

Differential serotonin transport is linked to the *rh5-HTTLPR* in peripheral blood cells

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The human serotonin transporter (*SERT*) gene possesses a 43-base pair (bp) insertion-deletion promoter polymorphism, the *h5-HTTLPR*. Genotype at this locus correlates with variation in anxiety-related personality traits and risk for major depressive disorder in many studies. Yet, the complex effects of the *h5-HTTLPR*, in combination with closely associated single-nucleotide polymorphisms (SNPs), continue to be debated. Moreover, although *SERT* is of high clinical significance, transporter function *in vivo* remains difficult to assess. Rhesus express a promoter polymorphism related to the *h5-HTTLPR*. The *rh5-HTTLPR* has been linked to differences in stress-related behavior and cognitive flexibility, although allelic variations in serotonin uptake have not been investigated. We studied the serotonin system as it relates to the *5-HTTLPR* in rhesus peripheral blood cells. Sequencing of the *rh5-HTTLPR* revealed a 23-bp insertion, which is somewhat longer than originally reported. Consistent with previous reports, no SNPs in the *rh5-HTTLPR* and surrounding genomic regions were detected in the individuals studied. Reductions in serotonin uptake rates, cell surface *SERT* binding, and 5-hydroxyindoleacetic acid/serotonin ratios, but not *SERT* mRNA levels, were associated with the *rh5-HTTLPR* short allele. Thus, serotonin uptake rates are differentiable with respect to the *5-HTTLPR* in an easily accessible native peripheral tissue. In light of these findings, we foresee that primary blood cells, in combination with high sensitivity functional measurements enabled by chronoamperometry, will be important for investigating alterations in serotonin uptake associated with genetic variability and antidepressant responsiveness in humans.

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Introduction

A number of polymorphisms in human genes coding for key proteins that regulate serotonin (5-HT) neurotransmission have been discovered. Among these are functional polymorphisms in the transcriptional control and noncoding regions of the serotonin transporter gene (*SERT*; *SLC6A4*).^{1–4} A 43-base pair (bp) insertion/deletion polymorphism in the promoter region of the human *SERT* gene, termed the 5-HT transporter-linked polymorphic region (*h5-HTTLPR*; Figure 1), has received considerable attention owing to its purported relationship with anxiety-related personality traits,^{5–7} stress-associated depression,^{8,9} amygdala activation in response to negative stimuli,^{10,11} and suicide.¹²

The *h5-HTTLPR* is thought to influence behavioral characteristics by driving allele-specific *SERT* promoter activity giving rise to two-fold variability in mRNA levels.^{1,13} Decreases in *SERT* protein binding in *postmortem* human brain and [³H]5-HT uptake in human platelets and

immortalized lymphoblasts have been reported to be associated with the *h5-HTTLPR* short 'S' allele.^{1,14,15} However, studies on human *SERT*-binding potential by positron emission tomography (PET)^{16–18} and mRNA levels in *postmortem* raphe tissue¹⁹ are not in agreement with earlier findings. Additional common noncoding polymorphisms thought to influence *SERT* transcription including an intron 2 VNTR, and rs25531 and rs25532 single-nucleotide polymorphisms (SNPs) in the *h5-HTTLPR* region, have been discovered, adding to the complexity of assessing variability associated with the human *SERT* gene.^{2,4,20} Nonetheless, although the timing²¹ and specific molecular effects of the *h5-HTTLPR* on *SERT* expression and transporter function in the human brain are unresolved, parallels continue to be drawn between this polymorphism and anxiety-related traits and susceptibility to depression.^{22–27}

Similar to humans, macaques express a *SERT*-linked polymorphic region (*rh5-HTTLPR*; Figure 1).^{28–30} The

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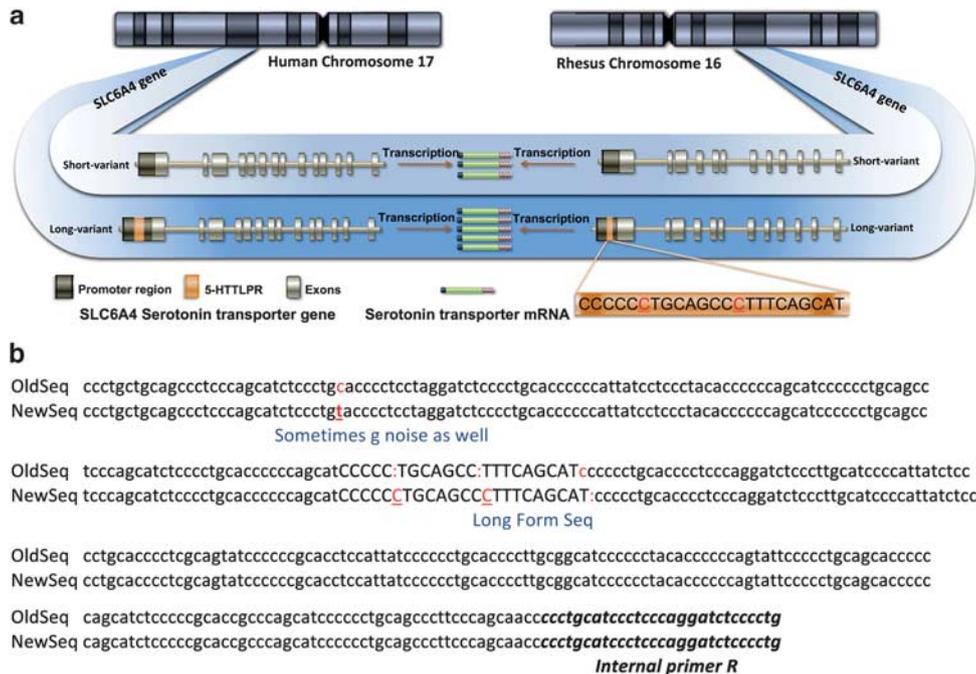


