PHYSIOLOGY

Delayed Behavioral Effects of β-Casomorphin-7 Depend on Age and Gender of Albino Rat Pups

V. A. Dubynin, I. V. Malinovskaya, Yu. A. Ivleva, L. A. Andreeva, A. A. Kamenskii and I. P. Ashmarin

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Systemic administration of β-casein heptapeptide β-casomorphin-7 (YPFPGPI, 1 mg/kg daily) to 10-23-day-old albino rat pups produced delayed anxiolytic effects, which were more pronounced in female than in male rats. Experimental findings confirm our assumption on the important role of nutritional opioids in brain development in newborn mammals.

Key Words: *β-casomorphin; opioids; behavior; ontogeny*

Previous studies demonstrated that alimentary opioid peptide β-casomorphin-7 (β-C-7) caused long-standing behavioral changes in albino rat pups [1,3]. Hence, biological effect of β-casomorphins are related not only to regulation of current behavior in newborns [11, 12], but also to hormone-like control of brain development. This assumption was confirmed by recent data on neurotrophic activity of β-casomorphins [10]. β-Casomorphins formed during enzymatic degradation of milk β-casein are rapidly transported into the circulation [13] due to facilitated transport of proteins and peptides from the intestine into the blood in mammalian newborns [2]. These substances interact with μ- and δ-opioid receptors in various tissues and cells (nerve cells, lymphocytes, smooth muscle cells, *etc.*) [12] and modulate maturation and sex differentiation of various systems, which essentially complicates analysis of experimental data. The aim of the present study was to evaluate delayed behavioral effects of β-C-7 administered during the early ontogeny.

MATERIALS AND METHODS

The most common representative of the β-casomorphin family, β-C-7, was synthesized at the Institute of Molecular Genetics (Russian Academy of Medical Sciences). Experiments were carried out on 98 outbred albino rats (males and females equally), of them 50 rats comprised the control group. β-C-7 was injected intraperitoneally in a dose of 1 mg/kg (aqueous solution, 1 ml/kg); control rats received equivalent volume of distilled water. The preparation was daily administered to 10-23-day-old rats). Motor activity, exploratory activity, and anxiety were evaluated in the open field test [4]: the horizontal (ambulation) and vertical (rearing) activities, the number of grooming acts and movements apart from the arena walls. At the age of 49 days the tests were performed for 4 min: 3 min under bright illumination and 1 min with a red lamp. At the age of 56 days the tests lasted 2 min (bright illumination, bell). Animal anxiety was evaluated by the transition latency in a shuttle box and a latency of exit from a transparent "house" [4]. The data were processed statistically using Statgraphics and Statistica software.

RESULTS

Open field test allows to analyze rat behavior in stress of varying intensity. Illumination with a red lamp provided mild conditions, when exploratory activity attained a maximum, while bright illumination and bell were the most potent stress-inducing factors. Inter-

Department of Human and Animal Physiology, Biological Faculty, M. V. Lomonosov Moscow State University. ***Address for correspondence:*** dubynin@5.human.bio.msu.ru. Dubynin V. A.

