Yonsei Medical Journal
Vol. 40, No. 2, pp. 124－130, 1999

Effects of Exposure Conditions to Footshocks Early in Life on Spontaneous Locomotor Activity at Maturity in Rats

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Abstract

Both genetic and environmental factors are involved in establishing a behavior. An animal study was done to determine the characteristics of interaction between genetic (nature) and environmental (nurture) factors. Delivery of footshocks (0.8 mA × 60 times, at random) early in life was used as the environmental stimulus. As the footshock was delivered repeatedly, a rat showed helplessness behavior and the number of shocks necessary to elicit helplessness was measured to quantify the trait of an animal in coping with the aversive environmental stimulus. The nocturnal ambulatory activity at adulthood was measured as a behavioral expression of the nature-nurture interaction. Although the experience of footshocks early in life did not significantly alter average activity levels at adulthood, the activity was positively correlated with the number of shocks necessary to elicit helplessness (nature) while receiving footshocks (nurture) on postnatal day 14. Additionally, a second exposure to identical shock parameters on postnatal day 21 reversed the relationship. These results clearly showed that an interaction between nature and nurture during infancy leads to substantial behavioral alterations later in life, and suggest that the nature-dependent determination of an adult behavior can be modified in different directions by the conditions of an environmental experience early in life.

Key Words: Footshock, helplessness, locomotor activity, nature, nurture, behavior

INTRODUCTION

It is generally believed that an organism's behavior is determined by both genetic and environmental factors (i.e. nature and nurture). However, the relative contributions of each of these variables upon behavior is still under debate[1-4] because dissecting a behavior down to the genetic level or integrating myriad genetic information at the behavioral level is virtually impossible. Most reports on the nature-nurture issue are retrospective human studies for abnormal behaviors or diseases.[5-8] These studies almost invariably came to the conclusion that both genetics and the environment were responsible for the magnitude of the dependent variables of the studies, and that the relative contributions of nature and nurture needed to be clarified. It is very difficult to study the relative contributions of nature and nurture in human studies because many confounding factors cannot be controlled for.

Judicious experimental design of animal studies can at least address and possibly eliminate many of the factors which tend to confound human studies.[9,10] Early manipulation may be a good way of studying the nature-nurture issue since altered behavior can be measured long after the environmental manipulation.[11] By analyzing animal behaviors, we report the example in which an interaction between nature and nurture during infancy leads to substantial alterations later in life, when spontaneous nocturnal ambulatory activity is used as a behavioral expression of the nature-nurture interaction and an electric footshock as the environmental stimulus early in life. Since the spontaneous ambulatory activity represents a fixed action pattern of an animal, we chose the activity as the target behavior. The learned behavior was avoided because we did not want to confound the target behavior by factors involved in learning and memory. Moreover, we were interested in nocturnal activity because natural living behavior occurred during this period in rats.

Received December 10, 1998
Accepted February 3, 1999

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This work was supported in part by Korean Ministry of Science and Technology under the Brain Science Research Program.

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MATERIALS AND METHODS

Animal breeding and grouping

To minimize and to standardize unwanted environmental stimulation from *in utero* life,[9,10] Sprague-Dawley rats were bred and offspring were reared in a controlled manner. The animals were supplied from the Division of Laboratory Animal Medicine, College of Medicine, Yonsei University. Animals were cared for in the SPF barrier area and the temperature (22 ± 1°C) and humidity (55%) were controlled constantly with a 12-hr light-dark cycle, lights on at 0700. Food (PMI) and tap water (RO water) were available *ad libitum*. Nulliparous female and proven breeder male Sprague-Dawley rats were used for breeding. Genetic heterogeneity was ensured by using rats from different litters. Twelve hours after confirming delivery, pups were divided by sex, weighed and culled. We used 9 litters in which there were 5 males and 5 females or more, and the pups were culled to 5 males and 5 females in each litter. Four pups of each sex in a litter were assigned to 4 different groups; the non-handled control, the non-shocked control, exposure to footshocks only on postnatal day (PND) 14 and exposure to footshocks on both PNDs 14 and 21. When offspring reached 14 days of age, two males and two females from each of 9 litters were subjected to 60 uncontrollable and unpredictable footshocks. One male and one female littermate of these pups were subjected to the same procedure but they did not receive footshocks (the non-shocked control group). Another male and female littermate were not handled, except for occasional weighing (the non-handled control group). On PND 21, the same footshock procedure was repeated in rats except those assigned for the exposure to the footshocks only on PND 14.

