Cognitive impact of genetic variation of the serotonin transporter in primates is associated with differences in brain morphology rather than serotonin neurotransmission

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Abstract

A powerful convergence of genetics, neuroimaging and epidemiological research has identified biological pathways mediating individual differences in complex behavioral processes and related risk for disease. Orthologous genetic variation in non-human primates represents a unique opportunity to characterize the detailed molecular and cellular mechanisms which bias behaviorally- and clinically-relevant brain function. We report that a rhesus macaque orthologue of a common polymorphism of the serotonin transporter gene (rh5-HTTLPR) has strikingly similar effects on behavior and brain morphology to those in humans. Specifically, the rh5-HTTLPR Short allele broadly impacts cognitive choice behavior and brain morphology without observably affecting 5-HT transporter or 5-HT$_{1A}$ concentrations *in vivo*. Collectively, our findings indicate that 5-HTTLPR-associated behavioral effects reflect genotype-dependent biases in cortical development rather than static differences in serotonergic signaling mechanisms. Moreover, these data highlight the vast potential of non-human primate models in advancing our understanding of human genetic variation impacting behavior and neuropsychiatric disease liability.

Introduction

A common functional genetic polymorphism believed to impact serotonin (5-HT; 5-hydroxytryptamine) signaling has been the focus of much interest in efforts to understand underlying biological mechanisms of individual differences in complex behavioral processes and related risk for neuropsychiatric disorders. Specifically, in comparison to the (L)ong allele of an insertion/deletion variant in the promoter region of the human serotonin transporter (5-HTT) gene (SLC6A4) (1), the (S)hort allele has been linked to relatively increased risk for the development of depressive disorders and alcoholism, especially in the context of environmental adversity and stress (2-4). Human imaging genetics studies indicate that this increased risk for neuropsychiatric disorders may be mediated by increased amygdala reactivity in S allele carriers (5, 6). More recently, allelic status at this 5-HTT gene-linked polymorphic region (5-HTTLPR) has been shown to affect cognitive function in humans. Specifically, S allele carriers exhibit improved performance on tasks involving visual episodic memory, set-shifting, and probabilistic reward learning (7-9). The improved cognitive performance is consistent with enhanced performance monitoring activity in the anterior cingulate cortex (ACC) of S allele carriers (10, 11). Although, the 5-HTTLPR S allele has been consistently linked with individual differences in behavioral, clinical and systems-level neural phenotypes, the underlying molecular mechanisms through which this variant biases brain circuit function and related behavioral processes remains unknown. Because the S allele is associated with reduced transcriptional efficiency *in vitro* (1), an obvious potential mechanism whereby variability in brain function and behavior could be mediated by 5-HTTLPR allelic variation is through differences in 5-HTT levels leading to differences in extracellular 5-HT (12, 13). However, evidence increasingly indicates no significant differences in 5-HTT levels associated with 5-HTTLPR allelic status *in vivo* (14-17). Decreased 5-HT_{1A} binding has also been linked to S carriers of the 5-HTTLPR (18), providing another potential mechanism for the behavioral and neural effects associated with S allele status. In addition, such individual differences in phenotype may reflect the developmental effects of 5-HTTLPR allele status on prefrontal brain regions as the S allele is associated with relatively reduced gray matter volumes in the amygdala as well as medial prefrontal regions including the ACC (19, 20).

Orthologous genetic variation in non-human primates has the potential to greatly expand our understanding of the detailed molecular and cellular mechanisms through which common human genetic polymorphisms, such as the 5-HTTLPR, bias behaviorally-relevant brain function. Rhesus monkeys carry L and S alleles orthologous to the human 5-HTTLPR (21). This rhesus orthologue (rh5-HTTLPR) has an impact on 5-HTT expression *in vitro* similar to that reported in humans (22) and also impacts socioemotional behaviors, especially in interaction with environmental stressors (23-25). Thus, there is some compelling convergent evidence that the rhesus monkey may provide a valuable model through which the molecular mechanisms of the human 5-HTTLPR can be studied (26, 27). However, additional research is necessary before the utility of this model for informing human brain function and behavior can be fully translated. Specifically, the potential impact of the rh5-HTTLPR on cognitive function, brain morphology, and 5-HT signaling pathways should be established. If parallel neurobiological effects are identified between the human and rhesus 5-HTTLPR

then additional studies only feasible in non-human primate models (e.g., *in vivo* microdialysis or stress manipulations) can be undertaken to identify specific molecular mechanisms mediating the effects of the genetic variants on brain function and behavior as well as acute reactivity to environmental manipulation.

