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Polymorphisms in the 3' UTR of the serotonin transporter are associated with cognitive flexibility in rhesus macaques

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Abstract

The serotonin system is an important neurophysiological mediator of many behavioral phenotypes. Genetic variation within this system is thought to contribute not only to the natural range of behavioral differences, but also to neuropsychiatric pathologies. Cognitive flexibility, the ability to change patterns of response as reward context shifts, is an important trait that underlies many complex social interactions. Environmental manipulations of the serotonin system have been shown to alter performance on tests measuring cognitive flexibility. Variation at the serotonin transporter promoter region (*5HTTLPR*) has recently been shown to associate with the performance of rhesus monkeys on an object discrimination reversal learning task [Izquierdo et al. 2007]. Here, we demonstrate that functional genetic variation at the serotonin transporter 3' untranslated region, independent of *5HTTLPR*, also associates with performance in an object discrimination reversal learning task in rhesus macaques. The polymorphisms comprising the T:G:T haplotype (T1970, G1991, and T2327) were associated with fewer errors on a reversal learning test and greater levels of cognitive flexibility. We have previously demonstrated that the T:G:T haplotype renders lower levels of gene expression *in vitro*, paralleling the functionality of human 3' UTR haplotypes, as well as the short allele of *5HTTLPR* found in both macaques and humans. The 3' UTR haplotypes are independent and in linkage equilibrium with the *5HTTLPR* locus. Together, these data lead to the intriguing possibility that differences observed in human cognitive flexibility, whether naturally or in pathological states, may be associated with genetic variation in the serotonin transporter 3' untranslated region also.

Introduction

The serotonin system is involved in numerous physiological processes, in particular those related with behavior and mood. Dysfunction in the system has been associated with many neuropsychiatric diseases including depression [Charney 1998; Owens and Nemeroff 1994], anxiety [Leonardo and Hen 2006], autism [Pardo and Eberhart 2007], schizophrenia [Prasad et al. 2002], and addiction disorders [Filip et al. 2005; Johnson 2004; Koob 2000; Muller et al. 2007]. One area of behavior in which the serotonin system is emerging as an important player is decision making, specifically cognitive flexibility with regards to assessment of reward value. Cognitive flexibility, the ability to change patterns of response as reward context shifts, is an important trait that underlies many complex social interactions. While it is often difficult to tease apart complex phenotypes that serotonergic dysfunction may affect in multiple ways (for instance suicide and depression/anxiety or drug abuse and the

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pharmacologic effects of the substances), variation in serotonergic genes has been associated with differences in decision making in suicide attempters [Jollant et al. 2005]; [Jollant et al. 2007].

Tryptophan is the amino acid precursor to serotonin and thus lowering tryptophan levels causes decreases in serotonin levels. Studies in which individuals are depleted of tryptophan have shown changes in decision-making in visual discrimination learning, attention control and gambling paradigms that are apparently modulated through reward perception and sensitivity to reward cues [Rogers et al. 1999; Rogers et al. 2003]. In humans, serotonergic dysfunction and the associated effects on reward-mediated learning have also been observed in pathological gamblers [Nordin and Sjodin 2006]. Accordingly, genetic variation that affects serotonergic tone, including variation at the serotonin transporter (*SLC6A4*, *SERT*) and monoamine oxidase A (*MAOA*), has been associated with pathological gambling and impulse-control disorders [Perez de Castro et al. 1999; Perez de Castro et al. 2002]. Additional associations have been found linking the serotonin system to eating disorders [Di Bella et al. 2000; Fumeron et al. 2001; Matsushita et al. 2002], compulsive shopping [Devor et al. 1999], impulsivity generally [Lee et al. 2003; Sakado et al. 2003], and numerous substance abuse disorders [Glatt and Reus 2003; Lin and Madras 2006]. It should be noted, however, that these studies show wide variability in replication, possibly reflecting population differences and the underlying genetic architectures of the disease (e.g., schizophrenia, [Fan and Sklar 2005; Schinka et al. 2004; Sen et al. 2004]).