Figure 1 Structure and sequence of the rhesus *5-HTTLPR*. (a) The serotonin transporter (*SERT*) gene in rhesus monkeys has been reported to contain a 21-bp insertion-deletion polymorphism in the promoter region termed the *rh5-HTTLPR*. A 43-bp polymorphism, the *h5-HTTLPR*, occurs in the human *SERT* gene. In humans, the presence of the *5-HTTLPR* short 'S' allele has been associated with decreased gene transcription and thus, reductions in *SERT* protein levels and uptake function. We investigated whether the *rh5-HTTLPR* 'S' allele is associated with decreased serotonin system parameters in PBCs, which natively express *SERT*. (b) Sequencing of the *rh5-HTTLPR* in the 15 animals studied revealed an insertion/deletion region consisting of 23 bp, as well as two additional sequence discrepancies (shown in red) compared with the originally published sequence.²⁸

rh5-HTTLPR was originally reported to consist of a 21-bp insertion/deletion involving repeat element 7 at a slightly shifted locus from that in humans.²⁸ In contrast to humans, no SNPs associated with the *rh5-HTTLPR* have been discovered.³⁰ Yet similar to humans, stress responsiveness, social behavior, and cognitive performance are influenced by *rh5-HTTLPR* genotype. For example, behavioral and hormonal responses to early life stress and sensitivity to alcohol are potentiated in macaques bearing a short 'S' allele.^{29,31–33} Moreover, rhesus with an 'S' allele showed greater anxiety and fearful behavior³⁴ and enhanced aversion to social threats.³⁵ Also similar to humans, rhesus short allele carriers perform better on a number of cognitive tasks supporting the idea that the short *5-HTTLPR* allele might be associated with evolutionary advantage.^{36–38} *SERT* immunoreactive fibers have been mapped to the central nucleus of the amygdala³⁹ and oxytocin-expressing neurons in the paraventricular nucleus⁴⁰ in rhesus suggesting that differences in *SERT* expression and uptake function modulate fear responses and social affiliation, respectively.

The *rh5-HTTLPR* is hypothesized to influence *SERT* gene transcription; the short allele has been associated with decreased reporter gene expression.²⁹ However, studies on *SERT* mRNA levels in blood cells⁴¹ or *SERT*-binding potential using PET^{37,42} have failed to find associations with respect to *rh5-HTTLPR* genotype. Otherwise, direct investigations into the effects of the *rh5-HTTLPR* on *SERT* function have been lacking. We recently reported on differences in uptake at a single concentration of serotonin in peripheral blood cells

(PBCs) in association with the *rh5-HTTLPR*.⁴³ Uptake rates for serotonin were reduced in macaques carrying one or two 'S' alleles compared with individuals homozygous for the long allele. In the present study, we investigated native blood cells as biomarkers for a wide range of serotonin system characteristics. We assessed the expression and function of *SERTs* in rhesus PBCs by analyzing mRNA levels, surface *SERT* binding (K_D and B_{max}), transporter kinetics (K_M and V_{max}), and cellular concentrations of serotonin and 5-hydroxyindoleacetic acid (5-HIAA) with respect to *rh5-HTTLPR* genotype. Investigating rhesus PBCs confers a number of key advantages. (1) Rhesus monkeys are closely related to humans in that they show a genetically similar but less complex form of the *5-HTTLPR*. (2) Macaques are animal models whereby *in vivo* measurements in the brain are possible to assess alterations in *SERT* function and extracellular serotonin levels directly.^{44,45} (3) Parallels can be drawn between *SERT* function in the brain vs PBCs in rhesus with relevance to diagnosing and treating psychiatric disorders in humans.

