Behavioural Brain Research 278 (2015) 280–285

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Cortisol responses to chronic stress in adult macaques: Moderation by a polymorphism in the serotonin transporter gene

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HIGHLIGHTS

- Rh5-HTTLPR exerted an influence on cortisol responses to stress in macaques.
- The *s*/*s* genotype was associated with increased cortisol responses to stress.
- In the absence of stress, no differences related to genotype were observed.
- This moderation was a genetic modulation of cortisol responses to stress.

ARTICLE INFO

Article history:
Received 4 August 2014
Received in revised form
30 September 2014
Accepted 4 October 2014
Available online 13 October 2014

Keywords:
Rhesus macaque
Adult
Female
Rh5-HTTLPR
Chronic stress
Cortisol

ABSTRACT

Accumulating evidence has shown that a polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) moderates the association between stress and depressive symptoms. However, the exact etiologies underlying this moderation are not well understood. Here it is reported that among adult female rhesus macaques, an orthologous polymorphism (rh5-HTTLPR) exerted an influence on cortisol responses to chronic stress. It was found that females with two copies of the short allele were associated with increased cortisol responses to chronic stress in comparison to their counterparts who have one or two copies of the long allele. In the absence of stress, no differences related to genotype were observed in these females. This genetic moderation was found without a genetic influence on exposure to stressful situations. Rather it was found to be a genetic modulation of cortisol responses to chronic stress. These findings indicate that the rh5-HTTLPR polymorphism is closely related to hypothalamus–pituitary–adrenal (HPA) axis reactivity, which may increase susceptibility to depression in females with low serotonin transporter efficiency and a history of stress.

1. Introduction

Mood disorders and major depressive disorder (MDD) are associated with disturbances of both the hypothalamus–pituitary–adrenal (HPA) axis [1] and the serotonergic system [2–4]. Anti-depressants, which are routinely prescribed for many depressed patients, serve to regulate the function of the HPA axis [5] and/or the serotonergic system [6].

The serotonin transporter (5-HTT), which transports serotonin (5-HT) back into the cell after its neurochemical message has been delivered, has been recognized to be a key regulator of serotonergic neurotransmission. The 5-HTT gene contains a 44 base pair deletion/insertion polymorphism in the promoter region (5-HTTLPR) and has received considerable attention as a regulatory mechanism by many researchers. This polymorphism in the 5-HTT gene consists of two functionally distinct promoter lengths; coined the short (*s*) and long (*l*) alleles, respectively. The short allele is

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http://dx.doi.org/10.1016/j.bbr.2014.10.001
0166-4328/

associated with lower transcriptional efficiency and thus has lower 5-HTT availability than the long allele [7].

Caspi and colleagues reported that individuals with one or two copies of the short allele exhibited more depressive symptoms, diagnosable depression, and suicidality in relation to stressful life events (SLEs) than individuals homozygous for the long allele [8]. Since then, the 5-HTTLPR has been the focus of intensive research, although some researchers who applied meta-analytic techniques to data from relevant published studies failed to replicate this interaction [9,10]. Nonetheless, a more recent meta-analysis including all relevant studies has since found strong evidence that 5-HTTLPR moderates the relationship between stress and depression [11]. Yet, the mechanisms underlying this moderation are still not well understood.

Findings from animal and human researches suggest that genetic moderation of HPA axis reactivity to stressors may be one possible mechanism [12–14]. Murphy et al. found that mice with diminished (5-HTT+/−, heterozygote mice) or absent (5-HTT−/−, homozygote knockout mice) 5-HTT function exhibited more fearful behaviors and displayed greater increases in stress hormone response to stress when compared to homozygous (5-HTT+/+) controls [15]. In the absence of stress, no differences related to genotype were observed in these animals [15]. Research in humans also supported that HPA axis reactivity moderated by a polymorphism in the serotonin transporter gene is involved in the development of depression. Individuals with two short alleles at the 5-HTT locus were more susceptible to the depressogenic effects of SLEs than those with one or two long alleles [16]. In addition, Miller et al. used a meta-analysis to further evaluate this HPA axis reactivity in humans, and found that individuals homozygous for the short allele displayed increased cortisol reactivity to acute psychosocial stress compared with individuals with one or two copies of the long allele [17]. This "stress sensitivity" hypothesis was further verified by the finding that individuals homozygous for the short allele exhibited greater amygdala activation in response to fearful stimuli than individuals who have at least one long allele [18,19]. In turn, elevated amygdala reactivity leads to a hyperactivity of HPA-axis and an exaggerated stress response [20].