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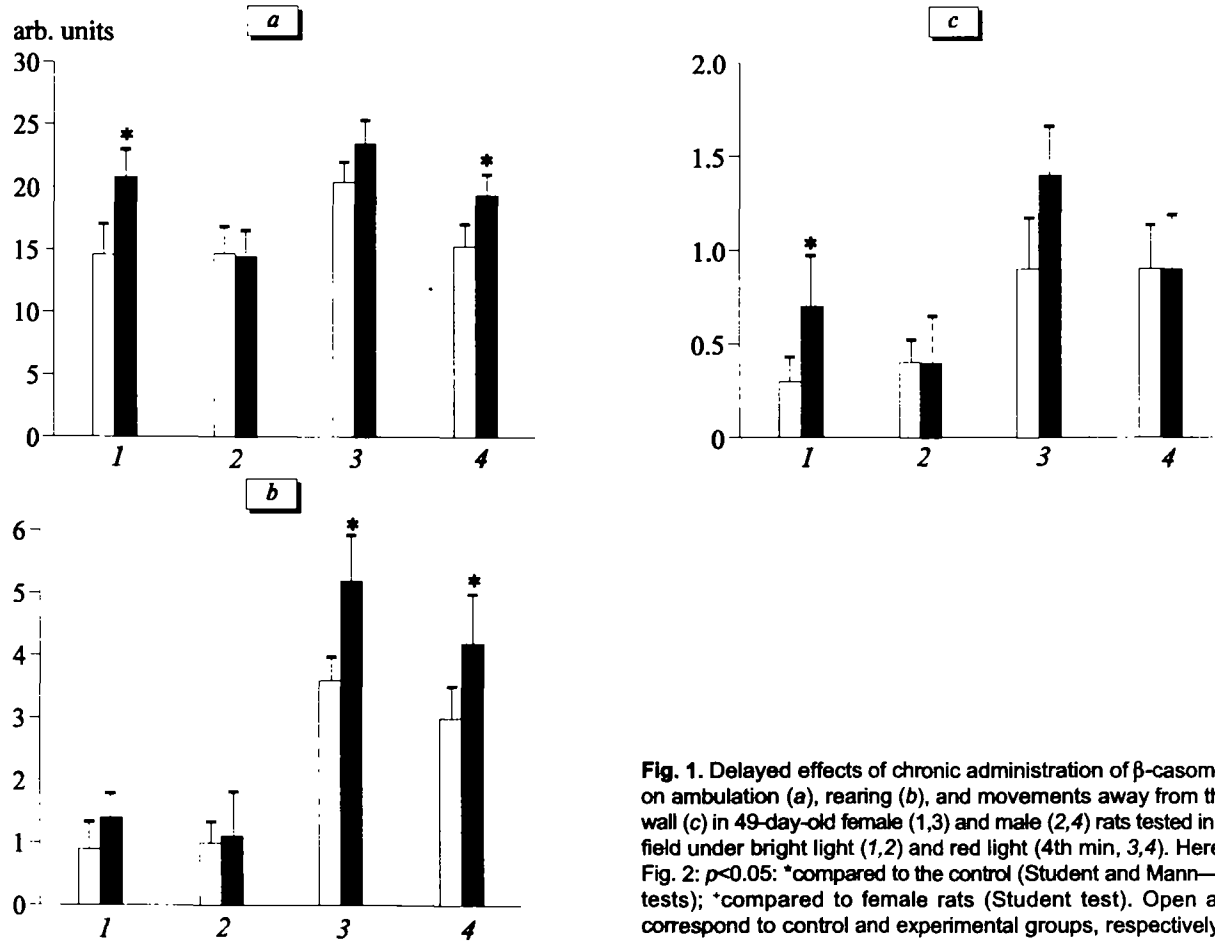

Fig. 1. Delayed effects of chronic administration of β-casomorphin-7 on ambulation (*a*), rearing (*b*), and movements away from the arena wall (*c*) in 49-day-old female (1,3) and male (*2,4*) rats tested in an open field under bright light (*1,2*) and red light (4th min, *3,4*). Here and on Fig. 2: $p<0.05$: *compared to the control (Student and Mann—Whitney tests); +compared to female rats (Student test). Open and bars correspond to control and experimental groups, respectively.

TABLE 1. Delayed Behavioral Changes in Albino Rat Pups Receiving Chronic Injections of β-C-7 at Various Terms in Early Ontogeny

Experimental conditions	Time of β-C-7 administration, days after birth		
	1-14 [1]	10-23	21-34 [3]
Red light			
females	+	++	++
males	++	++	0
Bright light			
females	0	++	++
males	++	0	0
Bright light and bell			
females	—	+	+
males	0	—	—

Note. 0 corresponds to the absence of significant changes, — denotes significant changes in only one parameter due to anxiogenic effect of the preparation, + and ++ denote significant changes in one and two parameters, respectively, related to anxiolytic effect of the preparation.

mediate conditions are the first 3 min of observations in 49-day-old rats. In male rats, the delayed behavioral effects of the peptide were observed only under red lamp illumination, while in females these effects were seen under both red and bright light. Moreover, in β-C-7-treated females, the number of movement away from the arena wall also increased compared to the control (Table 1). These changes confirm the anxiolytic effect of b-C-7.

