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Effects of Cigarette Smoke Exposure on Estrous Cycles and Mating Behavior in Female Rats

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While it is well known that cigarette smoke inhalation by pregnant females serves to increase perinatal mortality and the ratio of premature births, and to retard intrauterine growth in human and animals (Simpson 1957; Lowe 1959; Meyer et al. 1974; Tachi and Aoyama 1983; 1986), few studies have dealt with the effects of the smoke exposure during non-pregnant period upon the reproductive functions such as sexual cycles.

Either cigarette smoke, or nicotine, one of the constituents of the smoke, reportedly modifies or delays the proestrous surge of luteinizing hormone and prolactin (Blake et al. 1972; 1973; McLean et al. 1977). These findings could lead one to consider that smoke exposure in females makes the menstrual or estrous cycle irregular, due to the abnormal discharge in pituitary and subsequently sex hormones. The earlier observation that menstrual cycles in smoking women was less likely to be periodic than nonsmokers, may have proved the possibility (Hammond 1961). The study, however, failed to exclude other factors influencing the periodicity.

On the basis of the above findings, the present study was designed to evaluate the effects of cigarette smoke inhalation by female rats before pregnancy on the estrous cycle, mating behavior, and ovulation, and also to investigate how carbon monoxide exposure can affect the phenomena, compared with those by whole smoke exposure, because carbon monoxide has been suggested as one of the causes for the retardation of intrauterine fetal growth in smoking gravida (Tachi and Aoyama 1983; 1986).

MATERIALS AND METHODS

Female Wistar rats were purchased from Shizuoka

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Laboratory Animal Agricultural Cooperation (Hamamatsu, Japan), and maintained under the condition of lighting (12L12D, lights on 0600 h), and temperature at 22.5-26.5°C. Vaginal lavage was microscopically inspected every day throughout the experiment to determine the estrous cycle, after animals were accustomed more than a week to the room condition.

According to the manner previously described in pregnant rats (Tachi and Aoyama 1983; 1986), exposure to cigarette smoke, carbon monoxide at the concentration of 1200 ppm similar to that in cigarette smoke inhalation, or room air, started in females weighing 215-245 grams on the day of proestrus following observation of at least two consecutive 4-day estrous cycles. A preliminary result indicated that approximately 99% of female rats which had displayed two successive 4-day cycles repeated it again subsequently. Inhalation was accomplished twice daily in 81-minute exposures; the mainstream smoke of 14 cigarettes or 700 ml of carbon monoxide was introduced for 21 minutes into the chamber in which animals had been placed, and animals were exposed for another 60 minutes afterwards. The inhalation was continued until the day of proestrus, first appearing after the exposure for at least 21 days. A mating behavior was studied on the day of final exposure as follows; each female was placed in a cage with an experienced male around 1800 h, and the retention time for the first mounting was measured. The mountings and lordoses were counted for 30 minutes after the first mounting. In case the mounting did not occur within 40 minutes after pairing, the behavior was not evaluated. Rats were then mated overnight, and the female was killed at noon of the next day to record the number of ovulated ova in the oviduct.

Statistical analysis was performed using Duncan's new multiple range test, and a P value less than 0.05 was considered as significance.

RESULTS AND DISCUSSION

In accordance with the results of Hammond (1961), in which menstrual irregularity was more common among smoking women, the present study demonstrated that cigarette smoke inhalation by non-pregnant female rats modified the pattern of estrous cycles to prolong the period of diestrus (Figure 1, Table 1). Five out of 7 females showed a prolonged period of diestrus for more than 10 days, as soon as the cigarette smoke exposure began. Regular or irregular 4- or 5-day cycles were found in three of them after a prolonged diestrous period (Figure 1A), whereas two rats again entered a state of prolonged diestrus with one 5-day cyle

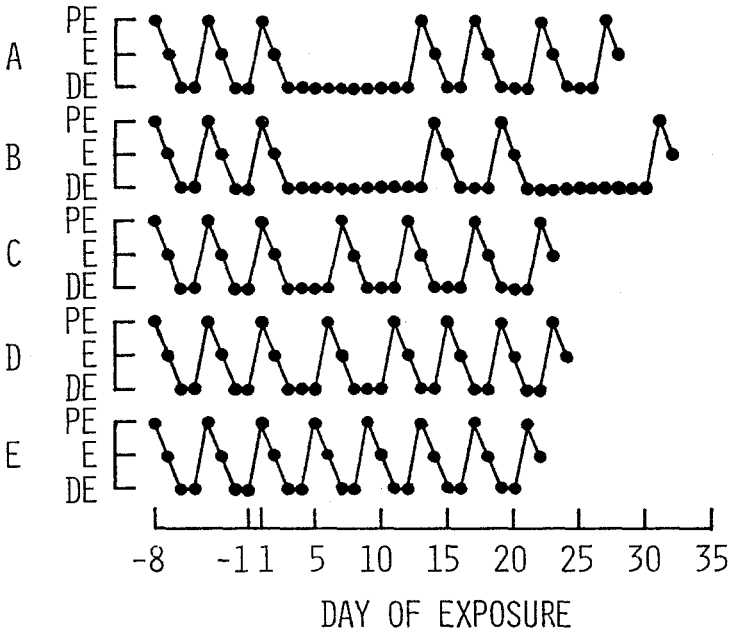

Figure 1. Representative pattern of estrous cycles in animals exposed to cigarette smoke, carbon monoxide, or room air.
PE, E, and DE mean proestrus, estrus, and diestrus, respectively.

