Genetic Influences on Behavior in Nonhuman Primates

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15.1 Introduction

The genetic basis for behaviors has been shown in a wide range of species, from singular cellular protozoans to human beings. The development of behaviors is driven by both nature and nurture: behavioral phenotypes are caused by the expression of genes within environments, and these genes change their expression patterns throughout the life of the organism in response to environmental stimuli. Experiences (especially during early ontogenic stages of life) can have long-lasting effects on the behavior patterns of that organism (Breed and Sanchez 2012).

Nonhuman primates are useful for the study of genetic influences of behavior because they have complex behaviors and social structures comparable to humans since they are closely related genetically (Blomquist and Brent 2013). Pedigreed populations have the added advantage in that confounding effects which might obscure the genetic control of behaviors such as diet and environment can be tightly controlled. Another advantage of working with nonhuman primates is that biological samples can be collected more frequently, and from tissues that can be difficult to collect from human subjects (e.g., spinal fluid) (Jasinska et al. 2012). Because of their genetic similarities to humans, nonhuman primates act as model organisms for studying human diseases, many of which—like anxiety, alcoholism and drug addiction—fall within the purview of behavioral genetics.

However, there are major difficulties inhibiting the study of behavior genetics in nonhuman primates. There are few suitable populations of captive or semi-captive animals which have known genetic relations. The majority of behavioral genetic investigations in nonhuman primates have involved studying a very limited set of species such as rhesus macaques (*Macaca mulatta*), baboons *(Papio hamadryas* and *Papio anubis*), vervets (*Chlorocebus aethiops sabaeus*), and chimpanzees (*Pan troglodytes*). These species mimic many aspects of human behavior in that they live in complex societies with defined social roles. They experience frequent social stressors; hence, biological adaptive measures have evolved, many of which mirror the adaptive measures that have evolved in humans. However, this focus on a limited number of species may have contributed to significant bias when attempting to generalize behavioral similarities and differences across all nonhuman primates. This is particularly affected by the fact that two phylogenic branches of the primate evolutionary tree (the prosimians and New World monkeys)

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have been broadly ignored. Thus, the research presented is limited to the species studied, which do not include representatives from all species available for comparison.

This chapter begins with a discussion of heritability in some of the published genes that have been demonstrated to have an effect on the behavior of nonhuman primates, and the mechanisms (if understood) by which the variants within those genes produce observable differences in primate behaviors. While this chapter is focused specifically on the genetic control of nonhuman primate behavior, evidence from other animal models such as mice and humans will be discussed to provide evolutionary context to the discussion. The chapter includes guidelines and recommendations to improve behavioral genetic research, and provides new tools and methods that will take the field into the future.

This is an exciting time for behavioral genetics on nonhuman primates, as the field is in its infancy and there is still much to discover.

15.2 Heritability: Is It Genetic?

Heritability (h^2) is a measure of the amount of a trait that may be genetic, and it is often used to determine whether there is sufficient genetic signal to be used to localize genes. A measure with a larger heritability score may give a higher chance of success of localizing a gene for that trait in that particular population. However, the number of genes involved, the trait architecture, and the effect size of each gene on the behavioral measure are important factors in determining the locus of the genes responsible for the behavioral variation. Linkage-based mapping techniques may have difficulty mapping the genetic loci of a trait, even if that trait has a large h^2, especially if the trait has a polygenic or oligogenic genetic architecture, and those genes each carry only a small effect size (Anderson et al. 2010; Göring et al. 2007).

More specifically, heritability is the proportion of variation attributed to genetics compared to the total variation seen in the phenotypic trait. The remainder of the variation in a given trait can then be explained by individual differences, and by differences in unique and shared environments. Heritability can be quantifiably estimated by decomposing the phenotypic variation using statistical methods such as variance component analyses. There are currently only a few nonhuman primate populations that have been studied for heritability of behavioral traits, due to the requirement of knowing the relatedness or the pedigree status of individuals. There are few 'pedigreed' colonies suitable for studies on the genetic effect of behaviors, so each population is studied for specific behavioral traits of interest to the researchers, using different methods and study populations for each study. Heritability results must be taken in context of the cohort under study, the complexity of the pedigree, and the number of individuals.