Delivery of footshocks

A rat was placed into the non-escapable chamber (20.5 × 17 × 20 cm) equipped with a grid floor of stainless steel bars (2.0 mm diameter, spaced 8 mm apart) within an opaque sound-attenuating cubicle. A constant current (0.8 mA) shock was delivered to the floor for a 5-sec period through the shock generator via a grid scrambler (ENV-410, ENV-412, Med Associates, Georgia, Vermont, USA). When the shocked group was exposed to footshocks, the non-shocked control group was also exposed to another chamber within an opaque sound-attenuating cubicle for the same period of the experiment. The opaque sound-attenuating cubicle enabled the non-shocked group not to be stressed by the simultaneously-occurring event of the footshock delivery to the shocked group. Daily sessions consisted of 60 trials on a random time schedule, ranging from 10 to 100 sec, with an average of 55 sec.

The number of shocks necessary to elicit the first behavioral expression of helplessness was defined as the number of the first of three consecutive shocks, all of which induced helplessness. Most rats continuously showed helplessness after this episode until the end of the shock session. If a rat did not show helplessness by the termination of the prescribed 60 footshocks, we regarded the rat's number of shocks necessary to elicit helplessness as 60.

Measurements of activity

After the experience of footshocks, rats were raised normally and their spontaneous ambulatory activity for 23 hours (13 : 30 – 12 : 30 on the next day) was measured at 112 ± 2 days of age by using activity meters (Opto-Verimex Mini, Columbus Instruments, Columbus, Ohio, USA). A rat was placed into the activity chamber (45 × 37 × 25 cm) at 1300 hr. After a 30 min acclimation period, activity of the rat was measured for 23 hours (1330 hr – 1230 hr on the next day) at 15-min intervals. Food and water were available *ad libitum* during the experiment and chambers were cleaned with alcohol (70%) after each use. The number of consecutive beam interruptions was counted as the ambulatory activity.

Statistical analysis

Data are presented as mean ± S.E. For the analysis of the data of weight gain during PND 14 – 15 (Fig. 1, a and b), the mean values of 2 males or 2 females in each of 9 litters were used for the shocks on the PND 14 group (n=9), instead of using data of individual rats (n=18). This procedure enabled the litter to be the unit of statistical analysis. The weight gain was analyzed by ANOVA and preplanned multiple comparisons were done by Fisher's protected LSD test.

RESULTS

Weight gain after experience of footshocks

The footshock did not cause immediate evidence of morbidity or mortality. However, a decreased weight gain was noted 24 hours after exposure to the footshocks (Fig. 1, a and b) demonstrating that such experience of footshocks caused biological changes shortly afterward. When the pups reached 21 days of age, one male and one female which had been exposed to footshocks on PND 14 were again exposed to the same footshocks. The footshock at this age likewise did not cause immediate noteworthy effects. Decreased weight gain was again noted 24 hours later, but the decrease was attenuated from 84.4±19.0%, 88.5±13.8% after the first exposure to 33.0±10.3%, 27.2±13.2% after the second exposure, compared to the non-shocked control group in males and females respectively (Fig. 1, c and d).

