EXPERIMENTAL BIOLOGY

Maternal Deprivation in Early Ontogeny Impairs Olfactory Learning with Mother's Grooming Imitation in 129Sv Mice

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Early experience, mediated by mother's care, exerts long-term effects on the formation of behavioral phenotype. However, there are no published data on the effects of such experience on the results of early learning. We investigated the effects of maternal deprivation associated with handling in 129Sv mice during postnatal days 3-6 on the results of olfactory learning with mother's grooming imitation used as the reinforcement on postnatal day 8. Mother deprivation and handling procedure are shown to impair early olfactory learning.

Key Words: *early olfactory learning; handling; maternal deprivation*

Early neonatal experience has long-term effects on the formation of behavioral phenotype. The balance between maternal care and environment plays the most important role in the development of behavior in altricial animals. The mother-pup interactions determine normal growth and behavior development in rodents [1,5] and humans [6,7]. Handling is a widespread technique to investigate the interactions between the mouse (and rat) dams and pups. It includes pup deprivation of different duration from mother, nest, and siblings and tactile stimulation. Repeated exposure to handling modulates maternal behavior [2,10] and leads to long-term changes in behavior and neuroendocrine parameters of offspring and impairs their learning in adulthood [8,9,11].

However, there are no data on the effects of handling on the results of early learning, an important factor of offspring development. To elucidate this issue, we chose the model of olfactory discrimination learning, in which mother's grooming (licking) imitation (served as reinforcement) was associated with novel odor. The model was based on the pup endeavor to approach the mother [12].

129Sv mice are frequently used to obtain transgenic mice, including directed mutations of genes that work in the nervous system; therefore, their behavioral features are of particular interest. Adult 129Sv mice demonstrate different performance in cognitive tests [3,4]; however, their ontogenetic features and maternal behavior have not described yet.

The objective of the study was to elucidate the effects of handling on the results of early learning in 129Sv mouse offspring in the model with mother's grooming imitation.

MATERIALS AND METHODS

The experiments were conducted in accordance with the Order No. 267 Ministry of Health of the Russian Federation (19.06.2003) and "Rules of Studies on Experimental Animals" (approved by the Ethics Committee of the P. K. Anokhin Institute of Normal Physiology; protocol No. 1, 3.09.2005).

Inbred 129Sv mice (1 male and 1-2 females) were housed in standard plastic cage 18×24×14 cm

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(W×L×H) in size with sawdust bedding, with water and food *ad libitum*, with natural daylight cycle, at 20-25°C. Females with visible pregnancy signs were housed individually in similar cages. Starting from several days to anticipated labour, the presence of newborn pups was checked daily at the same time. The day of their detection was considered as postnatal day 1 (PD1).

Before the start of daily procedures with offspring, the dam was transferred to an individual plastic cage (10×24×14 cm) with sawdust bedding, with water and food. On PD2, the pups were weighed, their sex was determined, individual marks were made and maintained thereafter using color varnish markers Edding780. Mouse pups of both sexes were used in the experiments.

Handling. The pups were exposed to handling (n=41) from PD3 to PD6. They were weighed and were immediately placed into individual boxes with fresh sawdust (7×7×5 cm) for 30 min. Thereafter each pup was placed on smooth plastic surface at room temperature for 5 min, and then was kept in hands with latex gloves and stroked for 1-2 min. After these manipulations, the animals were placed back into the nest and the dam was returned. Mouse pups not exposed to handling (n=24) were taken in hands (for weighing) on PD8; no manipulations were performed before that.

Training. On PD8, the handled and non-handled pups were transferred to individual plastic boxes (7×7×5 cm) with fresh sawdust for 30 min. The animals belonged to one of three experimental groups: training group, mint control group, and intact group. The training procedure was carried out in double-bottom boxes (10×10 cm) with 6-cm walls. The source of novel odor, sawdust moistened with peppermint extract (MosPharma), was placed under the perforated floor (perforation diameter 4 mm). Each animal from the training group (handled, n=10; and not handled, n=9) were placed into the box and were stroked with a paintbrush 10 trials for 30 sec each and with 30 sec intertrial intervals, preferentially in anogenital area and in the back and head areas (which mimics tactile stimulation that pup receives during mother's grooming).

Each pup from the mint control group (handled, n=11; and not handled, n=6) was placed into a similar double-bottom box for 10 min without stroking.

Each pup from the intact group (handled, n=20; and not handled, n=9) was placed into the double-bottomed box with fresh sawdust under perforated floor for 10 min.

All pups were tested in odor discrimination test 24 h later. Before the test, each pup was placed into individual box for 30 min. The test procedure was performed in a double-bottomed plastic chamber (20×20 cm) with 6-cm walls. There were two equal compartments: one with fresh sawdust and the other with sawdust moistened with peppermint extract (test compartment) under the perforated floor (perforation diameter 4 mm) with "neutral" zone 10 mm wide in the center of the floor.

