Neurosis-Associated Changes in the Granulocytic Hemopoietic Stem in Mice with Different Learning Capacity

E. G. Skurikhin, O. V. Pershina, L. A. Stavrova, N. I. Suslov, M. Yu. Minakova, A. M. Dygai, and E. D. Gol'dberg

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 8, pp. 136-141, August, 2005
Original article submitted November 9, 2004

We studied the local mechanisms of neurosis-associated changes in the granulocytic hemopoietic stem in CBA/CaLac mice with different learning capacity. It was found that in good learners hyperplasia of the bone marrow granulocytopoiesis during neurosis was related to enhanced proliferative activity and accelerated maturation of granulocyte-macrophage precursors resulting from increased formation of granulocytic and erythrogranulocytic hemopoietic islets. In poor learners primary suppression of the bone marrow granulocytopoiesis was associated with impaired formation of hemopoietic islets (conflict situation) and inhibition of differentiation of granulocyte-macrophage precursors (paradoxical sleep deprivation followed by T-maze learning). Then, recovered ability of bone marrow cells to form granulocyte complexes even against the background of inhibition of precursor differentiation (conflict situation) and accelerated maturation of granulocytic cells (paradoxical sleep deprivation and maze learning) lead to hyperplasia of the granulocytic hemopoietic stem.

Key Words: *granulocytopoiesis; individual reactivity; experimental neurosis; hemopoiesis-inducing microenvironment; regulation*

Much attention is now paid to individual resistance to psychoemotional stress. Evaluation of the orientation and exploratory activity in the open field help us to predict animal resistance or predisposition to emotional stress [8]. Specific features of cognitive function determine the type of stress-induced and neurotic changes. The division of animals by their open-field behavior is mainly based on nonassociative learning and, to a lesser extent, on individual characteristics of their higher nervous activity. Our previous studies revealed different changes in the erythroid hemopoietic stem during experimental neuroses in mice exhibiting good and poor learning in a 3-arm T-maze [4]. The observed intergroup differences were determined by functional activity of the local system of erythropoiesis regulation (hemopoiesis-inducing microenvironment). Published data show that different neurotic factors produce significant changes in the granulocytic hemopoietic stem in animals of the total population [1].

Here we studied changes in the granulocytic hemopoietic stem of mice with different learning capacity in a 3-arm T-maze under conditions of experimental neuroses.

MATERIALS AND METHODS

Experiments were performed on 120 CBA/CaLac mice (class I conventional mouse strain) aging 2.0-2.5 months and obtained from the collection of the Laboratory of Experimental Biological Modeling (Institute of Pharmacology, Tomsk Research Center).

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences

0007-4888/05/1402-0171

Conflict situation (CS, 10 min) and paradoxical sleep deprivation (PSD, 48 h) [9] followed by 2-day training in a 3-arm T-maze served as the models of experimental neuroses. Seven days before CS or PSD the animals were divided by their T-maze behavior into two groups: well and poor learners [7]; these animals are characterized by high and low locomotor activity in the open field, respectively.

The number of segmented neutrophils in the peripheral blood was estimated 24 h before and 1, 4, and 5 days after neurotic exposure. The mice were euthanized by craniocervical dislocation under ether anesthesia. The count of immature and mature neutrophilic granulocytes in the bone marrow was evaluated [2]. The content of granulocyte-macrophage colony-forming (CFU-GM) and cluster-forming units (ClFU-GM) in the bone marrow was determined by *in vitro* cloning of myelokaryocytes in methylcellulose culture [2]. Proliferative activity of granulocyte-macrophage precursors was evaluated by the method of cell suicide using hydroxyurea. The intensity of differentiation of granulocytic precursors was estimated by the index of maturation. It was calculated as the ratio between the numbers of clusters and colonies in a well [2]. Structural and functional organization of the bone marrow was determined by enzymatic isolation of hemopoietic islets and study of their quantitative and qualitative composition.

The results were analyzed by standard methods of variational statistics. The significance of differences was evaluated by parametric Student's *t* test and nonparametric Wilcoxon—Mann—Whitney *U* test [3].

