Published in final edited form as:
Mol Psychiatry. 2007 June ; 12(6): 544–555.

Serotonin Transporter Polymorphism, Memory, and Hippocampal Volume in the Elderly: Association and Interaction with Cortisol

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Abstract

The *s* allele variant of the serotonin transporter gene (5-HTT) has recently been observed to moderate the relationship of stress to depression and anxiety. To date no study has considered interactive effects of 5-HTT genotype, stress and HPA function on cognition in healthy, older adults, which may reflect developmental, functional, or neurodegenerative effects of the serotonin transporter polymorphism.

We investigated whether 5-HTT genotype interacts with cumulative life stress and HPA-axis measures of waking and diurnal cortisol slope to impact cognition in 154 non-depressed, older adults. Structural images of hippocampal volume were acquired on a subsample of 56 participants.

The 5-HTT *s* allele was associated with both significantly lower delayed recall and higher waking cortisol levels. Presence of the *s* allele interacted with higher waking cortisol to negatively impact memory. We also observed a significant interaction of higher waking cortisol and the *s* allele on lower hippocampal volume. Smaller hippocampi and higher cortisol were associated with lower delayed recall only in *s* allele carriers. No impact or interactions of cumulative life stress with 5-HTT or cortisol were observed.

This is the first investigation to identify an association of the 5-HTT *s* allele with poorer memory function in older adults. The interactive effects of the *s* allele and waking cortisol levels on reduced hippocampal volume and lower memory suggest that the negative effect of the serotonin polymorphism on memory is mediated by the HPA axis. Further, given the significant association of the *s* allele with higher waking cortisol in our investigation, future studies may want to evaluate the impact of the serotonin transporter polymorphism on any neuropsychiatric or behavioral outcome which is influenced by HPA axis function in older adults.

Keywords

Serotonin transporter; 5-HTT; Stress; HPA; Memory; Hippocampus; Aging

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Introduction

Current research has focused on the role of genetic variations in the promoter region of the serotonin transporter gene (5-HTTLPR) in depressive symptomatology, anxiety, and personality traits.[1–3] This polymorphism is a 44-base pair (bp) allelic insertion (long or *l* allele) or deletion (short or *s* allele) variant regulating the transcription of the 5-HTT.[4] The *s* allele is associated with lower levels of serotonin uptake and lower transcriptional efficiency of the serotonin transporter.[5]

A growing literature implicates the serotonin transporter gene in the development and integrity of neural systems which subserve complex emotional and cognitive processing.[6] Serotonergic activity is implicated in cognitive function, and is proposed to interact with the cholinergic system to impact memory and attention.[7–9] Schultz et al.[10] observed an association between increased phobic symptoms and lower global cognitive function in older adults, with a trend for this association to be exacerbated in those with the 5-HTT *s* allele. In healthy, nondepressed adults, Canli et al.[11] identified a range of functional and structural regional brain differences between *s* allele carriers and non-carriers, suggesting 5-HTT genotype affects neural systems controlling affective, motor, and cognitive processes.

In addition to impacting cognition, the serotonergic system is involved in the regulation of Hypothalamic-pituitary-adrenal (HPA) axis function.[12, 13] Animal studies indicate serotonin involvement in both activation and feedback control of the HPA axis, as well as a direct effect of serotonin on hippocampal glucocorticoid receptors.[14, 15] HPA axis dysfunction, particularly elevated levels of endogenous cortisol, are consistently associated with impairments in cognition.[16, 17] Exposure to higher levels of cortisol results in hippocampal damage by inhibiting synaptic transmission, decreasing dendritic branching, and causing neuronal injury and death.[18–20] These negative effects are proposed as potential physiological mechanisms underlying the documented association of higher cortisol levels with age-related cognitive decline and onset of dementia.[21–25]

Stressful life events have long been hypothesized to impair neuroendocrine function, with elevated cortisol levels associated with acute stress, and impaired diurnal rhythms of cortisol associated with chronic stress.[16, 26] Recent studies observe an interactive effect of the *s* allele variant of 5-HTT and stress on neuropsychiatric outcomes.[27, 28] Caspi et al. [27] were the first to report that the 5-HTT genotype moderates the influence of stressful life events on depression in a dose-dependent fashion, with young adults homozygous for the *s* allele being more sensitive to the depressogenic effect of stressful life events than those homozygous for the *l* allele. Kendler [28] similarly observed this relationship in a population-based sample of mostly middle-aged adult twins. But not all studies have replicated these findings. In an investigation of over 1000 Australian twins, Gillespie et al.[29] found a main effect of stressful life events on major depression but no main or interactive effect of 5-HTT genotype. This investigation differed from those with positive findings by virtue of a broader age range and a more limited reporting period for stressful life events (past 12 months). Other studies have observed gender differences in terms of the interactive effects of 5-HTT *s* allele and environmental factors on depression, with female *s* allele carriers being more prone to developing depression and male *s* allele carriers protected from this disorder.[30, 31] Although developmental stage, gender, and timing of stressful life events likely modify any impact of 5-HTT genotype, a significant literature is cumulating to support the perspective that the *s* allele interacts with stress to negatively impact a variety of behavioral outcomes,[32] including depression,[8,33, 34] alcohol and drug use[35] and psychotic symptoms in Alzheimer's Disease.[36]

While many studies have focused on the vulnerability of the 5-HTT *s* allele to psychological stressors, fewer have investigated the relationship of the 5-HTT gene to physiological markers

of stress such as cortisol. We are aware of no study that has considered an interactive effect of 5-HTT genotype and measures of HPA function on cognition.

To address whether 5-HTT genotype interacts with either physiological or psychological stress to impact cognitive function and hippocampal volume, we examined this issue in an ongoing investigation of the relationships among these variables in non-depressed, community dwelling older adults.

