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Association of *SLC6A4* Gene 5-HTTLPR Polymorphism with Parameters of Simple and Complex Reaction Times and Critical Flicker Frequency Threshold in Athletes during Exhaustive Exercise

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The incidence of *SLC6A4* gene 5HTTLPR polymorphism alleles was evaluated in 223 male athletes engaged in endurance sports, the results were compared with those in 177 male nonathletes. Association between 5-HTTLPR genotypes and the effect of exhaustive treadmill running on simple and complex visual reactions and critical flicker frequency threshold was studied. We found that the incidence of LL genotype was significantly higher in athletes in comparison to nonathletes; after exercise, the velocity of visual reactions and critical flicker frequency increased; exercise did not change the velocity of complex visual reaction in LL-carriers, and increased it in SS-carriers. We conclude that exhausting treadmill running leads to facilitation sensory information processing in athletes and that SS-carriers are more susceptible to the effect of exhaustive treadmill running than LL-carriers.

Key Words: *serotonin transporter; polymorphism; fatigue*

Serotonin (5-HT) system is involved in visceral regulation, motility control, modulation of cognitive, affective, and neuroendocrine functions; its activity is also closely related to fatigue, excitation, and reaction to stress [7,12]. It is known that physical exercises modulate the 5-HT system: acute exercise increases intracellular concentration of 5-HT [6], while regular endurance exercises modulate 5-HT [15]. There are contradictory data on the effect of 5-HT transmission on physical endurance. On the one hand, the 5-HT system mediates the decrease in working capacity under conditions of fatigue [8], but on the other hand, in overtrained athletes 5-HT transmission does not differ from that in nonathletes [14]. NaCl-dependent transporter (5-HTT) responsible for active 5-HT reuptake from the synaptic cleft is one of the key regulators of 5-HT neurotransmission. 5-HTT is encoded by *SLC6A4* gene containing a 5-HTTLPR polymorphism site affecting transcription activity of the gene and playing an important role in the regulation of the 5-HT system [2]. *SLC6A4* promoter is presented by a long (L-allele) and short (S-allele) forms. S-allele is associated with lower gene transcription and, hence, lower protein content on the presynaptic membrane compared to L-allele [2]. The incidence of S- and L-alleles in European population is about 40 and 60%, respectively [13]. Previous studies showed that the distribu-

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tion of 5-HTTLPR polymorphisms among athletes (synchronized swimming, football, and hockey) differs from that in nonathletes [13].

Here we compared the incidence of 5-HTTLPR alleles of *SLC6A4* gene among male athletes engaged in endurance sports and nonathletes and evaluated the influence of physical exercise on critical flicker frequency threshold (CFFT) and simple (SR) and complex visual reactions (CR) in carriers of different variants of 5-HTTLPR polymorphism. SR, CR, and CFFT were used as fatigue markers [10]; it is known that regular exercises improve, while fatigue impairs these parameters [3]. We hypothesized that the effect of physical exercise on these parameters can depend on the presence of this or that 5-HTTLPR allele.

MATERIALS AND METHODS

For genetic analysis we used DNA samples from 223 male athletes (72 rowers, 52 skiers, 37 bicyclist, and 62 biathlonists) and 177 male nonathletes. The mean age of athletes was 25±4 years, body weight 80.3±6.8 kg, height 182.6±6.2, maximum oxygen consumption 71.5±5.6 ml/kg×min; mean age of nonathletes was 25±4 years, body weight 75.3±4.8 kg, height 173.3±4.3, and maximum oxygen consumption 46.2±6.4 ml/kg×min.

In experiments aimed at evaluation of physical exercise on CFFT, SR, and CR we examined 165 of 223 athletes (mean age 22.3±3.2 years, body weight 84.4±7.6 kg; height 187.1±7.1; maximum oxygen consumption 69.5±4.6 ml/kg×min).

The examinees signed informed consent for participation in the study. The study protocol was approved by ethical committee of Russian Research Institute of Sport and Physical Education.

The athletes performed exhausting treadmill running test started at a velocity of 7 km/h followed by it increase by 0.1 km/h every 10 sec. The tests were performed during the first half of the day. The mean CFFT, SR, and CR were measured before and immediately after testing on a NS-Psikhotest device (Neirosoft).

DNA was isolated from the whole blood using Qiagen kits (Qiagen Inc., Hilden) according to manufacturer's instruction. *SLC6A4* gene 5-HTTLPR polymorphism was analyzed as described previously [1].

Conformity of empirical genotype frequency distribution to theoretically expected Hardy–Weinberg equilibrium was verified using Pearson χ^2 test. The frequencies of genotypes and alleles in groups were compared using z test for two fractions. The correlation between the genotype and changes in neurodynamic parameters under the effect of physical exercise were studied using *t* test for depended (effect of exercise) and independent (effect of genotype) samples. The differences were significant at $p<0.05$.