Footshock-induced helplessness behavior

Pups initially showed escape-like behavior when they received a footshock. As the footshocks were repeated, the pups froze regardless of the delivery of the shock. We determined the number of shocks presented before they displayed the freezing behavior (number of shocks necessary to elicit helplessness behavior) as an index of each animal's trait (i.e. genetic capacity) to cope with aversive stimuli because the pups were exposed to this environmental manipulation for the first time in their lives on PND 14. The number of shocks necessary to elicit helplessness behavior was 36.9±5.9 (males), 46.7±3.6 (females) during their first exposure. However, when they were exposed to the same environmental manipulation again, the number of shocks necessary to elicit help-

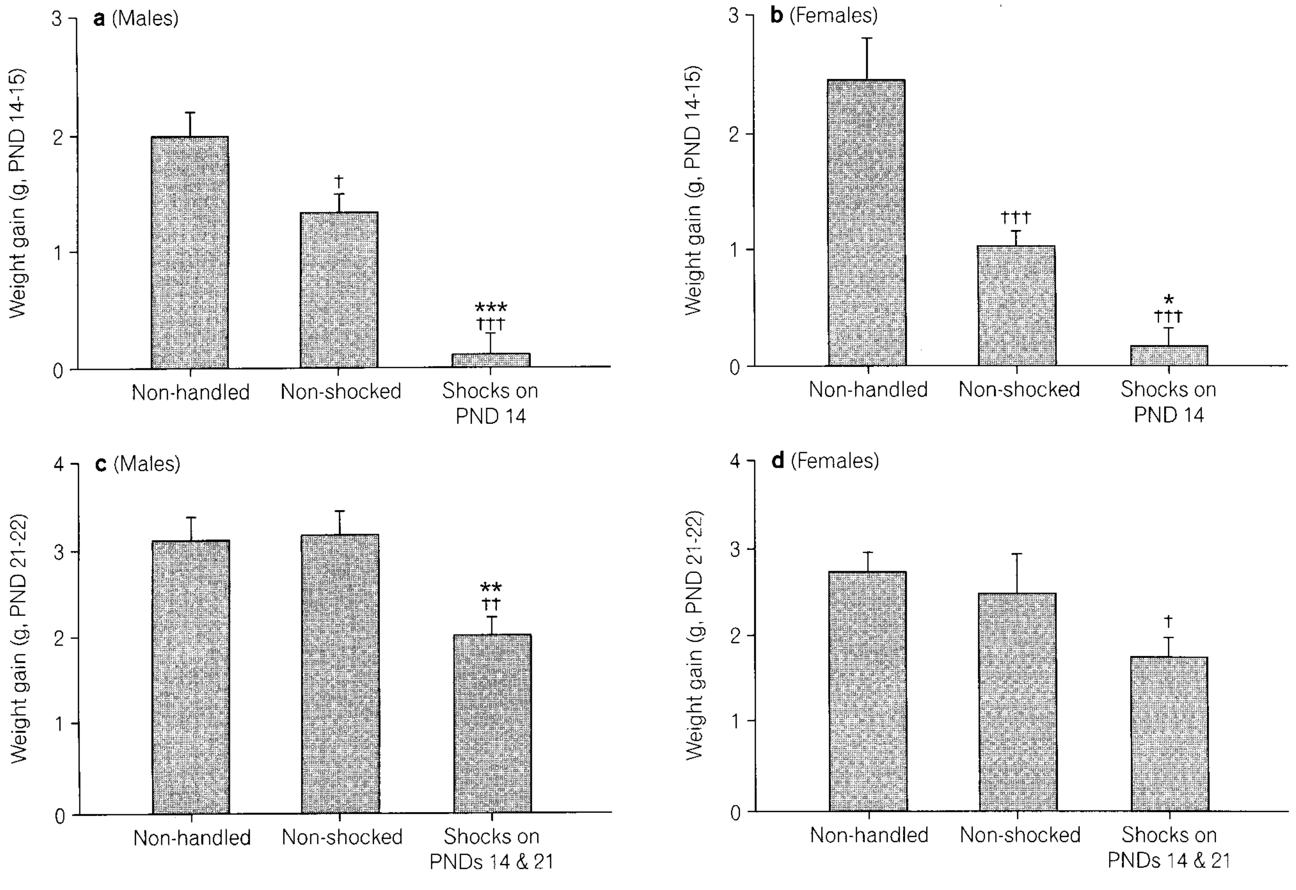

*Fig. 1. Weight gain after exposure to footshocks on PND 14 (a, b) or on PNDs 14 and 21 (c, d). Weight gain was measured 24 hours after exposure to 60 uncontrollable and unpredictable footshocks in a session. Animals in 3 groups were balanced by littermates from 9 litters ($n=9$). *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs. the