For odor preference testing, the pup was placed on the central "neutral" zone. In every new trial animal position was changed by 180°. The test consisted of five 60-sec trials with 60-sec intervals between them. The choice of compartment was assessed by crossing the "neutral" zone border with the head in the direction of certain compartment. The time over each compartment was registered in seconds.

For each animal, the mean percent of time over the test compartment was calculated from 5 trails.

The results were statistically processed using Statistica software, the data were assessed using two-way Student's t test; $M \pm SE$ were calculated.

RESULTS

Odor preference test showed that non-handled animals to spent more time over the test compartment following training with mother's grooming imitation than after exposure to novel odor: 62.6±16.8% in the training group and 40.6±18.2% in the mint control group (t=-2.4, p<0.05, Fig. 1). The time spent over the test compartment by non-handled animals in the training group (62.6±16.8%) insignificantly exceeded that in the intact group (48.4±17.4%; t=-1.8, p=0.097).

The time spent over the test compartment by handled animals in the training group (46.5±22.0%) did not differ from that in the mint control group (52.9±19.0%) and intact group (44.0±12.3%). There also was a trend towards higher percentage of time spent over the test compartment in the training group without handling in comparison with the training group with handling (t=1.78, p<0.093).

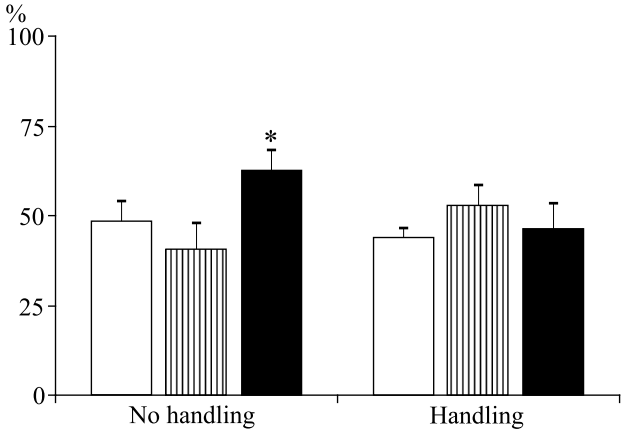

Fig. 1. Percent of time spent in the mint odor compartment (average over 5 trials; $M \pm SE$). Light bars: intact group, hatched bars: mint control, dark bars: training. *p<0.05 in comparison with mint control.

Thus, odor discrimination training was successful only in animals not exposed to handling.

The same duration of pup deprivation from mothers, siblings, and nest resulted in decreased ultrasound vocalization in 7-days old animals [13] and in altered maternal care [10], while the development of altricial animals in the early ontogeny is going under permanent maternal control [2]. In our study, pups exposed to handling developed no mother seeking associated with novel odor. These results indicated the need for further evaluation of the role of maternal behavior in the development of early behavior in the offspring exposed to handling.

REFERENCES

1. N. M. Cameron, F. A. Champagne, C. Parent, *et al.*, *Neurosci. Biobehav. Rev.*, **29**, Nos. 4-5, 843-865 (2005).
2. S. E. Claessens, N. P. Daskalakis, R. van der Veen, *et al.*, *Psychopharmacology* (Berl.), **214**, No. 1, 141-154 (2011).
3. C. Contet, J. N. Rawlins, and R. M. Deacon, *Behav. Brain Res.*, **124**, No. 1, 33-46 (2001).
4. J. N. Crawley, J. K. Belknap, A. Collins, *et al.*, *Psychopharmacology* (Berl.), **132**, No. 2, 107-124 (1997).
5. A. S. Fleming, D. H. O'Day, and G. W. Kraemer, *Neurosci. Biobehav. Rev.*, **23**, No. 5, 673-685 (1999).
6. D. W. Hedges and F. L. Woon, *Psychopharmacology* (Berl.), **214**, No. 1, 121-130 (2011).
7. C. Heim, C. B. Nemeroff, *Biol. Psychiatry*, **49**, No. 12, 1023-1039 (2001).
8. T. A. Kosten, H. J. Lee, and J. J. Kim, *Brain Res.*, **1154**, 144-153 (2007).
9. S. Levine, *Psychoneuroendocrinology*, **30**, No. 10, 939-946 (2005).
10. D. Liu, J. Diorio, B. Tannenbaum, *et al.*, *Science*, **277**, 1659-1662 (1997).
11. P. Meerlo, K. M. Horvath, G. M. Nagy, *et al.*, *J. Neuroendocrinol.*, **11**, No. 12, 925-933 (1999).
12. R. M. Sullivan, J. L. McGaugh, and M. Leon, *Brain Res. Dev. Brain Res.*, **60**, No. 2, 219-228 (1991).
13. B. Zimmerberg, A. J. Rosenthal, and A. C. Stark, *Dev. Psychobiol.*, **42**, No. 1, 52-63 (2003).