Methods

Subjects were 154 community-dwelling older adults participating in an investigation of cognitive decline. They were recruited through advertisements and local senior centers. Recruitment advertisements were general in nature, asking for individuals who were interested in participating in "A Study of Stress and Cognitive Functioning in Older Adults." All subjects provided informed consent in accordance with Stanford University IRB regulations.

Subjects were 103 females and 51 males, between 60 and 100 years of age (M = 71.13; SD = 8.8), with a mean 16.17 years of education (SD = 2.8). All subjects were Caucasian. An initial evaluation included demographics, self-reported current and past medical status, the Mini-Mental State Exam (MMSE) [37] and Structured Clinical Interview for DSM-IV-TR. Inclusion criteria were 60 years of age or over, capable of giving informed consent and sufficient visual and auditory acuity for the cognitive testing. Individuals were excluded if they had a MMSE less than 26, a diagnosis of possible or probable dementia, any serious medical illness or any Axis I disorders currently, or within the last two years. Participants were also excluded if they were currently using any systemic corticosteroids, psychotropic medication, short-acting anxiolytics, sedative hypnotics, or medications with significant cholinergic or anticholinergic side effects, or any FDA-approved medications for Alzheimer's disease (AD). Upon entry into the study, whole blood was drawn and transported immediately to the genetics laboratory, and DNA extracted upon arrival. The following measures were assessed, in order.

Cognitive Battery

As part of ongoing assessments at our laboratory, subjects are administered an extensive cognitive battery. For analytic purposes we identify a priori those cognitive domains most pertinent to the issues under investigation. We insure the measures are independent, and reduce those which are significantly correlated to yield one independent measure of the domain of interest. In the current investigation, we selected, a priori, four cognitive domains which we considered most relevant to the issues under investigation. The cognitive domains selected were 1) delayed recall from verbal memory (assessed by the Rey Auditory Verbal Learning Test (RAVLT)[38]); 2) information processing speed and attention (assessed by the Stroop Color and Word Test (SCW)[39]), 3) verbal naming (assessed by the Boston Naming Test (BNT)[40]), and 4) visuospatial ability (Judgement of Line Orientation (JLO)[41]). Prior work from our and other groups has consistently found lower performance on measures of delayed recall to be associated with increased age, elevated cortisol response, and reduced hippocampal volume.[42–44] Deficits in the other three domains are common in older adults and are associated with an increased risk of cognitive decline and onset of dementia.[45] One outcome measure from each test was selected. For the RAVLT we choose the delayed recall component, i.e. RAVLT 7. For Stroop Color and Word test we employed the time taken to complete the color/word component. Total number answered correctly served as the outcome measure for the Boston Naming Test, and also for the Judgment of Line Orientation. The MMSE[37] was included as a brief mental status examination to quantify global cognitive functioning. The selected measures are widely used and have proven reliability (further detail on the cognitive tests is provided in supplementary materials).

Assessment of Mood and Cumulative Life Stress

The Geriatric Depression Scale (GDS)[46] is a widely-used depression screening device specifically designed for the elderly.

The Life Stressor Checklist—Revised (LSC-R)[47], a 30-item questionnaire, assesses the number of traumatic stressors and negative events occurring over one's lifetime, the age at which the event occurred, the duration and perceived impact of these event. It was designed as a screen for life events that meet DSM-IV criteria for trauma. Total score for the perceived impact of stressful events across the lifespan was employed (further detail on our assessments of mood and cumulative life stress are provided in supplementary materials).

Cortisol measures

Directly following the cognitive assessment, each subject completed the following cortisol protocol on two consecutive days in their own home. Subjects collected saliva in salivette devices and samples were obtained upon waking, 30 minutes later, and at 1200h, 1700h, and 2100h. Samples were refrigerated immediately after collection. Our prior work comparing actual times recorded by participants at each saliva collection with the actual intervals between collection times recorded by a Medication Event Monitoring Units indicated that older adults were highly compliant with the protocol.[48] Full details of our protocol are provided in Kraemer et al..[48] Samples were stored at −80C prior to laboratory centrifugation and assays for salivary cortisol using luminescence immunoassay (LIA) reagents provided by Immuno-Biological Laboratories, Inc. Hamburg, Germany. Assay sensitivity was 0.015 μg/dl. Intra-assay variation on three saliva pools of the low, medium, and high controls were averaged 2.78%, 10.45%, and 4.79% respectively. The mean values of the low, medium, and high controls were .054 μg/dl, .228 μg/dl, and .863 μg/dl. The inter-assay coefficients of variation for the low, medium, and high controls were 10.9%, 10.5%, and 5.5%, respectively.

Two measures of cortisol response were identified a priori for investigation in the current study; namely waking cortisol level and diurnal slope. Waking cortisol has been associated with memory deficits,[49] and recent work has also suggested that the slope of diurnal cortisol may be an important measure of stress responsiveness.[26] Waking cortisol was calculated as the average of the waking cortisol values from each of the two days of collection. The slope of diurnal cortisol variation was calculated with log-transformed regression using the average of each cortisol collection time point from the two days of collection.

Genotyping

DNA was extracted from 200nl of frozen blood using the Qiagen DNeasy Kit (Cat.#69506). Oligonucleotide primers flanking the 5-HTT-linked polymorphic region[4] and corresponding to the nucleotide positions −1416 to −1397 (stpr5, 5'-GGC GTT GCC GCT CTG AAT GC) and −910 to −888 (stpr3, 5'-GAG GGA CTG AGC TGG ACA ACC AC) of the 5-HTT gene 5'-flanking regulatory region were used to generate 484bp or 528bp fragments. Polymerase Chain Reaction (PCR) amplification was carried out in a final volume of 30nl consisting of 50ng of genomic DNA, 50ng each of sense and antisense primers, 15nl of Taq PCR Master mix (Qiagen, Cat.#201445), 10% DMSO and 1M Betaine. Annealing was carried out at 61°C for 30s, extension at 72°C for 1 min, and denaturation at 95°C for 30s for a total of 35 cycles. The PCR products were electrophoresed through 5% Polyacrylamide gel (Acrylamide/bis-Acrylamide ratio 19:1) at 120 V for 60 min. A 100bp marker was used to measure the PCR product size for *l* and *s* allele. Two independent observers, blind to any information pertaining to the participants, assigned the alleles and genotypes.