Using cognitive tasks similar to those used in human clinical research (7-9), we have examined the impact of the rh5-HTTLPR on several touch screen-based behavioral tasks involving probabilistic reward learning, temporal discounting, stimulus discrimination and reversal, and visual working memory. Within the same subjects we also examined potential genotype-associated differences in three neurobiological mechanisms that might contribute to these cognitive differences and have been implicated in studies of the human 5-HTTLPR. Specifically, regional concentrations of the 5-HTT and 5-HT$_{1A}$ receptor were determined using PET imaging, and morphological differences in prefrontal regions were determined using high-resolution MRI and voxel based morphometry (VBM). These comprehensive data form an important validation of a primate model for the study of 5-HTTLPR variants orthologous to those in humans. Furthermore, they represent a comparison of multiple potential mechanisms contributing to traits associated with the rh5-HTTLPR in a single population and will thus inform future invasive studies designed to elucidate detailed cellular mechanisms whereby the allelic variants impact cognition and clinical risk for affective disorders in humans.

Materials & Methods

Subjects

Subjects were 8 pair-housed, 6-7 year old, male rhesus macaques weighing 8-11 kg. Animals were obtained from the NIH Animal Facility at Poolesville, MD. Water intake of the animals was regulated (25ml/kg/day) from Mon-Fri, with ad lib access to water during the weekend. Animals were fed sufficient monkey chow biscuits (Purina) to maintain healthy body weight plus fruit treats daily. Four animals were homozygous for the Long variant (LL) of a polymorphic repetitive element in the promoter region of the serotonin transporter (SERT) and 4 animals had a LS (Long-Short variant) genotype. The genotyping of the subjects was conducted as previously described (28). Animals were experienced in working on a touch screen in a sound-attenuated chamber for water rewards and trained on specific stimuli and task-specific criteria prior to each task (for a detailed description of the behavioral tasks, see Supplement). Animals were tested each day Mon-Fri. Data was collected and analyzed using E-prime (Psychology Software Tools: Pittsburgh, PA). Stimuli (200×200 pixels~61mm) were presented on the left and right side of the screen in randomized order to eliminate any side bias.

Data Analysis

The behavioral performance was compared between genotypes using t-tests or 2-way repeated measures ANOVAs using α=0.05. For post-hoc comparisons p-values were compared to critical levels adjusted for multiple comparisons according to Holm-Sidak (Sigmastat 3.5). If the assumptions for a standard ANOVA were not met, a non-parametric ANOVA on ranks was performed followed by posthoc comparisons corrected according to

Tukey. For both the 5-HT$_{1A}$ and 5-HTT PET-imaging studies, multiple brain regions were compared between both genotypes using multivariate regression (SPSS v15). To clarify the prominence of genotype effects over other potential contributors such as rearing differences or relatedness, separate analyses were run to evaluate their contribution. Within each genotype group, 2 animals were peer-reared and 2 animals were mother-reared using published procedures(29). The issue of relatedness was examined because some animals descended from the same sire. All behavioral, PET, and VBM data were separately analyzed by these conditions (rearing condition, or common vs different sire). Neither the rearing nor paternal analysis revealed any systematic differences between groups, further supporting the observed differences between genotype groups.

PET

PET imaging for the 5-HTT was conducted using a Siemens microPET P4 scanner and [^{11}C] 3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile ([^{11}C] DASB) as a radioligand. Following a transmission scan, approximately 6.0 mCi of [^{11}C] high specific-activity (>2.0 Ci / μmol) DASB was administered via an intravenous bolus injection. Dynamic PET emission data were collected for 90 min post-injection in 34 acquisition frames ranging in duration from 30 sec to 10 min.

PET imaging of the 5-HT$_{1A}$ receptor was conducted using [^{11}C] WAY100635 as radioligand. Following a transmission scan, approximately 9.0 mCi of [^{11}C] high specific-activity (>1.1 Ci / μmol) WAY100635 was administered via an intravenous bolus injection. Dynamic PET emission data were collected for 90 min post-injection in 23 acquisition frames ranging in duration from 30 sec to 10 min.

Parametric images of DASB or WAY100635 binding potential (BP$_{nd}$) were generated using a 2-parameter multilinear reference tissue model (MRTM2). Reconstructed PET images were coregistered to MR images for region-of-interest (ROIs) definitions which were drawn on coronal MRI sections for individual animals. Pmod 2.9 was used to define and quantify 5-HTT or 5-HT$_{1A}$ binding in ROIs for the anterior cingulate, ventral striatum, amygdala, hippocampus, and dorsal raphe.

