Behavioural Brain Research 292 (2015) 50–55

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Dopamine D4 receptor genotype variation in free-ranging rhesus macaques and its association with juvenile behavior

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HIGHLIGHTS

- We genotyped dopamine receptor D4 (DRD4) for first time in Cayo Santiago macaques.
- We investigated the association of DRD4 with juvenile impulsivity behaviors.
- Individuals with long DRD4 alleles are more restless and independent of mother.
- Individuals with long DRD4 alleles are more avoidant of other monkeys.
- Demonstrate utility of rhesus macaques as a model for ADHD research.

ARTICLE INFO

Article history:
Received 5 May 2015
Received in revised form 3 June 2015
Accepted 7 June 2015
Available online 11 June 2015

Keywords:
DRD4
Rhesus macaque
Juvenile
Social behavior
Impulsivity

ABSTRACT

A polymorphism in the dopamine receptor D4 (*DRD4*) gene has been associated with significant variation in behavioral impulsivity, novelty-seeking, and risk-taking in humans and other animals. Rhesus macaques are an excellent animal model for research on the genetic basis of behavior using the candidate gene approach. Little is known, however, about allelic variation in *DRD4* in large free-ranging populations of rhesus macaques and how this allelic variation relates to emotion regulation and behavior. In this study, we genotyped for the *DRD4* polymorphism 178 individuals of different age and sex categories in the free-ranging rhesus macaque population on the island of Cayo Santiago, PR. Moreover, we examined the possible association between DRD4 allelic variation and three measures of juvenile behavior (time spent in proximity to the mother, avoidance of other individuals, and behavioral restlessness). Five different *DRD4* alleles (5R, 5.5R, 6R, 6.5R, and 7R) were identified in the subject population. The most common allele was the 5R allele (78.5%), followed by the 7R allele (16.1%). Juveniles carrying the long form of the DRD4 allele (7R) spent less time in proximity to their mothers, avoided other individuals more often, and scored higher on behavioral restlessness than juveniles carrying the shorter alleles. Behavioral restlessness was also influenced by maternal *DRD4* genotype. These results highlight both similarities and differences in the relative occurrence of *DRD4* alleles and their association with behavior in this rhesus macaque population, other nonhuman primate species or populations, and humans.

1. Introduction

A large body of literature has shown that individuals who carry different alleles for genes that code for various aspects of monoaminergic (i.e., noradrenergic, serotonergic, and dopaminergic) function often exhibit differences in emotions or behavior (e.g., Gainetdinov and Caron [10]. A gene polymorphism that has received considerable attention involves a particular region (a 48-bp section of exon III, which encodes the third cytoplasmic loop of the receptor) of the gene that codes for the dopamine receptor

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http://dx.doi.org/10.1016/j.bbr.2015.06.014
0166-4328/

D4 (*DRD4*). This polymorphism has a variable number of tandem repeats (VNTR) [24]. In humans, the polymorphism can include anywhere from 2 to 11 repeats (2R–11R) [7], with the 7R being the most derived allele. This *DRD4* polymorphism has been shown to predict individual differences in impulsivity [22], risk-taking [4], and novelty-seeking [8] and has also been linked to clinical conditions such as Attention Deficit and Hyperactivity Disorder (ADHD; [23,17].

Non-human primates (NHPs) are ideal animal models for research on the genetic basis of normal and pathological behavior as they share with humans a variety of similarities in genetics, neuroanatomy, neurochemical and neuroendocrine function, and cognition and behavior (reviewed in Phillips et al [20]. Tandem repeats in the third exon of *DRD4* have already been identified in some NHPs such as vervet monkeys, macaques, and chimpanzees [13,12]. Studies reporting the frequency of occurrence of different *DRD4* alleles, however, have typically been conducted with relatively small captive populations. For example, *DRD4* alleles in rhesus macaques (*Macaca mulatta*) have only been investigated in one captive population housed at the National Institutes of Health Animal Center (NIHAC, [6]). Furthermore, little or no information exists about the potential association between the *DRD4* polymorphism and behavior in rhesus macaques. In fact, the only published studies so far have involved captive vervet monkeys (*Chlorocebus aethiops*). These studies have reported that carriers of low-function *DRD4* alleles are more prone towards novelty-seeking [3] and impulsivity [9].