While most research into the "stress sensitivity" hypothesis was focused on the relationship between acute SLEs and the 5-HTTLPR [12–14], research into the effects of chronic stress and the 5-HTTLPR has been more challenging due to the variable nature of human societal systems [21]. Non-human primates (NHP) afford an opportunity to study the associations between the 5-HTTLPR, chronic stress and HPA axis reactivity because of their genetic similarity to humans, and more importantly, because their rearing environments can be tightly controlled [22,23]. For example, the rhesus macaque polymorphism (rh5-HTTLPR) was found to affect HPA-axis reactivity in infant macaques and that this genetic influence on hormonal responses during stress was modulated by early life experience [24]. The study presented here was designed to measure adult macaques' cortisol responses to chronic stress generated in long-term dominant–subordinate relationships and aimed to demonstrate that HPA-axis reactivity depends on both: the rh5-HTTLPR genotype and chronic stress in an adult sample.

2. Methods

2.1. Animals

Twenty-nine female rhesus macaques (*Macaca mulatta*) living in the Kunming Primate Research Center of the Chinese Academy of Sciences were selected at random and observed in this study. The animals came from 13 breeding groups and ranged from 11 to 24 years of age (15.03 ± 3.51 years). They lived in colonies with access to a connected indoor ($2.61 \times 2.46 \times 2.58$ m)–outdoor ($2.67 \times 2.66 \times 2.67$ m) cage. All animals were given commercial monkey biscuits twice a day with tap water *ad libitum*, and were fed with fruits and vegetables once daily. The animals had lived in their respective social groups for at least 1 year prior to initial observation.

All animal procedures were approved by the National Animal Research Authority (P.R. China) and the Institutional Animal Care and Use Committee (IACUC) of Kunming Institute of Zoology, Chinese Academy of Sciences. All efforts were made to minimize the monkeys' suffering. For example, hair samples were taken from the back of the monkey's neck using an electric-razor without anesthetic and no animals were sacrificed in this study. Routine veterinary care was provided throughout the study by professional keepers and veterinarians.

2.2. Experimental design

Animal behaviors in social hierarchies were video recorded using a focal follow technique [25] and were analyzed to calculate the chronic stress that they experienced based on dyadic interactions. After completion of the video recordings, blood and hair samples were obtained to measure the rh5-HTTLPR genotype and cortisol levels, respectively. Then, associations among the rh5-HTTLPR, chronic stress and hair cortisol levels were studied.

2.3. Behavioral sampling

The monkeys were given seven days to acquaint themselves with the observers and cameras prior to recording and sampling. Then, a digital camera fixed on a tripod was set up in front of the colony to record one monkey at a time in the cage. The observers kept as far away as possible (at least 5 m) from the monkeys' cages in order to avoid disturbing the animals during video recordings. Fourteen 1-h recordings were collected for each monkey on separate days sequentially throughout a six month period. All video recordings were stored on a hard disk and interpreted by three technicians. The three viewers analyzed each video recording simultaneously using a standardized behavioral classification [26]. The inter-rater reliability was scored in the SPSS software package (SPSS Inc., Chicago, IL, USA), which found the interclass correlation coefficient (ICC) to be >0.99. Each technician was blind to the genotype and cortisol levels of the animals.

2.4. Chronic stress

Chronic stress were quantized as conflict behaviors, which included aggressive and submissive behaviors. Aggressive behaviors included a bite, slap, grab, stare threat, open-mouth threat, chase, and a forced displacement. Submissive behaviors included a scream, scream threat, crouching, fleeing, lip smack, grimace, submissive present, and moving away [26]. Each of the above behaviors was scored as initiating (displaying) or receiving from another female and the frequencies of each behavior were calculated per hour.

2.5. Genotyping

DNA was isolated using standard extraction methods from whole blood collected from the femoral vein under ketamine anesthesia (15 mg/kg, i.m.) [27]. Genotyping was performed as described in previous studies [7,24,27]. The rh5-HTTLPR was amplified from 25 ng of genomic DNA with flanking oligonucleotide primers (stpr5, 5′-GGCGTTGCCGCTCTGAATACC; intl, 5′-CAGGGGAGATCCTGGGAGGGA). Amplicons were separated by electrophoresis on a 2% agarose gel. The short and long alleles of the