

Effects of the Age of Pups and the Number of Days after Delivery on Retrieving in Mice

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Introduction

Several studies with rat and mouse have shown that retrieving behaviour, as well as other maternal behaviours, is induced by the stimulus from pups and further is stimulated more strongly by younger aged pups and smaller litter size.^{1~8)} In most of these studies, however, the maternal behaviour observed on certain day after birth should be also related to the number of days after delivery, since the age of foster mother is advanced together with that of pups.

In recent years, the procedure of cross-foster or foster nursing has been used for the approach to the analysis of maternal effect and maternal behaviour in rat and mouse.^{9~13)} It is difficult in these studies to distinguish between the effect of the age of pups on the maternal behaviour and that of the age of foster mother, because the age of pups is advanced with that of foster mother after fostering.

The continuous cross-foster nursing would become a new analyzing method for maternal behaviour, because it is possible to separate the combined effects of ages of pups and foster mother on the behaviour. The purpose of the present study was to determine whether maternal retrieving was affected by the age of pups or by the number of days after delivery, using the procedure of the continuous cross-foster nursing.

Materials and Methods

The animals used were virgin female CF 1 mice of full-sib mating for 10 generations in the laboratory. More than 35 females were divided into 7 groups. Each group consisted of at least 5 females which showed vaginal proestus on the same day. In order to obtain five deliveries on alternate days during 14 days, females of each group were mated every other day. Denenberg *et al.*⁹⁾ reported that the survival rate in foster nursing experiment was not reduced if the new pups remained with their natural mother for 12 hr before fostering. Therefore, all new pups had remained with their natural mothers for 24 hr before cross-foster nursing in this study. On the next day after birth, (1 day after delivery) all pups which were born on the same day were pooled and then randomized. Eight pups, four of each sex, were immediately returned to each foster mother.

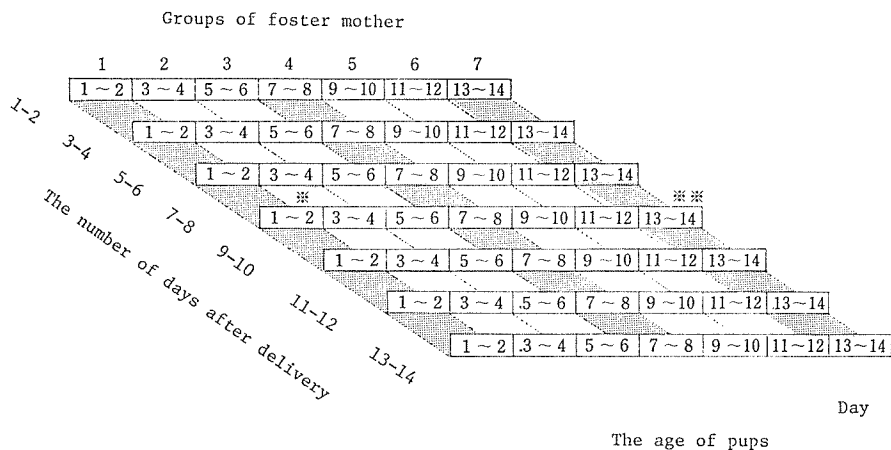


Fig. 1. The procedure of continuous cross-foster nursing.

Three groups of foster mother, reared 1-2, 7-8 and 13-14 day old pups repeatedly from 1 to 14 days after delivery, were used in the present study.

Each group of foster mother consisted of 5 foster mothers which delivered pups on the same day.

* indicated 1-2 day old pups which were born from a mother of No. 4 group and that were reared by a mother of No. 1 group of 7-8 days after delivery.

** indicated 13-14 day old pups which were born from a mother of No. 4 group and that were reared by a mother of No. 7 group of 7-8 days after delivery.