In this study, we investigated for the first time the *DRD4* polymorphism and its relationship with behavior in the free-ranging rhesus macaque population on the island of Cayo Santiago, PR. This population has been one of most valuable resources for primate behavioral and biomedical research over the past 50 years. Our aims were three-fold. First, we sought to verify that the rhesus macaques in this population display a VTNR polymorphism in exon III of the *DRD4* gene. Second, we investigated the extent to which the different *DRD4* alleles and their distribution in the Cayo Santiago rhesus macaque population are similar or different to those reported for captive rhesus macaques, as well as other nonhuman primates. Finally, we assessed whether the *DRD4* polymorphism in the Cayo Santiago rhesus macaque population shows an association with behaviors related to exploration and risky social responses to other individuals similar to those reported in captive vervet monkeys and in humans.

2. Methods

2.1. Study site and subjects

This study was conducted on Cayo Santiago, a 15.2 ha island located 1 km off the coast of Puerto Rico. A colony of rhesus macaques was established in 1938 with wild individuals captured in India [21]. At the time of the study, the population on Cayo Santiago included approximately 1200 animals split between 9 naturally formed social groups. The subjects of the behavioral study were 46 juveniles ($n = 24$ males) born between August–September 2011 from two of the nine social groups. The behavioral data presented here were collected from June to November 2013, when subjects were aged between 21.32 ± 0.08 and 26.89 ± 0.08 months. In addition to the 46 focal subjects, we were able to collect samples for genotyping from 132 individuals, including the mothers of the focal subjects, for a total of 178 individuals genotyped for the *DRD4* polymorphism.

2.2. Data collection

Behavioral data were collected five days a week from 0700 to 1430 h. Each subject was focally observed for 30 min once a week. Observations were counterbalanced biweekly between morning (0700–1030) and afternoon (1030–1430) observations to control for diurnal effects on behavior. A total of 497.5 h of data were collected on the 46 juvenile subjects across the 24 weeks of observations (on average, 21.6 weekly observations per subject). The following state behaviors were collected on a continuous basis: rest, feed/forage, travel, groom, play, and self-groom. The amount of time the subject was in proximity to the mother (within 3 m) or out of proximity from the mother (more than 3 m) was also recorded continuously. Finally, all agonistic behaviors directed at the subject as well as submissive behaviors initiated by the subject were recorded. Agonistic behaviors included: contact aggression (e.g., bite, slap), non-contact aggression (e.g., chase, lunge), and open-mouth threat. Submissive behaviors included submit-leave (i.e., individual gives submissive posture and moves away from another), submit-stay (i.e., individual gives submissive posture but does not move away from another), and fear-grin [16]. All data were recorded using the Behaviour software on a Psion Workabout. Data were parsed into an Access database (Microsoft Corp., Redmond, WA, USA) and queries were used to obtain frequencies and durations of behaviors.

2.3. Behavioral measures

The three juvenile behaviors studied were chosen due to their relevance to impulsivity or risk-taking. The first, time spent in proximity to the mother, is an indicator of willingness to engage in social and physical exploration. In most NHPs, including rhesus macaques, the mother is essentially the sole provider of resources and protection for the offspring [15]. Therefore, if an individual spends time away from the mother it is able to forge its own social network and explore its environment, but at the risk of being caught in an agonistic interaction without protection. The second variable, avoidant behavior, indicates an individual's risky or bold tendencies to "stand its ground" when approached by an older or more dominant individual. Avoidant behavior is easily and reliably identified and measured in macaques and often correlated with other fearful and submissive behaviors such as the fear grin or bared-teeth display [14,16]. Individual differences in avoidant behavior are relatively stable over time, as they are associated with differences in dominance rank and/or personality [16]. The use of avoidant behavior in our study is similar to the use of "flight initiation distance" (FID) in bird research on boldness. FID is the distance a bird allows a potential predator to approach before it flies away, with shorter distances indicating more risk-prone or bolder birds (e.g., Moller and Garamszegi [18]. Finally, we used a measure of behavioral restlessness, which was operationally defined as the rate at which individuals changed their behavioral state, e.g., from resting to traveling to resting to traveling again. This measure of behavioral restlessness has been used in one of our previous studies of rhesus macaques [11]. In this study, we showed that behavioral restlessness can be reliably measured, that individual differences in restlessness are stable over time, and that they are associated with physiological measures of energetic expenditure. We chose to use a measure of behavioral restlessness over simpler measures of activity levels such as frequency or% of time spent walking or running because our measure has direct parallels with measures of restlessness in human research and because, in humans, restlessness (e.g., fidgeting, being "on the go") is an established diagnostic criterion for ADHD (Diagnostic and Statistical Manual of Mental Disorders, 5th Edition [2]).