Neuroimaging Measures

Neuroimaging was conducted on a subset of our participants. To avoid selection bias, the first 60 subjects enrolled in this investigation were also approached for participation in the neuroimaging component. Fifty-six subjects fulfilled the eligibility criteria for participating in the MRI (e.g. no internal metal, not claustrophobic).

Following the cognitive and cortisol assessments, MRI scans were performed on a GE Signa 1.5 Tesla clinical scanner with a standard GE head coil. The pulse sequences were: (a) Spin-echo, sagittal localizer 20, 3 mm thick slices (acquisition time = 1 min 44 s); (b) Proton density and T2-weighted spin-echo MRI, TR/TE1/TE2 = 5000/30/80 ms, 51 contiguous axial slices (3 mm) covering the entire brain and angulated parallel to the long axis of the hippocampal formation, .976 x .976 mm2 in-plane resolution (acquisition time = 17 min); and (c) Three-dimensional spoiled GRASS (SPGR) MRI of entire brain, TR/TE = 9/2ms, 15o flip angle, perpendicular to the long axis of the hippocampi, .976 x .976 mm2 in-plane resolution, 1.5 mm slice thickness, no skip (acquisition time = 7 min 27 s). The double spin echo scan was axial, parallel to the long axis of the hippocampal formation. The T1/SPGR images were collected in the coronal plane, perpendicular to the long axis of the hippocampus.

Hippocampal volumes were evaluated using a semi-automatic, commercially available, high dimensional brain-mapping tool (Medtronic Surgical Navigation Technologies, Louisville, CO). First a rater manually places 22 reference points as local landmarks around the hippocampus. A landmark is placed at the hippocampal head and at the tail. Then the program divides the length of the hippocampus into five equally-spaced coronal images perpendicular to the long axis of the hippocampus. The rater completes the landmark placement with four landmarks per coronal image; i.e. at the superior, inferior, medial, and lateral boundaries. A fluid image transformation is used to register the individual MRI scans to a reference anatomy template which has the hippocampus carefully labeled. Pixels corresponding to the hippocampus are counted to obtain volume measurements. This method has been independently validated by comparing it with manual hippocampal tracing in AD patients and controls.[50, 51] Manual and semi-automated hippocampal volume measurements generally correlated better than 0.9[52] but semi-automated methods depend less on rater judgment, reducing potential rater bias. To adjust for differences in head size, all hippocampal volumes were normalized to total intracranial volume (TIV) by dividing total hippocampal volume by TIV. TIV was identified using a mask containing the brain as identified on the T2-weighted image, as described previously.[53]

Statistical Analysis

We employed a multiple regression analysis to test the impact of 5-HTT, HPA function, and life stress on cognition. 5-HTT status, waking cortisol, slope of diurnal cortisol, cumulative life stress, and all possible interactions served as independent variables, with performance on the four cognitive measures serving as the dependent measures. None of the outcome measures were significantly correlated, thus we considered these independent tests for the purposes of analyses. Since interactive effects were included, all independent variables were centered at the mean to insure interpretability of the regression coefficients. Further discussion of centering in regression analysis can be found in Kraemer and Blasey.[54] The distributions of the raw waking cortisol values and log-transformations for these values were sufficiently skewed that it was not clear they would fulfill the linearity assumptions necessary for conducting a multiple regression analysis. We divided the cortisol values into a 5-point scale giving 1 to those in the lowest quintile, 2 to those in the second quintile, etc., bringing the linearity assumptions closer in line with those required for employing a parametric procedure, yielding the same effect whether raw or log-transformed values for cortisol are employed. Residual analyses were conducted as the diagnostic procedure to ascertain whether there were any deviations from

either the linearity or the equal variance assumptions central to linear regression. No compromises were detected. Kruskal-Wallis analysis was used to compare the three genotypes with respect to HPA function. The same analytic approach was employed to consider the impact of 5-HTT genotype and cortisol on hippocampal volume in the 56 subjects who participated in the neuroimaging component of the study. All statistical tests were conducted at the two-tailed 5% level. Only 8 of the 56 subjects who underwent neuroimaging were homozygous for the *s* allele. This is not a sufficiently large sample to yield enough power to meaningfully consider the 5-HTT genotype groups separately for the neuroimaging component. Thus, in this analysis, *s* allele carriers were compared with *l* allele homozygotes. In secondary analyses, we included depressive symptomatology and all interactions in the multiple regression model. We also employed the same regression model using additional imaging measures as secondary outcome measures. These included Total Intracranial Volume, Total Gray Matter Volume, Total White Matter Volume, and CSF.

Results

Table 1 displays the distribution of 5-HTT genotype. The frequencies of the *s* and the *l* allele are .442 and .558, respectively, which is in line with frequencies observed in other Caucasian samples.[55] Genotypes do not deviate from Hardy-Weinberg equilibrium.

No significant differences were observed with respect to the 5-HTT genotype groups for any of the demographic variables. Kruskal-Wallis analysis revealed a significant difference among the 3 allele groups with respect to waking cortisol, but not slope of diurnal cortisol, with individuals with the *s* allele having significantly higher waking cortisol values (see Figure 1 and Table 1). No differences were observed among the allele groups on the GDS or the LSC-R.