Structural MRI

MRI images (0.5mm voxels size) were acquired using a Siemens 3T Allegra scanner with a custom-designed dual stereotaxic holder/secondary coil designed by Dr Seong Gi Kim and colleagues (Univ of Pittsburgh). Images were warped to a merged and fully segmented macaque monkey atlas, analogous to the Montreal Neurological Institute templates used in human imaging studies. A 5mm smoothing kernel was used in pre-processing. All MRI images from individual subjects were processed and analyzed using statistical parametric mapping software (SPM5;http://www.fil.ion.ucl.ac.uk/spm/software/spm5/) operating within Matlab (version 7.6.0, R2008a;MathWorks, Natick, MA) and the VBM toolbox developed by Christian Gaser (http://dbm.neuro.uni-jena.de/). Images were compared with a two-sample *t*-test design with absolute thresholding at 0.2, covaried for total gray matter volume. Thresholds were set at p=0.01 and 1000 voxel cluster extent (5mm cube), corrected for non-isotropic smoothness. To control for multiple comparisons, we empirically determined the

false positive detection rate (FPR) (30) using these criteria and Monte Carlo simulations implemented in AlphaSim, which account for spatial correlations between volume changes in neighboring voxels (31). Based on this analysis, the FPR using a threshold of p=0.01 and k=1000 voxels was found to be 0.001. ROI analysis of the pulvinar nucleus of the thalamus was performed using a threshold of p=0.025 for a specific ROI drawn based on the Atlas of the rhesus monkey brain (32) and the WFU Pick Atlas tool (http://www.fmri.wfubmc.edu/cms/software#PickAtlas).

RESULTS

Cognitive Performance

Probability Discounting Task—In the probability discounting task, two visual stimuli associated with a small guaranteed reward and a large probabilistic reward respectively, were presented on each trial. The probability of receiving the larger reward (versus no reward) was reduced stepwise (from an initial value of 100 to a final value of 10%) during each session (for detailed task description see supplement and Fig. S1). Consistent with the higher expected reward value, subjects initially continued to choose the high reward stimulus as reward probability decreased, despite not receiving a reward on all trials (Fig. 1A). Subsequently, at the lower probability levels of reward, subjects switched and predominantly chose the guaranteed small reward. Overall, S allele carriers chose and received the high reward on more trials (i.e. better choices adapted to probability level: p=0.021) and in terms of expected value, made more advantageous choices (p=0.044, Fig. 1B). Human studies have suggested an enhanced performance monitoring function, or augmented neural activation associated with error or feedback processing, in S allele carriers (10, 11). One indication of enhanced performance monitoring is an increase in RT following conflict or error trials (33, 34) and consistent with this notion, non-human primate (NHP) S allele carriers took significantly more time to make their choice following an unrewarded trial (as a result of no reward on prior trial) (Fig.1C). Repeated measures ANOVA of the choice RT using both genotype and reward level on the previous trial indicated a main effect of reward level (p=0.018) and a strong trend for a genotype by reward interaction (p=0.085). Based on this and the enhanced feedback monitoring in observed humans (10, 11), we conducted one way repeated measures ANOVAs on ranks of choice RT with reward outcome on the prior trial as factor and found a significant effect of reward on the previous trial (p=0.005) in S allele carriers but not in the LL group (p=0.125). Post-hoc comparisons, corrected for multiple comparisons (Tukey), between the reward levels in S allele carriers showed a significant difference between the high and the no-reward condition on the previous trial.

Delay Discounting Task—In the delay discounting task, two visual stimuli were presented, one of which was associated with a delayed but larger reward. With increasing reward delays (0-10 sec), subjects chose the immediate reward more often, which dynamically reduced the reward magnitude associated with this stimulus. There was a main effect of delay (p<0.001) and a strong trend for a right shift in the discounting curve for S allele carriers (genotype × delay interaction p=0.057 (Fig.2A). Post-hoc comparison showed that at the 5 sec delay, S allele carriers demonstrated a greater tolerance of delay whereas LL

subjects already discounted the reward value of the delayed stimulus to approximately 50% of the immediate reward (p=0.002). The hyperbolic discounting constants (LL 0.17±0.06, LS 0.07±0.03) were within the range of observations in other studies in humans and NHPs (35, 36). A strong trend for a genotype-associated difference was apparent for the log-transformed discounting constants (p=0.076) but failed to reach statistical significance due to large intra-individual variation similar to what is seen in humans (36).

Reversal Learning Task—A non-serial reversal learning task was employed which allowed a dissociation of the acquisition and reversal phases of association learning. All subjects readily learned to discriminate between different differentially rewarded stimulus pairs at a similar rate (Fig.2B **insert**). However, when the reward contingencies were reversed, S allele carriers more readily adapted and chose the previously less rewarded stimulus now associated with the larger reward (Fig.2B). Consequently, S allele carriers committed fewer errors in achieving criterion following contingency reversal (Discrimination p=0.22; Reversal p=0.05). There was a main effect of genotype on accuracy (p=0.034) and a significant trial by genotype interaction (p=0.003). Post-hoc comparisons demonstrated that significant differences in accuracy between the genotypes were confined to trials following contingency reversal.

Delayed-Match-to-Sample Task—When two visual stimuli were presented on the touch screen after a delay period, subjects selected the stimulus matching the original sample stimulus with high accuracy and this accuracy decreased with prolonged delays. The accuracy of LL subjects exhibited a steeper decline with increasing delays (0-40sec) compared to S allele carriers (genotype × delay interaction p=0.045) (Fig.2C). Post-hoc comparison demonstrated that S allele carriers responded more accurately at the 40 sec delay than LL subjects (p=0.025).

