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Changes of Exploratory Behaviour and Its Habituation in Rats Neonatally Treated with Monosodium Glutamate

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DUBOVICKY, M., D. TOKAREV, I. SKULTETYOVA AND D. JEZOVA. *Changes of exploratory behaviour and its habituation in rats neonatally treated with monosodium glutamate.* PHARMACOL BIOCHEM BEHAV **56**(4) 565–569, 1997.—In an attempt to elucidate mechanisms involved in adaptation to a novel environment, consequences of neurotoxic damage induced by administration of monosodium glutamate (MSG) to both male (n = 42) and female (n = 45) rats in the early postnatal period were studied. Rats treated with MSG and appropriate controls were tested on postnatal days 21 and 65 for alterations of exploration and the rapidity of habituation changes in an open field test. Compared with intact animals, a high dose of MSG (4 mg/g) increased exploratory behaviour, with a subsequent decrease in the rapidity of habituation of male rats. Neonatal stress represented by hypertonic saline injection in a vehicle-control group induced a slight increase of exploratory behaviour as compared with intact animals. Males proved to be more vulnerable to neonatal MSG treatment and handling than females. These results suggest a negative effect of neonatal stress and treatment with MSG on habituation to a new environment in male rats. © 1997 Elsevier Science Inc.

Rat Monosodium-L-glutamate Exploratory behaviour Habituation Open field Sex differences

HABITUATION as a simple cognitive process represents fundamental learning in which the organism learns not to respond to redundant nonsignificant stimuli (13). The neurochemical substrate of habituation is not fully understood, but the cholinergic system seems to be the most important component, as postulated several years ago (5). Recent studies have shown that acetylcholine release in the cortex and hippocampus is reliably activated by behaviourally relevant stimuli and that cholinergic neurons in the basal forebrain are involved in arousal and attentional processes (1). Moreover, glucose was found to modulate memory storage during habituation, and this effect was thought to be mediated via an enhancement of brain acetylcholine synthesis and its release (18). Next to the cholinergic system, other neurotransmitters such as histamine (2) or dopamine (19) modulate habituation and other cognitive and memory processes.

In an attempt to elucidate mechanisms involved in adaptation to a novel environment, we decided to study the influence of stressful insults on the course of habituation of exploratory behaviour (course of horizontal and vertical movements of the animal). One of the experimental models used was neurotoxic damage to the central nervous system (CNS) induced by administration of monosodium-L-glutamate (MSG) to rats in the early postnatal period. Neurotoxic lesions induced by glutamate are largely located in the arcuate nucleus of the hypothalamus, with main damage to the dopaminergic and cholinergic tuberoinfundibular neurons (23). Furthermore, some peptidergic systems, e.g., hypothalamic cells containing proopiomelanocortin-derived peptides (20), are also disturbed.

Our working hypothesis was that MSG-induced changes in neurotransmitter systems negatively modulate the course of habituation of exploratory behaviour. However, data on the intensity of exploratory behaviour in MSG-treated rats reported in the literature are very inconsistent. Therefore, in the present paper we describe the results of MSG model evaluation using a complex approach (age, gender, appropriate control groups, dose) involving assessment of the intensity of exploration together with a simple measurement of its habituation.

METHODS

Animals

Female SPF Sprague–Dawley rats (Charles River Laboratories, WIGA, Sulzfeld, Germany) were mated and housed

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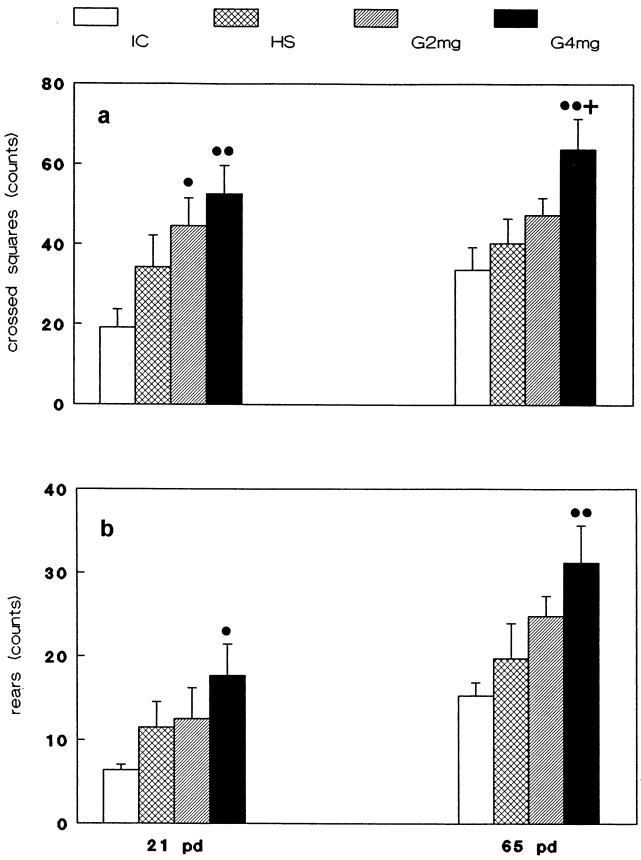