non- shocked control group (Fisher's protected LSD test after ANOVA). †$p<0.05$, ††$p<0.01$, †††$p<0.001$ vs. the non-handled control group (Fisher's protected LSD test after ANOVA).*

lessness was 33.2 ± 5.9 (males) and 29.9 ± 3.1 (females). Thus, in females, the number of shocks necessary to elicit helplessness decreased significantly (Fig. 3d).

Relationship between nocturnal ambulatory activity and the number of shocks necessary to elicit helplessness

Ambulatory activities of adult rats showed the typical pattern for nocturnal animals (Fig. 2). Nocturnal ambulatory activity was far higher than during the daytime. The mean nocturnal ambulatory activity of females was greater than that of males (Fig. 2) and the experience of footshocks early in life did not change their average activity scores (data not shown).

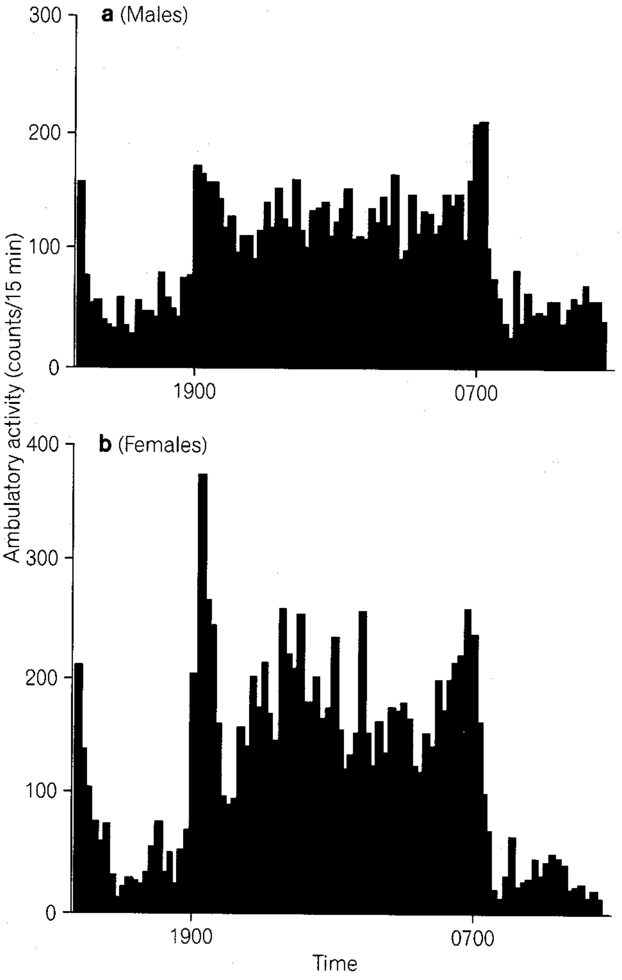

Fig. 2. Mean ambulatory activity of adult male (a) and female (b) rats for 23 hours. Data were collapsed across the groups in each sex since no group differences were noted. Activity was measured from 1330 hr to 1230 hr on the next day, with a 12 hr light-dark cycle, lights on at 0700 hr when rats were 112 ±2 days old.