Continuous performance task—In the continuous performance task, there were no differences in response times or accuracy of responses between genotypes. A two way repeated measures ANOVA on omissions showed however, a main effect of reward size (p=0.005) on frequency of omissions and strong trends for a main effect of genotype and a genotype by reward size interaction (p=0.070 for both). Based on this and the increased omission rate observed in human LL participants on an affective Go/NoGo task (37), we conducted post-hoc comparisons which demonstrated that LL subjects had more omissions than S allele carriers at the low reward level (p=0.012) but not at the high reward level (p=0.528).

In vivo 5-HTT binding

The primary mechanism responsible for effects of 5-HTTLPR allelic status on clinical or behavioral variables is generally thought to be altered extracellular 5-HT resulting from differing levels of functional 5-HTT. Though this interpretation is based on solid and reproducible effects of 5-HTTLPR allelic variation on *in vitro* expression of 5-HTT, the majority of reports show no effect of allelic status on *in vivo* 5-HTT binding in humans when selective radioligands are used (14, 15). Using [^{11}C] DASB, a highly selective 5-HTT radioligand used in human imaging studies, we observed large regional differences in 5-

HTT binding similar to those observed in humans (38), but no difference in binding across genotype (multivariate regression for genotype across all 5 regions p=0.695, Fig.3A), suggesting that the behavioral differences were not associated with a change in transporter binding.

In vivo 5-HT$_{1A}$ binding

The 5-HTTLPR has been associated with alterations in 5-HT$_{1A}$ binding with human LL subjects demonstrating higher binding in both the dorsal raphe and in cortical and sub-cortical projection areas (18). Using PET imaging with the radioligand [^{11}C] WAY-100635, a selective 5-HT$_{1A}$ ligand, and identical ROIs as used for the 5-HTT imaging, we observed large regional variations in 5-HT$_{1A}$ BP but no differences in binding across genotype (multivariate regression for genotype across all 5 regions p=0.116; all comparisons across genotype for each region p>0.25, except for the amygdala p=0.080; Fig.3B).

Evaluation of Structural Differences with Voxel-Based Morphometry

To determine whether differences in gray matter morphology were associated with genotype and cognitive performance, we used voxel-based morphometry of high resolution MR images. This analysis revealed that S allele carriers exhibited reduced gray matter volume in extensive bilateral prefrontal-, and ventrolateral temporal-, and posterior parietal cortices. These areas showed a remarkable overlap with previous observations in humans (19, 20). (See Fig.4 and table 1). Regions of the anterior cingulate, medial prefrontal, and orbitofrontal showing reduced volumes in S allele carriers included Brodmann areas 8, 9, 10, 11, 13, 24, 32 and 45. This is quite striking because of the critical role that these regions play in the type of cognitive function engaged by the reversal and probabilistic learning tasks we employed. Notably, S allele carriers also exhibited decreased volume in left amygdala at the whole brain level (previously only reported in humans using an *a priori* ROI analysis (20). Reduced volumes were also seen in medial parietal areas not previously reported. Based on post-mortem observations in humans (39), we specifically examined potential changes in the pulvinar nucleus of the thalamus using an a priori region of interest approach and observed an increased volume in this region in S allele carriers using a ROI analysis (p=0.002, k$_e$=98 voxels). There was no difference in total gray matter volume between genotypes (p=0.42). The peak *t*-values of the clusters in our study are larger compared to published reports of human data. It is likely that the reduced smoothing kernel size of our high resolution structural images and the standardized environmental conditions of our subjects permit greater sensitivity to detect differences and contribute to the higher *t*-values we observed.

Discussion

The present experiments demonstrate in rhesus monkeys that allelic variants of the rh5-HTTLPR orthologous to those in humans are associated with a similar impact on cognitive function, and strikingly similar morphological differences in multiple brain regions. *In vivo* PET imaging revealed no differences in 5-HTT or 5-HT$_{1A}$ binding characteristics. As proposed for humans(7), the overall better performance on an array of cognitive tasks may provide S allele carriers with an evolutionary advantage that offsets the increased

vulnerability to environmental insults, which in humans is manifested as an increased risk for affective and depressive disorders (2). Our results suggest that the rh5-HTTLPR has greater impact on brain morphology than on other implicated mechanisms, namely 5-HTT and 5-HT$_{1A}$ concentrations, suggesting that differences in brain structure may mediate the observed difference in cognition. Furthermore, these results support a developmental impact of rh5-HTTLPR status as proposed in humans (20, 40).