A uniting factor for all of these diseases and behaviors is a deficit in impulse control. It is unsurprising therefore that the serotonin system has generally and repeatedly been associated with changes in impulsivity and its associated measures [Clark et al. 2004; Soubrie 1986]. Impulsivity itself, however, is a difficult multi-faceted trait to analyze with constituent characters including not only the loss of inhibitory control of response, but also inability to delay gratification and/or poor attention skills [Winstanley 2007]. The role of serotonin in each of these dimensions of impulsivity is still a subject of debate. Indeed the interrelatedness of the individual traits has made dissection difficult. Nevertheless, there is an increasingly rich literature that suggests that serotonin has a specific effect on both inhibitory control [Clarke et al. 2004; Liu et al. 2004] as well as aversive signal processing [Evers et al. 2005; Graeff et al. 1986].

One specific measure of cognitive flexibility focusing on understanding changes in reward context is object reversal learning. In these tests, subjects are first taught to associate a reward with a specific stimulus before the association is switched to a previously unreinforced stimulus. The difficulty of the subjects in this reversal task is thus a measure of cognitive flexibility. The perseverative response effect seen in object discrimination tasks, especially in non-human primates, is generally believed to reflect losses in inhibitory control, though the absence of aversive cues precludes the specific association of serotonin to impulsivity through a solely inhibitory mechanism. Tryptophan depletion in both humans [Rogers et al. 1999] and non-human primate model systems [Clarke et al. 2004; Clarke et al. 2005; Clarke et al. 2007] is associated with impaired performance and similar results are also observed following damage to specific regions of the prefrontal cortex [Dias et al. 1996; Fellows and Farah 2003; Izquierdo et al. 2004; Jones and Mishkin 1972]. These studies, as well as neuroimaging studies, have firmly located control for decision-making processes in the prefrontal cortex and particularly in the orbitofrontal cortex [Lee et al. 2007; Murray et al. 2007; Rahman et al. 2001].

These findings are of particular interest because of their relationship with substance abuse and addiction problems generally. Users of illicit drugs show impaired performance on numerous cognitive tasks including those which measure impairment in decision-making

related to risk in the context of gambling tasks and these deficits correlate to aberrations in frontal lobe functional activation of abusers in response to error signals [Bechara et al. 2001; Garavan and Stout 2005; Grant et al. 2000; Rogers and Robbins 2001]. Similar patterns are also seen in non-human primate models of drug abuse [Jentsch et al. 2002; Liu et al. 2007; Verrico et al. 2008]. While many drugs of abuse have specific effects on brain systems that control cognitive flexibility, it is becoming more widely accepted that preexisting deficits in these systems may underlie an increasing vulnerability to addiction [Dom et al. 2005; Yucel et al. 2007]. This belief is buoyed by the fact that prefrontal cortex-mediated cognitive deficits are pervasive across addictions regardless of substance, including addictions without direct pharmacological effects such as gambling [Goudriaan et al. 2004]. More broadly, there appears to be an association between reward and learning which acts across neurotransmitter systems, including the serotonin system, and brain regions, including the regions associated with cognition [Fallon et al. 2007; Lajtha 2008].

Genetic variation has been identified in humans with functional consequences both *in vitro* and *in vivo*. In particular, studies have found functional polymorphisms in the primary serotonin synthesis gene tryptophan hydroxylase 2 (*TPH2*; [Brown et al. 2005; Chen et al. 2008; Zhang et al. 2005]), the serotonin transporter (*SLC6A4*, *SERT*; [Heils et al. 1995; Heils et al. 1996; Lesch et al. 1996]), the serotonin receptors (specifically *HTR1A* [Wu and Comings 1999] and *HTR2C* [Lappalainen et al. 1995]), and the serotonin catabolism gene monoamine oxidase A (*MAOA*; [Sabol et al. 1998]). Interestingly, for several of these genes, including the serotonin transporter, the functional variation in humans is paralleled by similar but not identical polymorphic variations in rhesus macaques [Bennett et al. 2002; Lesch et al. 1997; Newman et al. 2005; Trefilov et al. 2000; Trefilov et al. 1999].