It should be noted that despite similar horizontal activity during the 1st and 4th minutes of the test, the number of rearings and movements away from the wall under red light was higher than under bright illumination. This attests to a more strict correlation of these two parameters with exploratory motivation, which is more expressed under less stressful conditions. Horizontal activity also reflects defensive motivation, *i.e.* the animals try to get out of the test arena. Horizontal activity during the 1st min correlates with the corresponding parameter during the 4th min with a correlation coefficient of 0.14 (insignificant). Thus, horizontal activity during stress of different intensity depends of various factors (motivations). The corre-

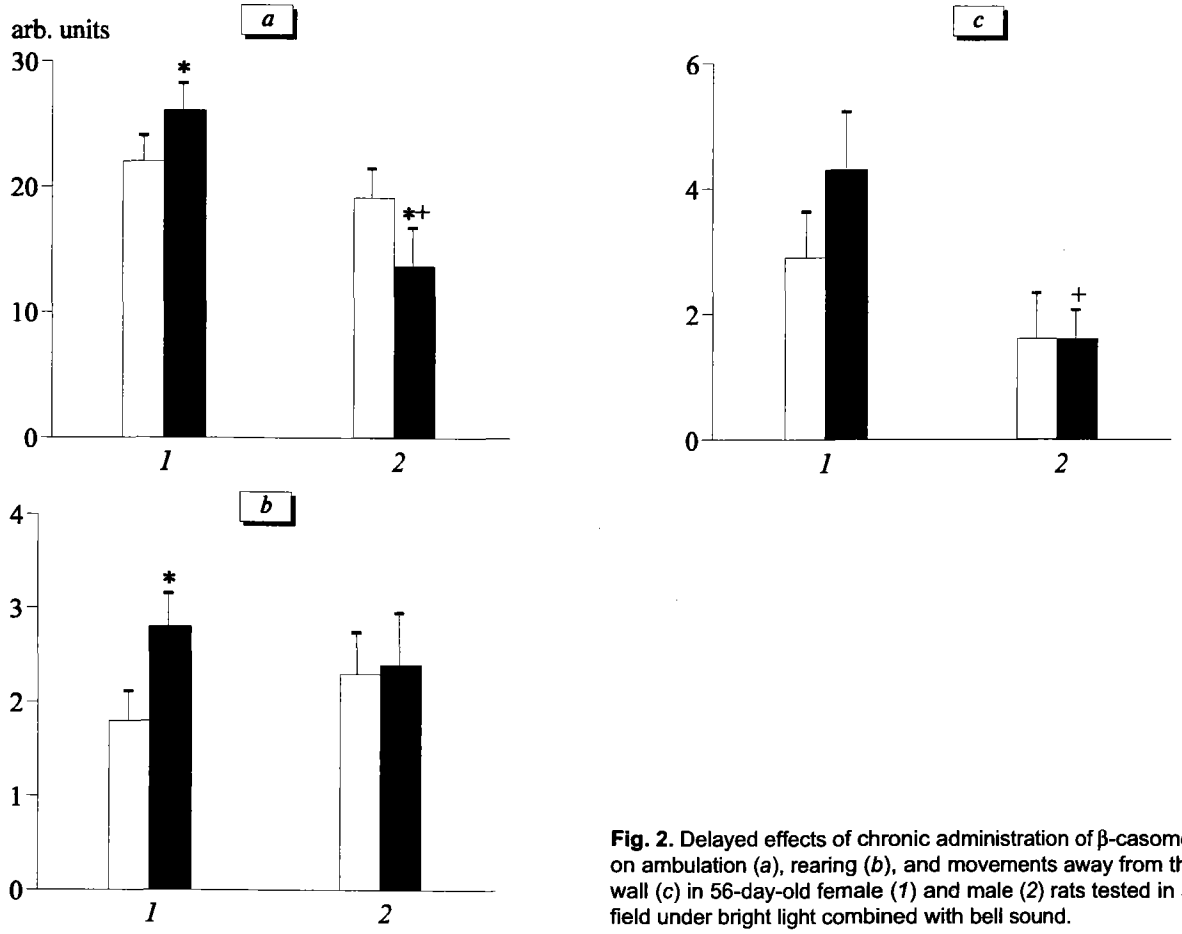

Fig. 2. Delayed effects of chronic administration of β-casomorphin-7 on ambulation (*a*), rearing (*b*), and movements away from the arena wall (*c*) in 56-day-old female (*1*) and male (*2*) rats tested in an open field under bright light combined with bell sound.