Cognitive function

Subjects carrying the S-allele of the 5-HTTLPR showed overall better performance on a series of cognitive tasks, paralleling clinical observations of the impact of allelic variant status on cognitive performance. Better performance (more advantageous choices) of S allele carriers on the probability discounting task is consistent with the increased attention to differences in probability of chosen gambles on a risky choice task observed in human S allele carriers (9). Although the effect of 5-HTTLPR allelic status on temporal discounting in humans is unknown, collectively these results suggest that S allele carriers are integrating feedback better across time to guide their behavior on subsequent choices. The improved performance of NHP S allele carriers on the delayed match to sample task (DMS) may parallel the improved performance of human S allele carriers in a delayed component of a pattern recognition memory test (41). Though performance on a specific DMS task in humans was unaffected by allelic variant status, the relatively short delays (0, 4, 12sec) used (9) may explain why no genotype by delay interaction was seen. Our observed differences were only significant at the longest (40 sec) delay but not at the 0 sec delay suggesting that both genotypes exhibit similar processing of sensory stimuli. To the extent that the DMS task engages an overlapping cognitive domain (e.g. working memory) as the Wisconsin Card Sort task, it is relevant that human S allele carriers show improved performance on that task (8).

On the stimulus discrimination/reversal task, the superior performance by the S allele carriers is seen only on the reversal, but not the discrimination component, again indicating specificity in the performance difference between LS and LL subjects. The present data appear consistent with the limited data obtained in humans demonstrating fewer errors committed by S allele carriers in a reversal task (42). Improved feedback integration is also suggested by the observation that S allele carriers committed fewer errors in a passive avoidance task (42). The present observations contradict the poorer reversal learning ability of NHP S allele carriers on a serial reversal learning task (24). The different nature (e.g. all or none food reward vs differential levels of water reward) or increased difficulty of the present non-serial reversal task may all have contributed to the discrepancy with this study.

There does not appear to be a single unifying cognitive difference between genotypes that would explain the broadly superior performance of the S allele carriers. An increased motivation by rewards, an increased ability to integrate feedback to guide future choices (performance monitoring), or an increased ability to focus on the task (greater attention) could all contribute to better performance on the multiple cognitive tasks used. A comparison of mean response times in a simple well-learned stimulus reward task is often used to probe for differences in motivation, but we found no differences in response time on

a continuous performance task. The behavioral analyses demonstrating a specific deficit during reversal but not the discrimination trials of the reversal task also suggests that the better performance of the S allele carriers is not a result of an enhanced motivation for reward.

Performance monitoring is a function repeatedly associated with the anterior cingulate. Enhanced neural activity and prominent morphological changes in the anterior cingulate have been reported in human S allele carriers (10, 11, 19, 20). The superior performance on the reversal learning task, and both the probabilistic and temporal discounting tasks could all be associated with enhanced performance monitoring in our S-carrier subjects. One potential mechanism via which the (dorsal) anterior cingulate might contribute to performance monitoring is the ability to integrate rewards across multiple previous trials (43, 44). Temporal reward integration over a longer time window could certainly account for the improved performance of S allele carriers on the delay and probability discounting tasks as a consequence of presumed enhanced ACC activity. It is however, inconsistent with the faster adaptation of S allele carriers following stimulus reversal. The orbitofrontal cortex (OFC) however, is a region most associated with an ability to rapidly adapt stimulus reward associations (44, 45). The OFC also exhibited prominent morphological alterations with genotype and differential functional activation of this region in response to environmental manipulation associated with the 5-HTTLPR has recently been reported in rhesus monkeys (26). Thus, while the ACC is involved in action-reinforcement representations and value based decision making (44), the difference in performance on the reversal task more likely represents a distinct contribution associated with morphological differences in the orbitofrontal cortex.

In terms of increased focus or attention, the continuous performance task revealed a trend for more omissions in the LL group, suggesting that attentional differences may contribute to their inferior performance. A similarly increased omission rate was also observed in human LL carriers on an affective Go-NoGo task (37). Increased focused attention in processing visual stimuli could also contribute to the enhanced performance of S allele carriers on the delayed match to sample task, similar to human S allele carriers who show better pattern recognition memory (7). We observed morphological differences in the pulvinar nucleus of the thalamus, similar to the impact of 5-HTTLPR genotype in human post-mortem observations (39). Because the pulvinar nucleus is associated with attention (46), the increased volume in this region may suggest a potential contributing mechanism for differences in attention observed in S allele carriers. Another brain region with a critical role in attention, which in addition, is part of an extended cortical control network, is the central parietal region (47). We observed large genotype-associated morphological alterations observed in this region (BA 5). Thus, these morphological alterations may contribute to the attentional effects, or improved cognitive control in general.

5-HTT and 5-HT$_{1A}$ imaging

Clear regional differences were found in ligand binding to 5-HTT, in general agreement with post-mortem autoradiography in humans and non-human primates (48-50) and *in vivo* PET studies (38). We observed no effect of genotype which is consistent with the majority

of human studies indicating a lack of effect of genotype on the 5-HTT binding (14-17) though some studies do report differences (12, 13). It is also consistent with a very recent report of NHPs (51). These findings do not rule out potential genotype-associated differences in ultrastructural localization or efficiency of the 5-HTT, but given the similarity with human and NHP findings, it suggests that differences in static levels of 5-HTT are not contributing to the differences in cognitive performance observed. The promise of a well-validated animal model such as this is that transient alterations in 5-HTT, e.g. following a stressor, can be investigated to evaluate the impact of the 5-HTTLPR on the brain's dynamic response to stress.