One of the colonies that has been studied for genetic inheritance on behavior in nonhuman primates is the Vervet Research Colony (VRC). The VRC pedigree consists of a 16 mutigenerational pedigree, matrilineal colony of vervet monkeys (*C. aethiops sabaeus*). All subjects are raised in social groups that are managed to reflect the natural social composition of vervet groups in the wild. Variance component analyses utilizing the genetic relatedness of each colony member has been used in this population to estimate heritability of several behavioral traits. Novelty-seeking was measured by using a novel (but unthreatening) object in the home enclosure, and is significantly heritable ($h^2 = 0.47 \pm 0.01$, $p < 0.0001$) implying that 47 % of the variation of the trait is under genetic control. (Bailey et al. 2007) Impulsivity and impulsive aggression, measured using the intruder challenge test developed at the VRC, uses the resident-intruder paradigm to assess the behavioral response of an individual to an unfamiliar conspecific on the periphery of the subjects' home enclosure. This challenge elicits species-typical reactions of interest, arousal, and aggression toward a social stranger (the 'intruder') for both males and females, and it amplifies individual differences in characteristic reaction tendencies. Animals scoring high on social impulsivity rush over to the

intruder immediately without taking the time to assess the situation. Social impulsivity is also found to be significantly heritable, (h^2 = 0.35 ± 0.11, $p < 0.0001$) (Fairbanks 2001). Subscales of the index are independently heritable: both impulsive approaching (h^2 = 0.25 ± 0.10, p = 0.0008) and aggressiveness (0.61 ± 0.12, $p < 0.0001$) (Fairbanks et al. 2004).

At the Harlow Primate Laboratory, alcohol consumption was studied using animals drawn from a large ongoing longitudinal study investigating genetic and environmental factors affecting neurobiology, including the behavior of alcohol consumption. In one study of 156 rhesus macaques belonging to a single pedigree who receive identical early rearing backgrounds, the heritability to consume alcohol was also significant, and 19.8 % of the variance was attributable to additive genetic effects (Lorenz et al. 2006).

Two hundred and eighty-five pedigreed rhesus monkeys (*Macaca mulatta*) from both the Harlow Primate Laboratory and the Wisconsin National Primate Research Center (Madison, WI) were studied for heritability of specific behavioral traits. Of the five behaviors studied, two were significant 'freezing duration' (behavioral inhibition) (h^2 = 0.38, p = 0.0120) and 'orienting to the intruder' (vigilance) (h^2 = 0.91, $p < 0.0001$). The other traits—'duration of locomotion', 'hostility', and 'frequency of cooing'—were not significantly heritable (Rogers et al. 2008).

Responses to novel and stressful environments were studied in 85 rhesus monkeys (*M. mulatta*) at the Oregon National Primate Research Center, which has a standard matriarchal colony (Williamson et al. 2006). The traits were tested using a set of temperament-testing paradigms, and heritabilities were estimated using variance component-based quantitative genetic analyses with much of the genetic information arising from paternal half-sibs. Significant heritabilities include latency to leave the mother during the initial 5-min observation period (h^2 = 1.00, $p \leq 0.05$), explore (h^2 = 1.00, $p \leq 0.01$, and movement (h^2 = 1.00, $p \leq 0.05$) during the alone-1 period and movement during the alone-2 period (h^2 = 1.00, $p \leq 0.05$). A factor analysis was also performed on the behaviors and seven factors emerged from the analyses. The only one statistically significant was factor 2, which consisted of movement during the test, and it was highly heritable (h^2 = 1.00, $p \leq 0.01$). Other factors (Factor 7, explore novelty) reached a heritability of 1 but were not significant. Nonsignificant factors included factor 1—distress vocalization, factor 3—distress cues, factor 4—delayed independence, factor 5—early independence, and factor 6—explore familiar environment. The nonsignificance of the higher heritabilities may be a reflection of the small sample size not being large enough for power, resulting in imprecise estimates.