FIG. 1. Motor activity (a) and vertical exploratory activity (b) after neonatal exposure to MSG treatment and handling in male rats (mean ± SE, $n \geqq 10$). IC, intact controls; HS, hypertonic saline controls; G2mg, dose of 2 mg/g of MSG; G4mg, dose of 4 mg/g of MSG; pd, postnatal day. ●$p < 0.05$, ●●$p < 0.01$ compared with intact controls; +$p < 0.05$ compared with saline controls.

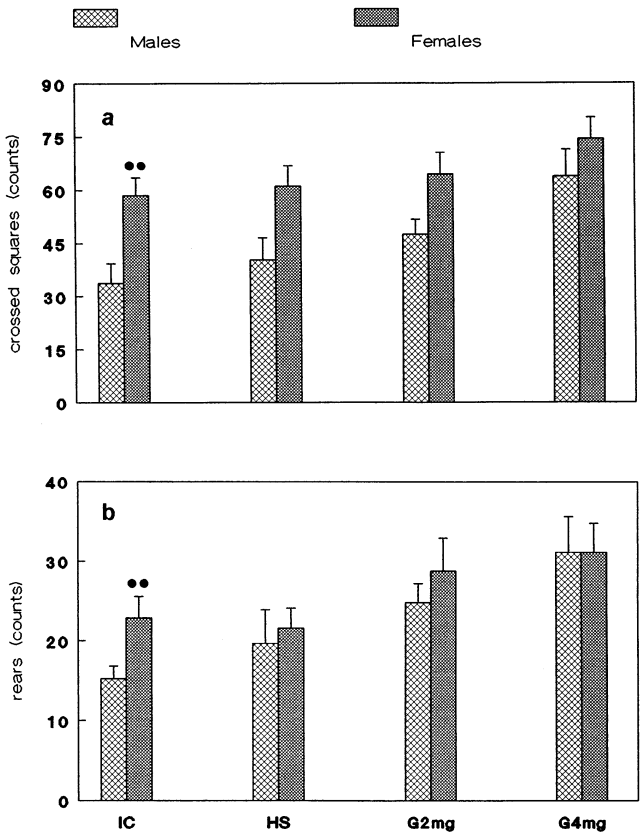

FIG. 2. Sex differences in motor activity (a) and vertical exploratory activity (b) in adult rats (65 pd) neonatally exposed to MSG treatment and handling (For further details, see Fig. 1.) ●●$p < 0.002$.

individually until delivery of pups. After weaning on postnatal day (pd) 21, the offspring were separated according to treatment and sex. Constant temperature (23–25°C) and a 12 L: 12 D cycle (lights on 0600–1800 h) were maintained in the animal room. Standard pelleted diet and tap water were provided ad lib.

MSG Treatment

The pups of both sexes (a total of 42 males and 45 females) were injected intraperitoneally with MSG (Merck KGaA, Darmstadt, Germany) dissolved in 0.9% NaCl in doses of 2 or 4 mg/g body weight on pd 2, 4, 6, 8, and 10. Littermate controls [hypertonic saline (HS) controls] received an equivalent volume of 10% NaCl (saline isoosmotic to MSG solution) on the same time schedule as MSG-treated rats. The other control group [intact controls (IC)] remained with their mothers without any handling.

Open Field Test

Spontaneous exploratory behaviour was evaluated in an open field test. All animals were exposed to the open field (36 × 48 cm, wall height 25 cm) for 6 min. Spontaneous motor activity (number of crossed squares) and vertical exploratory activity (number of rears: both forepaws lifted off the floor) were recorded. The first measurement was performed on pd 21 and the second on pd 65 in the same groups of rats.

The rapidity of habituation changes was evaluated in terms of a habituation index. It was expressed as a percentage of spontaneous motor activity within the first 90 s in relation to the total spontaneous motor activity [modified according to Klinberg et al. (16)].