No significant genotype-based differences in 5-HT$_{1A}$ binding were found although the the present study with a relatively small sample size may have been underpowered to detect small decreases in 5-HT$_{1A}$ binding reported clinically in S allele carriers (18). However, a new study conducted with a large sample of human subjects observed no difference in 5-HT$_{1A}$ binding (8). Given the prominent observations of widespread morphological differences in our subjects, but no differences in 5-HTT or 5-HT$_{1A}$ binding in the same subjects, we believe that the cognitive impact of the 5-HTTLPR is more likely a result of altered neuronal/cortical development and resultant morphological differences than to acute differences in serotonergic signaling.

Morphology

The regions exhibiting altered grey matter volume in NHP S allele carriers were strikingly similar to areas previously described in humans (19, 20, 39). The similarity between our findings and previous human findings obtained from much larger, independent populations further supports the notion that the present morphological changes are associated with the 5-HTTLPR rather than some other cohort—specific genetic difference.

It is likely that the morphological changes to the prefrontal and temporal areas contribute to the changes in choice behavior given previous studies demonstrating a critical role for the orbitofrontal cortex in reversal learning and temporal discounting behavior (52, 53), the anterior cingulate cortex in probabilistic choice behavior (44), and the medial and lateral prefrontal cortices and ventrolateral temporal cortex in the DMS task (54). It is surprising however, that both human and NHP S allele carriers exhibit improved performance on these choice tasks as well as the Wisconsin Card Sort Task (8) with smaller gray matter volume in these regions. However, gray matter volume is comprised of neuronal and extraneuronal elements, and provides no indication of the efficiency or connectivity of a neural network involved in cognitive function (20). In addition, it is highly likely that cognitive performance is affected by the compounding alterations in multiple, connected regions in associative and/or cognitive control networks such as formed between the frontal cortical, parietal, and temporal areas (53, 55, 56).

Although the distribution of the 5-HTT across the whole primate brain is not particularly well studied, the cortex in general has low levels relative to subcortical areas such as the amygdala and bed nucleus of the stria terminalis (57). Within the low cortical range of values, levels are relatively high in several areas with genotype-associated morphological changes, such as the anterior cingulate, parietal and inferotemporal cortex (38, 50).

Serotonin is known to play a prominent role in prenatal and neonatal cortical development as well as in adult neurogenesis (55, 58, 59). Transient expression of the 5-HTT during development in non-serotonergic neurons in primates (60, 61) raises the possibility that 5-HTTLPR may influence cortical development (and adult morphology) in primates via early perinatal influence of 5-HT. Clearly, further studies of the contribution of 5-HT to postnatal development and an impact of the 5-HTTLPR on expression of the 5-HTT during development are required to determine the potential developmental impact of 5-HTTLPR on cortical morphology.