Variation at the serotonin transporter promoter region (*5HTTLPR*) has recently been shown to associate with the performance of rhesus monkeys on an object discrimination reversal learning task [Izquierdo et al. 2007]. The results were not, however, in the predicted direction given the current understandings of the functional effects of the polymorphism and the role of serotonergic tone on cognitive flexibility; *5HTTLPR* short alleles rendering lower SERT expression and presumably higher extracellular serotonin levels were associated with poorer performance on reversal learning tasks. Recently, our laboratory has demonstrated an additional, independent, source of genetic variation in the 3' untranslated region of the serotonin transporter [Vallender et al. 2008]. This regulatory region is located approximately 40 kb downstream of, and is in complete linkage equilibrium with, the promoter variation. Three SNPs exist in this region in our population of rhesus macaques, all at intermediate frequencies: C1970T, G1991C, and T2327C. From these SNPs two haplotypes predominate, a high-expressing C:C:C allele and a low-expressing T:G:T allele. Interestingly, like the serotonin transporter promoter region variation, this rhesus functional variation at the 3' UTR appears to be mimicked by a similar, though not identical, high frequency polymorphism in the 3' UTR of humans in *in vitro* assays [Vallender et al. 2008].

In this study we attempt to further clarify these understandings. While we hypothesize based on tryptophan depletion and lesion studies that genetic variation that could be predicted to result in lower serotonin levels would associate with a greater number of perseverative errors on an object discrimination reversal learning task, previously described allelic variation at *5HTTLPR* associated with an opposite response [Izquierdo et al. 2007]. This difference may exist because of a compensatory developmental mechanism or epistatic genetic interactions. By considering a second regulatory region at the gene, we may address this question. If the 3' UTR variation behaves in the same fashion as the promoter variation, a common developmental mechanism is likely at work; but if the 3' UTR variation behaves in an opposite fashion, then it is likely that there are epistatic interactions within the gene which also effect the overall behavioral response.

Here we investigate the relationship between variation at the serotonin transporter gene, both at *5HTTLPR* as well as at the 3' UTR, and cognitive flexibility as measured by an object discrimination reversal learning task in rhesus macaques. Our findings offer insight into the genetic basis underlying decision making behaviors in rhesus macaques and, by extension through possible parallel functional genetic effects, in humans. This offers not only the ability to understand the normal range of primate and human behaviors, but also the deficits in cognitive flexibility that are associated with certain neuropsychiatric diseases and neurological pathologies.

Materials and methods

Subjects

15 male Indian-origin rhesus macaques (*Macaca mulatta*), weighing on average 15.7 ± 2.1 kg and with an average age of 16 ± 5 years, were individually housed in a shared room with a 12 hour light-dark cycle. The subjects were given unlimited access to water and maintained on a restricted diet of standard monkey chow (Teklad Monkey Diet, Harlan Teklad). The fifteen subjects were largely unrelated; the only exception being two animals who shared a single grandparent (139–84 and 246–94) and an additional animal with which they were first half-cousins twice removed (228–94). All animals were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and the Guide for Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, publication No. (NIH)85–23. The New England Primate Research Center is accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC).

The animals used in this study are part of a larger cohort aimed at understanding self-injurious behavior (SIB). They include both self-wounders and control animals. Animals were identified for inclusion in the larger cohort based on behavioral observation during routine maintenance. There was no correlation between SIB and results of the present study whether as regards the ability or willingness to take part in the behavioral tasks or in the ultimate results of those tasks. Behavioral and experimental histories of the animals did not vary systematically with either genotype or test performance. During the present study animals did not receive any pharmacological agent.

Cognitive testing was done between 1:00 and 6:00 PM. Highly palatable food rewards were used during testing ameliorating the need for restrictive diets (M&M's, Mars Inc.; Smarties, Ce De Candy Inc.; marshmallows, Kraft Foods Inc.; dried cranberries, Ocean Spray). Individual subjects showed preferences for specific rewards and these were used to ensure appropriate motivation. Testing was performed by a single researcher with previous experience and training on the apparatus [Verrico et al. 2008]. The behavioral researcher was blind to the genotypes of the animals.

Genotyping

Prior to the initiation of the cognitive study, 2–8 ml of venous blood was collected in EDTA-vacutainer tubes and genomic DNA was isolated using the Flexigene kit (Qiagen) following manufacturer's protocols. The animals were genotyped by individuals without knowledge of their performance on the object discrimination reversal learning test. The serotonin transporter promoter region length polymorphism, *5HTTLPR*, was PCR amplified using primers designed against the rhesus genomic sequence (SERT_pro_F: ataccagcacctaaccctaatgt; SERT_pro_R: ggaccgcaaggtggcgcgga). The PCR product was run on a 1.5% agarose gel with short (545 bp) and long (566 bp) bands visually discerned using ethidium bromide staining. Three serotonin transporter 3' UTR polymorphisms were

ascertained using fluorescent hybridization probe melting analysis with the LightCycler 2.0 (Roche Applied Science) and previously described methods [Chen et al. 2006; Vallender et al. 2008].