Materials and methods

Animals. Venous blood was collected under anesthesia from a group of mixed sex rhesus (*M. mulatta*) 6.9 ± 0.1 years of age. Whole blood was used for DNA isolation by established protocols. Animals were genotyped for the *rh5-HTTLPR* using previously described methods.²⁸

Genotypes were also determined for tryptophan hydroxylase-2 SNPs and a monoamine oxidase-A repeat length polymorphism³⁰ and are reported in the Supplementary Information (Supplementary Table S1). We had the opportunity to genotype a relatively large cohort of animals and thereby, were able to identify and to include a substantial number of individuals with the *S/S* genotype. However, animals were donated for the present study and constraints on the size of this donation dictated that only a subset of the genotyped cohort could be studied here. Thus, animals were selected to maximize *rh5-HTTLPR* genotype distributions, particularly *S/S* and *L/L* genotypes, and to control for sex and genotypes at the other loci where possible. Information on the final cohort appears in the Supplementary Information (Supplementary Table S1). The genotype distribution for the study cohort was $N=6$ for *L/L*, $N=3$ for *S/L*, and $N=6$ for *S/S*. Animals from China vs the United States (LABS of Virginia, Yemassee, SC, USA) are noted in Supplementary Table S1 and are distributed across genotypes. Animals were housed at the University of Pittsburgh in pairs, with the exception of large males who were housed singly. Individual blood samples (~40 ml) were collected and PBCs were isolated from genotyped subjects. Experiments for protocol development were conducted using pooled mixed genotype rhesus PBCs. All work involving animals was carried out in accordance with National Institutes of Health guidelines and was approved by the University of Pittsburgh School of Medicine Institutional Animal Care and Use Committee.

Cell survival and SERT function. Confocal microscopy and flow cytometry were carried out using IDT307 (4-(4-(dimethylamino) phenyl)-1-methylpyridinium iodide), a monoamine transporter substrate similar to ASP⁺ (4-(4-diethylaminostyryl)-*N*-methylpyridinium iodide).^{46,47} Following uptake, IDT307 fluoresces enabling transporter function in PBCs to be determined optically.

Serotonin uptake. PBCs (~10 million cell per ml) were thawed by adding assay buffer (12–15 ml) at room temperature. A small volume (200 μ l) of cells in solution was used for live cell counts using Trypan blue exclusion. Cells were centrifuged at 340 g for 7 min. Pellets containing PBCs were resuspended by gently vortexing in assay buffer to produce final concentrations of 2–4 million cells per ml. Chronoamperometry was carried out, as described previously,⁴³ using boron-doped diamond microelectrodes⁴⁸ to measure serotonin-uptake rates on a second-by-second basis over a range of serotonin concentrations to determine maximal uptake rates (V_{\max}) and affinity constants (K_M). We have shown that the use of chronoamperometry enables biologically important differences in uptake rates to be distinguished, which cannot otherwise be differentiated by radiochemical methods.⁴⁹

Cell surface SERT binding. SERT binding was performed using the cocaine analog (¹²⁵I)RTI-55 by previously published methods with minor modifications.^{50,51} As samples from genotyped animals were limited, we focused on determining surface SERT binding (as opposed to total

SERT) due to its greater relevance to serotonin uptake and antidepressant action. Cells (~10 million cells per ml) were thawed and centrifuged as described above. Pellets containing intact cells were divided to measure specific and nonspecific binding over a range of RTI-55 concentrations to determine maximal binding (B_{\max}) and dissociation constants (K_D).

SERT mRNA levels. Total RNA was isolated from PBCs (~8–9 million cells per sample) using isoamyl/chloroform phase separation and isopropanol precipitation. Real-time quantitative PCR (RT-qPCR) and TaqMan probes and primers specific to *SERT* and two control genes, *ACTB* (β -actin) and *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase), were used for amplifications. Sequences and efficiencies of the primer/probe sets are reported in Supplementary Table S2.

Neurotransmitter levels. Blood cell concentrations of 5-HT and its major metabolite, 5-HIAA, were determined using previously published procedures⁵² by high-performance liquid chromatography with electrochemical detection.

Statistics. All values are expressed as means \pm standard errors (s.e.m.s) with differences of $P < 0.05$ considered statistically significant. Significant differences are denoted in the figures as * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Additional information on electrochemical uptake, (¹²⁵I)RTI-55 binding, mRNA isolation, RT-qPCR, neurochemical analysis, chemicals, and statistics appears in the Supplementary Information.

Results

The *rh5-HTTLPR* comprises a 23-bp polymorphism. The genomic region 130-bps upstream and 227-bps downstream of the *rh5-HTTLPR* was sequenced for each of the 15 animals studied. Sequences were identical for all individuals (Figure 1b). A number of differences were noted compared with the original sequence published by Lesch *et al.*²⁸ One C/T discrepancy was identified upstream of the *rh5-HTTLPR* polymorphic region. Within the insertion region itself, two additional cytosines were detected suggesting that the *rh5-HTTLPR* consists of 23 bps, instead of 21 bps as originally reported. Additionally, the 3' region immediately flanking the *rh5-HTTLPR* contained one less cytosine than previously reported. Most discrepancies were associated with strings of cytosines, where sequencing errors commonly occur. However, we cannot rule out genetic heterogeneity in this region as possibly accounting for discrepancies with previously reported sequences.