To investigate the nature-nurture interaction, we determined the relationship between the nocturnal ambulatory activity after the experience of footshocks (nurture) and the number of shocks necessary to elicit helplessness (nature) when they received footshocks by using the data of individual rats. The nocturnal ambulatory activity was positively correlated with the number of shocks necessary to elicit helplessness for females (Fig. 3b) ($r=0.755$, $p<0.01$) in rats that received footshocks once, on PND 14. Males did not show a significant correlation (Fig. 3a) ($r=0.248$, $p>0.05$). We also determined the relationship between the number of shocks necessary to elicit helplessness and the nocturnal activity of rats that received footshocks twice, on PNDs 14 and 21. Unlike the rats that received footshocks once, these rats showed a negative correlation between their nocturnal ambulatory activity and the number of shocks necessary to elicit helplessness on PND 14 or 21. This negative correlation was evident in females (Fig. 3d) (PND 14: $r=0.816$, $p<0.01$, PND 21: $r=0.733$, $p<0.05$) but not in males (Fig. 3c) (PND 14: $r=0.231$. $p>0.05$, PND 21: $r=0.057$, $p>0.05$), similar to the lack of effect in males exposed to footshocks only on PND 14.

DISCUSSION

The results of this study clearly showed an interaction between nature and nurture to establish a behavior to a certain level. In determining nature (innate characteristics), nurture (environmental stimuli) should be involved because the innate characteristic of an organism can be identified only by the exposure to environmental stimuli. In the present study, nurture was the exposure to footshocks early in life and the quantification of nature was done by measuring the number of shocks necessary to elicit helplessness while receiving the footshocks.

We delivered footshocks according to the learned helplessness paradigm. In the classical experiment for the learned helplessness effect, animals which experience uncontrollable shocks in one situation later fail to learn to escape in a different situation, where escape is possible. In this case, the occurrence of helplessness behavior has been explained by two mechanisms, one motivational, the other associative-perceptual.[12] The motivational hypothesis argued that the cognition of no control reduces the animal's incentive to attempt escape. The associative-perceptual hypothesis argued that the previous learning

