direct actions of alcohol on the neonate. Behavioural changes induced in the offspring included an increase of exploratory activity together with a reduction of self-grooming.

Another effect seen in the present experiments was alteration in behaviour of the nursing female mice with increase in age of their pups. This effect occurred to a similar extent in alcohol-treated and in control animals. As pups became older the frequency of Maternal elements and Non-Social elements decreased although the time spent in these behaviours remained fairly constant. Social Investigation of the male partner and Sexual behaviour progressively declined in frequency and duration with time while females were nursing their pups. Maternal behaviour in rodents appears to be initiated by the endocrine changes that accompany parturition and lactation (Moltz, 1971), to be related to ultrasonic calling by the young (Noirot and Pye, 1969) and to be maintained by stimulation from the pups (Reisbick et al., 1975). Noirot (1966) found that adult females usually responded to calls from the pups by immediately stopping the activity in which they were engaged and switching to another. Ultrasonic calls from mouse pups usually disappear at the time when hearing develops and the eyes open (Noirot and Pye, 1969). These effects may explain the decline in frequency of maternal elements seen in the present experiments as mouse pups became older.

It has been suggested that mental as well as physical development may be retarded in children of alcoholic mothers, and psychiatric studies have revealed increased vulnerability in such children (Anthony, 1973; Barry and O'Nuallain, 1975; Jones et al., 1973). Such an effect could arise either from poor maternal care (Rhingold, 1963) or from direct actions of ethanol on the nervous system of the developing child. The present experiments using the laboratory mouse as an animal model have indicated that alcohol does influence behaviour of the offspring and that there are negligible effects on behaviour of their mothers.

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