Social stress impact on developmental stability of laboratory rat *Rattus norvegicus*

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Valetsky A. V., Dmitrieva I. L., Krushinskaya N. L. and Zakharov V. M. 1997. Social
stress impact on developmental stability of laboratory rat *Rattus norvegicus*. [In:
Developmental homeostasis in natural populations of mammals: phenetic approach.
V. M. Zakharov and A. V. Yablokov, eds]. Acta Theriologica, Suppl. 4: 27–32.

Developmental stability of the progeny of pregnant female rats *Rattus norvegicus*
Berkenhout, 1769 kept under different conditions was studied. In two experimental
groups, females were exposed to social stress by keeping them in crowded cages at
different stages of pregnancy, from the 1st to the 15th and from the 16th to the 21th
days, respectively. In the control group, pregnant females were kept under normal
conditions. Fluctuating asymmetry of 20 skull characters (number of foramina for
small blood vessels and nerves) was used as a measure of developmental stability.
Variance of the difference in foramen number between the left and right sides of the
skull (l–r) as well as mean number of asymmetrical traits per individual revealed an
increase of fluctuating asymmetry in both experimental groups compared with the
control group. These results imply the deterioration of developmental stability under
impact of social stress.

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Key words: *Rattus norvegicus*, non-metric traits, fluctuating asymmetry, developmental
stability, social stress

Introduction

Developmental homeostasis has been suggested as a sensitive estimate of an
organism's condition. From the morphogenetic view-point, homeostasis is charac-
terized by developmental stability measured by developmental noise or chance
variation that could not be interpreted as a result of genotype or environmental
dissimilarity. Intraindividual variation, as small non-directional differences be-
tween homologous structures that should be equal, provides us with an opportunity
to distinguish the developmental noise. As has been shown, fluctuating asymmetry
of various characters, even those uncorrelated with each other, could increase
under the impact of stress. This indicates an opportunity to reveal changes in
developmental stability through the study of the limited set of morphological
characters (Mather 1953, Zakharov 1987, 1989).

In this study, we tried to find out if social stress affects developmental stability. Social stress has already been suggested as a possible cause of disturbance of developmental stability revealed at the peak year in a cyclic population of small mammals (Zakharov *et al.* 1991). To get a more direct answer to the question we have modelled such stress in a laboratory strain of rats. To establish the deterioration of developmental stability under the impact of social stress seems to be important for two reasons. In a wide sense, it would be important evidence characterizing the disturbance of developmental stability as a non-specific reaction of an organism to any stress. In previous studies deterioration of developmental stability was registered under the stress impact of various chemical and physical factors that could be interpreted as a toxic effect (Siegel and Smookler 1973, Siegel and Doyle 1975, Doyle *et al.* 1977, Pankakoski *et al.* 1992). The social stress would be essential to establish the same reaction of an organism. In a narrow sense, it would be an experimental justification to interpret a decrease in developmental stability at the peak year in a cyclic population as a result of social stress. Negative correlation of developmental stability with density has already been established for a cyclic shrew population in central Siberia (Zakharov *et al.* 1991).

Material and methods

An experiment was conducted on laboratory rats *Rattus norvegicus* Berkenhout, 1769 of the strain "Tryon maze bright" (Wahlsten 1972). Social stress was modeled by keeping 2 pregnant females with 8 males in one cage ($32 \times 57 \times 20$ cm). In the first experiment (group 1) females were exposed to the stress during the first 15 days of pregnancy, and then each was kept in a separate cage. In the second experiment (group 2), each female was kept in a separate cage from the first day of pregnancy, but then they were exposed to the stress during the last 6 days of pregnancy (from the 16th to the 21st days of pregnancy). Each female in the control group was kept in a separate cage the entire time of the pregnancy. Offspring in all groups were kept with the mother in a separate cage for the first 14 days and then were kept separately, not more than 6 individuals of one sex in each cage, for 4 months.

Developmental stability was studied in three samples of the offspring. Experimental material consists of two samples: 14 individuals (progeny of 3 females) in the first experiment, and 17 individuals (progeny of 4 females) in the second experiment. A control sample was represented by 40 individuals (progeny of 8 females).

Twenty bilateral skull foramina (apertures through the bone for nerves and small blood vessels) were used for morphological analysis (Fig. 1). No directional asymmetry, antisymmetry, or sexual dimorphism was found for these characters. None of the correlation was revealed among the characters studied *per se*, (l+r) values, as well as between their asymmetries, (l−r) values. It has been established through the calculation of the coefficient of correlation (r) for all possible paired comparisons between the characters and their asymmetries.

Fluctuating asymmetry as difference in the number of foramina on the left and right sides of the skull (l−r) was used to assess developmental stability. Variance of (l−r) was calculated to estimate fluctuating asymmetry for each character. The mean number of asymmetrical characters per individual was used as an integrated measure of developmental stability.