Statistical Analysis

The data were analyzed statistically by one-way analysis of variance followed by post hoc tests of Dunn and Dunnett, as appropriate. Values for young versus adult and male versus female rats in intact controls were evaluated by Student's *t*-test. The results are presented as means ± SE.

RESULTS

Exploratory Behaviour

In the group of males (Fig. 1), an increase of exploratory behaviour related to treatment intensity was found on pd 21 and 65. In comparison with intact controls, a slight increase in both motor and vertical exploratory activity was observed in rats exposed to neonatal handling and injection of hypertonic saline. This increase was more pronounced in the group injected neonatally with the lower dose of MSG (2 mg/g) [significance in motor activity on pd 21: IC vs. MSG 2 mg, $F(3,$

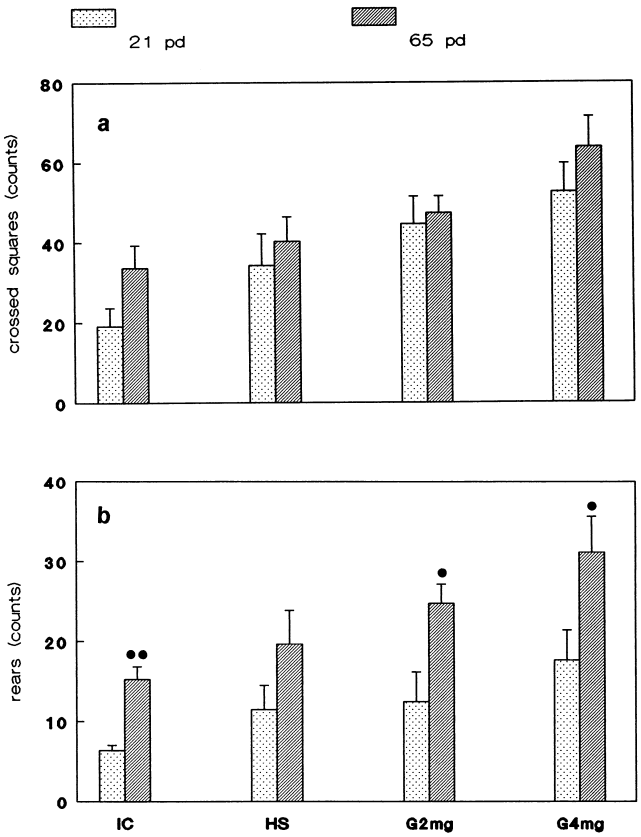

FIG. 3. Age differences in motor activity (a) and vertical exploratory activity (b) in male rats after neonatal exposure to MSG treatment and handling. (For further details, see Fig. 1.) ●$p < 0.05$, ●●$p < 0.01$.

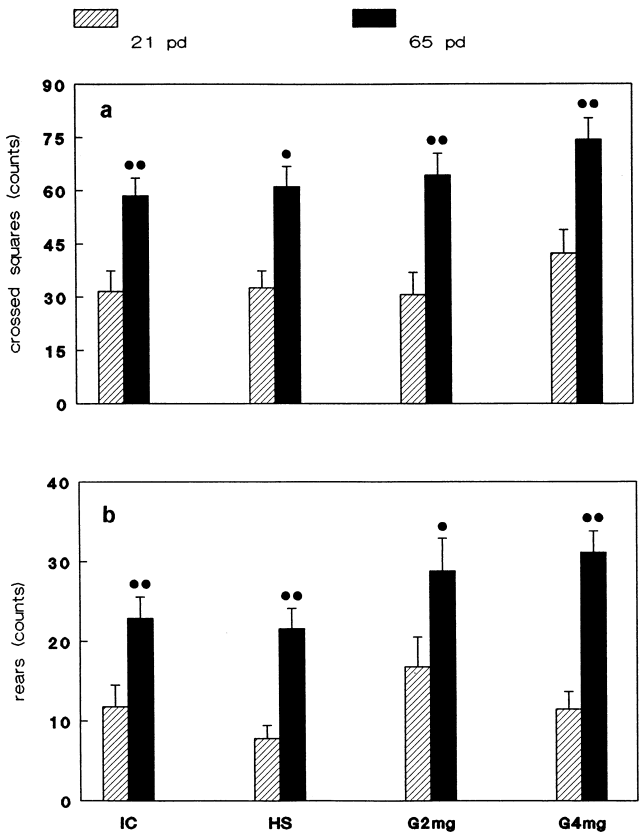

FIG. 4. Age differences in motor activity (a) and vertical exploratory activity (b) in female rats after neonatal exposure to MSG treatment and handling (For further details, see Fig. 1.) ●$p < 0.05$, ●●$p < 0.01$.