sponding coefficient for rearings was 0.31 (p=0.03). Therefore, vertical activity during the 1st and 4th minutes is determined by the same factor (exploratory motivation). In the experimental group, the corresponding correlation coefficients were higher than in the control: 0.34 for ambulation (p=0.02) and 0.43 for rearings (p=0.002), which suggested more uniform processes and lower contribution of additional factors (defensive motivation) in the regulation of animal behavior.

Shuttle-box and "exit from the house" tests revealed additional delayed anxiolytic effect of β-C-7 in females. Transition latencies in a shuttle box in the experimental and control groups were 25.5±11.8 and 17.2±8.4 sec, respectively, while the exit latencies were 46.9±12.0 and 25.6±9.8, respectively (p=0.04 for score values [4]). Correlation analysis showed that these parameters most closely correlated with parameters of animal behavior under red light.

In the most stressful situation (bright light and bell, Fig. 2), motor activity of experimental female rats increased, number of freezing responses was considerably lower than in the control (p=0.02), the number of rearing also slightly differed from the control (p=0.11). Moreover, grooming acts were more frequent in experimental females (Fig. 2, *b*). The frequency of grooming acts is usually related to competition between the main factors modulating animal behavior. In our experiments, the higher frequency of grooming acts suggest similar intensities of the exploratory and defensive motivation in rats treated with β-C-7. In control animals defensive motivation dominated over the exploratory one (decreased horizontal and vertical activities and low number of grooming acts).

Unlike female rats, in experimental males combination of acoustic stimulation and bring light decreased horizontal motor activity. Despite high exploratory activity under red light, these animals became more anxious under maximum sensory stimulation. Male and female rats demonstrated different behavior, while in the control these differences were insignificant (Fig. 2, *a* and *b*).

Thus, chronic administration of β-C-7 to 10-23-day old rats caused delayed anxiolytic effects and shifted the balance between the defensive and expoloratory motivations towards the latter one. These changes were more pronounced during mild stress and decreased with increasing stress intensity. The effect of β-C-7 in females was more potent and stable than in males, while in males exposed to maximum stress this effect can be inverted (anxiogenic changes).

These experiments continue our studies on the effects of β-C-7 at various terms of rat ontogeny [1,3]. Accumulated data obtained in different experimental series are presented in Table 1. Comparative analysis showed that the effect of β-C-7 increases in female rats and decreases in males with increasing the age of administration. Moreover, in female rats, behavioral response to β-C-7 is formed as soon as at the age of 10-23 days: the differences from rats receiving β-C-7 on days 1-14 of life are significant (p=0.01-0.05), while the differences from rats receiving β-C-7 on days 21-34 of life are insignificant. In male rats we observed an inverse and more smooth age dynamics of β-C-7 effects, which was confirmed by linear regression analysis (p=0.01-0.05).

These regularities are probably related to interaction between anxiolytic effect of β-C-7 and maturation of the cerebral opioid system in albino rats. Published data suggest that the number of opioid receptors in rat brain increases primarily during the second decade of life [8,14]. Under these conditions, administration of β-C-7 "supported" the endogenous opioid system. Moreover, the effects of β-C-7 coincided with a certain stage of sex maturation occurring during the second week of life. Intensification of androgen synthesis leads to partial suppression of the opioid system [5]. These events determine different delayed effects of β-C-7. Peculiarities of β-C-7 effects are important for the analysis of the ontogeny of different behavioral reactions as the factor of maturation and hormone-controlled imprinting of mammalian brain.

These conclusion to a some extent can be extrapolated to humans, because breast milk contains β-C-7-like fragment, which can be detected in newborns feeding breast milk. However, unlike rat pups, the opioid system in mammalian newborns is developed [6], which provide basis for realization of regulatory and modulatory effects of β-casomorphins. The delayed behavioral effects of β-casomorphins are probably realized in parallel with their acute (correctors of actual state) effects, but require lower amount of active substance.

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