Object Discrimination Reversal Learning Test

Apparatus—The apparatus used for testing reversal learning was designed and custom-built to be accessible from an individually housed monkey's home cage. This allowed for testing in a relaxed situation with minimal stresses associated with changes in the environment. The apparatus consisted of a tray with three food wells covered by removable objects of various shapes and colors and an opaque screen between the monkey and the tray. This allowed the experimenter to change the position of the test objects and rewards away from the view of the monkey subject. The elevation of the apparatus allowed the subject easy access to the testing platform, wells, and objects. A three-choice paradigm was designed and used to allow for the dissociation of reversal errors (any incorrect selection) into perseverative errors (selection of the object from the previously reinforced positions) and non-reinforced errors (selection of the object never associated with reward).

Acquisition Phase—Each monkey was habituated to the test apparatus and the experimenter. The experimenter had extensive contact with the subjects and experience in running object discrimination reversal learning tests using this equipment. The experimental design was double-blind and thus the trainer was unaware of the monkeys' genotypes during all cognitive testing. Habituation with the apparatus was accomplished through placing food rewards in each of the trays without objects covering them. When the subject had learned to consistently reach for the rewards in each of the wells, which usually occurred within one week of first exposure, the acquisition phase was begun.

During acquisition, the reward was placed in one of the three wells covered with an object of roughly similar size, but varying color and shape. At the beginning of each session, the opaque screen was lowered and the experimenter hid the reward under the positive stimulus. The screen was then raised and the animal subject was allowed to remove one object looking for the reward. The screen was then lowered and the positions of the three stimulus objects were altered while maintaining the association between the positive stimulus and the reward. Once the positive stimulus was identified by the subject, the number of errors ("acquisition errors") made during the next 24 trials was counted. This was repeated on three separate days with the same object as the positive stimulus. Positive stimuli placement across days and animals were randomly chosen in order to minimize well preferences and/or aversions.

Reversal Phase—Following the acquisition phase a reversal component was introduced. During this phase the session again commenced with 12 acquisition trials where the subjects came to associate the reward with a specific stimulus. Animals failing to acquire positive reinforcement at this point were scored as "failure-to-acquire" and dropped from future testing. This occurred for 2 of the 15 monkeys. Note that this was not necessarily due to a cognitive deficit in acquisition, but rather simply a failure of motivation or lack of interest by the animal. The two animals which failed to acquire showed differing genotypes and there does not appear to be any correlation between genotype and this ambivalence.

For animals demonstrating acquisition preceding reversal, the reward was then relocated beneath a previously non-reinforced stimulus for 24 trials. For each trial, the monkey could make the correct choice, the previously reinforced choice ("perseverative error"), or the incorrect, never-reinforced choice ("non-reinforced error"). Any incorrect choice was scored as a "reversal error". Reversal testing was run for five sessions across different days and values were recorded.

Object and Reward Placement—The nature and position of the objects as well as which object was the “positive stimulus” was determined using a random number generator (www.random.org).

Data Analysis—All data was analyzed following previously established methods [Verrico et al. 2008] using GraphPad Prism software (San Diego, CA). Parametric one-way ANOVA statistical analyses were used to assess association between genotype and test performance. The non-parametric Kruskal-Wallis test was also employed to alleviate potential confounds resulting from the non-homogeneous distribution.

Results

The genotypes of the animals at the four serotonin transporter polymorphic positions and the cognitive flexibility results were derived separately and independently, blind to one another. The allelic frequencies at the 3' UTR positions for the animals with behavioral information do not differ significantly from the broad New England Primate Research Center colony, though there are slightly more S alleles at the promoter present ($p \sim 0.09$) (Table 1). Moreover, similar frequencies of extended haplotypes in the 3' UTR are observed in the behaviorally-assessed cohort compared to the broad NEPRC colony. The three 3' UTR SNPs tend to fall out into two predominant haplotypes C:C:C and T:G:T with approximately 10% “others”. As is the case across the colony, promoter region and 3' UTR SNPs are in linkage equilibrium for the two regions separated by 40 kb. All polymorphic sites were in Hardy-Weinberg equilibrium both in the colony as a whole as well as in the specific behaviorally-assessed subset.