A limitation of the present study is the limited number of subjects. However, the present data do not exhibit any trend for a genotype-associated difference in 5-HTT binding which could be revealed with larger sample sizes. Furthermore, our conclusions are supported by the broad consistency across multiple domains of neurobiology and cognition of the present observations with previous observations in humans based on much larger sample sizes. Thus, the present data strongly support the rhesus macaque as an excellent translational model to further characterize the interaction between environmental variables and the anatomical, neurophysiological, and neurochemical impact of this genetic polymorphism thought to contribute to affective disorders, alcohol abuse, and aspects of temperament and cognitive function (27).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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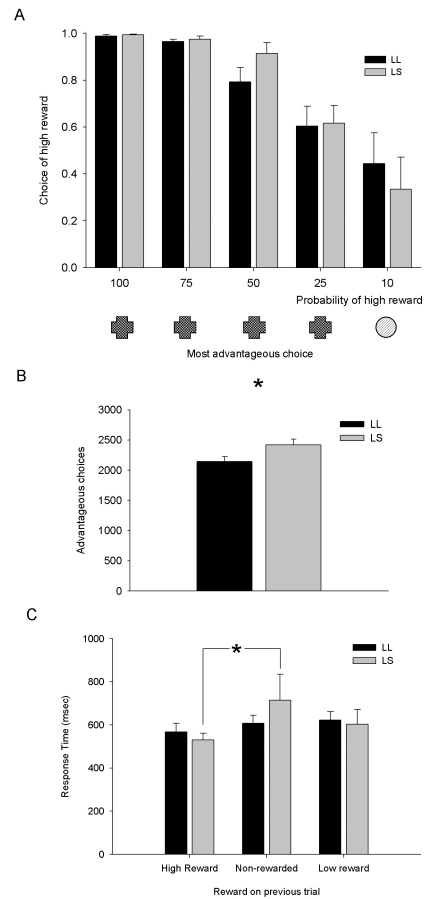
Figure-1.
S allele carriers make better probabilistic reward choices than LL subjects. (**A**) Both LS and LL subjects decrease the choice of the probabilistic reward when the reward probability is reduced during the task. The symbols below the graph refer to stimulus associated with the highest expected value at each probability level as described in further detail in the Supplement. (**B**) However, LS subjects make more advantageous choices (i.e. select the stimulus with the highest expected value) than LL subjects over the range of reward probabilities. *p=0.044 (**C**) The response time to choose between stimuli on a trial following reward omission is only increased in LS subjects. (main effect of reward outcome on previous trial LS p=0.005;LL p=0.125).
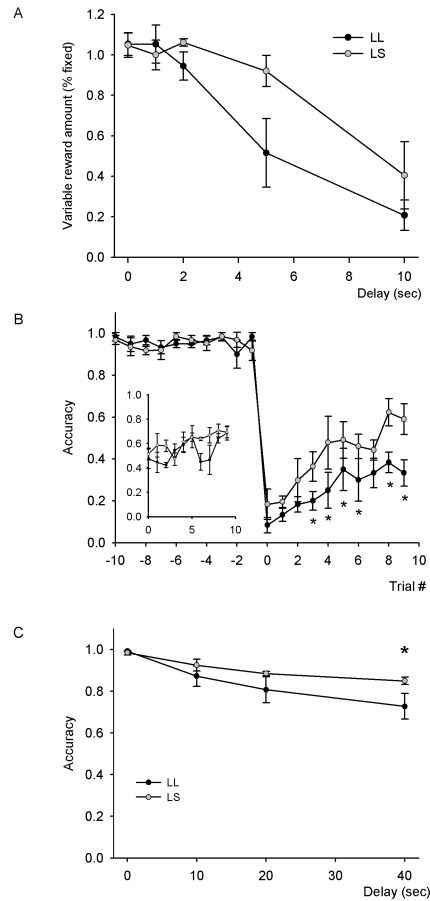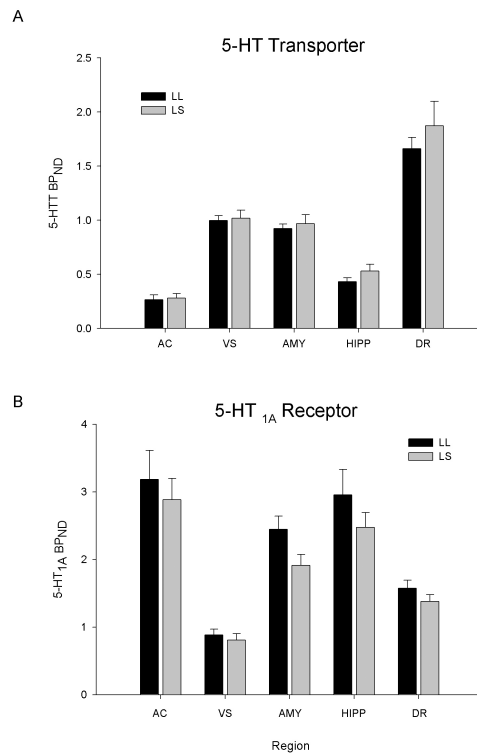
Figure-2.
S allele carriers exhibit better performance across multiple cognitive tasks. (**A**) S allele carriers are more tolerant of delay to obtain a larger reward than LL subjects. Despite the reduced reward magnitude of the immediate reward, LL subjects choose immediate reward more frequently than LS subjects, thereby further reducing its magnitude (genotype × delay interaction p=0.057). At 5 sec delay, S allele carriers choose the larger, delayed reward more often than LL subjects. *p=0.002 (**B**) S allele carriers more quickly adapt their choices to a new reward contingency on a non-serial reversal learning task. LS and LL subjects performed similarly during stimulus discrimination learning, but following reversal of the reward contingency LS subjects more rapidly switched to the new stimulus associated with the larger reward (performance based on 15 reversals; main effect of genotype p=0.034, trial by genotype interaction p=0.003. *post-hoc comparison between genotypes p<0.05. (*Inset*) Both groups learn at a similar following the start of a new set of stimuli). (**C**) S allele carriers exhibit better performance than LL subjects on a delayed match to sample task. The accuracy of LS and LL subjects declined with increasing delay, but the decline in performance of LL subjects was more severe (genotype × delay interaction p=0.046;* post-hoc comparison between genotypes p=0.025)

Figure-3.
PET imaging did not reveal genotype-associated differences.

(**A**) PET Imaging with [^{11}C] DASB demonstrated similar levels of 5-HT transporter binding potential in both LS and LL subjects (multivariate regression for all regions p=0.695) (**B**) PET Imaging using [^{11}C] WAY100635 revealed no difference in 5-HT$_{1A}$ binding potential between LS and LL subjects (multivariate regression for all regions p=0.116).