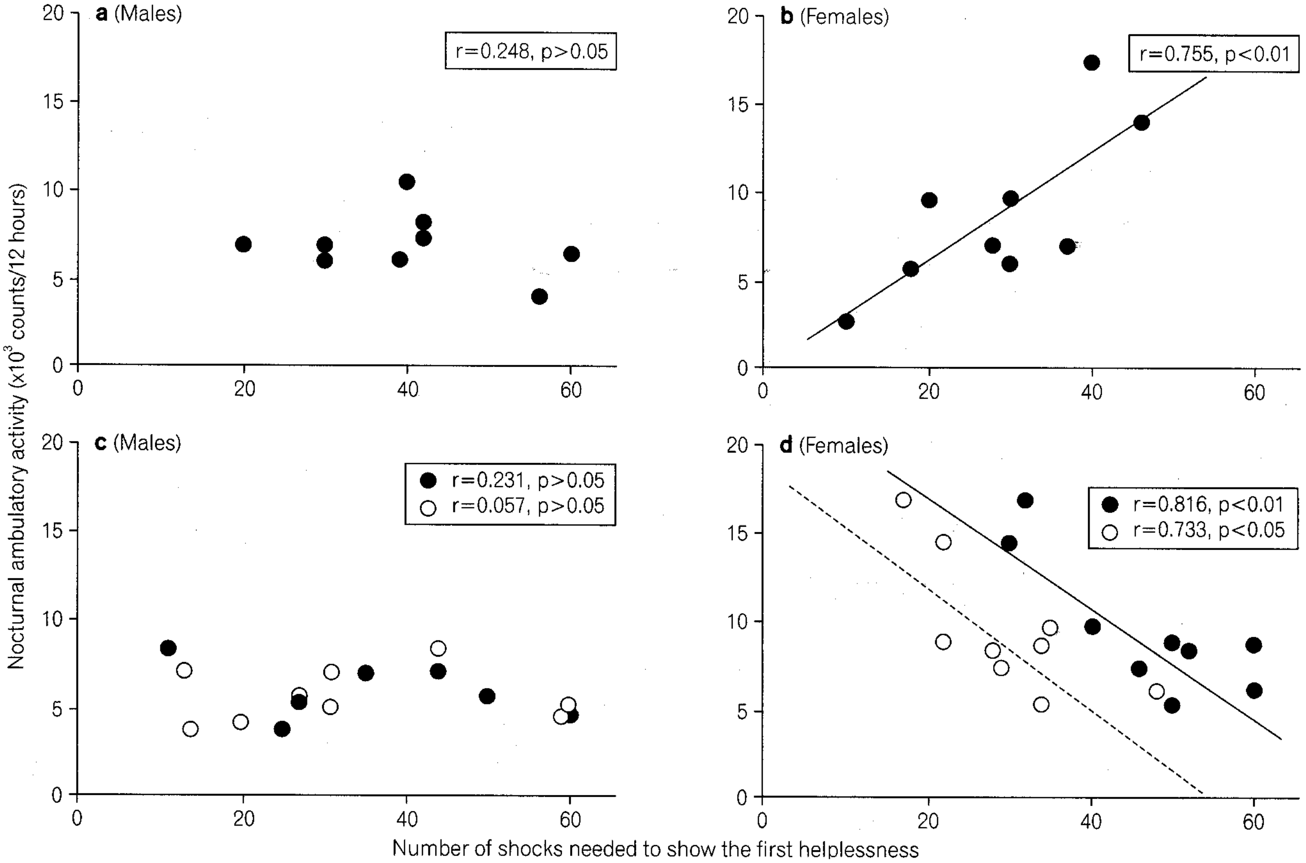

Fig. 3. Correlation between the nocturnal ambulatory activity at adulthood and the number of shocks necessary to elicit the first behavioral expression of helplessness during delivery of footshocks early in life. The nocturnal activity was measured for 12 hours during the dark period (1900 hr – 0700 hr on the next day). a,b, Rats were exposed to 60 footshocks on PND 14. c, d, Rats were exposed to 60 footshocks on PNDs 14 and 21. Thus, in c,d, the correlation was determined for the number of shocks necessary to elicit helplessness during the session on PND 14 (●, solid line) or 21 (○, dotted line).

about response-outcome independence interferes with the new learning that the response-outcome is related. In the present study, animals were only exposed to uncontrollable and unpredictable footshocks and they were not exposed to a situation where shock termination is possible. Therefore, the motivational hypothesis is more suitable for the mechanism of occurrence of helplessness behavior in the present study, and the number of shocks necessary to elicit helplessness behavior can be used as an index of the level of motivation to escape in each animal.

When we measured nocturnal ambulatory activity as the target behavior at adulthood, the average activity score was not changed by the exposure to footshocks, suggesting that the parameters of footshocks used in this study were not sufficient to change the average level of a behavior. However, the activity count of an individual was closely related to the number of shocks necessary to elicit helplessness while receiving the footshocks early in life. This result suggests that the exposure to an environmental stimulus elicits a trait-dependent response, and experience of this episode early in life can fix an adult behavior to a certain level even within the normal range. Subsequent analyses revealed that the relationship between two parameters was positively or negatively correlated in rats with exposure to footshocks once or twice, respectively. Regardless of the directions of the correlation, these data indicate, consistent with human studies,[6,8] that early experience of stress can establish an adult behavior to a certain level in cooperation with the trait to cope with the stress. Moreover, in the present study, the opposite relationship between two parameters according to the frequency of exposure to the same environmental manipulation was found, and this result indicates that the trait-dependent behavioral expression can be modified by the conditions of experiencing stress early in life. Since rats that received footshocks twice were littermates of rats which showed a positive correlation after a one-time exposure to footshocks, we expected an even stronger positive correlation between the two parameters in this group. However, to our surprise, there was a negative correlation. At this point, we can only speculate as to the mechanism of this totally different behavioral manifestation, according to the frequency of exposure to the same environmental manipulation. Two factors seem to be involved in the effects of frequency of exposure. One factor is the adaptation (learning) from the previous exposure, and another is the different age when the rat was exposed to footshocks. Although we can't speculate about the relative contribution of these two factors from the results of the present study, it is certain that the exposure conditions to footshocks early in life exert a critical role on the trait-dependent expression of behavior. Involvement of these two factors, adaptation and/or age, were also applicable to interpret observations that the number of shocks necessary to elicit helplessness and the attenuation of weight increase were less on the second exposure than on the first exposure. It is quite possible that steroid hormones are involved in the effect of conditions of experiencing stress early in life, as is the case of the neonatal handling studies in which the neonatal handling resulted in altered adult behavior via reduced hypothalamic-pituitary-adrenal (HPA) responses to stress.[13-16] However, more studies are needed to clarify genetic determinants of reactivity to and steps involved in determining the expression of behavior.

