# EXPERIMENTAL BIOLOGY

# Effect of Amitriptyline on Daily Variations in Cell Composition of Immune Organs in Rats with Experimental Desynchronosis

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We studied the effects of amitriptyline (preparation stimulating melatonin synthesis in the pineal gland) on daily variations of cell composition in immune organs of Wistar rats with experimental desynchronosis. Amitriptyline administered in the evening restored the daily dynamics of the number of thymus cells.

**Key Words:** *biorhythm; desynchronosis; lymphocytes; lymphoid organs*

Chronobiological approach extensively elaborated in biology and medicine allows us to understand the pathogenesis of various diseases and to optimize therapeutic and preventive procedures [5]. Functional disorders in the body are preceded by shifts in normal biological rhythm [4]. The pineal hormone melatonin, whose synthesis depends on the light/dark regimen [1], regulates circadian rhythm in living organisms [6,8,13] and has immunomodulatory properties [9, 12,14]. It was shown that various antidepressants, including monoamine oxidase inhibitors and tricyclic and atypical compounds, modulate melatonin synthesis. These drugs (in particular, amitriptyline) stimulate secretory processes in the pineal gland of experimental animals [2].

Here we studied the synchronizing effect of amitriptyline on the immune system of Wistar rats with experimental desynchronosis (constant light exposure for 14 days).

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## MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 150-200 g and living in a vivarium (Institute of Clinical and Experimental Lymphology). Group 1 rats were kept under normal light/dark regimen. Group 2, 3, and 4 rats were exposed to constant light for 14 days and then kept under normal light/dark regimen. Group 4 rats were intramuscularly injected with 0.06 mg amitriptyline in 0.2 ml distilled water at 18:00 for 3 days starting from day 1 of the normal light/dark regimen. Group 3 rats received 0.2 ml distilled water according to the same schedule. The animals (5 rats from each group) were decapitated at 10:00, 15:00, and 20:00, and the blood and lymphoid organs were taken. Cell suspensions were prepared from the thymus, spleen, and inguinal lymph nodes. Blood leukocytes and nuclear cells were counted in a Goryaev chamber. Blood smears were spread on slides, fixed with 96% ethanol, and stained by the method of Romanovsky—Giemsa. The leukocyte count was estimated. The results were analyzed by nonparametric Wilcoxon—Mann—Whitney test.

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## RESULTS

We observed significant daily variations in the number of blood leukocytes ($p<0.05$): this parameter was minimum at 10:00 and peaked at 20:00 (groups 2, 3, and 4) or 15:00 (group 1, Fig. 1, *a*). There were no intergroup differences in the number of blood leukocytes.

The absolute number of lymphocytes underwent significant diurnal variations in all animals (Fig. 1, *b*). In control rats, the number of lymphocytes per 1 ml blood was minimum at 10:00 and increased by 15:00. In group 2 and 3 rats, this parameter in the evening surpassed the morning and midday values. In group 4 rats, the absolute number of lymphocytes in the evening surpassed that in the morning ($p<0.05$). No intergroup differences in this parameter were revealed.

In group 1 rats, the number of thymocytes progressively decreased from 10:00 to 15:00 and then peaked at 20:00 ($p<0.05$: 15.00-20.00, Fig. 2, *a*). In group 2 and 3 rats, the number of thymocytes was maximum at 10:00 and minimum at 15:00; at 20:00, this parameter tended to increase. In group 4 rats, amitriptyline normalized daily variations in the number of thymocytes, which became similar to those in group 1 animals. This parameter decreased at 15:00 and reached the maximum at 20:00 ($p<0.05$ for all periods). The evening concentration of thymocytes in group 4 rats was higher than in groups 1, 2, and 3 ($p<0.05$).

The number of splenocytes underwent significant diurnal variations only in group 1 rats. At 20:00, the number of splenocytes in these animals was higher than in other rats ($p<0.05$, Fig. 2, *b*).

Cell count in the inguinal lymph nodes was minimum at 10:00. This parameter increased by 15:00 ($p<0.05$), but decreased by 20:00. There were no significant differences between the morning-midday and evening values in rats exposed to constant light (Fig. 2, *c*). Significant intergroup differences were observed only at 20:00 (Fig. 2, *c*).