Previously frozen PBCs are viable and transport serotonin. The effects of frozen storage on the quality of PBCs were assessed using flow cytometry and confocal imaging. Propidium iodide was used to identify dead cells. We found that ~75% of cells were alive after thawing and significant changes in cell viability did not occur when cells were

maintained at 4 °C for up to 4 h (Supplementary Figure S1). Studies using flow cytometry in conjunction with IDT307, a fluorescent monoamine transporter substrate,^{46,47} illustrated that PBCs retain transporter function after isolation, freezing and thawing (Supplementary Figure S2). Confocal imaging showed localization of IDT307 inside the majority of live cells (Figure 2), also indicating intact transporter function.

Using chronoamperometry to make highly time-resolved measurements, we previously demonstrated that serotonin uptake by rhesus PBCs is Na⁺-dependent.⁴³ Additionally, uptake is abolished after preincubation with the serotonin-selective reuptake inhibitor paroxetine. Here, we investigated the effects of oxygen on serotonin clearance rates in rhesus PBCs. Similar to synaptosomes,^{49,53} we observed a 40% increase in serotonin uptake in the presence of oxygenated assay buffer (Supplementary Figure S3). Changes in serotonin uptake rates were not observed after blocking norepinephrine transporters, dopamine transporters or organic cation type 3 transporters⁵⁴ (Supplementary Figure S3). These results, in combination with prior findings, suggest that extracellular serotonin clearance by rhesus PBCs is a SERT-mediated active uptake process.

Reduced serotonin uptake and surface SERT binding are associated with the *rh5-HTTLPR* short allele. Recently, we reported that PBCs isolated from individuals expressing the *rh5-HTTLPR* 'S' allele showed reduced uptake of 0.5 μM serotonin.⁴³ Here, we used chronoamperometry to investigate uptake over a range of serotonin concentrations to determine maximal uptake rates (V_{max}) and affinity constants (K_M). The 'S' allele of the *rh5-HTTLPR* was associated with significant decreases in maximal serotonin uptake rates (Figure 3a) but not the affinity of SERT for serotonin (Supplementary Table S3). Maximal uptake rates were 7.1 ± 0.4 , 5.1 ± 0.2 , and 4.9 ± 0.6 pmol per million cells per min and affinity constants were 0.45 ± 0.06 , 0.39 ± 0.05 , and 0.45 ± 0.1 μM for L/L, S/L, and S/S genotypes, respectively.

Binding of RTI-55 was used to investigate SERT protein localized at the plasma membrane in undisrupted cells. Surface SERT binding showed a trend toward a decrease in maximal binding in 'S' allele carriers (Figure 3b; $P < 0.12$). No differences in dissociation constants were detected (Supplementary Table S3). Maximal surface SERT binding was 3.5 ± 0.4 , 2.4 ± 0.4 , and 2.6 ± 0.4 fmol per million cells and dissociation constants were 0.27 ± 0.04 , 0.29 ± 0.08 , and 0.26 ± 0.07 nM in L/L, S/L, and S/S genotypes, respectively.