Multiple regression analysis revealed a statistically significant association of 5-HTT genotype with lower delayed recall scores on the RAVLT 7 ($t = -3.00$; $p<0.003$), with *s* allele carriers having significantly lower memory scores (see Figure 2 and Table 2). The mean delayed recall performance of those homozygous for the *s* allele was two-thirds of a standard deviation below the performance of those homozygous for the *l* allele. Also, the *ss* genotype group performed approximately one standard deviation lower than age and education appropriate normative values for the RAVLT 7 in the literature.[56] Performance greater or equal to one to one and a half standard deviations below age and education-matched normative values on a cognitive test can be indicative of impairment.[57] There was a main effect of waking cortisol ($t = -2.47$; $p<0.01$) and slope of diurnal cortisol ($t = -3.27$; $p<0.001$) with higher waking cortisol and steeper diurnal rhythms associated with lower delayed recall. 5-HTT genotype interacted with waking cortisol, but not slope of diurnal cortisol, on memory ($t=-1.98$; $p<.05$). Elevated waking cortisol was most strongly associated with lower memory in *s* allele carriers. Pearson product moment correlations between waking cortisol and delayed recall were $r=.12$, $r=-.21$, and $r=-.36$, for the *ll*, *ls*, and *ss* groups respectively (Figure 3). No main effect or interactions of the LSC-R with 5-HTT or cortisol were observed, and there were no associations of our predictor variables with any of the additional cognitive measures. Although a list learning measure of delayed recall from episodic memory and the Wechsler Memory Scale-II were not included in this study since we had identified the RAVLT 7 to serve as our primary measure of delayed recall, poorer performance on the delayed recall components of these tests were similarly negatively associated with the *s* allele and the *s* allele interacted with higher waking cortisol to result in lower performance on these tasks (data not shown).

With respect to hippocampal volume, there was no main effect of 5-HTT *s* allele on hippocampal volume. There was a significant main effect of waking cortisol ($t = -2.91$; $p<.01$), with higher levels associated with lower hippocampal volumes. A significant interaction of the

s allele and higher waking cortisol on lower hippocampal volume (t= −1.97; p<.05) was observed. This relationship was stronger among *s* allele carriers than non-carriers, with Pearson product moment correlations of r= −.49, and r= −.25, respectively (Figure 4). In an additional multiple regression with the RAVLT 7 as the outcome measure, lower hippocampal volume and higher waking cortisol were associated with lower delayed recall performance only in those with the *s* allele (t= −1.99; p<.05).

In secondary analyses, we included the GDS in the original multiple regression model and observed no interactive effect of depressive symptomatology, with 5-HTT genotype, stress, and cortisol on any cognitive measure. There were no differences in our findings when we covaried for age, gender or household income. Only 5 participants smoked, and only 17 consumed more than 2 drinks a week. Neither current nor past history of smoking or drinking impacted any of the observed relationships. Using the same multiple regression model approach, we examined for any contribution of the subcomponents of the LSC-R, including type of stressful event, age at which the event occurred and its associated impact. No significant independent or interactive effects were observed for any of these variables. We observed no significant impact or interaction of 5-HTT genotype, cortisol and cumulative life stress on any of the secondary neuroimaging measures (Table 3 and 4).

Discussion

In the current study, the 5-HTT *s* allele was significantly associated with lower memory performance in non-depressed, community-dwelling older adults. The poorest memory was exhibited by subjects homozygous for the *s* allele. Contrary to prior observations of interactive effects of psychosocial stress and 5-HTT on neurobehavioral outcomes,[27] we did not observe an interaction of 5-HTT and life stress on any cognitive domain. However, the 5-HTT *s* allele was associated with higher levels of waking cortisol, a physiological marker of stress. In addition to an independent association with both lower memory and higher waking cortisol, the *s* allele interacted with higher cortisol levels to result in lower delayed recall performance. Although we observed no impact of the 5-HTT *s* allele on hippocampal volume, higher waking cortisol significantly interacted with the *s* allele on lower hippocampal volumes. Higher waking cortisol and lower hippocampal volumes were associated with lower memory performance only among *s* allele carriers. Taken together, these findings go beyond the current perspective that the *s* allele variant of 5-HTT increases the risk for neuropsychiatric disorders in the presence of stress by implicating the 5-HTT *s* allele in cognitive function, specifically poorer delayed recall performance.

We are not the first to suggest a role for 5-HTT genotype in cognitive processing. In their fMRI investigation of healthy, non-depressed adults, Canli et al.[11] observed a range of functional and structural regional brain differences between *s* allele carriers and non-carriers during an emotional stroop paradigm leading the authors to suggest that 5-HTT genotype affects neural systems controlling affective, cognitive, and motor processes. Yet, they observed no performance differences according to *s* allele status on any condition of the emotional Stroop task, which draws on both affective and non-affective cognitive processes. Similarly, Hariri et al.[58] found in an fMRI investigation of healthy normal volunteers that *s* allele carriers and *l* allele homozygotes did not differ with respect to either reaction time or accuracy on an "n-back task", measuring working memory and attention. Also, performance was not associated with any brain regional activation differences according to genotype group.[58] The performance findings from these studies are consistent with our observed lack of association of 5-HTT genotype with the Stroop measure of attention, but contrary to our investigation they did not consider measures of delayed recall, and their findings regarding attention were secondary to a primary focus on emotion processing. Our observation of a significant negative impact of the 5-HTTLPR *s* allele on delayed recall and the observed interaction of waking cortisol and

the *s* allele on hippocampal volume speaks to a potentially important role of serotonin function in memory processing in older adults.