The procedure of continuous cross-foster nursing, shown in Fig. 1, was designed primarily to investigate the effect of suckling stimuli of pups on the lactation performance in CF 1 mice. The experimental procedure involved seven foster mother's groups in which each mother reared only one of 1-2, 3-4, 5-6, 7-8, 9-10, 11-12 and 13-14 day old pups repeatedly from 1 to 14 days after delivery.

Pups born from mothers of No. 1 group were fostered to their mothers, 1-2 days after delivery, from 1 to 2 days after birth. Then they were given to mothers of No. 2 group, 1-2 days after delivery, from 3 to 4 days after birth. But at that times mothers of No. 2 group reared their 1 day old pups. Therefore their pups and pups of No. 1 group were cross fostered to mothers of No. 1 group and to those of No. 2 group respectively. On 13 and 14 days after birth, pups born from mothers of No. 1 group were fostered to mothers of No. 7 group of 1-2 days after delivery. Thus these pups were reared always only by foster mothers of 1-2 days after delivery during 14 days after birth.

On the other hand, mothers of No. 1 group reared 1-2 day old their pups from 1 to 2 days after delivery. They reared again 1-2 day old pups which were born from mothers of No. 2 group from 3 to 4 days after delivery. Thus they reared only 1-2 day old pups repeatedly over the period of 14 days after delivery. Three groups of foster

mothers which reared 1-2, 7-8 and 13-14 days old pups were derived from the experimental materials mentioned above and were used for the present study.

All animals were housed individually in $30 \times 20 \times 15$ cm of metal cage and fed CE-2 pellets (Nihon CLEA Inc., Tokyo) and water *ad lib*. They were kept in the room maintained at the temperature of $20-25^{\circ}\text{C}$ under 12-hrs light-dark cycle.

The retrieving test was performed as follows. First the mother was gently removed from the cage and placed in a retaining cage at 2 minutes before the test. Secondly pups were removed from the nest and scattered about the place of 15 cm distant from the nest at 30 seconds before the test. The test started at 16:00 hr when the mother was gently returned to the empty nest. The number of seconds which a mother took to retrieve each pup to the nest were recorded. The latency and duration of retrieving were calculated from it. The maximum time allowed for the test was 10 minutes. Although the test was carried out daily during 14 days after delivery, the average values of two consecutive days, *i. e.* 1 and 2, 3 and 4, . . . , 13 and 14 days after delivery, were estimated. They were transformed to log units for statistical treatments.

Results and Discussion

The mean latency of retrieving in foster mothers reared 1-2, 7-8 and 13-14 day old pups for 14 days after delivery is shown in Fig. 2. One and two mothers reared 13-14 day old pups did not begin to retrieve, within 10 minutes, any pup on 4 and

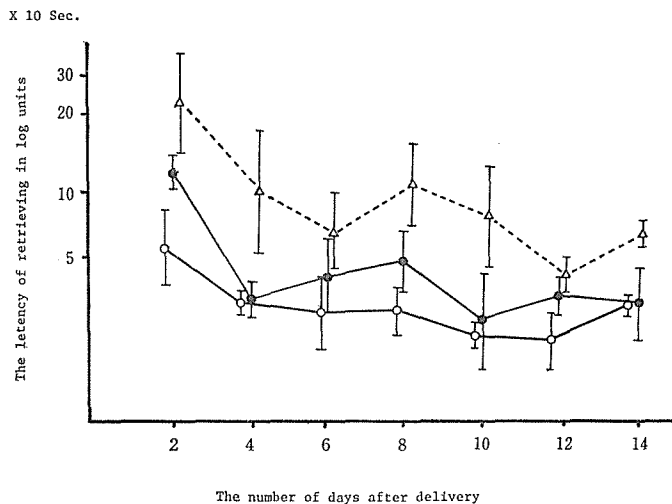


Fig. 2. The mean latency of retrieving in foster mothers reared 1-2 (○—○), 7-8 (●—●) and 13-14 (△··△) day old pups for 14 days after delivery. Vertical bars indicated S. E.