Zoo populations were used to study the heritability of personality on 145 chimpanzees from 13 zoos that participated in the ChimpanZoo Program of the Jane Goodall Institute. Heritability was estimated using the symmetric differences squared (SDS) technique (Weiss et al. 2000). SDS incorporates phenotypic differences among all possible pairs of subjects in the sample, whether related or unrelated of all the traits studied. Only dominance showed significant heritability (h^2 = 0.63, p = 0.0000). Shared zoo effects accounted for only a negligible proportion of the variance for all factors.

Genetic origins of social networks and social behaviors were studied in 107 of the free-ranging rhesus macaques on the island of Cayo Santiago (Brent et al. 2013). The animals were assessed for relatedness, and variance component analyses methods were used to assess heritability. When controlling for age, sex, dominance, rank, and social household effects, a significant heritability (h^2 = 0.84, p = 0.0250) was found with grooming betweenness, which is an index of affiliate social positioning (Brent et al. 2013).

Though calculated with different measures in different populations, these heritabilities show us that many of the behaviors have strong heritable genetic underpinnings and are under genetic control. These results also demonstrate that for many traits, the genetics is not the most important factor driving the variance, and that the environment is also significant. This would make gene detection difficult.

15.3 Understanding the Genetic Control of Behaviors

Genes that control behavioral traits adaptively tend to have broad systemic similarities: they tend to belong to diverse multigene families; they are expressed in the cells of the brain, sensory organs, or other nervous tissue; and they are involved in either the processing of environmental stimuli, the mediation of internal states such as hunger, affiliation, and emotions, or are associated with neuronal development and neuroplasticity necessary for learning (reviewed in Bendesky and Bargmann 2011). Since there are so many genes that participate in each pathway, polymorphisms or variants in several genes can be associated with similar phenotypes and can contribute additively to their severity. It is also probable that different polymorphisms or variants in the same gene can have differing effects on phenotypes. While an exhaustive list of these signaling molecules and the host of genes that interact with them is beyond the scope of this chapter, these three classes of genes neuroreceptors, and transporters will be discussed in the context of two neurotransmitters well-studied in nonhuman primates (dopamine and serotonin) and how they relate to behaviors in nonhuman primates.

15.3.1 The Dopamine and Serotonin System

Both dopamine (*DA*) and serotonin (*5-HT* or 5-hydroxytryptophan) are in a class of neurotransmitters called monoamines, which are simple organic molecules synthesized via enzymatic action from amino acids (tyrosine and tryptophan, respectively). These molecules conduct the action potential of the neuron across the synapse to other connected neurons, exciting or inhibiting their own potential to fire.

Dopamine has been broadly conserved in its role coordinating motor function and reward-based learning across most phyla of the animal kingdom (the arthropods appear to be the sole exemption to this rule). In vertebrates, dopaminergic neurons connect regions of the brain associated with reward-based learning, such as the ventral tegmental area and the nucleus accumbens. In response to primary rewards or stimuli that have become associated with rewards through conditioned learning, these neurons experience phasic activation (increased bursts of action potentials). Dopamine appears to encode a reward prediction error: dopamine release and the phasic activation of dopaminergic neurons strongly increase in respect to rewards that exceed expectation, and drop below the baseline level of activity if the expectations of rewards are not met. Furthermore the release of dopamine drives reward-seeking behavior, increasing the likelihood that the individual will repeat behaviors that have become associated with greater reward expectations (reviewed in Barron et al. 2010).

Increased dopamine signaling is associated with many interrelated behaviors, including reward-seeking, conditioned learning, social dominance and extroversion, aggression, voluntary physical activity and motor control, working memory and focus, and addictive and compulsive behaviors. Many stimulants, such as cocaine and amphetamines, act by increasing the level of dopamine available for signaling in the synaptic cleft, hence why the psychoactive effects of these drugs (arousal, confidence, extroversion, aggression, etc.) are similar to the effects of dopamine signaling. Meanwhile, drugs that reduce dopamine activity, such as neuroleptics, impair concentration, reduce motivation, and cause anhedonia (the inability to experience pleasure).