The involvement of 5-HTT genotype in modulating serotonin neuronal function is complex. The *s* allele has been associated with less transcriptional efficiency, reduced serotonin reuptake and less 5-HTT binding in the brain. However, recent neuroimaging assessments of 5-HTT binding potential observe no differences between *l* allele homozygotes and *s* allele carriers, producing a less consistent picture of 5HTT function in the in vivo brain.[59] 5-HTT knockout mice exhibit impaired clearance and marked elevation of extracellular levels of 5-HT in forebrain and hippocampal regions.[60, 61] While 5-HT is crucial for brain development, excess extracellular 5-HT has deleterious effects on neurogenesis and synaptogenesis, preventing or partially interfering with the development of the cortical projection areas during critical periods of brain development.[62–64] Lesch et al.[5] propose that this mechanism paves the way for impaired synaptic plasticity in adulthood, and sets the stage for later brain dysfunction. Others argue that *s* allele status confers increased vulnerability to a range of behavioral symptoms secondary to age-related neurodegenerative processes, particularly in response to stress.[65] Thus, the phenotype associated with the 5-HTT *s/l* alleles may result from developmental, functional, or neurodegenerative effects of the serotonin transporter polymorphism or a combination of these effects.

The higher cortisol levels and lower memory function observed in the *s* allele carriers in our study may independently reflect 5-HTT-associated impairments in neuronal development. If impairments in neuronal development were to contribute to smaller hippocampal volume this in turn could contribute independently or sequentially to HPA dysregulation and lower memory function. However, we did not observe a main effect of the *s* allele on hippocampal volume but we did observe a significant interaction of the *s* allele and higher cortisol on lower hippocampal volume. Thus, another interpretation is that neuronal development in *s* allele carriers increases vulnerability to dysregulation of HPA function with a subsequent negative effect on hippocampal volume and memory. Alternatively, given the overall correlation of elevated waking cortisol and lower memory function and reduced hippocampal volume, *s* allele carrier status may simply increase vulnerability to negative effects of higher cortisol levels on hippocampal volume and function, independent of the source of HPA dysregulation.

The serotonergic system is implicated in hippocampal regulation of HPA axis activity,[66] increasing hippocampal glucocorticoid receptors, specifically during certain developmental periods.[67] Developmental modification of the ascending serotonin system has been suggested to result in long-term dysregulation of HPA function.[12] Hippocampal regions are vulnerable to neurotoxic effects of dysregulation of the HPA system, specifically chronic elevated levels of glucocorticoids. Sapolsky et al.[25] proposed that the hippocampal damage caused by elevated cortisol levels alters the normal inhibitory control of corticosteroids on the hippocampal control of HPA activity, resulting in a forward cascade in which the elevated cortisol levels it causes further damage the hippocampus. The negative impact of elevated glucocorticoids on hippocampal formation is implicated in cognitive impairment,[22–25, 68] and our observed negative association of higher waking cortisol with memory performance is consistent with prior observations.[17, 42] Fewer studies have examined the relationship between HPA function and hippocampal volume in normal, older adults, and the variable findings may reflect the fact that moderating factors such as 5-HTT genotype were not considered.[16, 42, 69]

Andrews et al.[12] suggest that interactions between serotonin and glucocorticoids are likely bidirectional, thus any dysregulation of HPA function may also have a forward cascade effect on serotonin function. For example, prenatal exposure to glucocorticoids appear to influence serotonin production[70] and also 5-HTT expression.[12] Such effects may be exacerbated in those with the *s* allele. To date, a limited number of human studies have directly considered

the impact of 5-HTT genotype on measures of HPA function. These investigations found no association of 5-HTT genotype with plasma cortisol,[65, 71] although Smith et al.[65] observed a trend towards blunted cortisol and prolactin response to acute pharmacologic increase of serotonin in those with the *s* allele. Most of these investigations did not examine basal levels, and were conducted on small samples of younger adults, with limited power to test genotype differences. However, in one of the few large studies conducted, involving 139 middle-aged, adults, baseline plasma prolactin levels were found to be lower in *s* allele homozygotes.[72] Animal models have yielded mixed findings with some observing blunted neuroendocrine response, and others observing increased neuroendocrine response to stress in genetic variations hypothesized to be similar to *s* allele status.[73–76] The animal findings are also mixed regarding basal levels of cortisol.

In our study, the association of *s* allele status with higher waking cortisol levels did not appear to reflect cumulative life stress. Indeed, cumulative life stress, alone or interaction with 5-HTT genotype and cortisol, was not associated with any outcome. Given the likely developmental effects of the 5-HTTLPR on neural circuitry mediating stress reactivity and growing evidence that early life trauma plays a key role in modulating stress response on behavioral outcomes and hippocampal volume,[27, 77, 78] we examined individual items of the LSC-R, including the age at which the event occurred. The components of the LSC-R cover many of the same stressful life events assessed by other investigations of the relationship of 5-HTT genotype and stress on behavioral outcomes.[27–29] Only seven of the subcomponents of the LSC-R emphasized stressful events during childhood (see supplementary materials for complete details) but we found no item to be associated with cognition alone or in interaction with 5-HTT genotype or HPA measures. The impact of cumulative life stress was moderate across all three genotype groups, and may not have been sufficient to reflect vulnerability of the *s* allele to such stressors. HPA axis measures may be more sensitive indicators of mild stress. Indeed, the observed dose-dependent association of the *s* allele with higher waking cortisol values may account in part for previously observed vulnerability of this allele to a variety of neuropsychiatric outcomes, such as anxiety or depression, in response to stress.[27]

It is also possible that our retrospective self-report measure underestimated the level of life stress, especially in older adults who have recall difficulties. Further, distal traumatic events may be less relevant to older adults than chronic, ongoing daily hassles, and level of social support. Yet, Gillespie et al.,[29] in one of the few investigations to include older adults, observed no interactive effect of recent stressful events and 5-HTT genotype, albeit on depression. These authors did observe stressful events to be associated with increased depression, while we observed no impact of cumulative life stress on cognitive function. Our sample was healthy and well-educated, factors which may have modified any negative effects of stress exposure. For example, Manuck et al.[72] observed increased prolactin response to fenfluramine challenge in *s* allele carriers who had lower socioeconomic status. With a mean annual household income of $50k, few of our participants had low socioeconomic status. The 5HTT *s* allele has been negatively associated with smoking and drinking behaviors,[79, 80] but findings are considerably mixed.[81, 82] Increased smoking is also documented to affect HPA function.[83] In our study, neither current nor past history of smoking or drinking, nor household income impacted our findings. Our observed interaction between 5-HTT genotype and higher cortisol levels on hippocampal volume and memory is all the more striking given that our sample was healthy, well-educated, and had a high mean level of education.