RESULTS

The animals with different training capacity exhibited different reactions to CS. Well trained mice were characterized by increased number of mature neutrophilic granulocytes (day 5) and content of CFU-GM (days 1, 4, and 5) and ClFU-GM in the bone marrow (days 1 and 4). The development of neutrophilic leukocytosis in the peripheral blood (day 4) was preceded by neutrophilic leukopenia (day 1, Figs. 1 and 2). The number of bone marrow neutrophilic granulocytes in poor learners was below the basal level (days 1 and 4). By contrast, the count of mature neutrophilic granulocytes in these mice exceeded that in intact animals (day 5, Fig. 1). We revealed an increase in the content of CFU-GM in methylcellulose medium (days 1 and 4) and accumulation of neutrophilic granulocytes in the peripheral blood (day 4, Fig. 2).

PSD followed by T-maze learning stimulated bone marrow granulocytopoiesis in good learners. We observed an increase in the number of mature neutrophilic granulocytes (days 1, 4, and 5) and content of ClFU-GM in the bone marrow (days 4 and 5, Figs. 1 and 2). It should be emphasized that the count of segmented neutrophils in the peripheral blood decreased on day 1. In poor learners the increase in the content of CFU-GM and ClFU-GM (days 4 and 5), accumulation of immature neutrophilic granulocytes in the bone marrow (day 4), and development of peripheral blood neutrophilia were accompanied by a decrease in the number of immature neutrophilic granulocytes in hemopoietic tissue (days 1 and 4).

Evaluation of proliferative activity and differentiation of GM precursors showed that CS increased the number of GM precursors in S-phase of the mitotic cycle. These changes were revealed in good (days 1, 4, and 5) and poor learners (CFU-GM, days 1 and 4; ClFU, days 1, 4, and 5; Fig. 2). Proliferative activity differed in animals of these groups. On day 1 the number of precursor cells in poor learners was higher than in good learners. However, on days 4 and 5 the count of these cells in good learners surpassed that in poor learners. The intensity of cell differentiation increased on day 1, but sharply decreased in the follow-up period (good learners, day 5; poor learners, days 4 and 5).

PSD and maze learning significantly increased the number of GM precursors in S-phase of the mitotic cycle (CFU-GM, days 1, 4, and 5; ClFU-GM, days 1 and 5; Fig. 2). Proliferative activity of cells in poor learners was higher than in good learners. The intensity of granulocyte differentiation in good learners mice increased on days 1, 4, and 5. The index of maturation initially decreased (day 1), but then increased in poorly learning mice (day 5).

Study of structural and functional organization of the bone marrow showed that the number of macrophage-negative cell complexes increases in good learning mice on days 1, 4, and 5 after CS. Qualitative study showed that the count of granulocytic and erythrogranulocytic hemopoietic islets in these animals increases at various terms after treatment (Fig. 3). The content of cell complexes in poor learners initially decreased, but exceeded the basal level on days 5 (macrophage-negative and granulocytic hemopoietic islets) and 4-5 (erythrogranulocytic hemopoietic islets).

PSD followed by T-maze learning increased the number of various hemopoietic islets on days 1, 4, and 5 (Fig. 3). These changes were most pronounced in poor learners.

Our results show that hyperplasia of the bone marrow granulocytopoiesis in good learning animals with neurosis is related to the increase in proliferative activity and accelerated maturation of GM precursors. These changes result from increased formation of granulocytic and erythrogranulocytic hemopoietic islets. Initial suppression of the bone marrow granulocytopoiesis in poor learners under conditions of CS is as-