While dopamine is highly associated with the rewards centers of the brain, serotonergic projections are especially dense in the limbic system, a set of structures that are responsible for the regulation of mood, emotional learning, memory and fear response. Serotonin also plays an important role in development, and many studies into early-life adversity and stress have demonstrated long lasting effects on serotonin signaling in the brain (reviewed in Nordquist and Oreland 2010).

It is also an important modulator of appetite and sleep cycles, mood, and inhibitory control. Low levels of serotonin are associated with depression, anxiety, stress-reactivity, and aggression, as well as increased risk-taking in gambling tasks. All of these conditions share components of increased emotional-reactivity and impulse-control.

Cerebrospinal fluid (CSF) concentrations of the terminal metabolites of dopamine (*HVA*, homovanillic acid) and serotonin (*5-HIAA*, 5-hydroxyindoleacetic acid) have been used as proxy biomarkers (endophenotypes) of overall levels of dopaminergic and serotonergic metabolism. These studies have demonstrated that the variance observed in the concentration of these metabolites are highly heritable and are stable over time and across environment (Freimer et al. 2007; Kaplan et al. 2002).

15.3.2 Neuroreceptor Proteins

Neuroreceptor genes play an especially important role in the modification of behavior. This family of proteins localizes at the synapse of the neuron, and when they bind to their target ligands (e.g., a neurotransmitter, or an odor molecule), they stimulate a molecular cascade, exciting or inhibiting the action potential of the neuron. Most of these receptors are coupled to similar complexes of G-Proteins, and it is through this shared mechanism that both serotonin and dopamine activate or inhibit the activity of the neuron (reviewed in Barnes and Sharp 1999; Bendesky and Bargmann 2011; Callier et al. 2003). However, in the presence of too much of their specific ligand, the receptors can become desensitized to the synaptic signal. Over extended periods, this can lead to a long-term down-regulation of neuroreceptors available at the synapse and a significant decrease in synaptic efficiency, especially with the serotonin receptors, as we will see with the examples of early-life adversity models.

There are five known receptor proteins that bind to dopamine and translate the dopaminergic signal into neural activity, however only two of them, *DRD1* (dopamine receptor *D1*) and *DRD4* (dopamine receptor *D4*) have been studied in respect to nonhuman primate behavior.

DRD1 is the most highly expressed of the five dopamine receptors, and upon binding with its agonist, acts to increase the action potential of the neuron (reviewed in Callier et al. 2003). A single nucleotide polymorphism (SNP) in the 5′ UTR of *DRD1* has been associated with the alcohol consumption of adolescent, male rhesus macaques that had been maternally deprived and peer-reared (Newman et al. 2005). Maternal deprivation is frequently used as a model condition for early-life stress and adversity, and tends to produce anxious and impulsive behaviors during adolescence. Female rhesus macaques and maternally-reared male carriers of this allele did not show increased propensity to consume alcohol, and this allele exemplifies the effect confounding factors such as gender and early rearing experience have on behavior (Newman et al. 2005).

While *DRD1* excites the neuron, *DRD4* is an inhibitory receptor. Its expression throughout the brain is much lower than DRD1, but its binding affinity and selectivity to dopamine is much higher (reviewed in Callier et al. 2003). A VNTR (variable number of tandem repeats) in exon III of the *DRD4* gene has been linked to novelty-seeking behavior in vervet monkeys (*C. aethiops sabaeus*). Carriers of the rare, 5-repeat variant displayed significantly shorter latencies to approach a large and potentially threatening object with which they had no prior experience than were carriers of the more common 6-repeat variant. The variance observed was consistent across age-groups, the only other demographic factor that was shown to significantly account for the variance in observed novelty-seeking scores (Bailey et al. 2007). In addition, juvenile carriers of the 5-repeat variant also scored higher on the Social Impulsivity Index, as measured by the shortness of latency to approach an unfamiliar conspecific with risky, assertive, and aggressive behavior. Social-impulsivity scores were also

influenced by age and sex factors, but also by the genotype of the juvenile's mother, finding that the highest scores occurred in variant-carrying juveniles with variant-carrying mothers (Fairbanks et al. 2012). This illustrates two points: (1) the *DRD4* variant is a risk factor that is influenced by the developmental environment, and (2) almost everyone with any risk genotype also has one or both parents with the risk genotype, so they are likely to have both genetic and environmental influences operating. Similar repeat variations have been detected in humans, dogs, horses, and chimpanzees. In humans, variants have been associated with novelty seeking, risk taking behavior, and Attention Deficit Hyperactivity Disorder (Ptácek et al. 2011).