The non-parametric sign-test was used for the intersample comparison of variances for the whole set of characters. An *F*-test was used for pairwise comparisons of the variances and *t*-test for the intergroup comparison of the mean number of asymmetrical characters per individual (Sokal and Rohlf 1981).
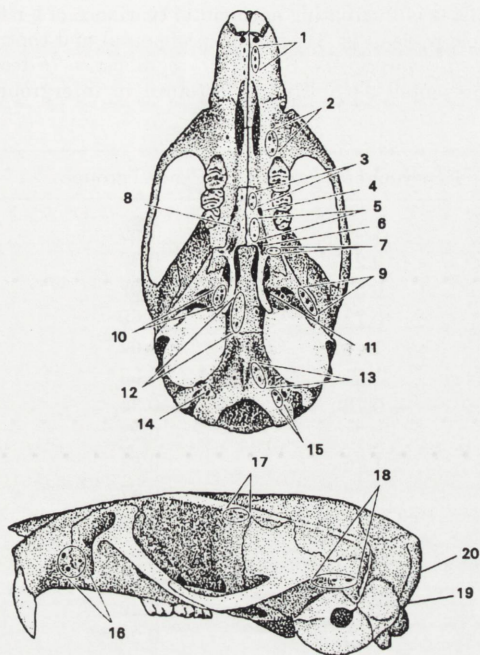
Fig. 1. Cranial characters (number of foramina) analyzed in the skull of the Norway rat *Rattus norvegicus*. 1 – *foramen praemaxillaris ventralis*, 2 – *foramen diastemae*, 3 – *foramen palatinae minor anterior*, 4 – *foramen palatinae*, 5 – *foramen palatinae posterior minor* I, 6 – *foramen palatinae posterior minor* II, 7 – *foramen palatinae posterior minor* III, 8 – *foramen palatinum major*, 9 – *foramen pterigoideum*, 10 – *canalis transversalis accessorius*, 11 – *canalis transversalis*, 12 – *foramina basisphenoidalis lateralis*, 13 – *foramen basioccipitalis*, 14 – *foramen hypoglossum accessorius externus*, 15 – *foramen hypoglossum accessorius internus*, 16 – *foramen praemaxillaris lateralis*, 17 – *foramen supraorbitalis*, 18 – *foramen postglenoidale*, 19 – *foramen occipitalis lateralis*, 20 – *foramen occipitalis medialis*.

Results

Because of the limited material available in this study, we used the general intersample comparison. We have revealed neither the significant proportion of interlitter differences (0.16 ± 0.21) nor the significant difference between the higher intralitter variance (2.60) and smaller interlitter variance (2.17) by ANOVA ($F = 1.20$, df = 25, 6, $p > 0.05$) for the total experimental material (31 individuals of 7 litters).

Comparison of two experimental groups exposed to stress at different stages of pregnancy did not reveal significant differences between them in fluctuating asymmetry. Mean number of asymmetrical characters per individual (NA ± SE) proved to be similar in both experimental groups: 14.28 ± 0.40 in the first experimental group stressed during the first 15 days of pregnancy and 13.41 ± 0.39

A. V. Valetsky *et al.*

Table 1. The values of fluctuating asymmetry (variance of l–r for 20 characters of skull morphology, see Fig. 1) in the experimental and control groups of the Norway rat *Rattus norvegicus*. Statistical significance (*F*-test) of intergroup difference is presented as *p*. Higher variance in intergroup comparison is underlined.

Character	Experimental group	Control group	*p*
1	1.00	1.60	ns
2	0.97	0.32	< 0.01
3	0.57	0.02	< 0.001
4	0.73	0.69	ns
5	1.18	0.02	< 0.001
6	1.51	0.53	< 0.01
7	0.70	0.36	< 0.05
8	0.71	0.22	< 0.001
9	1.76	0.81	< 0.05
10	3.06	0.87	< 0.001
11	1.46	1.52	ns
12	2.05	0.87	< 0.001
13	2.77	1.99	ns
14	1.23	0.40	< 0.01
15	1.09	0.26	< 0.001
16	1.25	1.34	ns
17	1.18	0.22	< 0.001
18	0.47	0.63	ns
19	2.53	2.19	ns
20	1.41	0.90	ns

in the second group stressed during the last 6 days of pregnancy ($p > 0.05$, t-test). Both groups had significantly higher mean number of asymmetric characters ($p < 0.001$) than in the control group (9.07 ± 0.34). This similarity allowed us to pool the two experimental groups into one sample to compare with the control group.

Variance of (l–r) was higher for the pooled experimental sample than for the control group in 16 of 20 characters under study ($p < 0.05$, sign-test). When treating the characters separately, significant differences ($p < 0.05$) were revealed for 12 characters (Table 1). Mean number of asymmetrical characters was significantly higher ($p < 0.001$) in the pooled experimental sample (13.80 ± 0.29) than in the control sample (9.07 ± 0.34).

Discussion

The general intersample comparison revealed neither the significant proportion of interlitter differences nor the significant difference between the higher intra-litter variance and smaller interlitter variance for all experimental rat pups. These

data corresponds to the conclusion established in many studies that heritability (h^2) of fluctuating asymmetry for various characters in different species, from insects to humans, does not differ from 0 (eg Reeve 1960, Bailit *et al.* 1970, Leary *et al.* 1985).

Stress impact of overpopulation was demonstrated in both natural populations and laboratory experiments (Christian and Davis 1964, Shilov 1977, Moshkin *et al.* 1990). Some biochemical, morphological and behavioral changes were observed for the progeny of females stressed during pregnancy (Thompson 1957, Naumenko *et al.* 1990, Pratt and Lisk 1990).

Our data on laboratory rats revealed an obvious developmental stability decrease in the progeny of females exposed to social stress at different stages of their pregnancy, comparing with a control group. The data indicate an adverse effect of stress on the pregnant female, and hence on the condition of her progeny. Previously, it was shown that prenatal and early postnatal stress from various physical factors could lead to decrease of developmental stability in mice and rats (Siegel and Smookler 1973, Siegel and Doyle 1975). In this study we demonstrate that social stress at different stages of prenatal development leads to the same adverse changes in an organism's condition that are revealed by a developmental stability decrease.

Thus, the study on the impact of social stress completes the previously obtained data on the impact of chemical and physical factors. Developmental stability disturbance seems to be a non-specific response of an organism to any stress impact. This study provides an experimental background to assess potential stress impact of overpopulation in population cycles of mammals. The results also support the assumption that developmental stability decrease during the peak year could be caused by the stressful impact of high density (Zakharov *et al.* 1991).

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