The genotypes of the individual animal subjects were associated with the scores on the object discrimination reversal learning task (Table 2). Grouping the animals according to genotype, we determined the association between the genotypes at each of the four variable positions and the number of acquisition, reversal, and perseverative errors (Figure 1). Parametric one-way ANOVA statistical analyses were used to assess significance between the genotypes. Because of the relatively low number of animals we also used the non-parametric Kruskal-Wallis test. These results did not differ from the parametric tests.

Errors during the acquisition phase did not correlate with genotype at any position. Only during the reversal phase did genotypic effects reveal themselves. Both total reversal errors as well as perseverative errors were found to associate with genotypes at the 3' UTR of the serotonin transporter. Specifically, fewer reversal and perseverative errors were associated with the T1970 allele, the G1991 allele, and the T2327 allele (Figure 1, Table 3). These three alleles are in tight linkage disequilibrium and a T:G:T haplotype represents 56.4% of all alleles in the population. Other haplotypes at lower frequencies in the population (including the ancestral C:G:C haplotype and the intermediate C:G:T haplotype) also exist however and complicate interpretation. While statistical power exists to say that alleles at each individual position associate with fewer reversal and perseverative errors, the addition of these rare haplotypes renders the study underpowered to state conclusively that it is the T:G:T haplotype that drives the effect.

At the same time, we see no evidence for association between promoter region genotype and performance on this test. A recent study has found just such an association [Izquierdo et al. 2007]. That work, however, found an association between the S/S genotype and an increase in reversal errors without any difference between the S/L and L/L genotypes. In our present study none of the animals had the S/S genotype that deviated in this prior group. Here, we recapitulate the observation that S/L and L/L genotypes are statistically indistinguishable as regards their number of reversal errors (S/L: 71.3 ± 20.1 , L/L: 55.8 ± 15.8) or

perseverative errors (S/L: 57.4 $\pm$ 19.8, L/L: 44.8 $\pm$ 16.2). Also, the lack of the S/S genotype in our animals may have averted its potential influence on 3' UTR polymorphism functionality.

We have also tested for other potential variables that may have confounded the study. These include age at time of testing, weight, behavioral and experimental history, order of testing, and relative location within the room. None of these associate in any way with any of the measures of the object discrimination reversal learning test in our animals (data not shown). This is not to say that these do not represent legitimate confounds, but rather that within the animals included here variation in these parameters was not sufficient to produce an effect.

Discussion

Cognitive flexibility as a concept encompasses many dimensions. Object discrimination reversal learning tasks, wherein a test subject is required to associate a novel object with a reward following a reversal in object-reward pairings, is believed to test a specific aspect of cognitive flexibility, inhibitory control [Evers et al. 2005; Soubrie 1986]. Serotonin effects on impulsivity have been studied in non-human primate model systems, with an eye towards understanding genetic and environmental effects as well as social and behavioral consequences [Fairbanks et al. 1999; Higley and Linnoila 1997]. By focusing efforts on understanding the effects of different genetic variation within the serotonergic system, it becomes possible to begin the work of understanding the genetic mechanisms linking genotype to phenotype.

We have recently demonstrated that, in addition to the commonly referenced serotonin transporter promoter region polymorphism *5HTTLPR*, variation at the 3' UTR in both human and rhesus monkey has effects on gene expression *in vitro* [Vallender et al. 2008]. The rhesus C:C:C 3' UTR haplotype rendered significantly greater expression than the T:G:T 3' UTR haplotype. Speculatively, a greater level of expression of the serotonin transporter could be hypothesized to correlate with a decrease in extracellular serotonin as reuptake would be increased. However, previous studies of the promoter variation effects in both humans and rhesus macaques have reported an association of lower expression alleles with lower serotonin metabolite concentrations in cerebrospinal fluid, seemingly contradicting the most parsimonious reasoning [Bennett et al. 2002; Heils et al. 1996]. The reason for this discrepancy is unclear, but has been suggested to reflect developmental consequences that compensate for the proximate effects [Bennett et al. 2002; Cools et al. 2008]. Based on the previous studies of tryptophan depletion and neurological injury, it is reasonable to conclude that genetic variation resulting in lower levels of serotonin would also lead to lower levels of perseverative error, though whether the relative effects produced by genetic variation would be enough to mimic the larger effects caused by environmental manipulations remains unclear.