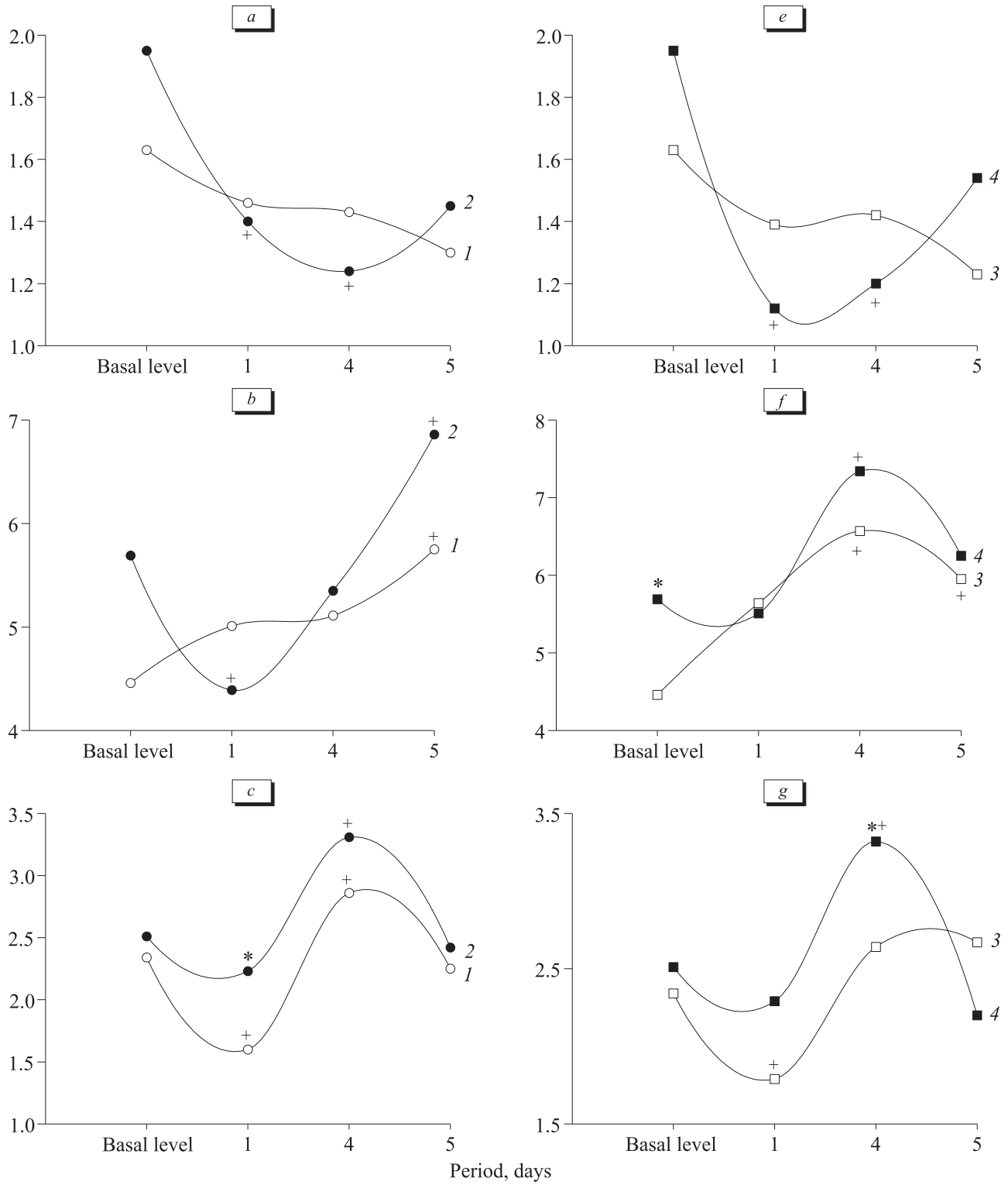

Fig. 1. Count of immature (*a*, *d*) and mature neutrophilic granulocytes in the bone marrow (*b*, *e*) and number of segmented neutrophils in the peripheral blood (*c*, *f*) of good (*1*, *3*) and poorly learning CBA/CaLac mice (*2*, *4*) subjected to conflict situation (*1*, *2*) or paradoxical sleep deprivation and training in a 3-arm T-maze (*3*, *4*). Ordinate: number of neutrophilic granulocytes in the bone marrow ($\times 10^6$ cells per femur, *a*, *b*, *e*, *f*). Here and in Figs. 2 and 3: $p<0.05$: *compared to good learners; +compared to the basal level.