The mean nocturnal ambulatory activity of females was greater than that of males. One factor which might contribute to the sex difference in activity was the weight of rats, since males (343.9±9.8 g) were heavier than females (224.7±3.9 g) at this age. The sex difference was again observed in the effect of conditions of experiencing footshocks early in life on locomotor activity at adulthood in which females showed a clear relationship between the activity and the number of shocks necessary to elicit helplessness, but not in males. One clue for this sex difference may be related to the method of applying footshocks. We used a procedure of the learned helplessness paradigm, an animal model of major depression for humans.[12,17] Because the incidence of depression is greater in women than men,[18,19] it is quite possible that the sensitivity to footshocks was higher in female rats than males in the present study. Animal studies also indicate that exposure to stress resulted in sex-dependent effects although the more-sensitive gender was reported controversially. Exposure to inescapable footshock disrupted the shuttlebox-escape performance of males, whereas that of females was unaffected.[20-23] Unlike the above studies which reported higher sensitivity in males than in females, most studies for the response of the HPA axis to stress have reported a sex-difference with females reacting more robustly than males.[24] Moreover, it was suggested that the circulating gonadal steroid hormone milieu played an important role for the sex differences in HPA function because testosterone could inhibit HPA function, whereas estrogen could enhance HPA function. The above studies showed that males and females were differentially sensitive to stress in HPA responding and subsequent behavioral performance. If we consider HPA function as an adapting response of the body to stress, this controversy can be solved. It can be interpreted that an altered behavior was shown only in males because an aversive stimulus (footshock) elicited significant neuronal damage to change behaviors only to males, but not to females which had a capacity to cope with the aversive stimulus effectively through a robust HPA responding. The age of rats also seems to exert a variability factor in sex-dependent effects. In aged rats, unlike adult rats, no sex-difference was noted in the body temperature rise after psychological stress induced by exposure to a novel environment.[25] In young rats, sex-dependent effects of stress were obvious. When the maternal adrenal gland was excised on gestation day 8, female offspring had significantly higher levels of corticotropin-releasing factor mRNA during PND 1−21, a period they studied, whereas no significant effects were observed in males.[26] We gave an aversive stimulus early in life and found behavioral effects at adulthood in females, but not in males. It is possible that the parameters of footshocks we used was not enough to elicit significant neuronal damage to both sexes and that early life experience of the robust HPA responding in females resulted in permanent functional alterations without apparent neuronal damages. This notion is supported by the fact that the central stress system is most easily modified and most permanently shaped by the experience of stress in early life.[27]

In conclusion, the results of this study provide evidence that the trait is the basic determinant of an adult behavior and that the environment can modify the expression of the behavior in a totally different direction according to the conditions of exposure. Therefore, nurture seems to be a more decisive determinant of behavioral expression, at least for this particular behavioral variable.

ACKNOWLEDGEMENTS

We thank Dr. S. B. Sparber for a critical review of the manuscript.

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