Another perspective is that the current findings reflect increased vulnerability in *s* allele carrier status to neurodegenerative processes rather than 5HTT-associated impairments in neurodevelopment. Smith et al.[65] suggest that 5-HTT genotype may interact with normal aging processes, in terms of reduced capacity for older adults with the *s* allele to adapt to age-related alterations in serotonin function, with the resulting emergence of behavioral symptoms,

particularly secondary to neurodegenerative diseases. Certainly, AD is associated with an extensive serotonergic deficit.[84] This has led investigators to consider the prevalence of the 5-HTT *s* allele in AD patients. Studies have been inconclusive, with some observing the *s/s* genotype to be more prevalent in late-onset AD[85, 86] and other larger studies observing no association.[87, 88] However, an increasing number of investigations suggest that diminished serotonergic function may contribute to cognitive impairment in both demented and non-demented patients.[89–91] Animal studies implicate reduced reuptake and excess extracellular 5-HT in hippocampal dendritic atrophy.[23] Thus, the impaired transcriptional efficiency of the 5-HTT *s* allele may contribute to or exacerbate the cognitive impairment and reduced hippocampal volume that reflects ongoing age-related changes in neuronal function in the elderly. The observed elevations in cortisol in our investigation may also independently reflect age-related neurophysiological changes in this cohort. However, the relationship of 5-HTT genotype and cortisol to brain structure and function did not covary with age in our study. Future studies are required to investigate whether our findings might generalize to younger or middle-aged adults.

Another consideration is that any negative impact of less effective serotonergic function on memory in older adults may reflect the lack of any benefit of serotonin on mood. None of our subjects had major depression. Yet even mild levels of depressive symptoms are associated with cognitive impairment,[92] and hyperactivity of the HPA axis is implicated in depression.[93] When we included level of depressive symptomatology, assessed by the Geriatric Depression Scale, in the model in secondary analyses we observed no impact of level of depressive symptomatology on the interaction of 5-HTT *s* allele and cortisol to memory. Thus, any association of the 5-HTT *s* allele to lower memory performance did not appear to reflect increased levels of even mild depression. However, given that the GDS is a screening instrument and the range of values for the sample under investigation was relatively narrow, this measure may not have captured more subtle depressive symptoms. The lack of any impact of depressive symptomatology on the interaction of 5-HTT *s* allele and cortisol to memory must be interpreted cautiously. Future studies should consider measures that better characterize mild depressive symptoms, which may not fulfill the criteria for Axis I depressive disorder but may still mediate the relationship between 5-HTT genotype and memory function.

There are several other limitations which constrain the interpretations that can be drawn from our study. The cross-sectional design limits the extent to which we can identify developmental or mediational relationships among 5-HTT genotype, HPA axis function and cognitive function and brain structure. Also, we focused only on hippocampal volume, and did not examine other brain regions related to stress and cognition, such as the amygdala, frontal and pre-frontal regions. Additionally, the moderate levels of life stress reported by our participants may have restricted the extent to which we could examine the 5-HTT stress-vulnerability hypotheses. Finally, although the 5-HTTLPR was first reported to consist of *s* and *l* alleles, Hu et al[94] reported a single nucleotide polymorphism which gives rise to two functional variants of the L allele. The L_G variant, occurs in approximately 6.5% of Caucasian Americans[95] and is associated with comparable levels of serotonin transporter expression levels to the *s* allele.[94] Presence of the L_G variant among the *l/s* genotype group may have accounted for some of the observed variability between waking cortisol and delayed recall scores in that group. Future studies with sufficient power to investigate the trialleleic subgroups should examine this possibility. Finally, all subjects were Caucasian and our findings may not be generalizable to non-Caucasian populations. Further investigations of this issue need to be conducted on other ethnic groups.

In conclusion, the current study finds the 5-HTT *s* allele to be a marker for poorer memory and higher wakening cortisol in older adults. The significant negative interaction of the 5-HTT *s* allele and cortisol on both lower memory and hippocampal volume may reflect independent

or combined neurodevelopmental, functional, or neurodegenerative effects of the serotonin transporter polymorphism. A clearer understanding of the involvement of the 5-HT system in contributing to HPA activity, hippocampal and cognitive changes in older adults, including increased understanding of the mediational pathways among these factors, could point to new therapeutic approaches for ameliorating memory impairment in older adults who may be at increased risk for memory decline conferred by 5-HTT *s* allele carrier status. Longitudinal studies are required to more fully elucidate the potential complex relationships among these factors. Future investigations should also consider whether the serotonin polymorphism impacts any neuropsychiatric or behavioral outcome or function, which is influenced by HPA axis function, independently or secondary to stress.

Acknowledgements

This work was supported in part by National Institutes of Health grants AG 18784; AG 17824; and MH70886, and the Department of Veteran Affairs, Sierra-Pacific Mental Illness Research, Education, and Clinical Center (MIRECC).