sociated with impaired formation of hemopoietic islets and during PSD and T-maze training with inhibition of differentiation of GM precursors. Then recovery of the ability of bone marrow cells to form granulocyte complexes under conditions of CS (even upon inhibition of precursor differentiation) and accelerated matu-

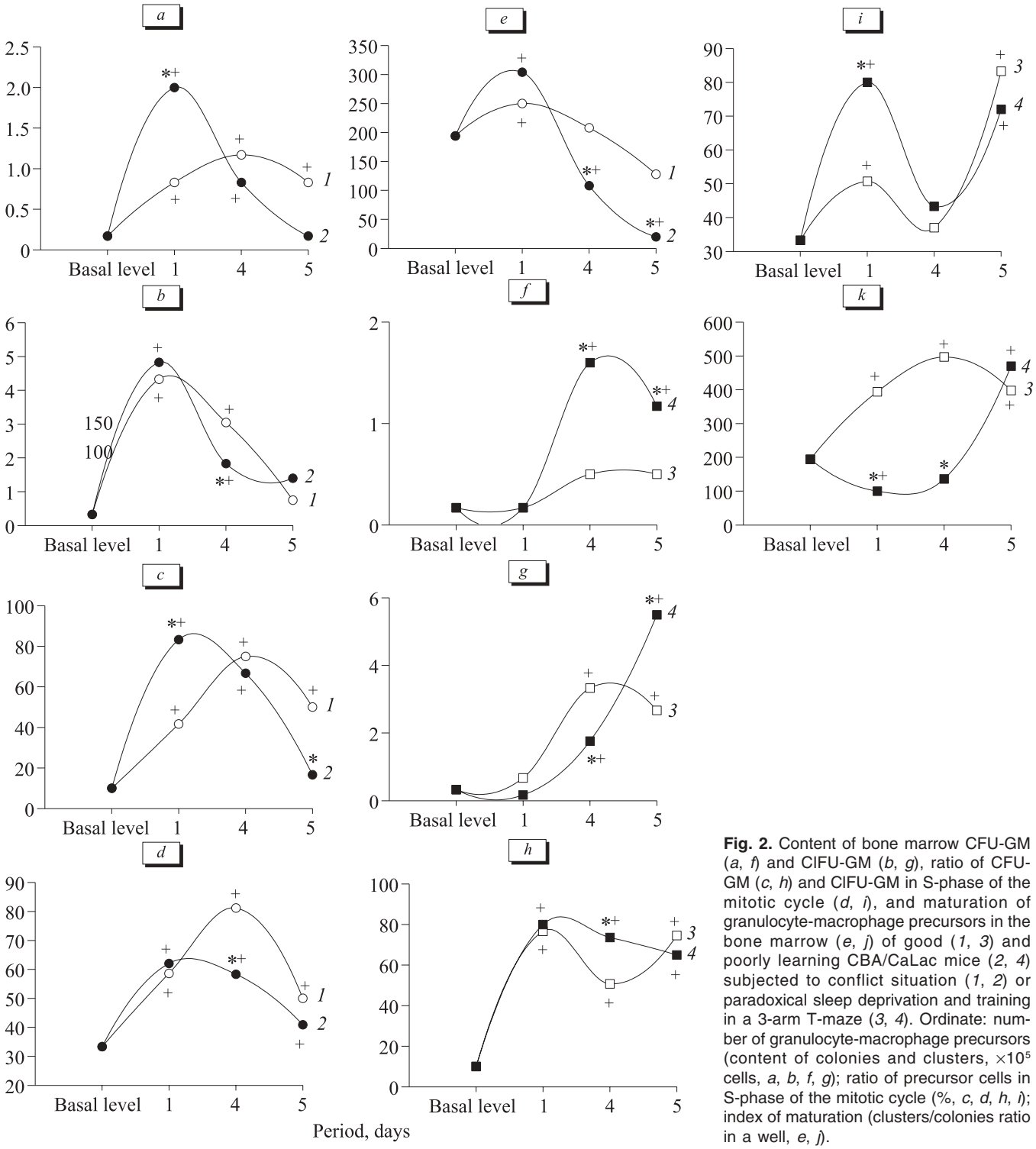

Fig. 2. Content of bone marrow CFU-GM (*a*, *f*) and ClFU-GM (*b*, *g*), ratio of CFU-GM (*c*, *h*) and ClFU-GM in S-phase of the mitotic cycle (*d*, *i*), and maturation of granulocyte-macrophage precursors in the bone marrow (*e*, *j*) of good (*1*, *3*) and poorly learning CBA/CaLac mice (*2*, *4*) subjected to conflict situation (*1*, *2*) or paradoxical sleep deprivation and training in a 3-arm T-maze (*3*, *4*). Ordinate: number of granulocyte-macrophage precursors (content of colonies and clusters, $\times 10^5$ cells, *a*, *b*, *f*, *g*); ratio of precursor cells in S-phase of the mitotic cycle (%, *c*, *d*, *h*, *i*); index of maturation (clusters/colonies ratio in a well, *e*, *j*).

ration of granulocytic cells after PSD and learning led to hyperplasia of the granulocytic hemopoietic stem.

The local mechanisms of regulation of proliferation and differentiation of hemopoietic cells during neuroses are realized via the adrenergic, serotoninergic, and cholinergic system [1]. Published data show that brain neurotransmitters determine behavioral pattern of intact animals [5,6]. For example, low adrenal concentration and high serotonin concentration correlate with low indexes of horizontal and vertical activity in the open field, and vice versa. Our experiments demonstrated that good and poor learners are characterized by high and low locomotor activity in the open field, respectively. These data suggest that dif-