While the other dopamine receptors have not been fully investigated in the behaviors of non-human primates, they have been studied in respect to other mammalian species. Polymorphisms in *DRD2* (dopamine receptor *D2*, also an inhibitory receptor) have been associated with increased risk for alcoholism (Noble et al. 1998), pathological gambling (Lobo et al. 2010), and other addictive/impulse-control behaviors in humans (Ariza et al. 2012).

While dopamine has five known neuroreceptors in primates, serotonin has fourteen. The sheer number demonstrates the complexity of studying candidate genes. All but one of the receptors operate through the same molecular mechanism of G-protein complexes as the dopamine receptors (reviewed in Barnes and Sharp 1999).

A couple of studies have investigated the impact that rearing-history has on the expression of these receptors in both rhesus macaques (*Macaca mulatta*) and marmoset monkeys (*Callithrix jacchus*). Parental deprivation during infancy in marmosets produces a pro-depressive state, increased stress-reactivity, and general anhedonia that can persist until adolescence. (Law et al. 2009) That same study found that peer-reared marmosets had decreased *5-HTR1A* mRNA (serotonin receptor 1A, as measured by in situ hybridization—ISH—and real-time polymerase chain reaction—RT-PCR–) and binding (via positron emission tomography—PET—imaging techniques) in the hippocampus, a region associated with memory formation that is disproportionately affected by long-term stress, and that *5-HTR1A* mRNA was correlated with cerebrospinal fluid (CSF) concentrations of cortisol, a biomarker for stress-response. Another study (Spinelli et al. 2010) duplicated the same study using rhesus macaques with both magnetic resonance imaging (MRI) and PET scans. They found that peer-reared monkeys had an overall decrease of *5-HTR1A* density and binding throughout the brain. In females, the receptor density in the dorso-medial prefrontal cortex (associated with cognitive decision-making and emotional control) was significantly higher in peer-reared subjects versus their maternally-reared counterparts.

15.3.3 Transporter Proteins

Another important category of neuromodular genes that affect behavior is a class of solute carrier proteins (SLC) or transporter genes associated with each neurotransmitter. These proteins moderate the signal transmission by reuptaking excess dopamine back into the presynaptic neuron and repackaging the neurotransmitter into synaptic vesicles, functionally terminating the neurotransmitter signal and resetting the neuron for the next time it needs to fire. These proteins are the target of several drugs, both therapeutic and illicit. Cocaine competitively binds with the dopamine transporter (*DAT*), preventing the reuptake of dopamine into the presynaptic neuron, while amphetamines reverse DAT activity, pumping dopamine back out into the synaptic cleft. Likewise, the serotonin transporter (*5-HTT* or SERT) is the target of a class of antidepressants called SSRIs (selective serotonin reuptake inhibitors), which functionally increases the amount of serotonin available for signaling. In macaques, both the genes that encode both the dopamine (*DAT*) and serotonin (*5-HTT* or SERT) transporters have alleles that differentially alter the pattern of expression in the brain.

In the *5'UTR* promoter region of *SLC6A3* (the gene that encodes the *DAT* protein), two single nucleotide polymorphisms (SNPs) associated

with transcription factor binding sites have been associated with social rank in cynomolgus macaques (Miller-Butterworth et al. 2007). One of those SNPs was also found in rhesus macaques, and is also associated with social dominance behaviors. While it is not clear how these variants alter the expression of the dopamine transporter, the reduced transcription of *DAT* mRNA correspondingly reduces the density of *DAT* within dopaminergic neurons. This would presumptively increase the concentration of synaptic dopamine available for signaling.