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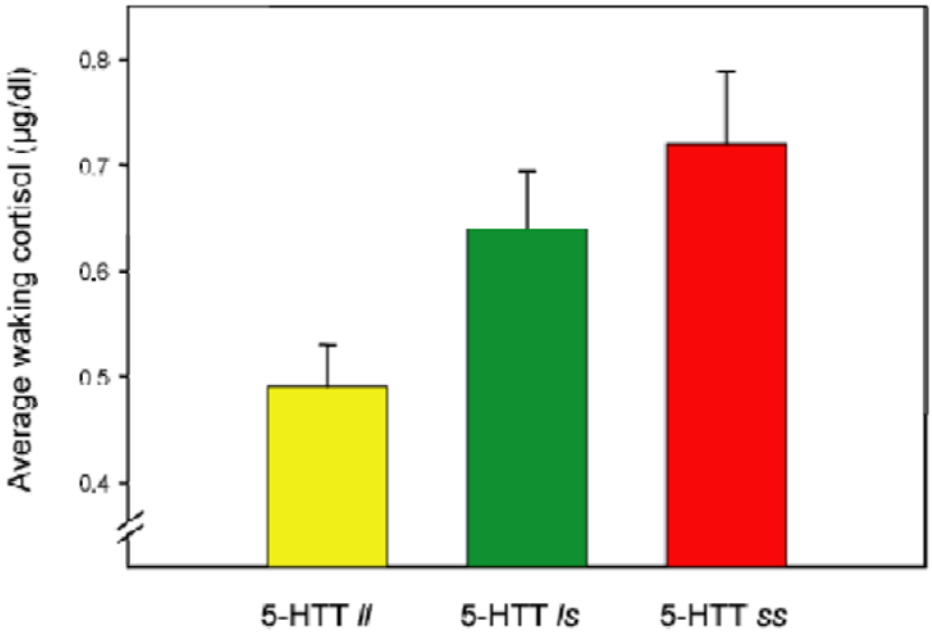

Figure 1.
Main effect of 5-HTT genotype on average waking cortisol (ng/dl).
Abbreviations: 5-HTT, serotonin transporter gene; *s*, short allele; *l*, long allele.

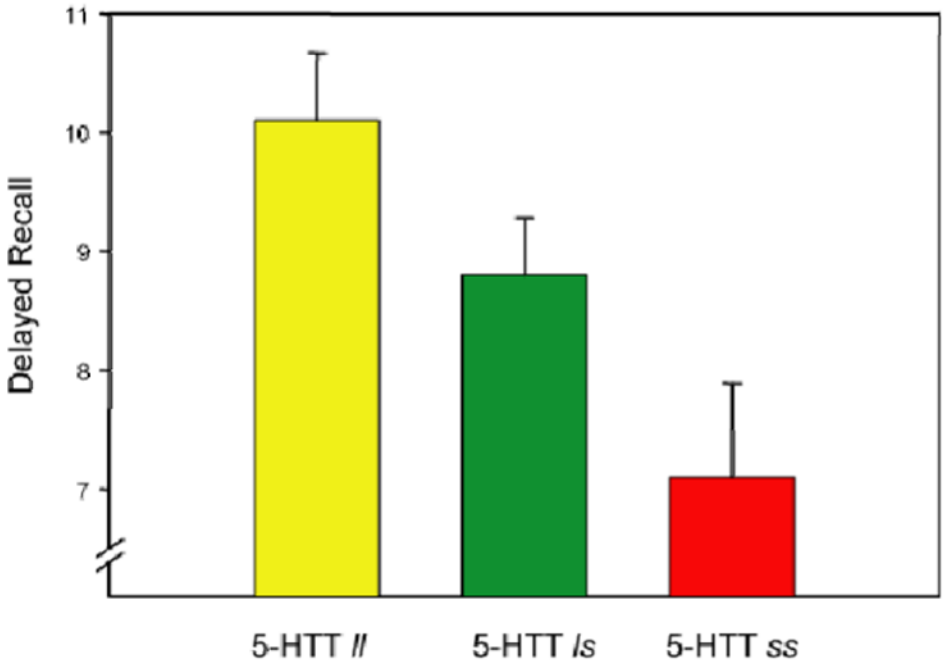

Figure 2.
Main effect of 5-HTT genotype on delayed recall performance as assessed by the Rey Auditory Verbal Learning Test- Delayed Recall.[38]
Abbreviations: 5-HTT, serotonin transporter gene; *s*, short allele; *l*, long allele.

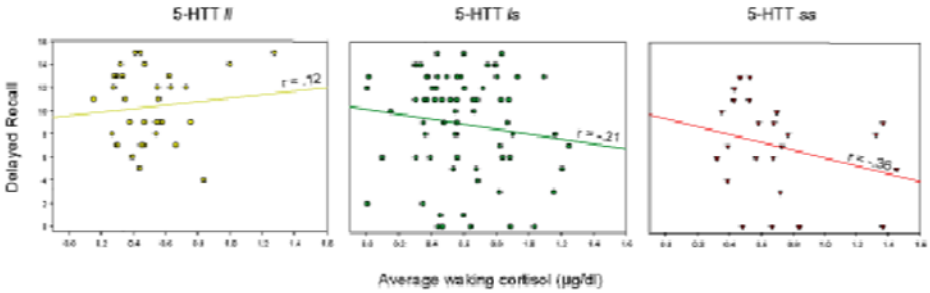

Figure 3.
Association of waking cortisol and delayed recall performance as assessed by the Rey Auditory Verbal Learning Test [38] as a function of 5-HTT genotype (*ll* vs. *ls* vs. *ss*).
Abbreviations: 5-HTT, serotonin transporter gene; *s*, short allele; *l*, long allele.