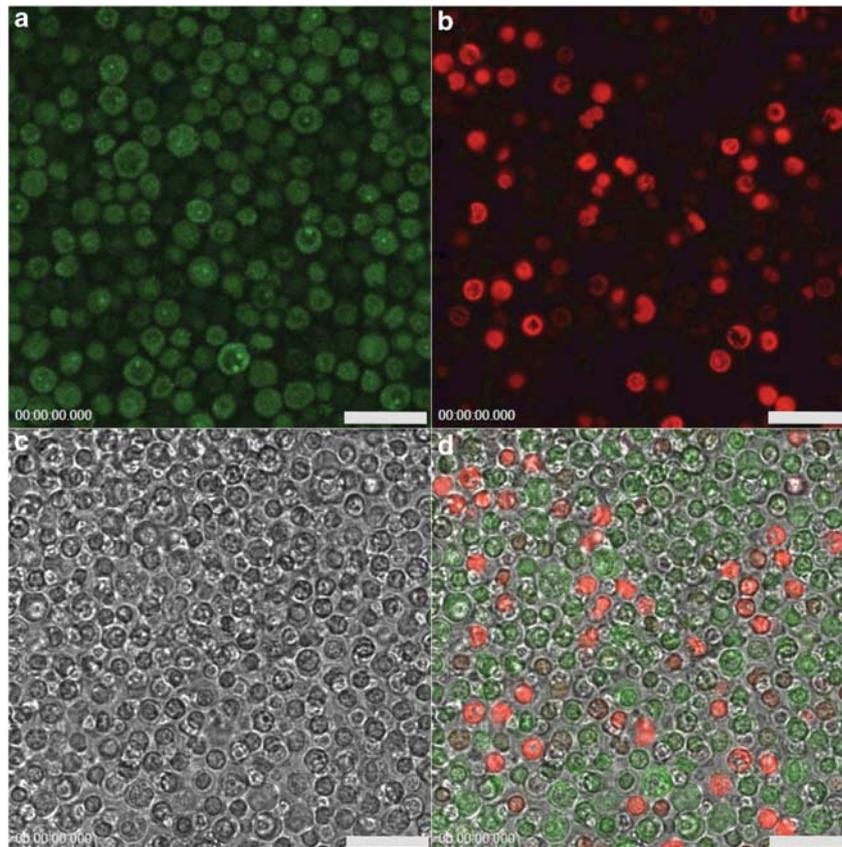


Figure 2 Confocal images of rhesus PBCs. Cells were incubated with (a) 1 μM IDT307 (30 min) and (b) propidium iodide (5 min). Propidium iodide (excitation 536 nm, emission 617 nm) stains dead cells, whereas IDT307 (excitation 485 nm, emission 520 nm), a substrate for SERTs, fluoresces after being taken up into live cells. (c) A differential interference contrast image shows all cells. (d) There is no overlap between cells predominantly labeled with IDT307 (green) vs propidium iodide-labeled cells (red) in the overlay of all three images demonstrating functional SERTs in living cells. Scale bars are 25 μm.

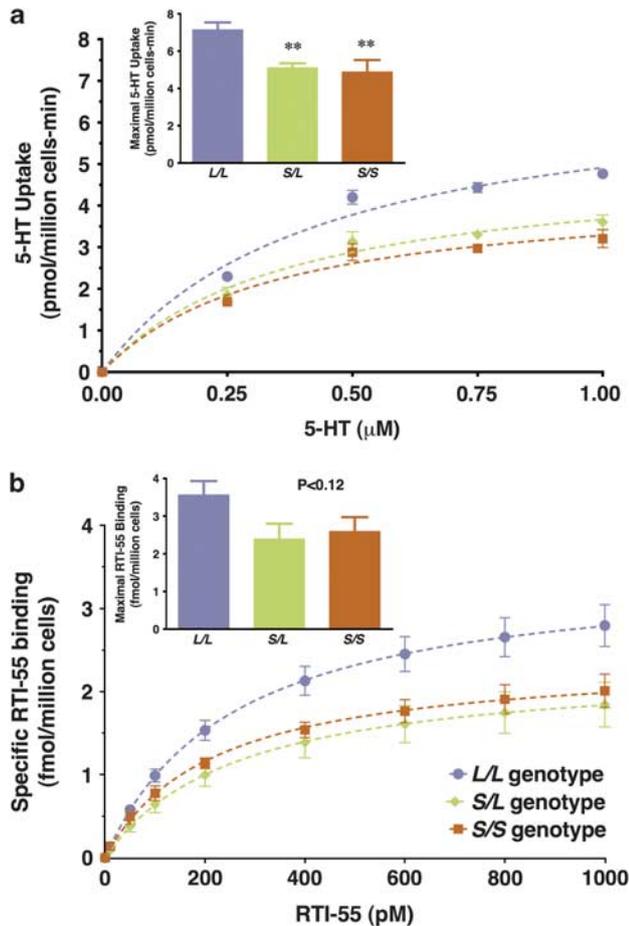


Figure 3 Serotonin uptake rates and surface SERT binding in rhesus PBCs. (a) Maximal uptake rates were calculated using nonlinear curve fitting for data from individual animals. Mean maximal uptake rates with respect to genotype are shown in the inset. One-way analysis of variance indicated that maximal uptake rates vary with respect to *rh5-HTTLPR* genotype ($F(2,12)=5.9$; $P<0.05$). *A priori* comparisons of uptake rates using one-tailed Student's *t*-tests showed significant decreases associated with the 'S' allele ($t=3.4$, $df=7$, $P<0.01$ L/L vs S/L and $t=3.0$, $df=10$, $P<0.01$ L/L vs S/S). (b) Binding of (125 I)RTI-55 to intact PBCs was used to determine the levels of SERT located at the plasma membrane. Maximal binding was calculated for data from individual animals by nonlinear curve fitting using one-site saturation isotherms. Mean maximal binding as a function of genotype is shown in the inset. There was a trend toward decreased SERT binding associated with the 'S' allele ($F(2,12)=2.5$; $P<0.12$). Data are means \pm s.e.m.s with $N=6$ for L/L, $N=3$ for S/L, and $N=6$ for S/S. ** $P<0.01$ vs the L/L genotype.

In addition to analyzing these data with respect to individual genotypes, we combined data for the S/L and S/S groups. Merging data from short allele carriers has been carried out previously due to a purported 'dominant' effect of the short allele and/or with smaller group sizes.^{1,37,55} Significant differences between animals homozygous for the *rh5-HTTLPR* 'L' allele and animals expressing one or two copies of the 'S' allele were present for serotonin uptake rates and cell surface SERT binding (Figures 4a and b). No significant differences in affinity constants for uptake (K_M) or binding (K_D) were found when the S/L and S/S genotypes were merged. Together, both modes of analysis suggest that reductions in serotonin uptake rates in rhesus PBCs from 'S' allele carriers are associated with decreases in surface SERT availability but not the affinity of SERT for serotonin or RTI-55.