RESULTS

Analysis of 5-HTTLPR genotype distribution revealed higher incidence of LL genotype (39 vs. 25% in the reference group) and lower incidence of SS genotype in athletes (Fig. 1). The observed genotype distribution conformed to theoretically expected Hardy–Weinberg equilibrium (χ^2=0.0014, p=0.97). We previously demonstrated predominance of LL genotype in athletes engaged in synchronized swimming, football, and hockey [1]. We also showed that the incidence of LL genotype in athletes was higher than in non-athletes, which was attributed to higher resistance of LL-carriers to stress [13]. Our findings are confirmed by the experiments demonstrating that affinity of 5-HHT protein increases after onset and remains high during the period of high physical activity [15]. Moreover, animal experiments showed that the decrease in free 5-HT in the CNS is an adaptive response of the organism to endurance training [9]. Thus, increased incidence of LL genotype in athletes engaged in endurance sports can be explained by the relationship between LL genotype with increased 5-HTT protein content on synaptic cells, which implies more rapid elimination of free 5-HT from extracellular space and can act as a factor facilitating adaptive reaction of CNS to endurance exercise. However, it should be noted that in a previous study [4] no associations between physical endurance and 5-HTTLPR polymorphism were detected in triathletes. Thus, further studies with participation of athletes engaged in other sports are required.

For more detailed understanding of increased incidence of LL genotype of 5-HTTLPR polymorphism in athletes, we analyzed possible associations between 5-HTTLPR variants and the effect of exercise on CFFT, SR, and CR [10]. To this end, we compared the groups of SS and LL carriers, because no differ-

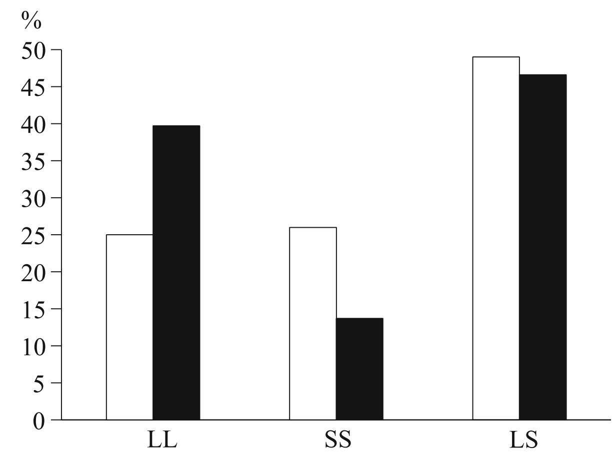

Fig. 1. Distribution of 5-HTTLPR polymorphism genotypes in athletes (dark bars) and in the reference group (light bars). The incidence of L-allele in athletes and references group was 63 and 49%, respectively. The differences are significant at $p<0.05$.

TABLE 1. Time of SR and CR and CFFT before and after Exercise (*M*±*SD*)

Parameter	Exercise		Significance of differences
	before	after	
SR, msec	216.1±19.9	213.3±20.5	*t*=1.14, *p*=0.25
CR, msec	321.3±31.1	308.9±33.5	*t*=3.71, *p*=0.0003*
CFFT, Hz	38.7±3.2	40.5±3.3	*t*=-6.10, *p*<0.0001*

Note. Here and in Table 2: *denotes significant changes.

ences were noted between athletes and nonathletes carrying LS genotype.

It was found that immediately after exercise SR remained unchanged, CR decreased (*t*=3.71, *p*=0.0003), and CFFT increased (*t*=-6.10, *p*<0.0001; Table 1) which attested to activation of nervous processes. This observation agrees with previous studies demonstrating that acute aerobic exercise activates cognitive processes and increases the rate of stimulus recognition and decision making [11].

No significant differences in the mean time of SR were revealed in LL and SS carriers before and after exercise (Table 2). No significant differences between the groups in the mean time of CR before exercise were found; after exercise, CR remained unchanged in LL carriers, but significantly decreased in SS carriers (*t*=2.33, *p*=0.0446; Table 2). No significant differences between the groups in the mean time of CFFT before exercise were found; after exercise, CFFT remained unchanged in carriers of LL (*t*=-4.10, *p*=0.0004) and SS (*t*=-3.19, *p*=0.0150; Table 2).

Thus, the effect of exhausting exercise on sensory information processing in SS carriers was more pronounced than in LL carriers. Intensification of nerve processes (increase in CFFT) was observed in both groups and in SS carriers it was accompanied by CR acceleration. These findings suggest that nervous processes in LL carriers are more resistant to the modulating influence of exercise than in SS carriers. It was demonstrated that S-allele is associated with enhanced lability of nervous processes compared to LL genotype, due to which carriers of S-allele are more susceptible to stress factors [5]. Thus, the presence of S-allele is associated with higher activity, lability, and velocity of nervous processes, which, at first glance can positively affect sport achievements, but carriers of this low-active allele of 5-HTT gene are less resistant to external influences (stress, fatigue) [13]. Higher stability of nervous processes in LL carries can be a factor determining advantages of this genotype for athletes in endurance sports. Further studies with participation of athletes engaged in other sports are required for verification of this assumption.

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TABLE 2. Time of SR and CR and CFFT before and after Exercise in Carriers of LL and SS Genotypes (*M*±*SD*)

Parameter	5-HTTLPR polymorphism genotype	Exercise		Significance of differences
		before	after	
SR, msec	LL	218.2±19.8	221.7±22.5	*t*=-0.46, *p*=0.65
	SS	212.8±16.2	211.6±25.6	*t*=-0.56, *p*=0.58
CR, msec	LL	319.1±29.3	323.3±34.2	*t*=-0.36, *p*=0.72
	SS	331.7±31.3	314.2±36.8	*t*=2.33, *p*=0.0446*
CFFT, Hz	LL	39.4±3.5	41.3±3.2	*t*=-4.10, *p*=0.0004*
	SS	39.9±2.4	42.7±3.4	*t*=-3.19, *p*=0.0150*

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