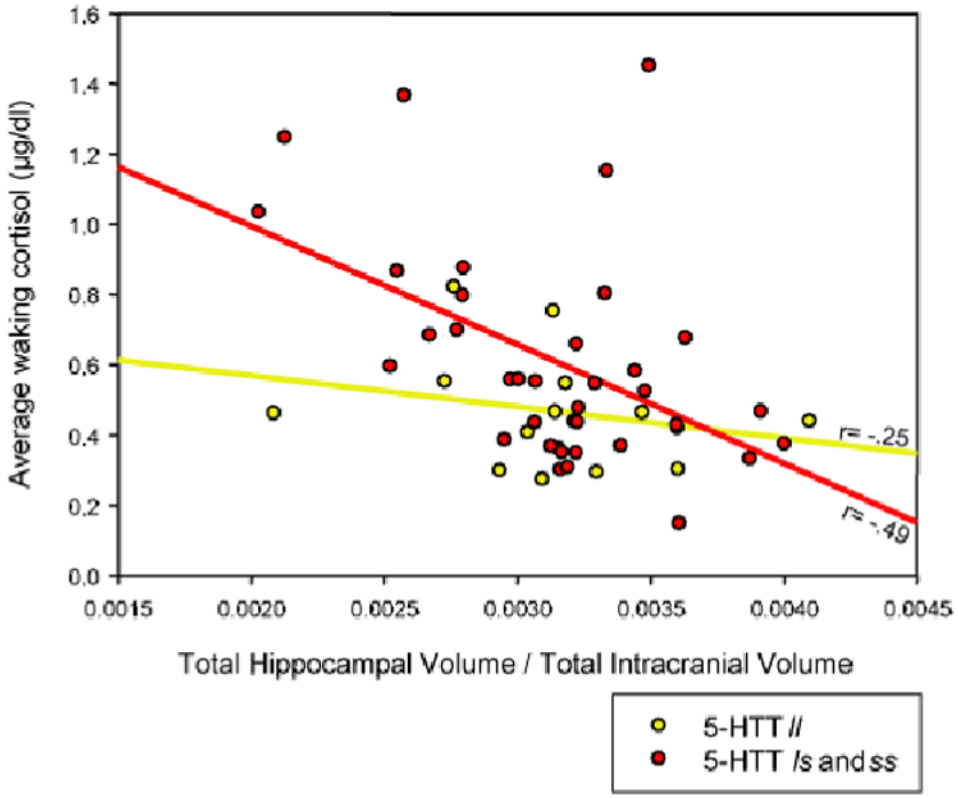

Figure 4.
Association of waking cortisol and hippocampal volume as determined by structural MRI (total hippocampal volume corrected for total intracranial volume) as a function of 5-HTT genotype (*ll* vs. *ls*/*ss*).

Table 1

5-HTT Genotype Distribution and Mean Values for Demographics and Predictor Variables

	***ll* (n=45)**	***ls* (n=82)**	***ss* (n=27)**	***P* Values**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**p**
Age, y	70.1 (9.09)	71.9 (8.9)	70.4 (8.2)	.38
Years of Education, y	16.3 (3.6)	16.0 (2.44)	16.4 (2.82)	.67
GDS	6.2 (6.0)	6.8 (5.2)	6.3 (6.0)	.42
Life Stress	11.9 (10.36)	9.7 (7.21)	11.7 (10.04)	.55
Waking Cortisol, μg/dl	0.49 (0.24)	0.64 (0.39)	0.72 (0.35)	.01
Cortisol Slope	−0.12 (0.07)	−0.13 (0.07)	−0.15 (0.05)	.20

Abbreviations: *l*, long allele; *s*, short allele; GDS, Geriatric Depression Scale.

Table 2

Means and Standard Deviations for Cognitive Tests by 5-HTT Allele Group

	***ll* (n=45)**	***ls* (n=82)**	***ss* (n=27)**	***P* Values**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**p**
RAVLT Delayed Recall	10.1 (3.68)	8.8 (4.29)	7.1 (4.08)	.003
SCW	145.45 (43.36)	155.96 (42.38)	166.741 (96.24)	.30
JLO	24.1 (3.82)	23.6 (3.73)	24.3 (3.88)	.51
BNT	56.7 (3.94)	56.5 (3.94)	57.3 (3.89)	.35

Abbreviations: 5-HTT, serotonin transporter; *l*, long allele; *s*, short allele; RAVLT, Rey Verbal Learning Test; SCW, Stroop Color and Word; JLO, Judgement of Line Orientation; Boston Naming Test.

Table 3

Mean Values for structural MRI variables dependent on *s* allele carrier status.

	***ll* (n=16)**	***ls* and *ss* (n=40)**	***P* Values**
	Mean (SD)	**Mean (SD)**	**p**
Total Hippocampal Volume (cc)	4.356(.587)	4.316 (.571)	.82
Total Intracranial Volume (cc)	1414.312 (110.031)	1387.135 (128.985)	.46
Gray Matter Volume (cc)	628.311 (52.408)	628.159 (66.176)	.99
White Matter Volume (cc)	427.780 (35.581)	424.417 (49.415)	.81
Cerebrospinal Fluid (cc)	286.106 (48.260)	271.358 (50.128)	.34

Abbreviations: *l*, long allele; *s*, short allele; MRI, Magnetic Resonance Imaging.

Table 4

Multiple Regression with RAVLT Delayed Recall as the Dependent Variable

	Coefficient	Std. Error	t	p
Waking Cortisol	−0.699	0.282	−2.48	0.01
Cortisol Slope	−20.565	6.285	−3.27	0.001
5-HTT genotype	−1.910	0.637	−3.00	0.003
Life Stress	0.012	0.048	0.24	0.81
Waking Cortisol X Cortisol Slope	−3.977	4.056	−0.98	0.33
Waking Cortisol X 5-HTT genotype	−0.937	0.474	−1.98	0.05
Waking Cortisol X Life Stress	−0.041	0.038	−1.07	0.29

Abbreviations: RAVLT: Rey Auditory Verbal Learning Test; 5-HTT, serotonin transporter.