2 days after delivery respectively. The mean latency in foster mothers of the first 2 days after delivery was longer than those of 4 days and over in all groups ($p < .05$). The result was in agreement with that shown by Smith and Berkson⁸⁾ and probably demonstrated from no experience of retrieving in primiparous mothers.

Therefore the analysis of variance, given in Table 1, was applied to the data of 6–14 days after delivery in three groups in order to examine the effects of the age of pups and the number of days after delivery on the latency of retrieving. The results indicated that the difference in the latency of retrieving among the age of pups was significant ($p < .01$) but that among the number of days after delivery was not significant. Consequently the difference in mean latency between groups was tested. The result showed that the mean latency of the mother reared 13–14 day old pups was longer significantly than those of mothers reared 1–2 and 7–8 day old pups ($p < .05$), as shown in Fig. 2.

Table 1. The analysis of variance showing effects of the age of pups and the number of days after delivery on the latency of retrieving.

Source	d. f.	Mean Squares
Age of pups	2	84.3**
No. of days after delivery	4	8.5
Interaction	8	4.3
Error	60	8.9

** $P < .01$

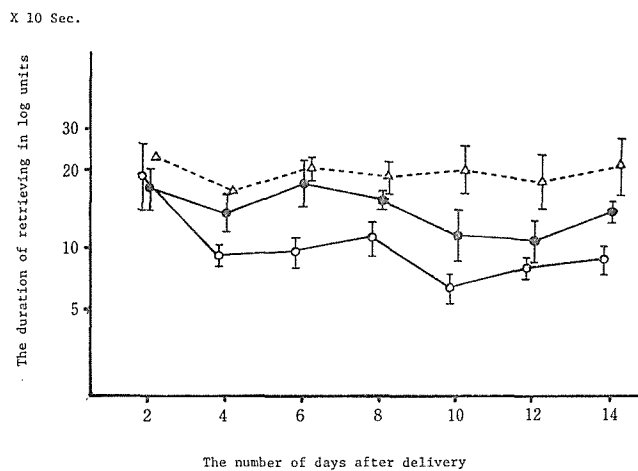


Fig. 3. The mean duration of retrieving in foster mothers reared 1–2 (○—○), 7–8 (●—●) and 13–14 (△...△) day old pups for 14 days after delivery. Vertical bars indicated S. E.

Smith and Berkson⁸⁾ reported that there was no difference in the latency of retrieving over the age of pups in rat. The discrepancy of the results between the report and the present study might be attributable to differences in the procedures of fostering or the animals used.

Fig. 3 shows the mean duration of retrieving in foster mothers reared 1-2, 7-8 and 13-14 day old pups for 14 days after delivery. In the foster mother which reared 1-2 day old pups, the mean duration of retrieving on 2 days after delivery was longer than that on another days ($P < 0.5$). This was also likely due to no experience of the retrieving in the mother as described above. Furthermore, as mentioned previously, any mother reared 13-14 day old pups did not begin to retrieve within 10 minutes on 2 days and 4 days after delivery.

Table 2. The analysis of variance showing effects of the age of pups and the number of days after delivery on the duration of retrieving.

Source	d. f.	Mean Squares
Age of pups	2	74.0**
No. of days after delivery	4	5.0
Interaction	8	1.9
Error	60	3.4

** $P < .01$

Therefore the analysis of variance, given in Table 2, was applied to the data of 6-14 days after delivery in all groups in order to examine the effects of the age of pups and the number of days after delivery on the duration of retrieving. The result indicated that the difference in the duration of retrieving among the age of pups was significant ($P < .01$) but that among the number of days after delivery was not significant. The test of the difference in the mean duration of retrieving among three groups showed that there were significant differences in the mean durations between each two groups ($P < .05$); the mother reared 13-14 day old pups spent significantly more time in retrieving all pups than did that reared 7-8 day old pups and the latter spent significantly more time in retrieving than did that reared 1-2 day old pups. The mean duration of retrieving of the mother reared 13-14 day old pups was about two times longer of 1-2 day old pups over the period.