Table 1. Distribution of animals which displayed each pattern of estrous cycle.

	Type A[1)]	Type B	Type C	Type D	Type E
CIG[2)]	3/7[3)]	2/7	2/7	0/7	0/7
CO	0/8	0/8	0/8	6/8	2/8
RA	0/8	0/8	0/8	2/8	6/8

1): Patterns of estrous cycles. See Figure 1.
2): CIG, CO, and RA represent the groups of cigarette smoke, carbon monoxide, and room air exposure, respectively.
3): The number of rats displaying the pattern used in the experiment.

between two long periods of diestrus (Figure 1B). The rest inhaling cigarette smoke displayed one 6-day cycle followed by regular 5-day cycles (Figure 1C). Except for one rat, none of the rats exposed to the smoke reverted to the periodic 4-day cycles seen before inhalation. In those breathing carbon monoxide or room air, the cycle was not so drastically modified as observed in the smoke exposed group, and females drew the pattern of regular 4-day cycles throughout the experiment (Figure 1E), except for the duration of the first or second cycle immediately after the beginning of exposure. During this time, the cylce was prolonged to 5 days in several animals (Figure 1D). Transient 5-day cycles were often observed with light stress such as by transferring animals to another room. In this way, it could be supposed that the estrous cycle in females exposed to either carbon monoxide or room air was within the normal range. Therefore, the modification in the cycle by cigarette smoke was presumed not to be induced by carbon monoxide in smoke, which has been considered one of the important factors in reducing intrauterine fetal growth in pregnant females exposed to smoke (Tachi and Aoyama 1983; 1986).

Some possibilities were considered to explain the change in estrous patterns. First, a shortage in energy intake or some subsequent metabolic disorder may have been responsible for the event, since animals which had inhaled cigarette smoke lost about 8% of their weight, at maximum, in relation to before inhalation (data not shown); this in addition to the fact that the food intake has been reported to decrease in pregnant rats exposed to cigarette smoke (Tachi and Aoyama 1986). Secondly, the change could be considered to be due to hormonal disadvantage. In rats, a proestrous surge of luteinizing hormone and prolactin has been indicated to be delayed or modified by cigarette smoke inhalation or administration of nicotine, one of the major components in smoke (Blake et al. 1972; 1973; McLean et al. 1977). The exact reason(s), however, remain unclear at present.

Only four out of 7 females exposed to the smoke accepted the male within 40 minutes after pairing with extended retention, compared to the other two groups in which any female permitted mounting by the male within 3 minutes on average (Table 2). This finding did not mean that no copulation was achieved in females exposed to cigarette smoke, but that it was so delayed that it could not be seen during the observation period, because sperms were discovered in the vaginal smear in all rats on the following morning. This seemed likely to be associated with the delay in the surge of pituitary hormones (McLean et al. 1977) controlling the

Table 2. Indices of mating behavior, and the number of ovulated ova.

	Rate of male acceptance	Retention time (min.)	Lordosis quotient (%)	Number of ovulated ova
CIG[1]	4 / 7[2]	15.8±8.0*(4)[3]	72±18 (4)[4]	13.0±0.4 (7)[5]
CO	8 / 8	2.9±1.0 (8)	72±13 (8)	11.4±0.6 (8)
RA	8 / 8	2.9±0.7 (8)	84± 6 (8)	13.0±0.5 (8)

1): CIG, CO, and RA represent the groups of cigarette smoke, carbon monoxide, and room air exposure, respectively.
2): The number of animals which accepted the male during the observation period used in the experiment.
3), 4), and 5): Mean±S.E. Numerals in parentheses represent the number of animals observed. An asterisk shows a significant difference from the groups of CO, and RA.

gonadal steroid release, which in turn regulates the mating behavior (Lisk 1962).

Once a female accepted the male, there was no difference in the lordosis quotient (the ratio of the number of lordoses to that of mountings) which reflected the mating behavior.

All rats ovulated a similar number of ova on the following day (Table 2), in contrast to the earlier observation in which ovulation was partially blocked in the smoke-exposed group (McLean et al. 1977). This disagreement between two studies may have resulted from the difference in the day of observation, which was after a prolonged exposure for at least 21 days in the present study, whereas it was only after one-day inhalation in the earlier one. The period immediately after the beginning of inhalation revealed the greatest effect on estrous cycles as shown in this study, and ovulation may have been affected as well.

The present study indicated that cigarette smoke exposure in female rats could disturb the estrous cycle, and delay the time of male acceptance. These findings lend support to the theory that women who smoke tend to be rather infertile, compared with nonsmokers (Tokuhata 1968; Baird and Wilcox 1985; Howe et al. 1985).

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