The serotonin transporter (*5-HTT*, encoded by the *SLC6A4* gene [Solute Carrier, family 6, member 4]) together with its linked polymorphic region (*5-HTTLPR*) is probably the most studied gene in nonhuman primate behavior. In both humans and rhesus macaques, there is an analogous 21 bp length variant *rh5-HTTLPR* which is located in the same region as the serotonin transporter gene promoter polymorphism identified in humans. However, it is not in the same precise location. Therefore, two major alleles segregate in both species, descriptively called *Long* (*L*) and *Short* (*S*) for their relative sizes. The core sequence is similar, (*C*)*7 AGCAT*(*C*)6, but there is difference in the variation in allele length for the *L* allele that is usually attributed to the association with human behaviors; humans have 17 repeat units while rhesus macaques have 24 repeat units (Trefilov et al. 2000). Functional studies show similar effects between humans and rhesus polymorphisms in that the *S*-allele results in decreased transcriptional efficiency of the serotonin transporter. Variation in the serotonin transporter gene promoter has been shown to be related to several behavioral traits in humans including anxiety and depression (Goenjian et al. 2012).

In nonhuman primates, these two variants have been associated with observable differences in development and reproductive timing. Studies performed on the free-ranging macaques of Cayo Santiago in Puerto Rico, found that the number of *S*-alleles carried by each monkey was predictive of the age at which male macaques left their natal group. Homozygous S-carriers dispersed approximately 6 months earlier than heterozygotes and 14 months earlier than homozygous L-carriers (Krawczak et al. 2005; Trefilov et al. 2000).

The serotonin transporter has also been implicated in several social behaviors, notably the construction of social dominance hierarchies. Social dominance hierarchies represent a collection of behaviors observed in many species of captive and free-ranging nonhuman primates. Dominant individuals tend to be more aggressive, initiate agonistic encounters, display attack gestures and vocalizations, and consistently defeat lower ranking conspecifics. Subordinates display gestures and vocalizations associated with submission and flight, and tended to flee or cower when placed in agonist encounters with a dominant individual (Miller-Butterworth et al. 2007). While many factors can influence social dominance hierarchies such as personality, early-life history, physiological traits such as size, and the immediate social environment, both male and female macaques tended to retain the same relative dominance status even when assigned to different social groups (reviewed in Miller-Butterworth et al. 2008). As stated before, CSF *5-HIAA* concentrations (a biomarker of overall serotonin metabolism) have been positively associated with increased social status in female cynomolgus macaques and negatively associated with social status in males, and *5-HTTLPR* genotypes and early-rearing experience have been shown to affect CSF *5-HIAA* concentrations. (Bennett et al. 2002) In a study of psychosocial stress in the form of social reorganization and subordinate social status, 40 females were drawn from middle ranking genealogies of several large social grounds and reorganized into groups: those dependent on *5-HTTLPR* genotypes; those with only *LL*-homozygote individuals; and those in which all individuals had at least one *S* allele. Most of the measures (morning cortisol concentrations, glucocorticoid negative feedback, weight loss, and abdominal fat loss) were not significantly associated with genotype. There appeared to be an interaction with social status, genotype, and changes in serum concentrations of leptin and triiodiothyronine. Dominant *LL*-homozygote females had the highest levels while subordinate

S-variant females had the lowest level (Jarrell et al. 2008).

Watson et al. (2009) found that male rhesus *S*-allele carriers spent less time looking at the eye region of faces, and had larger pupil diameter when gazing at photographs of familiar high-status males from the same cohort. They also experienced higher risk-aversion on gambling tasks when presented in conjunction with another high-status individual. In the same activity, *LL*-homozygotes demonstrated increased risk-seeking behavior.

In the study of social networks in free-ranging rhesus macaques on the island of Cayo Santiago (Brent et al. 2013), one measure of sociality was associated with serotonergic genes profiles. Specifically the 'grooming eigenvector', which represented the tendency of individuals to spend a lot of time in grooming behaviors, was associated with an interaction of the *5-HTTLPR* and *TPH2* genotypes (tryptophan hydroxylase 2 is the rate-limiting enzyme required for serotonin biosynthesis in the brain).