Thus, constant light exposure for 2 weeks attenuates (but not abolishes) diurnal variations of all studied parameters, which confirms the major (but not sole) role of light/dark regimen in circadian rhythms in the immune system. These data are consistent with our previous findings [3]. The desynchronizing effect of constant illumination is probably realized via suppression of diurnal rhythms of melatonin synthesis in the pineal gland [11]. It should be emphasized that complete recovery of circadian rhythms of cell composition after amitriptyline-induced stimulation of melatonin synthesis in the evening time was observed only in the thymus (central organ of T lymphopoiesis). In the inguinal lymph nodes containing many T cells, this parameter only tended to normal. The effects of amitriptyline on the spleen containing various cells (T and B lymphocytes, macrophages, and hemopoietic cells) and blood (site of migration of lymphoid subpopulations) did not differ from the influence of injection stress. These data suggest that the T and B cell-dependent immune mechanisms have various neuroendocrine synchronizers. Diurnal biorhythms of T cell populations are primarily regulated by melatonin. This assumption is confirmed by the data that pinealectomy leads to involution and disorganization of the thymus [7] and that T cells (mainly T helper cells) are the main targets for melatonin [10]. The synchronizing effect of amitriptyline on cell composition of the thymus can be mediated by more complex neuroendocrine interrelations between melatonin and somatotropin [15], gonadal steroid hormones [7], and opioid peptides [10]. Thus, administration of amitrip-

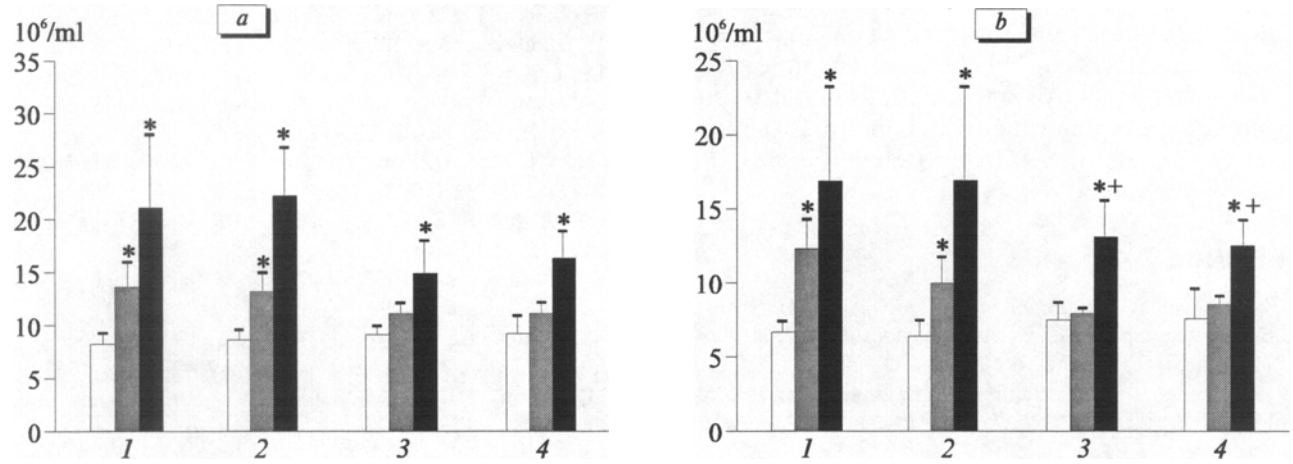


**Fig. 1.** Diurnal variations of the total number of leukocytes (*a*) and absolute number of lymphocytes (*b*) in intact rats (*1*) and in animals with experimental desynchronosis (*2*) injected with distilled water (*3*) or amitriptyline (*4*). Here and in Fig. 2: 10:00 (light bars), 15:00 (dark bars), and 20:00 (shaded bars). $p<0.05$: *compared to 10:00 (intragroup differences) and +compared to 15:00 (intragroup differences).