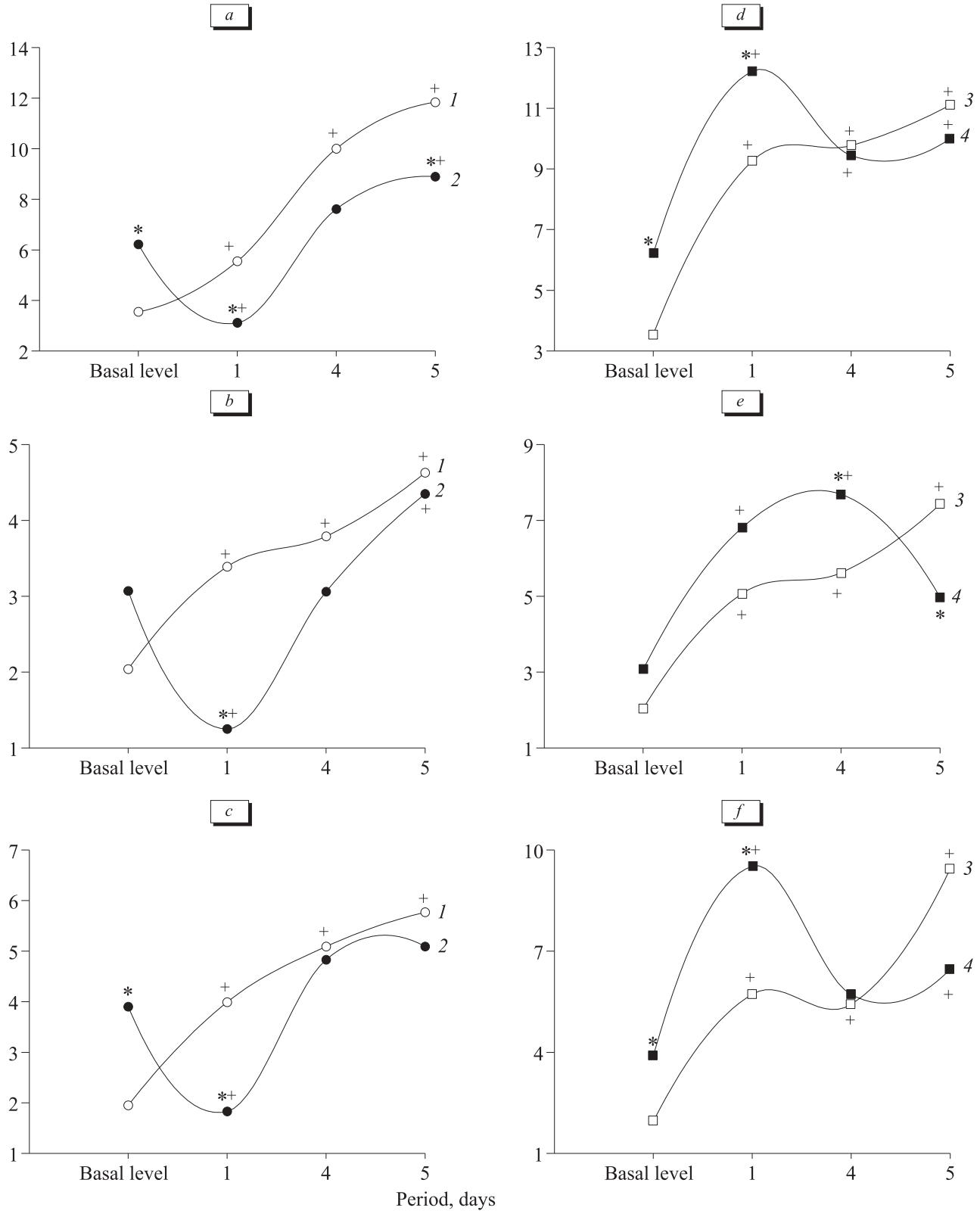

Fig. 3. Number of macrophage-positive (*a*, *d*), granulocytic (*b*, *e*), and erythrogranulocytic hemopoietic islets (*c*, *f*) in the bone marrow of good (*1*, *3*) and poorly learning CBA/CaLac mice (*2*, *4*) subjected to conflict situation (*1*, *2*) or paradoxical sleep deprivation and training in a 3-arm T-maze (*3*, *4*). Ordinate: number of hemopoietic islets in the bone marrow, $\times 10^3$.

ferences in the central neurotransmitter-mediated mechanisms of hemopoiesis regulation in good and poor learners determine different changes in the granulocytic hemopoietic stem (activation and dysregulation, respectively) and system of local regulation of granulocytopoiesis during neuroses.

This work was supported by the Regional Public Foundation of Russian Medicine Support.

REFERENCES