A study on the prevalence of social dominance behaviors in respect to seven different species of macaque showed that the relation to social organization may be more controlled by genetic factors than by environmental ones. They found that species which displayed relaxed patterns of dominance, open relationships, and higher levels of conciliatory behavior tended to be monomorphic in the upstream promoter region of the *rh5-HTT* gene. Rhesus macaques (*Macaca mulatta*), the most stratified species of macaques, had three variants. This relationship of hierarchical social dominance to the amount of allelic variation was also linked to the polymorphic region in the monoamine oxidase A (MAO-A) gene, an enzyme required for the degradation of several monoamine neurotransmitters including both dopamine and serotonin (Wendland et al. 2006).

The last group of related behavioral traits associated with the serotonin transporter has to do with stress-reactivity and anxiety, two traits which show a strong gene-environment interaction between the *5-HTTLPR* and the prior experience of stress (usually modeled in experiments by maternal-deprivation and peer-rearing) (Barr et al. 2003, 2004). The limbic-hypothalamus-pituitary-adrenal (LHPA) axis is the central mechanism by which the nervous system and endocrine system modulate the reaction to stress and the fight-or-flight response. Because the LHPA axis is so well understood, studies frequently use serum concentrations of many of the hormones described above as endophenotypes for these behaviors. The reaction to stress is quantified by a baseline (nonstressed) measure, and by another reading following the exposure to stress. Researchers have used several methods to model ethologically-relevant stressors including social separation, threat (usually introducing a plastic snake or a fake predator), intrusion by an unknown conspecific, intrusion by a human researcher (nonthreatening), and relocation. Each of these stressors produce distinct behavioral responses organized through different parts of the limbic system, and produce similar interactions with the LHPA axis.

The *5-HTTLPR* variants have been associated with differences in the serum concentrations of the stress hormones, and this interacted with a history of environmental stressors (peer-reared vs. mother-reared). Rhesus macaques with the *LS*-genotype and peer-reared macaques each showed increased adrenocorticotropic hormone (ACTH) release in response to stress, and together these conditions increased the release of ACTH synergistically. (Barr et al. 2004) Another study found that mother macaques with the *LL*-genotype had consistent serum cortisol levels over the course of 6 months of study, while mothers with the *LS*-genotype showed significantly greater fluctuations in this trait over the same period. These *LS* mothers were also found to be more likely to be abusive to their infants (McCormack et al. 2009).

In addition, studies using PET scans and fMRI (functional magnetic resonance imaging) have found that *rh5-HTTLPR* S-carriers demonstrated increased limbic reactivity in response to specific aversive stimuli. For example, S-carriers displayed increased metabolic activity (measured using fluorodeoxyglucose PET imaging) in the

amygdala in response to relocation stress, and the bed nucleus of the stria terminalis (BNST) in response to threat (Kalin et al. 2008). The metabolic activity of the BNST has been shown to be highly predictive of the "freezing" response of monkeys in response to threats (Oler et al. 2010; Rogers et al. 2008). Oler et al. (2009) used PET imaging to demonstrate that *5-HTT* availability (an index of its density and binding affinity) in the amygdala, hippocampal, and BNST regions correlated positively with several behavioral and neuroendocrine measures of anxious temperaments.

15.3.4 Interaction of Dopamine and Serotonin Signaling

The story becomes even more complex when considering the fact that many genes affect both dopamine and serotonin. There are many other genes important in the dopamine pathway, such as *COMT* (*catechol-O-methyl transferase*), *DBH* (*dopamine β hydroxylase*), and Tyrosine hydroxylase (*TH*), which is an enzyme responsible for catalyzing the rate-controlling step in dopamine biosynthesis. Most genes and variants have not been thoroughly studied in humans, let alone in nonhuman primates.

In cynomolgus macaques (*Macaca fascicularis*), social dominance rank illustrates the interaction between these two system. Both dominant males and females had significantly higher HVA concentrations than subordinates. Dominant males (but not females) had significantly lower CSF 5-HIAA concentrations (Kaplan et al. 2002; Riddick et al. 2009). In a study of free-ranging rhesus macaques, low CSF *5-HIAA* concentrations early in life were associated with delayed migration from the natal group, and increased aggression and premature death, but the individuals who survived were more likely to attain higher social ranks (Howell et al. 2007).