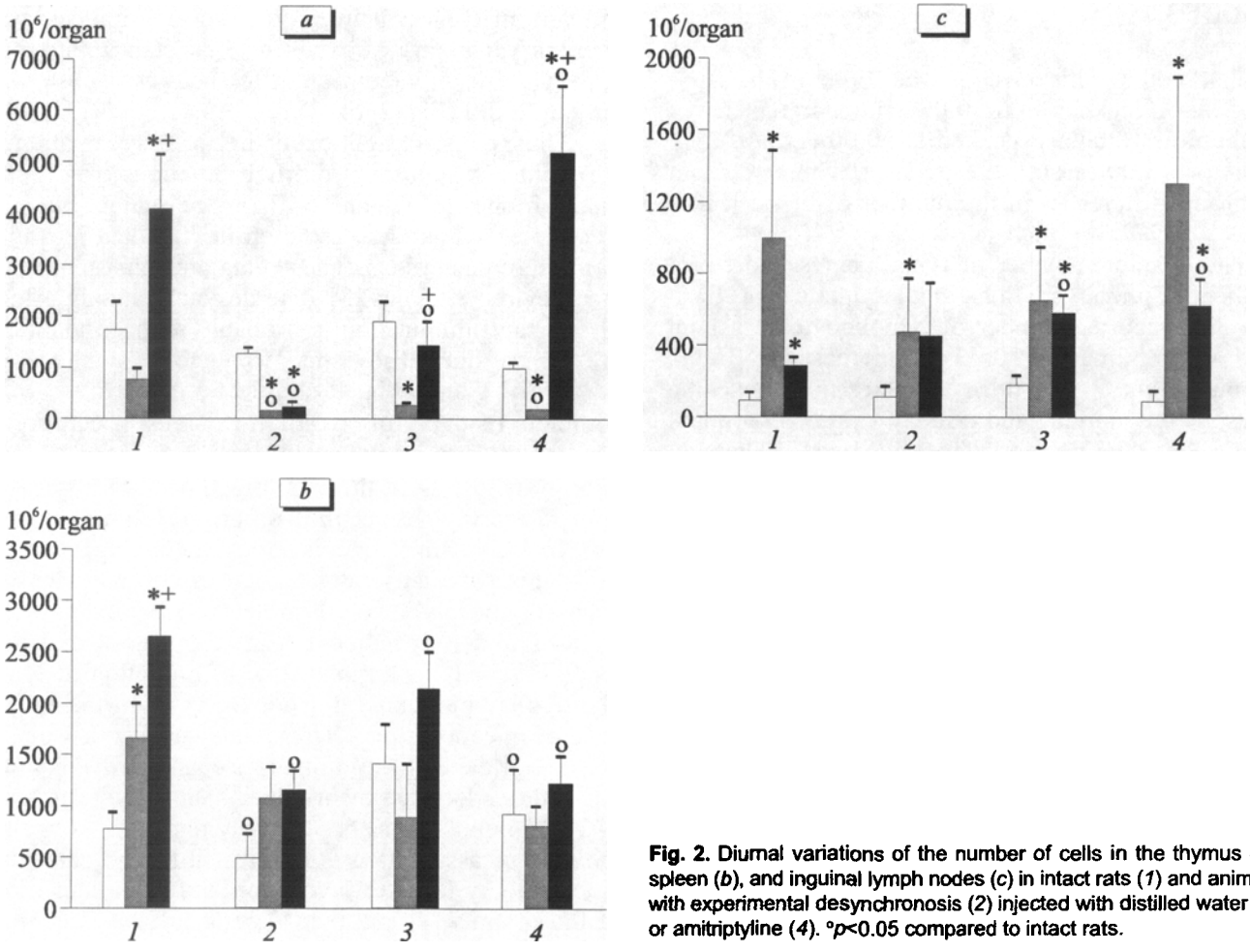


**Fig. 2.** Diurnal variations of the number of cells in the thymus (*a*), spleen (*b*), and inguinal lymph nodes (*c*) in intact rats (*1*) and animals with experimental desynchronosis (*2*) injected with distilled water (*3*) or amitriptyline (*4*). °$p<0.05$ compared to intact rats.

tyline in the evening time normalizes diurnal biorhythms of T cell proliferation and differentiation probably by enhancing the nocturnal melatonin peak. Migration of immune cells is less sensitive to amitriptyline, because the number of leukocytes and lymphocytes in the peripheral blood undergoes only insignificant changes under these experimental conditions. It should be emphasized that the most drastic differences between the control and experimental rats were observed in the evening time, which indicates the importance of chronobiological approach in studying the effects of immunomodulatory factors.

## REFERENCES

1. E. B. Arushanyan, *Usp. Fiziol. Nauk*, **27**, No. 3, 31-50 (1996).
2. E. B. Arushanyan and L. G. Arushanyan, *Eksp. Klin. Farmakol.*, **60**, No. 6, 71-77 (1997).
3. Yu. I. Borodin, V. A. Trufakin, A. Yu. Letyagin, and A. V. Shurlygina, *Circadian Biorhythms of the Immune System* [in Russian], Novosibirsk (1992).
4. F. I. Komarov, S. I. Rapoport, V. P. Karp, *et al.*, *Klin. Med.*, No. 9, 4-7 (1996).
5. L. M. Makarov, *Ibid.*, No. 2, 22-26 (1997).
6. S. M. Armstrong and J. R. Redman, *Med. Hypotheses*, **34**, No. 4, 300-309 (1991).
7. G. Csaba and P. Barath, *Endocrinol. Exp.*, No. 9, 59-67 (1975).
8. M. Humlova and H. Illnerova, *Neurosci. Res.*, **13**, No. 2, 147-153 (1992).
9. P. Lissoni, S. Bami, and A. Ardizzoni, *J. Biol. Regul. Homeost. Agents*, No. 7, 121-125 (1993).
10. G. J. M. Maestroni and A. Conti, *Neuroimmunomodulation*, No. 3, 325-332 (1996).
11. I. Mahvoud, S. S. Salman, and A. al-Khateeb, *J. Anat.*, **185**, No. 1, 9-15 (1994).
12. K. M. Morrey, J. A. McLanchlan, C. D. Serkin, and O. Bakomche, *J. Immunol.*, **153**, No. 6, 2671-2678 (1994).
13. R. J. Reiter, *Exp. Gerontol.*, **30**, No. 5, 199-212 (1995).
14. J. E. Roberts, *J. Photochem. Photobiol.*, **29**, No. 1, 3-15 (1995).
15. M. Zeman, J. Buyse, D. Lamosova, *et al.*, *Domest. Anim. Endocrinol.*, **17**, No. 2-3, 199-207 (1999).