Individually, the T1970 allele, the G1991 allele, and the T2327 allele associated with better performance (i.e. fewer errors) on the reversal and perseverative portions of the object discrimination reversal learning task. That the three should all go hand-in-hand is unsurprising. The T:G:T haplotype makes up 56.4% of the SERT 3' UTR alleles in the population while the C:C:C haplotype forms and additional 38.5%. Minor haplotypes, including the ancestral C:G:C haplotype and the intermediate C:G:T haplotype, represent the remaining 5%. The inclusion of these rare haplotypes, however, increases the number of degrees of freedom in statistical tests and renders the study underpowered at the haplotypic level.

Interestingly, the strongest difference was observed between individuals homozygous for the T1970 allele. This also appears to be the final mutation to occur creating the T:G:T haplotype, which then went on to increase rapidly in the population to greater than 50%. While greater work is required to establish the impact of selection on this region, the suggestive data that does exist points towards the T1970 allele as the substrate. That this position should also be the most strongly associated with a cognitive flexibility phenotype in a species like rhesus macaques known for its environmental adaptability offers an intriguing story. Nevertheless, this story is far from proven. Not only is more work required to establish the evolutionary history of the locus and the possible role of selection in shaping it, but also significant work is required to demonstrate that indeed C1970T is the causative SNP. *In vitro* work can offer clues, but the most informative data set would include a greater number of animals with the rare haplotypes that dissociate T1970 from G1991 and T2327.

A previous study showed a correlation between the rhesus serotonin transporter promoter region length polymorphism and cognitive flexibility [Izquierdo et al. 2007]. In that study the S/S genotype, which confers lower expression of the serotonin transporter, was associated with decreased levels of cognitive flexibility. This finding is in accordance with the hypothesis that lower serotonin levels associate with greater numbers of perseverative errors, illustrated by serotonin depletion studies [Clarke et al. 2004; Clarke et al. 2005; Clarke et al. 2007; Rogers et al. 1999]. It remains a paradox however that lower SERT expression should result in lower serotonin levels. The authors suggest that perhaps this is the result of a compensatory mechanism during development.

This would explain the differences between persistent lower extracellular serotonin levels through development, such as those conferred by genetic factors, and acute serotonin depletion as can be manipulated environmentally. These hypotheses are not invalidated by the current study. Rather, we agree that there are likely developmental effects of persistent lower levels of synaptic serotonin that makes comparisons between genetic and environmental effects difficult. What is possible, however, is that there is additional genetic variation affecting serotonergic tone that is contributing to cognitive flexibility.

The serotonin transporter C:C:C 3' UTR haplotype, like the promoter region L haplotype, results in higher expression *in vitro*, while the T:G:T haplotype has similar effects to the S allele. In the measuring of perseverative errors on a reversal learning task, however, these similarities are not borne out. We found that the individual SNPs composing the T:G:T haplotype are associated with significantly greater cognitive flexibility than those composing the C:C:C haplotype. This is opposite to the pattern predicted by previous promoter region work [Izquierdo et al. 2007], but more in keeping with the understanding that lessened extracellular serotonin is associated with cognitive inflexibility. There are several possibilities that present themselves to explain this seeming paradox. The first and most central perhaps, is that we do not, as of yet, have a good understanding of the relative effects of the promoter region variation and the 3' UTR variation. It is possible that the effects of one of the two may overwhelm the other. Without any animals with the S/S promoter region genotype with genotypic information available at the 3' UTR positions we cannot yet say with certainty. This is obviously an area of ongoing study. It is also possible that one of the two studies presents a false positive as a result of small sample numbers. Very likely too, there is additional functional variation in the *SERT* gene that has yet to be identified.