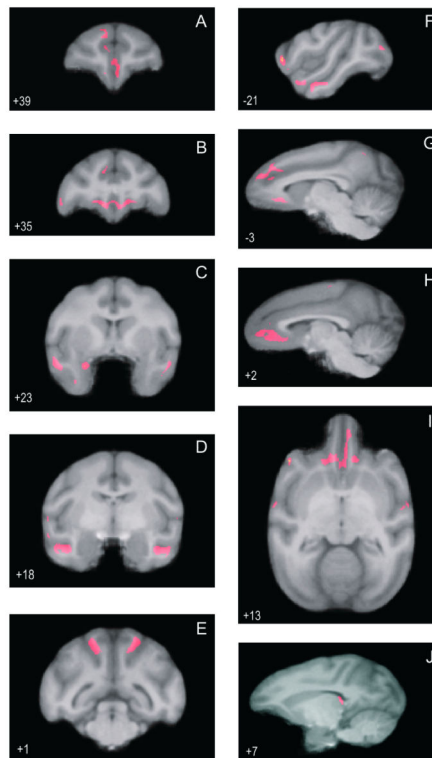AC: anterior cingulate, VS: ventral striatum, AMY: amygdala, HIPP: hippocampus, DR: dorsal raphe

Figure-4.
Multiple clusters of decreased gray matter volume in S allele carriers in frontal, temporal, and parietal cortices using a whole brain analysis. (**A-E**) Coronal views of clusters encompassing (**A**) Brodmann areas 8, 9, 10, 11, and 24;(**B**) areas 10, 13, 24, and 45;(**C**) amygdala, inferotemporal cortex area TE, and temporal pole;(**D**) area TE;(**E**) area 5. (**F-H**) Sagittal views of clusters in (**F**) left Brodmann area 45, temporal pole, and area TE;(**G**) areas 8,9, and 24;(**H**) areas 10, 13, and 32. (**I**) axial view highlighting that the clusters of reduced gray matter volume in S allele carriers are concentrated in the ventral frontal corical regions (Brodmann areas 10, 11, 13, and 45). (**J**) Based on post-mortem observations in humans, an ROI analysis was performed for the pulvinar nucleus of the thalamus, which revealed larger grey matter volume in S allele carriers.The values in the left bottom corners indicate the distance (in mm) from a reference location in the brainstem (negative values in sagital sections refer to the left side of the brain).

Table 1

Summary of VBM data

Reported are cluster extent, peak voxel t-value, associated p-value, and coordinates, and Brodmann areas (BA) of brain regions demonstrating a greater grey matter volume in LL-homozygotes compared to LS-heterozygotes based on a whole brain, 2-sample t-statistic analysis by genotype. Total grey matter was used as a covariate. Data were corrected for non-isotropic smoothness and thresholded at p=0.01 and k=1000 voxels. Coordinates refer to the distance (in mm) from a reference point in the brainstem in the anterior-posterior (x), medial-lateral (y), and dorsal-ventral (z) direction. Negative coordinate values correspond with the ventral, left, and caudal direction, respectively. The final columns list the corresponding panel in Fig. 4, and the analogous brain region in published human studies on genotype-associated morphology.

ACC anterior cingulate cortex, L Left, LR bilateral, R Right, SFG superior frontal gyrus

equivk	t-value	x	y	z	Area (rhesus)	Figure 4	Human Analog
5560	26.18	20	20	0	R inferotemporal cortex area TE	D	Inferior Temporal Gyrus IT G[1]
4697	10.89	−5	34	13	LR BA10, LR BA13, LR BA32, R BA11	A, B, H, I	Rectus[1], Medial SFG[1], ACC[2]
4364	15.78	6	−1	35	R somatosensory BA5	E	
3953	15.35	−3	38	28	L BA8, L BA9, L BA24	A, B, G	Med. SFG[1], ACC[2]
3148	24.17	−9	3	34	L somatosensory BA5	E	
2961	17.2	24	15	15	R anterior lateral, belt region auditory cortex	I	
2223	7.08	−22	26	3	L temporal pole	C, F	
1911	12.11	−22	19	2	L inferotemporal cortex area TE	D	Inferior Temporal Gyrus IT G[1]
1902	9.18	−27	16	18	L anterior lateral, belt region auditory cortex	I	
1798	13.49	−21	34	14	L BA45	B, F	Inferior Frontal Gyrus IFG[1]
1365	10.42	−11	23	3	L Amygdala	C	Amygdala[2]
1337	7.09	−20	−16	20	L Occipital area V1		
1191	5.73	1	−14	17	Dorsal cerebellum		Vermis[1]
1155	7.06	1	11	37	R BA4		

Height threshold: T = 3.36, p = 0.010 (1.000) {p<0.01 (unc.)}

Extent threshold: k = 1000 voxels, p = 0.000 (0.000)

Volume: 79020 µl; 632160 voxels; 4251.5 resels

[1]Canli et al (2005)

[2]Pezawas et al (2005)