These results in the latency and duration of retrieving confirmed that retrieving behaviour in mice was affected by the age of pups and was also stimulated more strongly by younger pups over the period of 14 days after delivery. These findings were similar to those of the previous reports essentially. However, the results showed that the number of days after delivery did little influence upon the retrieving behaviour in each group of

foster mother except 2 days after delivery.

The variance of the latency of retrieving was larger than that of the duration of retrieving, as shown in Fig. 2 and 3. It could be considered from the fact that the time to begin retrieving was influenced by many environmental conditions, such as sight, noise and odor¹⁴⁾ and that the time to retrieve all pups could be scarcely influenced by them.

The author observed in the same materials that there was no difference in lactation performance between the mothers which reared 1-2 day and 13-14 day old pups (unpublished). It was shown, however, in the present study that there was a significant difference in retrieving behaviour between the mothers which reared 1-2 day and 13-14 day old pups. Although there was still no direct evidence in regard to the relationship between the lactation performance and the retrieving behaviour, it was probable to say that the retrieving behaviour could not be related directly to the lactation performance in mice.

Summary

Effects of the age of pups and the number of days after delivery on retrieving behaviour were examined using the procedure of continuous cross-foster nursing. The procedure included the foster mother which reared either 1-2, 7-8, or 13-14 day old pups repeatedly from 1 to 14 days after delivery. Results obtained were as follows.

- 1) The difference in the latency of retrieving among the age of pups was significant but that among the number of days after delivery was not. The mean latency of retrieving of the mother reared 13-14 day old pups was longer than those of the mothers reared 1-2 and 7-8 day old pups.
- 2) The difference in the duration of retrieving among the age of pups was significant but that among the number of days after delivery was not. The mean duration of retrieving in the mother reared younger pups was shorter than that reared older pups.
- 3) Mothers of 2 days after delivery was slower to begin retrieving than those of above 4 days after delivery in all groups.
- 4) The variance of the latency was larger than that of the duration of retrieving. The fact demonstrated that the time to begin retrieving was more influenced by many environmental conditions.
- 5) It was concluded from these results that retrieving behaviour observed over the period of 14 days after delivery, was effected by the age of pups and was stimulated more strongly by younger pups, independently with the age of the foster mother.

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マウスの retrieving におよぼす子の日令 および分娩後の母の日令の効果

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マウスの retrieving (連れ戻し) 行動が子の日令により決定されるのかそれとも母の分娩後の日令によって決定されるのかを、1～2, 7～8, 13～14日令の子のいずれかを分娩後14日間常に哺育する母を作る連続的交換哺育法を用いて調べた。得られた結果は次の通りである。

1) retrieving 潜伏期においては母の分娩後の日令による差異はなく、子の日令において差異が認められた。13～14日令の子を育てた母は、1～2および7～8日令の子を育てた母よりも retrieving 開始迄の潜伏時間が長かった。

2) retrieving 所要時間においても、母の分娩後の日令による差異はなく、子の日令による差異が認められた。13～14日令の子を育てた母は7～8日令の子を育てた母より retrieving するのに長時間を要し、又7～8日令の子を育てた母は1～2日令の子を育てた母よりも長時間を要した。

3) 分娩後2日令では、どの日令の子を育てた母においてもそれ以降より、retrieving 潜伏期および所要時間ともに長時間を費やした。

4) retrieving の潜伏期および所要時間の分散を比較すると、潜伏期の方が常に大であり、retrieving の行動を開始する迄の時間にはより多くの環境条件が関与しているのではないかと考えられた。

5) 以上の結果から、分娩後14日迄の間ではマウスの retrieving 行動は、母の分娩後の日令とは関係なく、子の日令によって決定され、若い日令の子の方が retrieving 行動を母によくひきおこさせると結論した。