SERT mRNA levels do not differ with respect to *rh5-HTTLPR* genotype. The *h5-HTTLPR* is hypothesized to influence SERT mRNA levels whereby the presence of the 'S' allele is associated with reduced transcriptional efficiency.^{1,56} We investigated whether the *rh5-HTTLPR* confers effects associated with differential transcription. In contrast to surface SERT binding and function, we did not find differences in SERT mRNA levels in rhesus PBCs with respect to *rh5-HTTLPR* genotype (Supplementary Figure S4A) or when the S/L and S/S genotypes were merged (Figure 4c).

Ratios of 5-HIAA/5-HT are decreased in association with the rhesus 'S' allele. We measured serotonin and 5-HIAA concentrations in PBCs to investigate alterations associated with the *rh5-HTTLPR*. We found that 5-HT levels were not different with respect to genotype (Supplementary Figure S4B). However, we observed a significant decrease in 5-HIAA levels in association with genotypes having one or two copies of the 'S' allele (Supplementary Figure S4C). Significant decreases in 5-HIAA to 5-HT ratios were observed for S/S vs L/L groups (Supplementary Figure S4D) and when the S/L and S/S genotypes were combined (Figure 4f).

Discussion

The human *5-HTTLPR* is postulated to drive allele-specific *SERT* promoter activity leading to differences in mRNA and protein levels, and functional serotonin uptake (Figure 1).¹ Here, we investigated each of these aspects of SERT expression and function, in addition to serotonin and 5-HIAA concentrations, to elucidate the effects of the *rh5-HTTLPR* in native (untransformed) PBCs. We find that the short allele of the *rh5-HTTLPR* is associated with reduced surface SERT binding, which is correlated on an individual basis with serotonin uptake rates (Figure 5a). By contrast, differences in mRNA levels with respect to genotype were not detected; nor was there a correlation between individual SERT mRNA levels and surface SERT protein binding (Figure 5b).

Lesch and co-workers reported lower promoter activity associated with the *h5-HTTLPR* short variant.⁵⁶ Decreases in SERT mRNA, SERT binding, and serotonin uptake have also been associated with the *5-HTTLPR* short allele in human lymphoblastoid (transformed) cell lines.¹ Subsequent studies of serotonin uptake in human platelets support these findings^{15,57,58} with one exception.⁵⁹ By contrast, binding studies in platelets measuring total SERT protein report variable results with respect to the *h5-HTTLPR*.^{15,58–60} *Postmortem* brain tissue and *in vivo* brain-imaging studies are similarly associated with conflicting results.^{14,16,18,61–64} Contradictory findings in human studies are attributable to a number of factors such as small samples sizes, subject genetic variability, the influence of environmental/developmental factors, and the use of insufficiently sensitive analytical methods.^{38,43,49,65}

Notably, the interpretation of the results of investigations into the human *SERT* gene are complicated by additional common noncoding *SERT* gene polymorphisms thought to influence transcription. These and other factors make direct