38) = 5.32, $p < 0.05$] and most pronounced in the group treated with the higher dose of MSG (4 mg/g) [significance in motor activity on pd 21: IC vs. MSG 4 mg, $F(3, 38) = 5.89$, $p < 0.01$; on pd 65: IC vs. MSG 4 mg, $F(3, 38) = 4.32$, $p < 0.01$; HS vs. MSG 4 mg, $F(3, 38) = 4.72$, $p < 0.05$; vertical exploratory activity on pd 21: IC vs. MSG 4 mg, $F(3, 38) = 2.62$, $p < 0.05$; on pd 65: IC vs. MSG 4 mg, $F(3, 38) = 4.53$, $p < 0.01$]. In the group of females, no significant changes in relation to postnatal treatment and handling were observed (data not shown). Figure 2 illustrates the results concerning sex differences. On pd 65, significantly higher values of both activities studied were observed in females in the group of intact controls ($p < 0.002$, Student's t-test). In female rats postnatally handled and treated with hypertonic saline or MSG, no statistically significant changes were recorded, though the values of motor activity exhibited an insignificant increase compared with males. On pd 21, no significant differences between males and females were found (data not shown). In both males and females, motor as well as vertical exploratory activity was higher on pd 65 than on pd 21. These differences were less pronounced in males compared with females. In males (Fig. 3), significantly higher values of vertical exploratory activity were found in adults compared with young animals in the group of intact controls ($p < 0.01$, Student's t-test) and in both groups of MSG-treated rats [MSG 2 mg, $F(7, 76) = 6.52$, $p < 0.05$; MSG 4 mg, $F(7, 76) = 6.49$, $p < 0.05$], whereas for motor activity these differences failed to be statistically significant. In females (Fig. 4), the age-related rise in both activities was statistically significant in all experimental and control groups [motor and vertical exploratory activities: IC, $p < 0.01$, Student's t-test; motor activity: HS, MSG 2 mg, and MSG 4 mg, $F(7, 82) = 7.93$, 8.19, and 8.32, $p < 0.05$–0.01; vertical exploratory activity: HS, MSG 2 mg, and MSG 4 mg, $F(7, 83) = 9.60$, 8.93, and 9.76, $p < 0.05$–0.01].

Habituation

On pd 21, the values of habituation index in males (Fig. 5a) were lower in all treatment groups in comparison with intact controls, although statistical significance was reached only after the dose of 2 mg/g of MSG [$F(3, 38) = 2.56$, $p < 0.05$]. On pd 65, no changes in habituation index compared with the values in intact controls were noticed in any of the experimental groups. In the group of females (Fig. 5b), no significant changes in habituation index were observed on both pd 21 and 65. No differences between males and females were observed in the rapidity of habituation, although the values of habituation index were somewhat lower in females in the group of intact controls. As for age differences, the values of habituation index in males (Fig. 5a) were significantly lower ($p < 0.01$, Student's t-test) in adult rats (32.70 ± 4.94) compared with young animals (67.81 ± 8.85) in the group of intact controls. No age-related differences were observed in rats treated neonatally with saline or MSG. In females (Fig. 5b), the values of habituation index tended to be lower in adulthood, but the changes failed to be statistically significant.