15.3.5 Caveats and Guidelines

While studying genetic influences on behaviors in nonhuman primates is very promising, there are several caveats of which we must be aware. Primary among them is the question of whether the comparison of phenotypes between species is valid and meaningful.

There are issues with cross-species analyses and analogous behavioral traits. Some traits, such as aggression, dominance, and extroversion, may be more comparable than others like anxiety. It is unclear if the anxieties found amongst different nonhuman primates are comparable. Is the anxiety of a social primate the same as the anxiety of a nonsocial primate? Does it matter if the trait is measured directly or is a composite or endophenotype? It is not inconceivable to think there would be differences at least in the manifestation of social anxiety. In a genetic sense, these traits are phenocopies of each other, and have different genetic mechanisms. So this leads us to question whether we can expect replication in genetics of primate behaviors if the phenotypes under study are not similar.

Many of these traits are obviously present in humans; however, it is unclear exactly how to map them back for specific comparison. This would be necessary to detect similar genetic mechanisms. For example, there are several types of anxiety defined by psychiatrists in the DSM (Diagnostic and Statistical Manual): generalized anxiety; social anxiety; and 'anxiety disorders' such as posttraumatic stress disorder (PTSD) and obsessive compulsive disorder (OCD). It is suspected that in humans, these anxieties have unique and shared genes (Domschke and Deckert 2012), though it is unclear how to map or to correlate the excitability/anxiety found in nonhuman primates to any or all of these anxiety disorders.

As mentioned previously, the current results in this field are biased because of species and phenotypes studied. Species bias is an issue because most research is performed on a very small subset of primate species, as there are few controlled populations where genetic relations between individuals are known.

Associated with this is another issue of variant detection bias. Most variants that are tested are genotyped using specific PCR primers, such as for the *5-HTTLPR* variants that have already been associated with some easily quantified

behavioral phenotype. This is the "low-hanging fruit", and it only tests for one particular variant within a given gene of interest, ignoring other variants that might also contribute to the phenotype. Also the PCR'd genotype does not necessarily mean that the specific variant that was studied is disease-causing. It could be possible that the PCR'd genotype and the actual-disease causing variant are close together and are in high degree of linkage disequilibrium.

Association studies require large, out-bred populations (such as humans) to detect possibly causal variants. Most of the primate populations used for research are controlled, and are thus at least somewhat inbred, which can dramatically reduce the power of the method to detect phenotype affecting variants, unless methods such as linkage analysis that exploit the relatedness in pedigrees are used.

Another way to discover genes is to perform linkage analyses, either of specific genes or chromosomal regions or a 'genome scan' that searches markers over all the chromosomes. An advantage of a 'genome scan' over candidate gene studies is that it allows for the discovery of novel genes, since in a candidate gene study one has to first have a candidate gene. In a genome scan one can detect new candidate genes. In order to do linkage genome scans, however, one needs a genetic marker map of the species under study.

15.4 Conclusions and Future Directions

The study of Genetic Influences on Behavior in Nonhuman Primates is in its infancy, and there is much room to grow. Up to this point, most of the work has been in calculation of determining traits, and in calculation of heritabilities, and in examining a limited number of candidate genes. In order for the field to develop, there is a need to have reliable, valid phenotypes, and to thoroughly test each gene hypothesis, including all potential variants. Researchers in the field need to be cognizant that the phenotypes and genotypes may not be comparable between species (or even subspecies) and that this problem is probably even more complex than hypothesized.

There are many new tools emerging from the human genome project (as described elsewhere in this volume) which will prove to be very useful in the study of the genetic influences on behaviors in nonhuman primates. Chief among these are the databases of comparative genomics, of proteomics, and of all the sequenced species. New maps will be developed, including new sequenced maps. New techniques like Next Generation or Deep Sequencing will allow us to study the genomes in depth and to more exactly determine genetic variants responsible for observable behavioral variation in nonhuman primates.

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