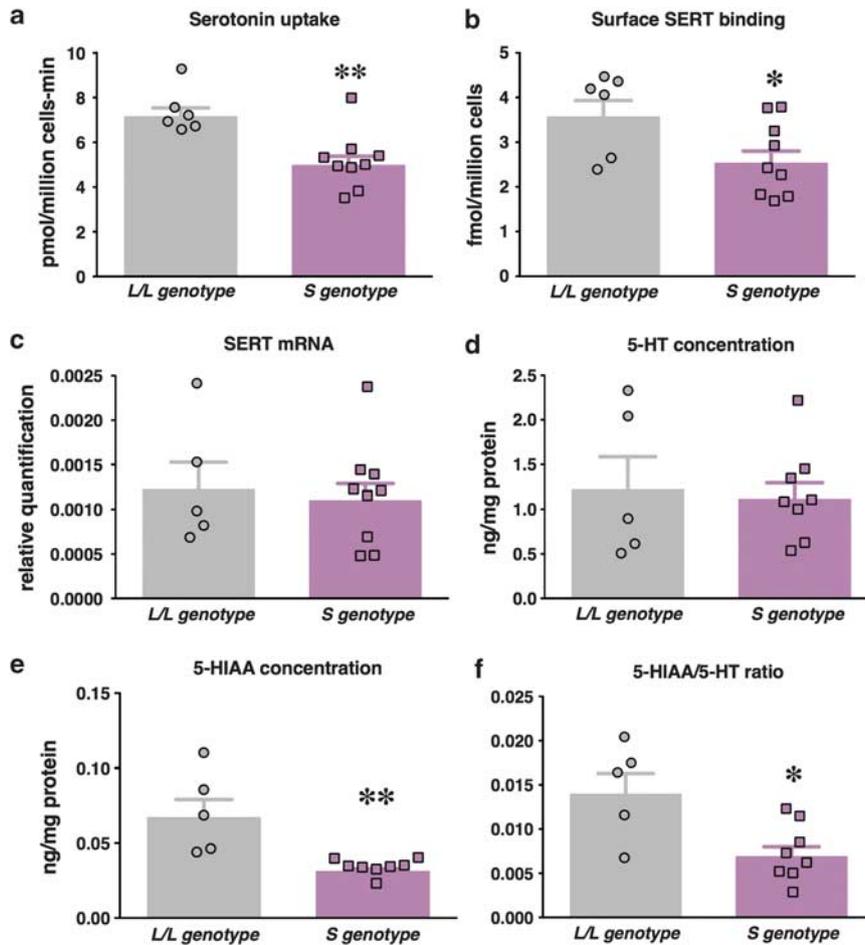


Figure 4 Serotonin system characteristics associated with the *rh5-HTTLPR* short allele in PBCs. Experimental data from *S/L* and *S/S* genotypes were merged and means \pm s.e.m.s are shown for (a) maximal serotonin uptake, (b) maximal surface SERT binding, (c) SERT mRNA levels, (d) 5-HT concentrations, (e) 5-HIAA concentrations and (f) 5-HIAA to 5-HT ratios. Data for animals having one or two 'S' alleles (*S/L* and *S/S*) were compared with data from animals with the *L/L* genotype using one-tailed *t* tests ($t=3.6$, $df=13$, $P<0.01$ for maximal serotonin uptake, $t=2.3$, $df=13$, $P<0.05$ for maximal SERT binding) or two-tailed *t* tests ($t=3.7$, $df=11$, $P<0.01$ for 5-HIAA concentrations, and $t=3.0$, $df=11$, $P<0.05$ for 5-HIAA/5-HT ratios). Individual values for each animal are shown as scatter plots overlaid on the respective bar graphs. Data are means \pm s.e.m.s with $N=5-6$ for *L/L* and $N=8-9$ for 'S' genotypes. ** $P<0.01$ and * $P<0.05$ vs the *L/L* genotype.

correlations between the *h5-HTTLPR* and changes in serotonin system expression and function challenging (see Singh *et al.*⁴³ for further discussion). Recent controversies have arisen regarding the role of the *h5-HTTLPR*, particularly with regard to stress-associated depression.^{24,25,66-69} The results of the current study present a comprehensive assessment of the cellular phenotype of the short vs long alleles providing solid evidence for a functional effect of the *rh5-HTTLPR*. One of the major advantages of the nonhuman primate model studied here is that unlike in human studies, subjects' environments can be highly regulated and manipulated over various periods in the lifespan.^{29,32,33,70,71} Furthermore, data can be collected from subjects whose genetic relatedness is knowable and controllable. Neither early life environment nor the pedigrees of the animals in the present study were highly homogeneous; animals from China and the United States were represented across genotypes. Nonetheless, *rh5-HTTLPR* short allele carriers were characterized by reduced serotonin uptake rates, cell surface SERT binding, and intracellular 5-HIAA levels in PBCs.

In the present study, SERT mRNA levels measured by RT-qPCR in rhesus PBCs were not significantly different with respect to *rh5-HTTLPR* genotype. This finding is consistent with a previous report on SERT mRNA in PBCs from a large cohort of rhesus (~ 80 animals).⁴¹ *Postmortem* human brain studies highlight the variability in SERT mRNA levels such that within the same genotype, 10-fold differences in SERT mRNA are observed.¹⁴ Similarly, in human lymphoblasts, SERT mRNA levels vary by 5-10-fold even when controlling for triallelic genotype with only 8% of the variance arising from measurement contributions.³ Variability in SERT mRNA could be due to a number of factors unrelated to *5-HTTLPR* genotype that influence mRNA synthesis, stability and degradation. Most studies in native tissues or cells from humans or rhesus fail to find associations between SERT mRNA and *5-HTTLPR* genotype, suggesting that mRNA levels are a poor indicator of variability associated with the *5-HTTLPR*.

The *h5-HTTLPR* has not yet been precisely modeled in rodents, although there are similarities with SERT-deficient mice and rats, whereby the low-functioning *h5-HTTLPR* allele is hypothesized to confer similar reductions in SERT expres-

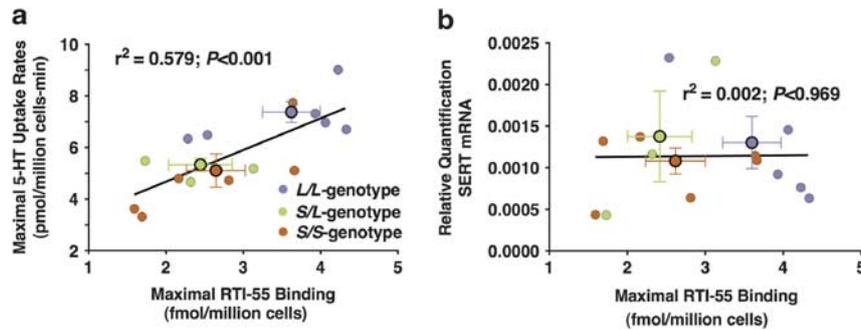


Figure 5 Correlations between surface SERT binding vs serotonin uptake or SERT mRNA levels. Correlations are shown with respect to individual animals between (a) SERT function and surface SERT binding and (b) SERT mRNA levels and surface SERT binding. Mean values with respect to genotype are shown as larger symbols with s.e.m.s for each variable indicated. Only SERT function and surface SERT binding are correlated, such that 60% of the variance is shared with a low probability of chance correlation ($P < 0.001$).