1. E. D. Gol'dberg, A. M. Dygai, N. V. Provalova, *et al.*, *Role of the Nervous System in Regulation of Hemopoiesis* [in Russian], Tomsk (2004).
2. E. D. Gol'berg, A. M. Dygai, and V. P. Shakhov, *Tissue Culture Methods in Hematology* [in Russian], Tomsk (1992).
3. G. F. Lakin, *Biometry* [in Russian], Moscow (1990).
4. O. V. Pershina, E. G. Skurikhin, L. A. Stavrova, *et al*., *Byull. Eksp. Biol. Med*., **138**, No. 11, 499-505 (2004).
5. S. B. Seredenin, B. A. Badyshtov, G. G. Neznamov, *et al*., *Eksper. Klin. Farmakol*., **64**, No. 1, 3-12 (2001).
6. S. B. Seredenin, B. A. Badyshtov, G. G. Neznamov, *et al*., *Ibid*., **64**, No. 2, 3-10 (2001).
7. N. I. Suslov, O. V. Pershina, E. G. Skurikhin, and N. V. Provalova, *Byull. Eksp. Biol. Med.*, Suppl. 2, 45-49 (2003).
8. *Emotional Stress: Theoretical and Clinical Aspects*, Eds. K. V. Sudakov and V. I. Petrov, Volgograd (1997).
9. M. Thakkar and B. N. Mallick, *Sleep*, **16**, No. 8, 691-694 (1993).