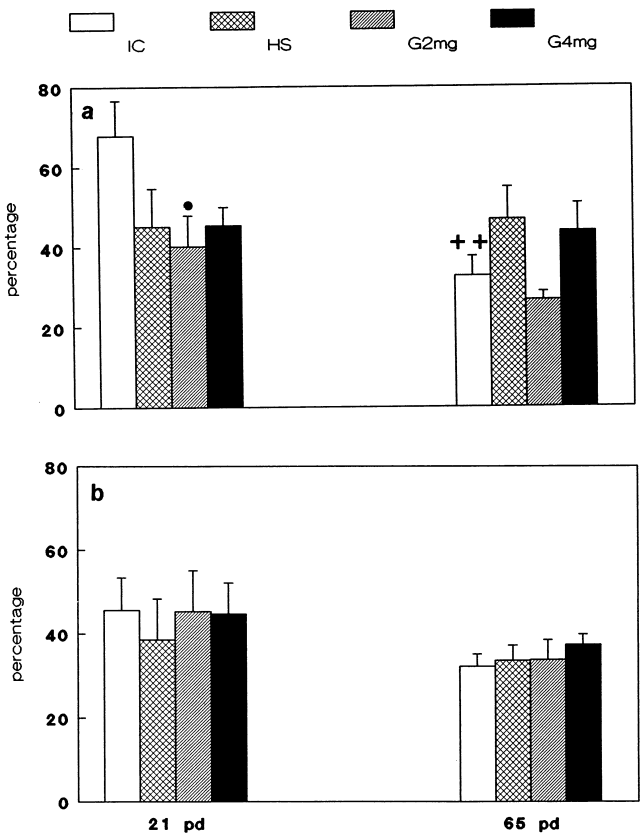

FIG. 5. Values of habituation index after neonatal exposure to MSG treatment and handling in male (a) and female (b) rats. (For further details, see Fig. 1.) ●$p < 0.05$ compared with intact controls; ++$p <$ 0.01 compared with intact controls on pd 21.

DISCUSSION

Results regarding changes of exploratory behaviour after neonatal MSG treatment are controversial. Some authors have described increased exploratory behaviour in rats (15,25) as well as in mice (6). However, Grimm and Frieder (12) reported reduced exploratory behaviour, with a more robust effect in males. Fisher et al. (10) found decreased activity in an open field test in rats, and Lorden and Caudle (21) observed hypoactivity in mice after neonatal treatment with MSG.

The results of our experiments confirmed that the changes in behaviour depended on methodological details. Neonatal treatment with MSG, but also with hypertonic saline, produced an increase in exploratory behaviour and a subsequent decrease in the rapidity of habituation of male rats. Males appeared to be more vulnerable to neonatal MSG treatment and handling than females.

Data obtained in our study underline the importance of using appropriate controls to avoid misinterpretation of the results obtained. Short handling of rats and administration of a hypertonic solution are known to be stressful (7,14). Although the postnatal period from days 2 to 10 of life is considered to be stress-hyporesponsive (26), neonatal handling as short as 15 min daily was found to result in altered behaviour in adulthood (24,28). The neonatal handling in this study, represented by intraperitoneal treatment with hypertonic saline, induced a slight increase of exploratory behaviour as compared with intact animals. Nevertheless, the most pronounced changes in behavioural parameters were observed in rats neonatally exposed to MSG, which might be due to changes in neurotransmitter systems.

Exploratory behaviour in rats was described to be sexually dimorphic, with females expected to show more ambulation and rearing in the open field than males (3,22). Exploratory behaviour of females in this study was not influenced by MSG treatment. However, in an other study we observed increased motor activity in females treated with MSG in a test of social memory (Glatz, Duncko and Jerová, unpubl. data). More marked changes of exploratory behaviour in males than in females were observed by Grimm and Frieder (12) but, in contrast to our study, the activities were reduced after MSG treatment. We noticed a different sensitivity to stressful situations in males and females after prenatal and early postnatal exposure to a neuroleptic derivative, stobadine (8). It is not known which factors are responsible for sex differences in behaviour-disrupting effects after neonatal MSG treatment. More severe and widespread neuron damage in the basomedial hypothalamus in MSG-treated male rats than in females (27) as well as the effect of sex hormones may contribute to these gender differences (9,11,17,22).

It is generally known that locomotor activity in adult rats is higher than in pups about the time of weaning (4). In females, we found marked differences between young and adult animals in all experimental and control groups in both activities. In males, motor activity of neonatally treated rats was increased at weaning without a further increase in adulthood. Consequently, no differences were found between young and adult males. In young animals, no marked gender differences in activities were reported (4), which is in accordance with our findings on day 21 of age. At the age of 65 days, gender differences were observed in intact controls but not in treated rats.

Changes in the rapidity of habituation of young males treated with MSG might have been related to their increased motor activity. In intact controls, habituation significantly decreased on pd 65. On the other hand, in animals handled and treated with hypertonic saline and those treated with MSG, approximately same values of the habituation index were observed during the second exposure. We cannot exclude the possibility that a different extent of remembering the new environment from the first open field exposure could have played a role in this phenomenon. These results suggest a negative effect of neonatal stress and treatment with MSG on the habituation to a new environment in male rats. However, measurements of both interrupted and uninterrupted courses of habituation are needed to confirm this suggestion. These studies are currently under evaluation.

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