It is also important to note that while there is genetic comparability between human and rhesus macaque genetic variation at the serotonin transporter locus, there remain significant developmental and neuroanatomical differences. The observation that genetic influences at the transporter can affect cognitive flexibility in the rhesus macaque cannot be *a priori*

extended into humans. It is likely that from a systems perspective there are numerous modulatory influences on all aspects of cognition and learning, and that the relative impact of these changes in rhesus compared to humans may be different.

Nevertheless, two separate studies of variation at the serotonin transporter gene now exist that report an association with variable success in an object discrimination reversal learning task that measures cognitive flexibility in rhesus macaques. Coupled with the findings of studies that alter either serotonin levels environmentally or serotonergic signaling more broadly through injury, it seems likely that the ability to shift understandings of reward context is mediated by this system and likely can be influenced by genetic factors. This offers an initial glimpse at a genetic influence on a basic and far-reaching behavior and raises questions on its implications for not only disease, but for differences in healthy behaviors as well.

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Figure 1. Cognitive measures grouped by genotype at each of four polymorphic positions. **Left.** Acquisition errors: No significant differences seen. **Center.** Reversal errors: Significantly ($p < 0.05$) fewer errors for T/T, G/G, and T/T genotypes (1970, 1991, and 2327 respectively) compared to others. **Right.** Perseverative errors: Same pattern of significance ($p < 0.05$ by ANOVA) as seen for reversal errors.

Table 1

Frequency of the four polymorphic variants at the serotonin transporter locus in the NEPRC colony and in the behaviorally-assessed cohort presented here.

	Frequency	
	Colony	Cohort
n	200	15
5-HTTLPR		
long	82.2%	70.0%
short	17.8%	30.0%
C1970T		
C	44.9%	46.7%
T	55.1%	53.3%
G1991C		
G	56.0%	53.3%
C	44.0%	46.7%
T2327C		
T	58.2%	53.6%
C	41.8%	6.4%

Table 2

SERT promoter (5-HTTLPR) and 3'-UTR (C1970T, G1991C, T2327C) genotypes and cognitive measures for 15 animals. Acquisition errors were over 72 trials, reversal and perseverative errors were over 120 trials. Note that 228-94 and 533-99 failed acquisition tests prior to reversal.

Animal	5-HTTLPR	SERT 3' UTR			Errors		
		C1970T	G1991C	T2327C	Acquisition	Reversal	Perseverative
139-84	SL	CT	GC	TC	15	56	55
155-90	SL	CT	GC	TC	27	88	58
158-84	SL	CT	GC	TC	20	61	43
173-95	LL	CT	GC	TC	14	60	46
228-94	SL	CC	CC	CC	29		
245-82	SL	CC	CC	CC	23	87	79
246-94	LL	CT	CC	TC	21	81	72
285-88	SL	CC	CC	CC	9	57	44
533-99	LL	CT	GG	TT	27		
540-99	SL	CT	GG	TC	23	76	51
569-92	LL	TT	GC	TT	33	49	31
570-92	SL	TT	GG	TT	29	43	35
573-92	SL	CT	GC	TC	18	102	94
574-92	LL	TT	GG	TT	17	40	35
578-92	LL	TT	GG	TC	22	49	40

Table 3

Means and standard deviations for acquisition, reversal, and perseverative errors across genotypes. Significant ($p < 0.05$) differences in reversal and perseverative errors are observed for T/T, G/G, and T/T genotypes (1970, 1991, and 2327 respectively) compared to other genotypes.

	5-HTTLPR		C1970T		G1991C			T2327C		
	L/L	S/L	C/C	C/T	T/T	G/G	G/C	C/C	T/T	C/C
Acquisition	21.4	20.5	20.3	20.6	25.3	23.6	21.2	20.5	26.5	20.3
	± 7.2	± 6.5	± 10.3	± 4.9	± 7.1	± 4.7	± 7.4	± 8.4	± 6.8	± 10.3
Reversal	55.8	71.3	72	54.9	45.3	52	69.3	75	44	72
	± 15.8	± 20.1	± 21.2	± 16.9	± 4.5	± 16.4	± 20.8	± 15.9	± 4.6	± 21.2
Perseverative	44.8	57.4	61.5	59.9	35.3	40.3	54.5	65	33.7	61.5
	± 16.2	± 19.8	± 24.7	± 17.8	± 3.7	± 7.5	± 21.6	± 18.5	± 2.3	± 24.7