sion and function to those occurring with constitutive loss of one functional *SERT* allele in rodents.^{53,65,72–74} Mice with constitutive reductions in *SERT* gene expression show elevated anxiety-related behavior,⁷⁵ enhanced stress reactivity,^{76–78} and in some background strains, increased depressive-like behavior.⁷⁹ Increased anxiety-like behavior⁸⁰ and amygdala over-activity⁸¹ are associated with constitutive reductions of *SERT* in rats.

In mice, life-long absence of *SERT* is associated with decreased brain tissue serotonin concentrations.^{50,82,83} However, mice with a 50% constitutive loss of *SERT* expression show little to no change in brain tissue serotonin. Here, rhesus PBCs showed no significant differences in serotonin levels. Yet, 5-HIAA was reduced in PBCs from rhesus ‘S’ allele carriers. In light of decreased serotonin uptake in rhesus PBCs associated with the ‘S’ allele, these data suggest that a feedback mechanism might be at work to conserve available serotonin. Antidepressant administration in rhesus has been associated with decreased 5-HIAA concentrations in cerebrospinal fluid.⁸⁴ Investigation of serotonin synthesis and degradation rates with respect to the *rh5-HTTLPR* will be required to elucidate the underlying nature of the differences in 5-HIAA levels in rhesus PBCs.

Altered stress and anxiety responses exhibited by *SERT*-deficient mice resemble phenotypic characteristics of humans and macaques associated with the short form of the *5-HTTLPR*. Studies in rodents show that disruption of *SERT* function during a key postnatal period results in changes in emotional behaviors in adulthood that share some similarities with constitutive reductions in *SERT* expression.^{85,86} Developmentally sensitive changes in anxiety-related behavior in rodents, in combination with negative findings in association studies on the *5-HTTLPR* in adult humans, have led to the idea that the effects of the *h5-HTTLPR* on serotonin transmission predominate during key developmental periods.²¹ Here, we show that changes in the serotonin system associated with the *rh5-HTTLPR* are present during adulthood, alternately suggesting that this gene variant influences *SERT* function and serotonin neurochemistry throughout life, at least in the periphery. Moreover, gene \times environment interactions between *rh5-HTTLPR* genotype and peer vs mother rearing, with respect to CSF 5-HIAA levels further implicate central effects of the *5-HTTLPR* beyond early development.²⁹

The present findings suggest that PBCs might be used to study genetic and pharmacologic alterations in serotonin transmission directly and more accessibly than measurements in the central nervous system. PBCs are obtained using minimally invasive methods and can be viably frozen for later study. Additionally, PBCs are native cells; they do not suffer from potential problems associated with altered gene expression associated with immortalization, for example, in lymphoblasts.⁸⁷ Investigating *SERT* in the brain *in vivo* in humans is currently only possible via PET imaging. However, a lack of association between *SERT* binding by PET and *5-HTTLPR* genotype is reported in humans^{16–18,63} (discussed in greater detail in Singh *et al.*⁴³), as well as in rhesus.^{37,42} Before using PBCs as biomarkers of alterations in the brain serotonin system, additional research clarifying the relationship between the brain and peripheral blood serotonin systems is needed. For instance, there might be important differences between PBCs and the central nervous system in terms of regulatory mechanisms affecting *SERT* function. Also, PBCs constitute a mixed population of cells consisting of a number of different cell types expressing *SERT*, that is, mononuclear cells including monocytes and lymphocytes, and small numbers of platelets. Determining the contributions of each of these kinds of cells to uptake rates measured in PBC preparations is the subject of ongoing studies. In any case, the findings of the present study were the same regardless of whether the data were expressed and analyzed with respect to numbers of lymphocytes or total protein levels, the latter of which reflect all relevant cell types (Supplementary Table S3).

Native peripheral cells hold promise as candidates to elucidate central nervous system function at the molecular and genetic levels and with respect to drug mechanisms and efficacy. Furthermore, developing clinically applicable methods to perform functional measurements in PBCs that are sensitive to genetic influence represents a step toward an approach whereby cells readily accessible from human blood samples might be used to predict drug responses, thus initiating the concept of individually tailored therapeutic interventions.

Conflict of interest

SJ Rosenthal and ID Tomlinson declare financial interests in commercial products involving IDT307. All other authors declare no conflict of interest.

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